AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

8000 Westpark Drive, Suite 130
McLean, Virginia 22102 (until 12/31/93)

60 Revere Drive
Suite 500
Northbrook, Illinois 60062 (effective 1/1/94)

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Editor, *Tropical Medicine and Hygiene News*	Karl A. Western
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Martin S. Wolfe

Sub-Committee on Computer Applications

George Childs
John Huggins

ASTMH STANDING COMMITTEES

•**Awards:** John R. David, MD (Chair); Scott B. Halstead, MD; and Donald J. Krogstad, MD.

•**Commemorative Fund Lectureship:** Barnett L. Cline, MD, PhD, Chair.

•**Continuing Medical Education:** Jay Keystone, MD, Chair (1992-1994); David Hill, MD, (1991-1993); Phyllis Kozarsky, MD (1991-1994); Douglas McPherson (1994-1996); Mary E. Wilson (1994-1996); Ralph Bryan (1994-1995); and Elaine Jong, MD (1993-1995).

•**Corporate Liaison Committee:** Joseph Perrone, PhD, Chair: (1993-1995); Kent Campbell, MD (1993-1994); John Horton (1993-1994); Robert Lennox, PhD (1993-1994); Thomas Monath, MD (1933-1996); Francine Perler, PhD (1993-1996); Donald J. Krogstad, MD *ex officio* (1993-1995); and Richard Burk, Jr. *ex officio* (1993-1994).

•**Editoral Board,** *American Journal of Tropical Medicine and Hygiene*: McWilson Warren, PhD (Editor); Mark Eberhard, PhD (Associate Editor); Allison Kitfield (Associate Editor); Ronald L. Anthony, PhD; Abram S. Benenson, MD; Donald S. Burke, MD; Charles H. Calisher, PhD; Allen W. Cheever, MD; Daniel H. Connor, MD; James L. Hardy, PhD; Stephen L. Hoffman, MD; James M. Hughes, MD; David J. Jacobus, MD; Rodney C. Jung, MD; David Kaslow, MD; Jay S. Keystone, MD; Llewellyn J. Letgers, MD; Steven R. Meshnick, MD, PhD; Franklin A. Neva, MD; Steve Reed, PhD; Robert E. Shope, MD; Mette Strand, PhD; Diane Taylor, PhD; Bryce C. Walton, PhD; and Thomas Yuill, PhD.

•**Education:** Dickson Despommier, PhD, Chair (1993-1995); Peter Schantz, VMD, PhD (1991-1993); and Jay Keystone, MD (1991-1993).

•**Gorgas Memorial Institute Fellowship:** Stephanie L. James, PhD, Chair (1993-1995).

•**Honorary Membership:** Louis H. Miller, MD, Chair; Franz von Lichtenberg, MD; and Karl M. Johnson, MD.

•**Lecture (Fred L. Soper and Charles F. Craig):** Barry Beaty, PhD, Chair (1991-1993); Kenneth Bart, MD; Larry Laughlin, MD; and Leon Jacobs, PhD.

•**Local Organizing:** Peter Schantz, DVM, PhD (Chair).

•**Membership:** Cynthia L. Chappel, PhD, Chair (1993-1995); Rosamund Wendt (1993-1994); Gerhard Schad, PhD (1993-1994); David O. Freedman, MD (1993-1994); Peter F. Weller, MD (1993-1995); Jose Ribeiro, MD, PhD (1993-1995); and Barbara Herewaldt (1993-1995).

•**Nominations:** Thomas E. Wellems, MD (Chair); Dickson Despommier, PhD; Stephanie L. James, PhD; Steven R. Meshnick, MD, PhD. Carol A. Nacy, PhD; Steve Reed, PhD, and Mette Strand, PhD.

•**Public Affairs, Legislative Action:** John R. David, MD (Co-Chair) and Stephanie R. Sagebiel, MS (Co-Chair).

•**Scientific Program:** William A. Petri, MD, PhD (Chair).

•**Young Investigator:** Ronald Blanton, MD, Chair (1993-1995); Thomas Unnasch, PhD (1990-1993); William O. Rogers, MD (1991-1993); and James LeDuc, PhD (1991-1993).

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- **Certification in Tropical Medicine and Travelers' Health:** Michele Barry, MD, Chair; Donald J. Krogstad, MD; Richard L. Guerrant, MD; Leonard J. Marcus, DVM, MD; Jay Keystone, MD; and Steven L. Hoffman, MD.

- **Governance:** Peter F. Weller, MD, Chair, Joseph A. Cook, MD; Donald J. Krogstad, MD; Robert E. Shope, MD; and Richard Burk, Jr.

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ASP STANDING COMMITTEES

- **Awards:** Timothy Y. Yoshino (Chair), Albert O. Bush, Daniel G. Colley, Wieslaw J. Kozek, and Peter W. Pappas.

- **Bueding-Von Brand Memorial Endowment Fund:** George A. Conder (Chair), Janine N. Caira, William C. Campbell, W. Eugene Foor, and Judy Sakanari.

- **Business Advisory:** Jane A. Starling (Chair), Alan A. Marchiondo, Gerald W. Esch, Danny Pence, and Lillian F. Mayberry *ex officio*.

- **Editorial Board,** *The Journal of Parasitology*: Martin L. Adamson, Daniel R. Brooks, William C. Campbell, Bruce M. Christensen, D.W.T. Crompton, William L. Current, Terry A. Dick, Gerald W. Esch, Jacob K. Frenkel, Robert B. Grieve, W. Michael Kemp, Raymond E. Kuhn, J. Ralph Lichtenfels, Philip T. LoVerde, Austin J. MacInnis, John S. Mackiewicz, Leo Margolis, Janice K. Moore, Miklos Muller, Robin M. Overstreet, Danny B. Pence, Marilyn E. Scott, Clarence A. Speer, Jane A. Starling.

- **Education:** Thomas R. Platt (Chair), David Bruce Conn, George D. Cain, Jaqueline C. Fernandez, Eugene G. Hayunga, John A. Oaks.

- **Honorary & Emeritus Members:** Paul P. Weinstein (Chair), Annie Katherine Prestwood, Howard J. Saz.

- **In Memoriam:** Jeffrey M. Lotz (Chair), Mary E. Doscher, Eric S. Loker.

- **Meeting Site Selection:** Louis S. Diamond (Chair), Lee Couch, Martin L. Adamson.

- **Membership:** Thomas R. Klei (Chair), Patricia C. Augustine, Eain M. Cornford, Richard W. Komuniecki.

- **Newsletter:** George L. Stewart (Chair), Karl A. Western, Sherwin S. Desser, K. Darwin Murrell, H. Ray Gamble, Lillian F. Mayberry, J. Richard Seed.

- **Nominating:** Leo Margolis (Chair), Patricia C. Augustine, George A. Conder, Mike Moser, Charles R. Sterling, Albert O. Bush.

- **Priorities of ASP:** J. Ralph Lichtenfels (Chair), Ann Adams, Timothy G. Geary, Peter J. Hotez, Eric P. Hoberg, Patricia R. Komuniecki, Mike Moser.

- **Public Responsibilities:** J. Richard Seed (Chair), W. Michael Kemp, Gerhard A. Schad, Gerald W. Esch.
- **Special Awards:** John C. Holmes (Chair), David R. Thompson, John M. Hawdon.

- **Student Awards:** James W. Tracy (Chair), John M. Aho, Miodrag Belosevic, Malcom R. Powell, Robert L. Rausch.

- **Stoll-Stunkard Memorial Endowment Fund:** Philip T. LoVerde (Chair), Marilyn E. Scott, George D. Cain, Raymond E. Kuhn.

- **Tellers:** Eugene G. Hayunga (Chair), John H. Cross, Willis A. Reid, Jr.

- **Translations:** Maurice D. Little, Hisao P. Arai, Leo Margaolis, Robert L. Rausch, Clive Schiff.

Ad Hoc Committees

- **Biodiversity:** James L. Mau (Chair), John R. Barta, George Davis, Mary Hanson Pritchard.
- **Clinical Laboratory Standards:** Lynne S. Garcia (Chair), John H. Cross, Robert L. Edwards, Ruth Leventhal.
- **Local Arrangements Committee:** Victor C.W. Tsang (Chair), Floy Brandt, Annie Katherine Prestwood, Byron Blagburn.
- **Outreach:** Michael V.K. Sukhdeo (Chair), Byron L. Blagburn, Joseph F. Urban, Jr., Mark C. Jenkens.
- **Industrial Relations:** George A. Conder (Chair), Joseph A. Cook, Ann R. Donoghue, Jorge Guerrero, Alan A. Marchiondo, Wesley L. Shoop.
- **Student Travel Grants:** Bernard Fried (Chair), J.P. Dubey, Ann R. Donoghue, Donald W. Hosier.
- **By-Law Review:** Steve G. Kayes (Chair), Austin J. MacInnis, Robert R. Short.
- **Keynote Speaker Program:** Gerald W. Esch (Chair), Judy Sakanari, Austin J. MacInnis, Dennis J. Minchella, Dennis A. Thoney, John R. Bristol, Rick L. Tarleton, Janice K. Moore, Timothy Goater.
- **Animal Rights Issues:** Gerhard A. Schad (Chair), W. Michael Kemp, Philip T. LoVerde, Ronald Fayer.
- **ASP Fellows Program:** J. Ralph Lichtenfels (Chair), Jay P. Farrell, William C. Campbell, Barbara L. Doughty.
- **Special Events Program:** Eric P. Hoberg (Chair), Daniel R. Brooks, James H. McKerrow, Donald L. Wassom, Edwin C. Rowland.
- **Parasitic Genetic Resources:** Daniel R. Brooks (Chair), Steven A. Nadler, Wesley L. Shoop, Thomas A. Nerad, J. Ralph Lichtenfels.
- **Parasitology Literature for Foreign Scientists:** Eugene G. Hayunga (Chair), Peter W. Pappas, Robert L. Rausch.
- **Stedmans ASP Parasite Words Book:** John E. Ubelaker (Chair), J. Ralph Lichtenfels, Mary Hanson Pritchard, Robin M. Overstreet.

REGISTRATION INFORMATION

Foyer Area, Hyatt Regency

Sunday	October 31	2:00 PM - 7:00 PM
Monday	November 1	7:30 AM - 5:00 PM
Tuesday	November 2	7:30 AM - 5:00 PM
Wednesday	November 3	7:30 AM - 12:00 N

SPONSORS

U.S. Army Medical Research & Development Command
The Rockefeller Foundation
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EXHIBITORS

Academia Book Exhibit
Berna Products Corp.
Biorad Labs
Circadian Travel Technologies, Inc.
Dako
Immunetics
Integrated Diagnostics
Kirkegaard & Perry Labs, Inc.
Seradyn
Smith Kline Beecham

TIME AND PLACE OF EXHIBITS

Regency Ballroom - Phoenix, Terrace Level

Sunday	October 31	3:00 PM - 7:00 PM
Monday	November 1	9:00 AM - 3:30 PM
Tuesday	November 2	9:00 AM - 3:30 PM
Wednesday	November 3	7:30 AM - 12:00 N

ARCHIVE EXHIBIT

The 1993 ASTMH Archives exhibit, in the Flemish Suite will be open from 8-5, Monday thru Thursday. The exhibit will commemorate the 100th anniversary of the Walter Reed Institute of Research. Historic photographs, artifacts and videotapes from the WRAIR collection, as well as items from the ASTMH archives, will highlight the contributions of military scientists in diseases from yellow fever to smallpox to AIDS. This exhibit is designed to complement the Walter Reed seminar scheduled for 8-12 Thursday.

LATE BREAKERS IN BIOLOGY AND MOLECULAR BIOLOGY

This Session is specifically designed for brief presentation of important, new data obtained after the closing date for abstract submission. Presentations are restricted to 5 minutes plus 5 minutes discussion time. Submit abstracts of 200 words or less to:

Dr. B. Kim Lee Sim
Department of Immunology
WRAIR
Washington DC 20307 - 5100
Phone: (202) 576-0867 Fax: (202) 576-0748

prior to the Meeting or at the Meeting, but no later than 5:00 PM, Tuesday, November 2, 1993. The list of presenters will be posted Wednesday morning. Check the bulletin board at the Meeting for further information.

AUDIOVISUAL FACILITIES

Slide preview and submission facilities are provided in the Spanish Suite beginning 1:00 PM on Sunday, October 31. Speakers scheduled for AM sessions should preview slides and place them in carousels on the afternoon before their scientific presentation. Speakers scheduled for afternoon sessions should prepare slides on the morning of their presentation.

MESSAGES AND EMERGENCY CALLS

A message board will be available near the Registration Desk, Hyatt Regency Hotel. Emergency calls should be directed to (404) 577-1234, the main switchboard of the hotel.

EMPLOYMENT OPPORTUNITIES

Bulletin boards for posting employment opportunities will be available in the Registration area.

POSTER PRESENTATIONS

Poster Sessions on Wednesday, November 3 and Thursday, November 4 will be located in the Regency Ballroom (Falcon/Condor) on the Terrace level of the Hyatt. A Continental Breakfast will be served for the Wednesday session and a buffet lunch for the Thursday session. Posters may be set up beginning at 7:00 PM on the night before the session or in the morning before the session begins. Numbers on poster boards correspond to abstract numbers in the Program Booklet. Authors should be in attendance for the entire 2 hour period at each poster session. Posters should be taken down during the lunch hour or afternoon following the session.

Poster Boards are 4 x 8 ft in size. Pins and numbers will be available in the presentation hall.

CONTINUING MEDICAL EDUCATION

INFORMATION ON CME FOR PRE-MEETING WORKSHOP IS LISTED IN THE WORKSHOP PROGRAM

The American Society of Tropical Medicine & Hygiene is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians.

The American Society of Tropical Medicine & Hygiene designates this continuing medical education activity for up to 30 credit hours in Category 1 of the Physician's Recognition Award of the American Medical Association.

Attendees desiring CME credit are advised to preregister for this activity (see materials mailed in the Registration Package) or to register at the ASTMH Registration Desk whereupon they will receive instructions and required forms. Documentation fees will be collected. Certificates based on recorded attendance at medical education activities will be mailed within one month of the meeting.

NOTICES

Badges must be worn to attend all functions.

Smoking will be permitted only where specifically authorized. This rule is in compliance with the Resolution on Smoking adopted at the ASTMH Annual Business Meeting on November 5, 1976. The cooperation and thoughtfulness of smokers is requested to minimize embarrassment and discomfort for all persons.

The time and/or location of all activities are subject to change. Change notices will be posted in the Registration area.

Suggestions for changes in the Annual Meetings may be directed to members of the Scientific Program Committee.

ASTMH 1994 ANNUAL MEETING

The 1994 Annual Meeting of the American Society of Tropical Medicine and Hygiene will be held November 13-17 at the Hyatt Regency Hotel, Cincinnati, Ohio.

The Deadline for Abstract submission will be 1 June 1994. A revised Abstract Submission computer software package will be available in early 1994.

The executive offices for ASTMH starting in 1994 will be moved to the Sherwood Group, where requests for abstract discs and questions about the 1994 meeting should be referred to at 60 Revere Drive, Suite 500, Northbrook, Illinois 60062 (phone 708/480-9080; FAX 708/480-9282). Watch for announcements and order forms, which will be mailed with the Journal and in the Call for Abstracts booklet in the winter of 1994.

ASP 1994 ANNUAL MEETING

The 1994 Annual Meeting of the American Society of Parasitologists will be held August 8-13 in Fort Collins, Colorado.

ASTMH TRAVEL GRANT AWARDEES

Sponsored by

U.S. Army Medical Research and Development Command

Dr MJ Doenhoff	University of Wales, Bangor, United Kingdom
Dr T Lehmann	University of Arizona, Tucson
Dr AL Omara-Opyene	University of Calgary, Canada
Dr UR Rao	University of South Florida, Tampa
Dr P Kittayapong	Yale University, New Haven, Connecticut
Dr CS Toebe	Tulane University, New Orleans, Louisiana
Dr L Povinelli	Tulane University, New Orleans, Louisiana
Dr F Balderrama	Univ. Mayor de San Simon, Cochabamba, Bolivia
Dr J. Bermudez	Univ. Mayor de San Simon, Cochabamba, Bolivia
Dr F. Torrico	Univ. Mayor de San Simon, Cochabamba, Bolivia
Dr P Prociv	University of Queensland, Brisbane, Australia
Dr J Hernandez-Sanchez	Cntr. Research & Advanced Studies, Mexico City
Dr S Green	University of Massachusetts, Worcester
Dr C-A Lobo	Tata Institute, Bombay, India
Dr O Tomori	University College Hospital, Ibadan, Nigeria
Dr G Calderon	Inst. Enferm. Virales Humanas, Buenos Aires, Argentina

ASP STUDENT TRAVEL GRANT AWARDEES

Sponsored by

The American Society of Parasitologists

Behrouz Ahvazi	McGill University, Montreal, Quebec, Canada
Ruth Barratt	Johns Hopkins University, Baltimore, MD
Elida G. Campos	McGill University, Ste-Anne de Bellevue, Quebec, Canada
Rebecca A. Cole	Auburn University, Auburn, AL
Barbara J. Davids	University of Wisconsin, Madison, WI
Philip M. Manger Jr.	University of Wisconsin, Madison, WI
John C. Mullican	University of Nebraska Medical Center, Omaha, NE
James F. Smothers	Idaho State University, Pocatello, ID
Gabrielle A. Stryker	Johns Hopkins University, Baltimore, MD
Denise M. Toney	Virginia Commonwealth University, Richmond, VA
Surang Triteeraprapab	Johns Hopkins University, Baltimore, MD
Kristy Wolff	Johns Hopkins University, Baltimore, MD

SUMMARY OF

SCIENTIFIC PROGRAM

SATURDAY 30 OCTOBER

8:00- 5:00	Tropical Medicine Update	Lancaster

SUNDAY 31 OCTOBER

8:00 - 1:00	Tropical Medicine Update	Lancaster
8:00 - 5:00	ASTMH Council Meeting	Essex A
8:00 - 5:00	ASP Council Meeting	Essex B
1:00 - 5:00	SIRACA Subcommittee of ACAV	Italian Suite
1:00 - 4:00	**Coccidiosis Conference:** Parasite-Host Interactions	York/Stuart
2:00 - 5:00	Tour of Centers for Disease Control and Prevention	Meet in Foyer
2:00 - 7:00	**Registration**	Foyer Area
3:00 - 7:00	**Exhibits**	Regency Ballroom (Phoenix)
4:00 - 5:30	ACAV Executive Council	Austrian Suite
4:30 - 5:30	ACME Council	English Suite
4:00 - 4:30	Graduate student mixer	Lancaster D
4:30 - 6:30	**Student workshop:** Beyond Journals & Articles - Writing and Publishing Books	Lancaster B/C
6:00 - 9:00	**Opening Halloween Reception** (Costumes Encouraged!)	Regency Ballroom (Falcon/Condor)

MONDAY NOVEMBER 1 - MORNING

6:30 - 7:45	Scientific Program Committee Breakfast	Grecian Suite
7:30 - 5:00	**Registration**	Foyer area
8:00 - 11:00	**ASTMH-ASP Plenary Symposium**	Regency Ballroom (Falcon/Condor)
9:00 - 3:30	**Exhibits**	Regency Ballroom (Phoenix)
11:10 - 12 :00	**Commemorative Fund Lecture**	Regency Ballroom (Falcon/Condor)
11:10 - 12 :00	**Stoll-Stunkard Lecture**	Lancaster B/C

MONDAY NOVEMBER 1 - AFTERNOON

1:00 - 5:00	**Symposium**: Endemic Parasites in the US	Essex A/B
1:00 - 4:30	**American Committee on Medical Entomology**	Lancaster D
1:00 - 3:00	**Scientific Session A:** Amebiasis	English Suite
1:00 - 3:00	**Scientific Session B:** Filarial Biology	Lancaster C
1:00 - 5:45	**Scientific Session C:** ASP Students I	Lancaster E
1:00 - 4:15	**Scientific Session D:** Malaria Molecular Biology	York
1:00 - 3:00	**Scientific Session E:** Malaria Epidemiology and Diagnosis	Stuart
1:00 - 3:30	**Scientific Session F:** Arbovirus Vaccines	Austrian Suite
1:30 - 5:30	**Symposium**: Granulomatous Inflammation in Schistosomiasis	Lancaster A/B
1:30 - 5:00	**Symposium**: Cysticercosis and Taeniasis	Tudor Room
3:30- 5:15	**Scientific Session G:** Kinetoplastidae: Molecular Biology	English Suite
3:30 - 5:30	**Symposium**: Update on Hepatitis A	Lancaster C
5:15 - 6:30	**ICTDR Directors' Meeting**	Italian Suite
5:15 - 6:30	**Francophone Investigators Social Hour**	French Suite

MONDAY NOVEMBER 1 - EVENING

7:00 - 10:00	**Annual Parasitology Auction**	Regency Ballroom (Falcon/Condor)

SUMMARY OF SCIENTIFIC PROGRAM

TUESDAY NOVEMBER 2 - MORNING

7:00 - 9:00	American Journal of Tropical Medicine and Hygiene Editorial Board Breakfast	Italian Suite
7:00 - 8:00	Past Presidents Breakfast	French Suite
7:30 - 5:00	**Registration**	Foyer area
8:00 - 12:00	**Symposium:** Drug Development for Opportunistic Infections in AIDS	Lancaster A/B
8:00 - 12:15	**Scientific Session H:** Schistosomiasis: Immunology	Lancaster C
8:00 - 11:45	**Scientific Session I:** Filariasis: Therapy and Immunology	Lancaster D
8:00 - 9:45	**Scientific Session J:** Rickettsia and Bacterial Diseases	English Suite
8:00 - 11:15	**Scientific Session K:** Molecular Entomology	York
8:00 - 11:30	**Scientific Session L:** Malaria Immunology - I	Stuart
8:00 - 12:00	**Scientific Session M:** ASP Students II	Lancaster E
8:00 - 9:45	**Scientific Session N:** Arbovirus Epidemiology	Tudor
8:00 - 12:00	**Scientific Session O:** Development, Life History, and Systematics of Parasites	Austrian Suite
9:00 - 12:00	**Symposium:** Transfection	Essex A/B
9:00 - 3:30	**Exhibits**	Regency Ballroom (Phoenix)
10:30 - 12:00	**Scientific Session P:** Lyme Disease	English Suite
10:30 - 12:30	**Scientific Session Q:** Arbovirus Pathogenesis and Molecular Virology	Tudor

TUESDAY NOVEMBER 2 - AFTERNOON

1:30 - 2:30	**ASTMH Presidential Address**	Regency Ballroom (Falcon/Condor)
1:30 - 3:30	**Symposium:** Molecular Parasitology	Lancaster A/B
2:30 - 4:30	**ASTMH Awards/Business Meeting**	Regency Ballroom (Falcon/Condor)
3:45 - 5:45	**Scientific Session R:** ASP Students III	Lancaster A/B
4:30 - 5:30	**Discussion of Diploma Program**	Regency Ballroom

WEDNESDAY NOVEMBER 3 - MORNING

7:30 - 5:00	**Registration**	Foyer area
7:30 - 10:00	**Poster Session I** (Coffee and donuts provided)	Regency Ballroom (Falcon/Condor)
7:30 - 12:00	**Exhibits**	Regency Ballroom (Phoenix)
9:30 - 12:30	**Scientific Session S:** Filariasis: Immunopathology and Protection	York
9:30 - 12:00	**Symposium:** Case Management of Pediatric Illness	English
9:30 - 12:00	**Symposium:** Emerging Viruses	Tudor
9:30 - 11:45	**Scientific Session T:** Schistosomiasis: Molecular Biology and Biochemistry	Stuart
9:30 - 12:00	**Scientific Session U:** Clinical Tropical Medicine I	Lancaster A/B
9:30 - 11:45	**Scientific Session V:** Opportunistic Protozoa	Lancaster C
9:30 - 12:00	**Scientific Session W:** Kinetoplastidae - Epidemiology and Chemotherapy	Lancaster D
9:30 - 12:00	**Scientific Session X:** Severe Malaria	Essex A/B
9:30 - 12:00	**Scientific Session Y:** Parasite Biochemistry and Physiology	Lancaster E
10:00 - 12:00	**Symposium:** Parasitology and Biodiversity	Italian Suite

SUMMARY OF SCIENTIFIC PROGRAM

WEDNESDAY NOVEMBER 3 - AFTERNOON

1:30 - 2:15	**ASTMH Soper Lecture**	Regency Ballroom (Condor)
1:30 - 2:30	**ASP Presidential Address**	Regency Ballroom (Falcon)
2:30 - 6:30	**American Committee on Arbovirology:** Hantavirus ARDS in North America	Regency Ballroom (Condor)
2:30 - 5:00	**Symposium:** Protozoan-Host Cell Interactions	Lancaster D
2:30 - 6:00	**American Committee on Clinical Tropical Medicine and Travelers Health**	Lancaster A/B
2:30 - 4:45	**Scientific Session Z:** Giardiasis and Cryptosporidiosis	Lancaster C
2:30 - 4:15	**Scientific Session AA:** Kinetoplastidae: Immunology andPathology	York
2:30 - 5:00	**Scientific Session BB:** Malaria Chemotherapy	Tudor
2:30 - 4:15	**Scientific Session CC:** Malaria Biology	Essex A/B
2:30 - 3:30	**ASP Ward Medal, Student Awards**	Regency Ballrooom (Falcon)
3:30 - 4:30	**ASP Business Meeting**	Regency Ballroom (Falcon)
4:30 - 5:30	R.B. McGhee Memorial Lecture	Regency Ballroom (Falcon)

WEDNESDAY NOVEMBER 3 - EVENING

7:00 - 10:00	**ASP-ASTMH Banquet**	The Carter Center

THURSDAY NOVEMBER 4 - MORNING

7:30 - 12:00	**Registration**	Foyer area
7:00 - 9:00	ASTMH Council Breakfast	French Suite
8:00 - 10:30	**Scientific Session DD:** Cestodes	Lancaster D
8:00 - 12:00	**Symposium:** 100th Anniversary of The Walter Reed Army Institute of Research	Essex A/B
8:30 - 12:00	**Symposium:** Cytokines & Infection	Lancaster A/B
8:00 - 12:00	**Scientific Session EE:** Late Breakers	Tudor
8:00 - 12:00	**American Committee Medical Malacology**	Lancaster C
8:00 - 11:45	**Scientific Session FF:** Filariasis: Epidemiology and Diagnosis	English
8:00 - 11:30	**Scientific Session GG:** Immunology of Parasites	York
8:00 - 11:30	**Scientific Session HH:** Malaria Immunology II: Vaccines	Stuart
8:00 - 10:15	**Scientific Session II:** Viral Diseases	Grecian Suite
8:00 - 12:00	**Scientific Session JJ:** Schistosomiasis - Epidemiology and Clinical aspects	Lancaster E

THURSDAY NOVEMBER 4 - AFTERNOON

12:00 - 2:00	**Poster Session II** (Lunch provided; Recognition of ASTMH Young Investigators)	Regency Ballroom

DETAILED

SCIENTIFIC PROGRAM

CONTINUING MEDICAL EDUCATION COURSE

TROPICAL MEDICINE UPDATE - 1993

A Bench to Bedside Perspective on New and Re-emerging Tropical Infectious Diseases

Saturday, October 30, 1993 — Lancaster A/B/C
8:00 AM - 5:00 PM

Sunday, October 31, 1993 — Lancaster A/B/C
8:30 AM - 1:00 PM

Sponsored by the American Committee on Clinical Tropical Medicine and Travelers' Health (ACCTMTH) in association with the National Center for Infectious Diseases (NCID), Centers for Disease Control and Prevention (CDC).

Course Directors: Ralph T. Bryan, David O. Freedman
Administrative Assistant: Susan L. Stokes (404) 639-3670

In a one and a half day program, the latest developments in pathogenesis, diagnosis, management, epidemiology, and prevention of a spectrum of new and re-emerging tropical infectious diseases will be revealed. Familiarity by participants with basic concepts in tropical medicine would be helpful. Lectures will be presented by leading experts from the Society and NCID/CDC in addition to invited experts from overseas. The course will begin and end with one hour keynote addresses, each presented by a pre-eminent leader in the field of tropical diseases. This advanced level pre-meeting workshop is intended for tropical medicine practitioners, infectious diseases consultants, clinical microbiologists, and any interested medical students and physicians-in-training.

The American Society of Tropical Medicine & Hygiene is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians.

The American Society of Tropical Medicine & Hygiene designates this continuing medical education activity for 10.5 credit hours in Category 1 of the Physician's Recognition Award of the American Medical Association.

Attendees desiring CME credit are advised to preregister for this activity (see materials mailed in the Registration Package) or to register at the ASTMH Registration Desk whereupon they will receive instructions and required forms. Documentation fees will be collected. Certificates based on recorded attendance at medical eduction activities will be mailed within one month of the meeting

To register for the course, please contact Ms. Susan Burk (Tel.: (703) 790-1745, Fax (703) 790-9063) or refer to the August isue of *Tropical Medicine and Hygiene News*.. Registration is $95 for ACCTMTH members, $110 for ASTMH/ASP members at large, $200 for non-ASTMH/ASP members, and $60 for students and PGY-1-3 housestaff (with letter of verification).

COCCIDIOSIS CONFERENCE
PARASITE-HOST INTERACTIONS: IMMUNITY AND EVASION MECHANISMS

Sunday, October 31, 1993 York/Stuart
1:00 - 4:00 PM
Chair: MC Jenkins

1:00 S1 ALTERNATIVE PATHWAYS OF IFN-γ PRODUCTION AND PROTECTIVE IMMUNITY INDUCED BY AN ATTENUATED *TOXOPLASMA GONDII* VACCINE. Gazzinelli RT*, Denkers E, Wysocka M, Trinchieri G, and Sher A. Immunology and Cell Biology Section, Laboratory of Prasitic Diseases, NIAID - NIH, Bethesda, MD; and Wistar Institute, Philadelphia, PA.

1:30 S2 INDUCTION OF IMMUNITY TO *TRICHURIS SUIS* IN SWINE USING EXCRETORY/SECRETORY PRODUCTS FROM ADULT WORMS. Hill DE* and Urban JF. Biosystematic Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

2:00 S3 CASEIN KINASE II IS CONSTITUTIVELY EXPRESSED IN BOVINE LYMPHOCYTES TRANSFORMED BY INTRACELLULAR PROTOZOAN PARASITE *THEILERIA PARVA*. ole-MoiYoi OK, Brown WC, Iams KP, Nayar A, and Maclin MD. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya; Department of Microbiology and Parasitology, Texas A&M university, College Station, TX; Department of of Physiology, University of Alberta, Calgary, Alberta, Canada; Department of Chemistry, Indiana University, Bloomington, IN; and Agracetus, Middleton, WI.

2:30 S4 THE ROLE OF NATURAL KILLER CELLS, IL-12 AND IFN-γ IN RESISTANCE TO *LEISHMANIA MAJOR*. Scott P*. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.

3:00 S5 IMMUNIZATION OF MICE WITH IRRADIATED *PLASMODIUM YOELII* SPOROZOITES. Sedegah M*, Weiss WR, Charoenvit Y, Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda MD.

3:30 S6 VACCINATION WITH RADIATION-ATTENUATED CERCARIAE OF *SCHISTOSOMA MANSONI*: ROLE OF CYTOKINES IN THE PROTECTIVE IMMUNE RESPONSE. Wilson A. Department of Biology, University of York, York, UK.

STUDENT WORKSHOP: BEYOND JOURNALS & ARTICLES - WRITING AND PUBLISHING BOOKS

Sunday, October 31, 1993 Lancaster B/C
4:30 - 6:25 PM
Chairs: N. Hollebeke, P. Olson, W. Mosier

4:30 WELCOME. Hollebeke N. University of Texas, El Paso.

4:40 S7 WRITING SCHOLARLY BOOKS: DON'T QUIT YOUR DAY JOB. Brooks D. Department of Zoology, University of Toronto.

5:00 S8 A TASTE OF WRITING TEXTBOOKS. Roberts L. Department of Biological Sciences, Florida International University, Miami.

5:20 S9 THE TRADE BOOK MARKET: DOORSTEP TO THE WORLD'S MIND. Janovy J. School of Biological Sciences, University of Nebraska, Lincoln.

5:40 S10 THE PUBLIC-PUBLISHING PARASITOLOGIST. Desowitz R. Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu.

6:00 PANEL DISCUSSION

6:20 CLOSING . Hollebeke N. University of Texas, El Paso.

PLENARY SYMPOSIUM

Monday, November 1, 1993 Regency Ballroom
8:00 AM - 11:00 AM (Falcon/Condor)
Chairs: W. Petri, Jr. and G. Esch

8:00 RECOGNITION OF ASTMH & ASP TRAVEL GRANT AWARDEES. LeDuc JW and Duszynski DW

8:05 S11 WHAT CAN GENETICS AND TRANSFECTION DO FOR THE PERSON INTERESTED IN PARASITE **BIOLOGY**: A CASE STUDY WITH *TOXOPLASMA GONDII.* Boothroyd JC. Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA

8:30 S12 VECTOR COMPETENCE IN MOSQUITO-BORNE FILARIASIS. Christensen BM. College of Agriculture and Life Sciences, University of Wisconsin, Madison WI. *(Introduced by MG Peck, Burroughs Wellcome Fund).*

8:55 S13 HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE: A PARADIGM FOR SELECTIVE ANTIPARASITIC CHEMOTHERAPY. Ullman B. Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR. *(Introduced by MG Peck, Burroughs Wellcome Fund).*

9:20 S14 ON BEING A PARASITE IN AN INVERTEBRATE HOST: A SHORT COURSE IN SURVIVAL. Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM.

9:45 S15 BIOLOGY OF THE MALARIA VECTOR *ANOPHELES GAMBIAE* IN BAMAKO AND BETHESDA. Vernick KD*, Lanzaro GL, Toure YT, Traore S, and Miller LH. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD; and National School of Medicine and Pharmacy, Bamako, Mali, West Africa.

10:10 Coffee Break

10:30 S16 PREGNANCY AND TROPICAL MEDICINE: A MULTI-DISCIPLINARY APPROACH. Lucas AO. Department of Population and International Health, Harvard School of Public Health, Boston MA.

COMMEMORATIVE FUND LECTURE

Monday, November 1, 1993
11:10 AM
Chair: E. Otteson
S17

Regency Ballroom
(Falcon/Condor)

LYMPHATIC FILARIAL DISEASE:
THE POWER OF THE CLINIC TO UNRAVEL THE MYSTERIES

Dreyer G.
Centro de Pesquisas Aggeu Magalhaes, Cidade Universitaria
Recife, Brazil.

STOLL-STUNKARD LECTURE

Monday, November 1, 1993
11:10 AM
Chair: G. Cain
S18

Lancaster B/C

PARASITIC PROTISTS AND
THE SEARCH FOR OUR MICROBIAL ANCESTORS

Sogin M.
Center for Molecular Evolution, Marine Biological Laboratories
Woods Hole, Massachusetts

SYMPOSIUM:
ENDEMIC PARASITES OF IMPORTANCE IN THE UNITED STATES–AN EMERGING REALIZATION

Monday, November 1 Essex A/B
1:00 - 5:00 PM
Chair: T. Nash

1:00 INTRODUCTION. Nash T. National Institutes of Health, Bethesda, MD

1:15 S19 CRYPTOSPORIDIOSIS: WHO'S AT RISK. Juranek DD. CDC, Atlanta.

1:35 S20 RECENT ADVANCES IN THE IMMUNOBIOLOGY OF CRYPTOSPORIDIOSIS. Riggs M. University of Arizona, Tucson, AZ.

1:55 S21 EPIDEMIOLOGIC FEATURES OF *GIARDIA* INFECTIONS IN THE UNITED STATES. Addiss DG. CDC, Atlanta.

2:10 S22 *GIARDIA*: WHAT'S IT ALL ABOUT. Nash TE. NIH, Bethesda, MD.

2:30 S23 *CYCLOSPORA*: THE NEW BUG ON THE BLOCK. Sterling CR. University of Arizona, Tucson, AZ.

2:50 Coffee Break

3:20 S24 *TRICHOMONAS VAGINALIS*: EPIDEMIOLOGY OF A STEALTH PATHOGEN. Cotch MF. Research Triangle Institute, Rockville, MD.

3:40 S25 MOLECULAR AND CELLULAR BIOLOGY OF THE HUMAN-INFECTIVE PARASITE *TRICHOMONAS VAGINALIS*. Johnson P. UCLA School of Medicine, Los Angeles, CA.

4:00 S26 MICROSPORIDIOSIS IN THE UNITED STATES: AN EPIDEMIOLOGIC AND CLINICAL UPDATE. Bryan RT. CDC, Atlanta.

4:30 S27 TOXOPLASMOSIS IN THE 90'S. Kovacs JA. NIH, Bethesda, MD.

9th ANNUAL MEETING
AMERICAN COMMITTEE ON MEDICAL ENTOMOLOGY

MALARIA VECTOR BIOLOGY

Monday, November 1 Lancaster D
1:00 - 5:00 PM
Chairpersons: J. Beier and M. Shahabuddin

1:00 1 CRITICAL PARAMETERS OF CULTURED *PLASMODIUM FALCIPARUM* GAMETOCYTES THAT AFFECT INFECTIONS IN *ANOPHELES GAMBIAE* MOSQUITOES. Noden BH, Beadle P, Vaughan JA, and Beier JC. Department of Immunology and Infectious Diseases, The Johns Hopkins University, Baltimore, MD.

1:15 2 SPOROGONIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN SIX SPECIES OF: LABORATORY-INFECTED *ANOPHELES* MOSQUITOES. Vaughan JA*, Noden BH,

and Beier JC. Immunology & Infectious Diseases, School of Hygiene & Public Health, Johns Hopkins University, Baltimore, MD.

1:30 3 FACTORS AFFECTING THE INFECTIVITY OF MALARIA SPOROZOITES. Beier JC*, Pumpuni CB, and Mendis C. Department of Immunology and Infectious Diseases, The Johns Hopkins University, Baltimore MD.

1:45 4 LONG-TERMSURVIVAL OF *PLASMODIUM* SPOROZOITES *IN VITRO*. Pumpuni CB* and Beier JC. Department of Immunology and Infectious Diseases, The Johns Hopkins Univeristy, Baltimore MD.

2:00 5 PERITROPHIC MATRIX (PM) AS A DETERMINANT OF MALARIA PARASITE SPECIFICITY FOR MOSQUITO VECTOR. Shahabuddin M* and Kaslow DC. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; and National Institutes of Health, Bethesda, MD.

2:15 6 GENETIC ASPECTS OF SUSCEPTIBILITY OF *AEDES AEGYPTI* TO *PLASMODIUM GALLINACEUM*. Thathy V*, Severson DW, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

2:30 7 CHROMOSOMAL MAPPING OF GENETIC LOCI ASSOCIATED WITH *PLASMODIUM GALLINACEUM* SUSCEPTIBILITY IN *AEDES AEGYPTI*. Severson DW*, Thathy V, Mori A, Zhang Y, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

VECTOR FIELD BIOLOGY

2:45 8 FIELD RELEASES TO INTRODUCE TROPICAL TRAITS INTO A TEMPERATE POPULATION OF *AEDES STEGOMYIA ALBOPICTUS*. Mutebi JP*, Craig Jr. GB, and Novak RJ. Vector Biology Lab., Department. of Biological Science, University of Notre Dame, Notre Dame, IN; and Illinois Natural History Survey, Champaign, IL.

3:00 Coffee Break

3:30 9 VECTOR COMPETENCE OF *AEDES ALBOPICTUS* FROM PINE BLUFF, ARKANSAS, FOR A ST. LOUIS ENCEPHALITIS VIRUS STRAIN ISOLATED DURING THE 1991 EPIDEMIC. Savage HM*, Smith GC, and Mitchell CJ. Medical Entomology-Ecology Branch, DVBID, NCID, Centers for Disease Control and Prevention, Fort Collins, CO.

3:45 10 APPARENT CHANGES IN THE DISTRIBUTION AND ABUNDANCE OF MALARIA VECTORS IN GRENADA. Manguin S*, Peyton EL, James AC, and Roberts DR. Preventive Medicine & Biometrics, Uniformed Services University of Health Sciences, Bethesda, MD; Walter Reed Biosystematics Unit, Department of Entomology, Walter Reed Army Institute of Research, Washington DC; and Ministry of Health, St George's, Grenada.

4:00 11 THE USE OF REMOTE SENSING AND LANDSCAPE FEATURES TO ACCURATELY PREDICT THE PRESENCE AND ABUNDANCE OF TWO MALARIA VECTORS IN FOOTHILL AREAS OF BELIZE. Roberts DR*, Paris JF, Manguin S, Harbach RE, Woodruff R, Rejmankova E, Polanco J, and Legters LJ. Uniformed Services University of the Health Sciences, Bethesda, MD; Department of Biology, California State University, Fresno, CA; Smithsonian Institution, Washington, DC; University of California, Davis, CA; and Ministry of Health, Belize City, Belize.

4:15 12 PROPOSAL FOR EXPERIMENTAL FIELD EVALUATION OF ENVIRONMENTAL METHOD TO CONTROL VECTOR-BORNE DISEASES AROUND MANANTALI RESERVOIR IN WESTERN MALI. Jobin WR*. Director of Blue Nile Associates, Foxboro, MA.

4:30 ACME Business Meeting

SCIENTIFIC SESSION A: AMEBIASIS

Monday, November 1 English Suite
1:00 - 3:00 PM
Chairpersons: S. Stanley and S. Reed

1:00 13 *ENTAMOEBA HISTOLYTICA* STIMULATES TNF-α AND C-FOS GENE EXPRESSION IN MACROPHAGES THROUGH PROTEIN KINASE C SIGNAL TRANSDUCTION. Seguin RM*, Keller K, and Chadee K. Institute of Parasitology, McGill University, Quebec, Canada.

1:15 14 THREE ISOFORMS OF AMOEBAPORE, THE PORE-FORMING PEPTIDE OF PATHOGENIC *ENTAMOEBA HISTOLYTICA*. Leippe M*, Andrä J, Tannich E, and Müller-Eberhard HJ. Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, F.R. Germany.

1:30 15 CLONING OF AN *ENTAMOEBA HISTOLYTICA* GENE (EHVMA3) ENCODING THE PUTATIVE PROTEOLIPID OF A VACUOLAR MEMBRANE-ATPASE. Descoteaux S, Yu Y, Lohia A, and Samuelson J *. Department. of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department. of Biochemistry, Bose Institute, Calcutta, India.

1:45 16 EXPRESSION OF AN ACTIVE, RECOMBINANT CYSTEINE PROTEINASE OF *ENTAMOEBA HISTOLYTICA*. Reed SL*, Que X, Herdman DS, Hirata KK, Torian BE, and McKerrow JH. Departments of Pathology and Medicine, University of California, San Diego, CA; Department of Pharmaceutical Sciences, Idaho State University, Pocatello, Idaho; and Department of Pathology, VA Medical Center, San Francisco, CA.

2:00 17 IN VITRO GALACTOSE SPECIFIC BINDING OF RECOMBINANT *ENTAMOEBA HISTOLYTICA* ADHESION LECTIN MAPS TO THE CYSTEINE-RICH REGION OF THE 170 KDA HEAVY SUBUNIT. Wan P *, Ravdin JI, and Kain KC. Tropical Disease Unit, Division of Infectious Diseases, The Toronto Hospital, Toronto, Canada; and Department of Medicine, Case Western Reserve University School of Medicine and VA Medical Center, Cleveland, OH.

2:15 18 RECOMBINANT AND NATIVE *ENTAMOEBA HISTOLYTICA* 29KDA ANTIGEN DEMONSTRATES SPECIFICITY FOR AMEBIC LIVER ABSCESS. Soong G*, Abd-Alla MD, Kain KC, Jackson TF, Torian BE, and Ravdin JI. Case Western Reserve University and the Cleveland VA, Cleveland, OH; University of Toronto and The Toronto Hospital, Toronto, Canada; Medical Research Council (Natal), Natal, South Africa; and Idaho State University, Pocatello, ID.

2:30 19 ANTIBODIES TO THE SERINE RICH *ENTAMOEBA HISTOLYTICA* PROTEIN (SREHP) PREVENT AMEBIC LIVER ABSCESS IN SCID MICE. Tonghai Z, Cieslak PR, Foster L, Kunz-Jenkins C, and Stanley, Jr. SL*. Department of Medicine, Washington University School of Medicine; Departments of Medicine and Molecular Microbiology, Washington University School of Medicine.

2:45 20 EPIDEMIOLOGY OF INVASIVE AMEBIASIS IN A FAVALA IN FORTALZA, BRAZIL. Braga LL*, Mann BJ, Sears C, Wuhib T, Newman R, Lima A, Guerrant R, and Petri WA. Universidade Federal do Ceara, Fortaleza, Brazil; Johns Hopkins University School of Medicine, Baltimore, MD; and University of Virginia, Charlottesville, VA.

SCIENTIFIC SESSION B: FILARIAL BIOLOGY

Monday, November 1 Lancaster C
1:00 - 3:00 PM
Chairpersons: A. Bianco and R. Chandrashekar

1:00 21 HOMOLOGUES OF SMALL HEAT SHOCK PROTEINS THAT ARE CONSTITUTIVELY EXPRESSED IN FILARIAE. Lillibridge D* and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

1:15 22 SUPPRESSION OF REPRODUCTIVE CAPACITY IN MALE *DIROFILARIA IMMITIS* BY PROPHYLACTIC TREATMENT WITH MILBEMYCIN OXIME. Lok JB*, Knight DH, Selavka CM, and Bergman RN. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; and Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

1:30 23 MOLECULAR PHYLOGENETIC STUDIES ON FILARIAL PARASITES. Xie H*, Bain O, and Williams SA. Program in Molecular and Cellular Biology, University of Massachusetts at Amherst, MA; Laboratoire de Zoologie-Vers, Museum National d'Histoire Naturelle, Paris, France; and Department of Biological Sciences, Smith College, Northampton, MA.

1:45 24 MOLECULAR CLONING AND NUCLEOTIDE SEQUENCING OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT OF *ONCHOCERCA VOLULUS*. Egwang TG* and Ajuh PM. Centre Internationale de Recherches Medicales de Franceville (CIRMF), Franceville, Gabon.

2:00 25 IDENTIFICATION OF POTENTIAL NUCLEAR HORMONE RECEPTORS IN *DIROFILARIA IMMITIS* AND *CAENORHABDITIS ELEGANS*. Richer JK*, Hough DM, and Maina CV. New England Biolabs, Beverly, MA.

2:15 26 CYCLOPHILIN-LIKE PROTEIN OF THE FILARIAL NEMATODE *BRUGIA MALAYI*. Page AP* and Carlow CK. New England BioLabs, 32 Tozer Road, Beverly, MA.

2:30 27 CHARACTERIZATION OF THE GENE AND CDNA FOR *ONCHOCERCA VOLVULUS* EXTRACELLULAR SUPEROXIDE DISMUTASE. James ER* and McLean DC. Department of Ophthalmology, Medical University of South Carolina, Charleston, SC.

2:45 28 EFFECTIVENESS OF CGI 18041 AGAINST *BRUGIA PAHANGI* IN BEAGLES WITH INDUCED LYMPHATIC INFECTIONS. Dzimianski MT*, McCall JW, Supakorndej P, and Jun JJ. Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, GA.

SCIENTIFIC SESSION C:
ASP STUDENT PRESENTATIONS - I

Monday, November 1 Lancaster E
1:00 - 5:45 PM
Chairpersons: J.R. Barta and L. Couch

1:00 29 MORPHOMETRICS OF THE LYME DISEASE VECTOR, *IXODES SCAPULARIS* SAY, 1821, INCLUDING THE JUNIOR SUBJECTIVE SYNONYM, *I. DAMMINI* SPIELMAN *ET AL*, 1979. Hutcheson HJ*, Oliver JH. Inst. of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

1:15 30 A COMPARISON OF YOLK PROTEINS FROM THE EGGS OF A HARD TICK, *AMBLYOMMA AMERICANUM* AND A SOFT TICK, *ORNITHODOROS PARKERI*. Dudley CK*, James AM, and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

1:30 31 ABILITY OF THE TICKS *IXODES SCAPULARIS, AMBLYOMMA AMERICANUM* AND *DERMACENTOR VARIABILIS* TO TRANSMIT *BORRELIA BURGDORFERI* FROM FLORIDA. Sanders FH* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, Georgia.

1:45 32 CHARACTERIZATION AND LOCALIZATION OF VITELLIN FROM THE LYME DISEASE VECTOR, *IXODES SCAPULARIS* SAY (ACARI: IXODIDAE). James AM* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, Georgia.

2:00 33 FACTORS INFLUENCING THE EIMERIAN COMMUNITY IN A FREE-LIVING POPULATION OF TOWNSEND'S GROUND SQUIRRELS (*SPERMOPHILUS TOWNSENDII*). Wilber PG*, Hanelt B, and Duszynski DW. Department of Biology, The University of New Mexico, Albuquerque, NM.

2:15 34 DEVELOPMENT OF IMMUNITY TO *EIMERIA ARIZONENSIS* BY DEERMICE (*PEROMYSCUS MANICULATUS*): A COMPARISON OF LABORATORY AND FIELD RESULTS. Fuller CA*. Department of Zoology, Oregon State University, Corvallis, OR.

2:30 35 TRANSPLACENTAL TRANSMISSION OF *NEOSPORA CANINUM* IN BITCHES INFECTED DURING EARLY PREGNANCY. Cole RA*, Lindsay DS, Dubey JP, Sorjonen DC, and Blagburn BL. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL; AL and ARS, USDA, Beltsville, MD.

2:45 36 *GIARDIA LAMBLIA* INFECTION IN CHILDREN FROM THE STATE OF TAMAULIPAS, MEXICO: A PRELIMINARY STUDY. Faulkner CT* and Patton S. Department of Environmental Practice, University of Tennessee College of Veterinary Medicine, Knoxville, TN.

3:00 Coffee Break

3:30 37 THE EFFECTS OF ODONATE INTERMEDIATE HOST ECOLOGY ON THE LEVELS OF LARVAL TREMATODE INFECTION: IN THE WRONG PLACE AT THE WRONG TIME? Wetzel EJ* and Esch GW. Biology Department, Wake Forest University, Winston-Salem, NC.

3:45 38 MARKOVIAN CHAIN DYNAMICS OF PARASITE PREVALENCE IN THE KANGAROO RAT, *DIPODOMYS MERRIAMI*. Patrick MJ*. Department of Biology, The University of New Mexico, Albuquerque, NM.

4:00 39 THE STRENGTH OF SPATIAL AND TEMPORAL HETEROGENEITY AS STRUCTURING FORCES OF THE PARASITE COMMUNITIES IN *HELISOMA ANCEPS* AND *PHYSA GYRINA*. Sapp KK* and Esch GW. Biology Department, Wake Forest University, Winston-Salem, NC.

4:15 40 SEASONAL RECRUITMENT AND MATURATION OF *BOTHRIOCEPHALUS ACHEILOGNATHI* IN LOUISIANA MOSQUITOFISH *GAMBUSIA AFFINIS*. Mars CL* and Font WF. Department. of Biological Sciences, Southeastern Louisiana University, Hammond, LA.

4:30 41 PYLORIC CECA VS. ANTERIOR INTESTINE AS SUITABLE HABITAT FOR *LEPTORHYNCHOIDES THECATUS* (ACANTHOCEPHALA) IN GREEN SUNFISH (*LEPOMIS CYANELLUS*). Richardson DJ*. School of Biological Sciences, University of Nebraska, Lincoln, Nebraska.

4:45 42 THE USE OF A GEOGRAPHIC INFORMATION SYSTEM (GIS) TO ANALYZE THE DISTRIBUTION OF THE HUMAN SCHISTOSOME-TRANSMITTING SNAILS IN KENYA. Boyce TG*, Rizor DD, and Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM; and University of New Mexico Technology Application Center, Albuquerque, NM.

5:00 43 TRANSMISSION OF *LEPTORHYNCHOIDES THECATUS* (ACANTHOCEPHALA) THROUGH BASS AND GREEN SUNFISH POPULATIONS. Olson PD*. School of Biological Sciences, University of Nebraska.

5:15 44 HOST-SPECIFICITY AMONG SPECIES OF *HAEMATOLOECHUS*. Snyder SD*. School of Biological Sciences, University of Nebraska, Lincoln, NE.

5:30 45 RETINOIDS IN NEMATODE DEVELOPMENT. Wolff KM*, Scott AL. Immunology and Infectious Diseases, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

SCIENTIFIC SESSION D:
MALARIA MOLECULAR BIOLOGY

Monday, November 1 York
1:00 - 4:15 PM
Chairpersons: D.J. Bzik and D.C. Kaslow

1:00 46 SEQUENCE VARIATION IN THE CS GENE OF *PLASMODIUM VIVAX*. Mann VH*, Huang TY, Cheng Q, Bustos D, Huang YM, and Saul A. Malaria and Arbovirus Unit, Queensland Institute of Medical Research, Brisbane, Qld. Australia; Guizhou Provincial Institute of Parasitic Diseases, Guiyang, Guizhou, P. R. China; and Research Institute for Tropical Medicine, Metro Minila, Philippines; and Guang Xi Institute of Parasitic Diseases Control, Nanning, Guang Xi, P. R. China.

1:15 47 IMMNOCLONING OF A LIVER STAGE ANTIGEN OF *PLASMODIUM VIVAX* CROSS-REACTING WITH ANTI-*PLASMODIUM CYNOMOLGI* LIVER STAGE ANTIBODIES.

Yang C*, Nelson C, Collins WE, Pieniazek NJ, David PH, and Millet P. Department of Pathology, Emory University School of Medicine, Atlanta, GA; Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Department of Immunology, Institut Pasteur, Paris, France.

1:30 48 BINDING DOMAINS OF THE DUFFY ANTIGEN BINDING PROTEINS OF *PLASMODIUM VIVAX* AND *P. KNOWLESI*. Chitnis CC* and Miller LH. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; National Institutes of Health, Bethesda, MD.

1:45 49 THE MICRONEME PROTEIN-1 (DUFFY BINDING PROTEIN) OF *PLASMODIUM VIVAX* IS POLYMORPHIC IN CLINICAL ISOLATES FROM PAPUA NEW GUINEA. Tsuboi T, AL-Yaman F, Alpers MP, and Adams JH*. Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana; Papua New Guinea Insitute of Medical Research, Madang, Papua New Guinea; and Papua New Guinea Insitute of Medical Research, Goroka, Papua New Guinea.

2:00 50 BINDING DOMAIN OF EBA-175, A *PLASMODIUM FALCIPARUM* LIGAND FOR INVASION INTO ERYTHROCYTES. Sim KL*, Chitnis C, and Miller LH. Laboratory of Malaria Research, National Institute of Allegy & Infectious Diseases, National Institutes of Health, Bethesda, MD.

2:15 51 THE LARGEST SUBUNITS OF *PLASMODIUM FALCIPARUM* NUCLEAR RNA POLYMERASE HAVE UNIQUE FEATURES. Bzik DJ* and Fox BA. Department of Microbiology, Dartmouth Medical School, Hanover, NH.

2:30 52 ADENINE NUCLEOTIDE TRANSLOCATOR OF *PLASMODIUM FALCIPARUM*. Dyer M *, Wong H, Huynh P, Jackson M, and Mikkelsen RB. Departments of Radiation Oncology and Microbiology/Immunology, Medical College of Virginia, Richmond, VA.

2:45 53 SEQUENCE ANALYSIS AND PHYLOGENETIC MAPPING OF A *PLASMODIUM VIVAX* PROTEIN KINASE. Dimayuga FO and Levitt A. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY.

3:00 Coffee Break

3:30 54 IDENTIFICATION OF A NOVEL *PLASMODIUM FALCIPARUM* SEXUAL STAGE PROTEIN WITH HOMOLOGY TO Pgs28. Duffy PE and Kaslow DC. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD; and Department of Immunology, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

3:45 55 CLUSTERED AMINO ACID VARIATION IN PFS230 FROM DIFFERENT ISOLATES OF *PLASMODIUM FALCIPARUM*. Williamson KA* and Kaslow DC. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; and National Institutes of Health, Bethesda, MD.

4:00 56 TRANSFECTION OF *PLASMODIUM GALLINACEUM* ZYGOTES AND EXPRESSION OF FIREFLY LUCIFERASE. Goonewardene R, Daily J*, Kaslow D, Sullivan TJ, Duffy P, Carter R, Mendis K, and Wirth D. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Malaria Research Unit, Department of Parasitology, University of Colombo, Colombo, Sri Lanka; National Institutes of Health, Malaria Section, Bethesda, Maryland; and Institute of Animal Genetics, Edinburgh, Scotland.

SCIENTIFIC SESSION E:
MALARIA EPIDEMIOLOGY AND DIAGNOSIS

Monday, November 1 Stuart
1:00 - 3:00 PM
Chairpersons: J. Zucker and K. Baird

1:00 57 KINETICS OF MATERNAL ANTIBODIES TO MSP-1 OF *PLASMODIUM FALCIPARUM* IN INFANTS BORN IN A MALARIA ENDEMIC AREAS OF PAPUA NEW GUINEA. Kramer K, Sehgak V, Alpers M, Hui G, and Chang S.

1:15 58 ACQUISITION OF *PLASMODIUM FALCIPARUM* INFECTION AND DEVELOPMENT OF ANEMIA IN INFANTS IN WESTERN KENYA. Bloland PB*, Ruebush TK, Boriga DA, Nahlen BL, Oloo AJ, and McCormick JB. Malaria Branch, Division of Parasitic Disease, NCID, Centers for Disease Control, Atlanta, GA; and Vector Biology and Control Research Center, Kenya Medical Research Institute, Kisumu, Kenya.

1:30 59 EVALUATION OF ILLNESS INDICATORS IN A PEDIATRIC POPULATION-KENYA, 1993. Paxton LA*, Zucker JR, Steketee RW, Nahlen BL, Olongo C, Oloo A, and Campbell CC. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta, Georgia; and Kenya Medical Research Institute; and Siaya District Hospital, Kenya.

1:45 60 COST-EFFECTIVENESS OF ANTIMALARIAL REGIMENS DELIVERED THROUGH ANTENATAL CLINICS TO PREVENT MALARIA DURING PREGNANCY. Schultz LJ*, Steketee RW, Wirima JJ, Macheso A, and Chitsulo L. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta, GA; and Malawi Ministry of Health, Malawi.

2:00 61 PRESUMPTIVE VS DIRECTED TREATMENT OF UNCOMPLICATED MALARIA IN ADULT MALAWIANS: RELATIVE COSTS. Jonkman A, Chibwe RA, Khoromana CO, Liabunya UL, Chaponda ME, Kandiero SE, and Taylor TE*. Queen Elizabeth Central Hospital, Blantyre, Malawi; and College of Osteopathic Medicine, Michigan State University, East Lansing, MI.

2:15 62 MALARIA CONTROL AND INFANT AND CHILD MORTALITY: COMPARING THE IMPACT OF DECREASING TRANSMISSION VERSUS PREVENTION OF SERIOUS DISEASE. Courval JM* and Singer B. Program in Epidemiology, Michigan State University, East Lansing, MI; and School of Public Health, Yale University, New Haven, CT.

2:30 63 FIELD EVALUATION OF THE SENSITIVITY AND SPECIFICITY OF A RAPID DIPSTICK ANTIGEN-CAPTURE ASSAY FOR THE DETECTION OF *PLASMODIUM FALCIPARUM*. McElroy PD*, Long GW, Beadle C, Maret SM, Weiss WR, Oloo AJ, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Johns Hopkins University School of Hygiene and Public Health; Becton Dickinson Co., Baltimore, MD; Naval Medical Research Institute-Detachment, Kisumu, Kenya; U.S. Army Medical Research Unit-Kenya, Nairobi, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya

2:45 64 A SIMPLE METHOD FOR THE DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA IN RURAL SETTINGS. THE RAPID MANUAL PARASIGHT®-TEST. Premji J*, Minjas JN, and Shiff CJ. Muhimbli University College of Health Sciences. Dar es Salaam, Tanzania; and The Johns Hopkins University, Baltimore, MD.

SCIENTIFIC SESSION F: ARBOVIRUS VACCINES

Monday, November 1 Austrian Suite
1:00 - 5:00 PM
Chairpersons: T.P. Monath and S.J. Green

1:00 65 CLINICAL TRIALS OF LIVE ATTENUATED DENGUE VACCINE CURRENT STATUS. Bhamarapravati N*, Yoksan S, and Angsubhakorn S. Mahidol University, Bangkok, Thailand.

1:15 66 FEASIBILITY OF A DENGUE VACCINE EFFICACY TRIALS IN NORTHERN THAILAND. Vaughn DW*, Nisalak A, Kozik CA, Snitbhan R, Kunasol P, Suntayakorn S, Pinyopornpanich S, Poopatanakul W, and Innis BL. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Communicable Diseases, Ministry of Public Health, Bangkok, Thailand; and Kamphaeng Phet Provincial Hospital and Department of Health, Kamphaeng Phet.

1:30 68 ANALYSIS OF A MINIMAL CTL EPITOPE ON DENGUE VIRUS NS3 PROTEIN RECOGNIZED BY MURINE DENGUE-SPECIFIC T CELLS. Rothman AL*, Kurane I, Dai L, and Ennis FA. Division of Infectious Diseases & Immunology, University of Massachusetts Medical Center, Worcester, MA.

1:45 69 RECOGNITION OF DENGUE VIRUS ENVELOPE (E) PROTEIN BY SEROTYPE-SPECIFIC CD4+ CD8- CYTOTOXIC T LYMPHOCYTES. Livingston PG, Kurane I*, Lai CJ, and Ennis FA. Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical Center, Worcester, MA; and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

2:00 70 AN ANALYSIS OF DENGUE VIRUS-SPECIFIC CD4+ CYTOTOXIC T CELL CLONES DERIVED FROM A RECIPIENT OF AN EXPERIMENTAL LIVE-ATTENUATED DENGUE 1 VACCINE. Green S*, Kurane I, Edelman R, Tacket CO, Zeng L, Brinton M, Pincus S, Paoletti E, and Ennis FA. Division of Infectious Disease and Immunology, University of Massachusetts Medical Center, Worcester, MA, Center for Vaccine Development, University of Maryland, Baltimore, MD, Department of Biology, Georgia State University, Atlanta, Georgia, Virogenetics Corporation, Troy, NY.

2:15 71 CYTOKINE RESPONSES TO DENGUE INFECTION AMONG PUERTO RICAN PATIENTS. Kuno G* and Bailey RE. Division of Vector-Borne Infectious Diseases, CDC, San Juan, PR; Division of Vector-Borne Infectious Diseases, CDC, Ft. Collins, CO.

2:30 72 A CANDIDATE VACCINE AGAINST ROSS RIVER VIRUS. Yu S, and Aaskov J*. WHO Colaborating Centre for Arbovirus Reference and Research, Centre for Molecular Biotechnology, School of Life Science, Queensland University of Technology, Brisbane, Queensland, Australia.

2:45 73 ALPHAVIRUS INTERACTIONS: SUPPRESSION OF IMMUNE RESPONSE TO VEE VACCINE IN PERSONS PREVIOUSLY VACCINATED WITH VEE OR WEE VACCINES. Pittman PR*, Makuch RS, Cannon T, and Gibbs P. Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Biometrics & Information Management Division, USAMRIID, Fort Detrick, Frederick, MD.

3:00 74 DOSE-SEEKING STUDY WITH A LIVE, ATTENUATED JUNIN VIRUS VACCINE CANDID #1, LOT 2, IND 2257. Makuch RS*, Barrera-Oro J, Lewis T, Rossi C, Higgins Y, Mangiafico J, Schmaljohn A, and Sjogren MH. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; and Salk Institute, Clearwater, PA.

SYMPOSIUM: *TAENIA SOLIUM* CYSTICERCOSIS AND TAENIASIS: COMMUNITY BASED STUDIES AND INTERVENTION

Monday, November 1, 1993 Tudor Room
1:30 - 5:00 PM
Chairs: F. Beltran and A. Flisser

1:30 S28 THE IDRC RESEARCH NETWORK ON THE EPIDEMIOLOGY OF CYSTICERCOSIS. AN INTRODUCTION. Wijeyaratne P. IDRC, Ottawa, Canada.

1:35 S29 IMMUNOBLOT SURVEYS SHOW *TAENIA SOLIUM* CYSTICERCOSIS TO BE A SIGNIFICANT GLOBAL PUBLIC HEALTH PROBLEM. Tsang V. CDC, Atlanta.

1:55 S30 WHAT ARE WE LEARNING FROM COMMUNITY-BASED EPIDEMIOLOGIC STUDIES OF TAENIASIS AND CYSTICERCOSIS? Schantz P. CDC, Atlanta.

2:15 S31 FIELD APPLICATION OF AN ELISA FOR *TAENIA SOLIUM* COPRO-ANTIGEN DETECTION. Allan J. University of Salford, UK.

2:35 S32 DEVELOPMENT OF HEALTH EDUCATION INTERVENTION FOR THE CONTROL OF HUMAN TAENIASIS AND CYSTICERCOSIS IN RURAL MEXICO. Bronfman M. El Colegio de Mexico, Mexico D.F.

2:55 Coffee Break

3:30 S33 EVALUATION OF TWO INTERVENTION STRATEGIES FOR THE CONTROL OF TAENIA SOLIUM IN THREE RURAL COMMUNITIES IN MEXICO. Sarti E. Direccion General de Epidemiologia, SSa, Mexico D.F.

3:30 S34 THE COMPLEX EPIDEMIOLOGY OF TAENIASIS/CYSTICERCOSIS IN PERUVIAN COAST, JUNGLE AND HIGHLANDS. Garcia H. Universidad Peruana Cayetano Heredia, Lima, Peru.

3:50 S35 PREVALENCE, DISTRIBUTION AND MORBIDITY OF *TAENIA SOLIUM* IN AN ANDEAN COMMUNITY IN ECUADOR. Cruz M. Academia Ecuatoriana de Neurociencias, Quito, Ecuador.

4:30 DISCUSSION

SYMPOSIUM:
INDUCTION AND REGULATION OF GRANULOMATOUS INFLAMMATION IN SCHISTOSOMIASIS

Monday, November 1 Lancaster A/B
1:30 - 5:30 PM
Chairs: D.G. Colley and M.J. Stadecker

1:30 INTRODUCTORY REMARKS. Colley DG. CDC, Atlanta.

1:40 S36 MOLECULAR SIGNALS FOR GRANULOMA FORMATION AND LOCALIZATION IN SCHISTOSOMIASIS. McKerrow J. University of California--San Francisco, San Francisco, CA.

2:00 S37 CHARACTERIZATION OF HUMAN T CELL CLONES THAT INITIATE IN VITRO GRANULOMA FORMATION. Doughty B. Texas A & M University.

2:20 S38 EARLY EVENTS IN THE HOST RESPONSE TO SCHISTOSOME EGG ANTIGENS. Pearce E. Cornell University, Ithaca, NY.

2:40 S39 CYTOKINES AND THE SCHISTOSOMAL EGG GRANULOMA. Boros D. Wayne State University.

3:00 S40 EVOLVING CYTOKINE EXPRESSION IN *SCHISTOSOMA MANSONI* EGG-INDUCED GRANULOMA FORMATION. Chensue S. Michigan.

3:20 Coffee Break

3:50 S41 REGULATION OF EGG-INDUCED TH-2 CYTOKINE RESPONSES BY IL-12. Oswald I. NIH, Bethesda, MD.

4:10 S42 THE SCHISTOSOMAL EGG GRANULOMA: ANTIGENS, ANTIGEN-PRESENTING CELLS AND T CELLS. Stadecker M. Tufts University, Boston, MA.

4:30 S43 THE ROLE OF SPECIFIC OLIGOSACHARIDES N DIRECTING CD4+ T CELL SUBSETS. Harn D. Harvard University, Boston, MA.

4:50 S44 NEUROPEPTIDE REGULATION OF GRANULOMATOUS INFLAMMATION. Weinstock J. Iowa.

5:10 DISCUSSION.

SCIENTIFIC SESSION G:
KINETOPLASTIDAE: MOLECULAR BIOLOGY AND BIOCHEMISTRY

Monday, November 1 English Suite
3:30 - 5:15 PM
Chairpersons: M.E. Wilson and S. Jeronimo

3:30 75 RNA VIRUSES OF *LEISHMANIA BRAZILENSIS*: THE COMPLETE cDNA SEQUENCE OF LRV1-4. Scheffter SM*, Widmer G, and Patterson JL. Infectious Diseases, Children's Hospital, Boston MA; Microbiology and Molecular Genetics, Harvard

Medical School, Boston, MA; and Tufts University School of Veterinary Medicine, N. Grafton, MA.

3:45 76 HETEROGENEITY OF gp63 PROTEINS IN *LEISHMANIA CHAGASI*. Roberts SC, Donelson JE, Streit JA, and Wilson ME. Department of Biochemistry, The University of Iowa, Iowa City, IA; Department of Medicine, The University of Iowa, Iowa City, IA.

4:00 77 *LEISHMANIA* AMASTIGOTES ADHERE TO HEPARAN SULFATE PROTEOGLYCANS ON MAMMALIAN CELLS. Love DC* and Mosser DM. Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA.

4:15 78 EGF-SENSITIVE PHOSPHORYLATION OF THE 90 KD EGF-RECEPTOR HOMOLOGUE OF *TRYPANOSOMA CRUZI* AMASTIGOTES. Freeman-Junior P* and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

4:30 79 DETECTION OF MINICIRCLE DNA FROM THE NATURALLY DYSKINETOPLASTIC *TRYPANOSOMA EVANSI* BY PCR. Lun ZR*, Lu LX, and Desser SS. Department of Biology, Zhongshan University, Guangzhou, P.R. China; and Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

4:45 80 CHARACTERIZATION OF *LEISHMANIA DONOVANI* CATION TRANSPORTING ATPASE TRANSCRIPTS. Meade JC*, Kong L, and Hicock PI. Division of Parasitology, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS.

5:00 81 CLONING AND CHARACTERIZATION OF TWO DISTINCT CYSTEINE PROTEASE GENES IN *LEISHMANIA DONOVANI CHAGASI* . Omara-Opyene AL*, Ismail SO, Bhatia A, and Gedamu L. Department of Biological Science, University of Calgary, Canada.

SYMPOSIUM
UPDATE ON HEPATITIS A: DIAGNOSIS AND PREVENTION

Monday, November 1 Lancaster C
3:30-5:30
Chair: E. Jong

3:30 S45 INTRODUCTION. Jong E. University of Washington School of Medicine, Seattle, WA.

3:40 S46 HEPATITIS A--A CLINICAL OVERVIEW. Koff RS. Boston University School of Medicine, Boston, MA.

4:10 S47 EPIDEMIOLOGY OF HEPATITIS A AND GROUPS AT RISK IN THE US. Shapiro C. CDC, Atlanta.

4:40 S48 HEPATITIS A AND THE TRAVELER: RISK AND PREVENTION (ISG VS. VACCINE). Wolfe M. George Washington University Medical School, Washington, DC.

5:00 PANEL DISCUSSION.

SYMPOSIUM:
DRUG DEVELOPMENT FOR OPPORTUNISTIC INFECTIONS ASSOCIATED WITH AIDS

Tuesday, November 2, 1993 Lancaster A/B
8:00 - 12:00 Noon
Chairs: A.S. Fairfield, C. Lambros, and B. Laughon

8:00 S49 OVERVIEW OF SYMPOSIUM GOALS. Laughon B. Division of AIDS, NIAID, NIH.

8:15 S50 CRYPTOSPORIDIOSIS: NEW TARGETS FOR CHEMOTHERAPY. Nelson RG*. Medicine and Pharmaceutical Chemistry, San Francisco General Hospital, San Francisco, CA .

8:45 S51 *PNEUMOCYSTIC CARINII* PNEUMONIA: RECENT ADVANCES IN EXPERIMENTAL AND CLINICAL CHEMOTHERAPY. Gutteridge WE*. Wellcome Research Laboratories, Langley Court, Beckenham, Kent, UK.

9:15 S52 TOXOPLASMOSIS: RECENT ADVANCES IN CHEMOTHERAPY. Derouin F*. Lab. Parasitol-Mycologie, Hopital Saint Louis, Paris, France.

9:45 Coffee Break

10:15 S53 THE MYCOBACTERIA: RAPID *IN VITRO* DRUG SCREENING OF SYNTHETIC AND NATURAL PRODUCTS. Franzblau S*. GWL Hansen's Disease Center, Baton Rouge, LA.

10:45 S54 MICROSPORIDIOSES: PREVALENCE AND PROSPECTS FOR TREATMENT. Canning EU*, Hollister WS, Colbourn NI, and Silveira H. Department of Biology, Imperial College of Science, Technology and Medicine, London, UK.

11:15 S55 NEW THERAPIES FOR OPPORTUNISTIC PATHOGENS: NIAID RESOURCES AND OPPORTUNITIES. Laughon BE*, Fairfield AS, and Lambros C. Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda MD.

11:45 Discussion.

SCIENTIFIC SESSION H:
SCHISTOSOMIASIS: IMMUNOLOGY

Tuesday, November 2 Lancaster C
8:00 AM- 12:15 PM
Chairpersons: E. Secor and B. Doughty

8:00 82 THE CERCARIAL GLYCOCALYX OF *SCHISTOSOMA MANSONI* IS A LYMPHOCYTE MITOGEN. Xu XF*, Holm MJ, Devens BH, and Caulfield JP. Syntex Discovery Research, Palo Alto, CA.

8:15 83 *SCHISTOSOMA MANSONI*: ACTIVE IMMUNIZATION WITH WORM MEMBRANE ANTIGENS ENHANCES PRAZIQUANTEL EFFICACY. Fallon PG*, Ripley BA, Riley SL, and Doenhoff MJ. School of Biological Sciences, University of Wales, Bangor, Gwynedd, U.K.

8:30 84 DEVELOPMENTALLY REGULATED PHOSPHORYLATION OF SMIRV1, A 90 KDA *SCHISTOSOMA MANSONI* PROTEIN RECOGNIZED BY SERA OF MICE

VACCINATED WITH IRRADIATED CERCARIA. Hawn TR* and Strand M. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore MD.

8:45 85 ELEVATED TH1 CYTOKINE AND NO SYNTHASE RNA EXPRESSION IN THE LUNGS OF MICE VACCINATED WITH IRRADIATED CERCARIAE DURING CHALLENGE INFECTION. Wynn TA*, Oswald IP, Eltoum I, Lewis FA, James SL, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Biomedical Research Institute, Rockville, MD.

9:00 86 IDENTIFICATION AND CHARACTERIZATION OF *SCHISTOSOMA MANSONI* ANTIGENS RECOGNIZED BY T AND B LYMPHOCYTES OF HUMANS WITH EARLY ACTIVE SCHISTOSOMIASIS. Al-Sherbiny MM*, El Ridi RA, Guirguis NI, and Dean DA. Zoology Department, Faculty of Science, Cairo University; Research Department, VACSERA, Cairo; and Naval Medical Research Unit No. 3, Cairo, Egypt.

9:15 87 EFFICIENCY OF CLASS II MHC EXPRESSING EOSINOPHILS AS ANTIGEN PRESENTING CELLS TO CD4+ T CELLS. Mawhorter SD*, Kazura JW, and Boom WH. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; Department of Medicine, Case Western Reserve, OH.

9:30 88 INCREASED TH2 CYTOKINE RESPONSES IN PATIENTS INFECTED WITH *SCHISTOSOMA MANSONI*. Williams ME, Wynn TA*, Montenegro S, Doningues AL, Teixeira K, Coutinho A, and Sher A. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and Centro de Pesquisas Aggeau Magalhaes, FIOCRUZ, Recife, PE, Brasil.

9:45 89 *SCHISTOSOMA MANSONI* EGG ATTACHMENT TO VASCULAR ENDOTHELIAL CELLS. Ngaiza JR*, Doenhoff MJ, and Jaffe EA. Division Hematology Oncology, Cornell University Medical College, New York, NY; and School of Biological Sciences, Bangor, Gwynedd, UK.

10:00 Coffee Break

10:30 90 *SCHISTOSOMA MANSONI* EGG DEPOSITION RESULTS IN INCREASED ICAM-1EXPRESSION IN LIVER TISSUE. Ritter DM, Rosen S, Singer M, and McKerrow JH. University of California at San Francisco, San Francisco, CA, and Department of Veterans Affairs Medical Center, San Francisco, CA.

10:45 91 SOLUBLE INTERCELLULAR ADHESION MOLECULES IN HUMAN SCHISTOSOMIASIS: CORRELATIONS WITH CLINICAL FORM OF DISEASE AND PROLIFERATIVE RESPONSES. Secor WE*, Reis MG, Ramos EA, Carno TM, Reis EA, Mattos EP, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, Salvador, Bahia, Brazil.

11:00 92 OLIGOSACCHARIDE INTERACTIONS WITH MONONUCLEAR CELLS FROM MICE INFECTED WITH *SCHISTOSOMA MANSONI* OR *TRYPANOSOMA CRUZI* MAY LEAD TO CD4+ T CELL SUBSET REGULATION. Velupillai P *, Pereira M, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department. of Geographic Medicine, Tufts Medical School, Boston, MA.

11:15 93 PURIFICATION, CHARACTERIZATION, AND IDENTIFICATION OF A *SCHISTOSOMA MANSONI* EGG ANTIGEN RECOGNIZED BY A

GRANULOMATOUS T CELL CLONE. Chikunguwo SM*, Secor WE, Stadecker MJ, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Geographic Medicine, Tufts Medical School, Boston, MA.

11:30 94 STRATEGIES FOR THE DOWN-REGULATION OF GRANULOMATOUS INFLAMMATION IN SCHISTOSOMIASIS. Stadecker MJ*, Flores-Villanueva PO, Ricklan DE, and Harris TS. Tufts University School of Medicine, Boston, MA.

11:45 95 THE IMMUNOREGULATION OF GRANULOMA FORMATION AND FIBROSIS IN SCHISTOSOMIASIS BY ANTIGEN-SPECIFIC IMMUNOCONJUGATES. Ali MR*, Farid AG, Gabr NS, Shi S, and Phillips SM. Parasitology Department, Faculty of Medicine, Minia University, Minia, Egypt; and Allergy and Immunology Section, School of Medicine, University of Pennsylvania, Philadelphia, PA.

12:00 96 IDIOTYPIC REGULATION IN SCHISTOSOMIASIS: II. RELATIONSHIP BETWEEN LEVEL OF IDIOTYPES AND MORBIDITY OF DISEASE IN *SCHISTOSOMA HAEMATOBIUM*-INFECTED PATIENTS. Shata MT*, Helmy A, Badary MS, Deaf EA, Mohamed AM, Naser AM, Nafi MA, Napi AK, Elrehawy N, and Sercarz E. Faculty of Medicine, Assuit University, Assuit, Egypt; and Department of Microbiology, University of California, Los Angeles, CA.

SCIENTIFIC SESSION I:
FILARIASIS: THERAPY AND CLINICAL IMMUNOLOGY

Tuesday, November 2 Lancaster D
8:00 - 11:45 AM
Chairpersons: S. Mahanty and G. Dreyer

8:00 97 RADIONUCLIDE LYMPHOSCINTIGRAPHY IN 45 SYMPTOMATIC AND ASYMPTOMATIC SUBJECTS WITH BANCROFTIAN FILARIASIS. Almeida Filho P*, Besh S, Silva MC, Braga C, Maciel MA, Furtado AF, and Freedman DO. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; Nuclear Medicine Unit, Laboratorios Cerpe, Recife, Pernambuco, Brazil; and Centro des Pesquisas Aggeu Magalhaes, Recife, Pernambuco, Brazil.

8:15 98 DIETHYLCARBAMAZINE VS IVERMECTIN FOR TREATMENT OF BANCROFTIAN FILARIASIS IN PAPUA NEW GUINEA. Kazura JW*, Greenberg J, Perry R, Weil G, Day K, and Alpers M. Case Western Reserve University, Cleveland, OH; Imperial College, London, England; Washington University, St Louis, MO; and Papua New Guinea Institute of Medical Research, Papua, New Guinea.

8:30 99 DRUG DOSAGE AND INTAKE PERIODICITY FOR MASS CHEMOPROPHYLAXIS OF BANCROFTIAN FILARIASIS. Moulia-Pelat JP, Glaziou P*, Nguyen NL, Chanteau S, Martin PM, and Cartel JL. Institut Territorial de Recherches Medicales Louis Malarde, Tahiti, French Polynesia.

8:45 100 ADVERSE REACTIONS FOLLOWING IVERMECTIN TREATMENT IN HYPERENDEMIC LOIASIS AREA. Chippaux JP*, Garcia A, Ranque S, Schneider D, Boussinesq M, Cot S, Le Hesran JY, and Cot M. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon; and Antenne ORSTOM, OCEAC, Yaounde, Cameroun.

9:00 101 ANTIFILARIAL IMMUNE RESPONSES IN INDIVIDUALS EVALUATED OVER A 17-YEAR PERIOD ON AN ISLAND ENDEMIC FOR BANCROFTIAN FILARIASIS. Ottesen EA*, Steel C, Guinea A, McCarthy J, and Poindexter RW. Laboratory of

Parasitic Diseases, National Institutes of Heath, Bethesda, MD; and Health Department, Mauke, Cook Islands.

9:15 102 THE EFFECT OF PRENATAL EXPOSURE TO MATERNAL MICROFILAREMIA ON THE IMMUNE RESPONSIVENESS TO PARASITE ANTIGENS IN ADOLESCENTS. Steel C*, Guinea A, McCarthy JS, Zimmerman PA, and Ottesen EA. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD; and Mauke Hospital, Mauke Island, Cook Islands.

9:30 103 REACTIVITY OF HUMAN SERA WITH RECOMBINANT *BRUGIA MALAYI* MICROFILARIAL CHITINASE. Piessens WF*, Perler FB, Southworth MW, Dissanayake S, Xu M, Wang SH, Chen GH, Morin PM, Deng BJ, Watawana L, Zheng HJ, and Fuhrman JA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; New England Biolabs, Beverly, MA; Guizhou Provincial Institute of Parasitic Diseases, Guiyang, China; Shanghai Medical University, Shanghai, China; Faculty of Medicine, Peradeniya, Sri Lanka; and Department of Biology, Tufts University, Medford, MA.

9:45 104 FILARIASIS WITHOUT MICROFILAREMIA. Weil GJ*, Liftis F, Chandrashekar R, Gad AM, Faris R, and Ramzy RM. Washington University School of Medicine, St. Louis, MO; and Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt.

10:00 Coffee Break

10:30 105 DIFFERENTIAL TH1 ACTIVATION IN FILARIAL ANTIGEN-NEGATIVE AND ANTIGEN-POSITIVE INDIVIDUALS. Dimock KA*, Lammie PJ, and Eberhard ML. Division of Parasitic Diseases, Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA.

10:45 106 *BRUGIA MALAYI* MICROFILARIAE - COSTIMULATION AND MODULATION OF HUMAN T LYMPHOCYTE RESPONSES. Weller PF*, Liu LX, and Kim J. Beth Israel Hospital, Harvard Medical School, Boston, MA.

11:00 107 ELEVATED IL-10 PRODUCTION BY CIRCULATING MONONUCLEAR CELLS IN INDIVIDUALS WITH LYMPHATIC FILARIASIS MANIFESTED AS ASYMPTOMATIC MICROFILAREMIA. Mahanty S*, Mollis SN, Ravichandran M, Jayaraman K, Kumaraswami V, Abrams JS, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; Centre for Biotechnology, Anna University, Madras, India; Tuberculosis Research Centre, Madras, India; and DNAX Research Institute, Palo Alto, CA.

11:15 108 IL-12 REGULATION OF PARASITE ANTIGEN-DRIVEN IGE PRODUCTION IN HUMAN HELMINTH INFECTIONS. King CL*, Stupi RJ, Shata T, Saad M, Nafeh M, and Medhat A. Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH; Department of Microbiology, Assiut University, Assiut, Egypt; and Department of Tropical Medicine, Assiut University, Assiut, Egypt.

11:30 109 ONCHOCERCIASIS IN ENDEMIC AND NONENDEMIC SUBJECTS: DIFFERENCES IN CLINICAL, LABORATORY, AND IMMUNOLOGICAL FINDINGS. McCarthy JS*, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

SCIENTIFIC SESSION J: RICKETTSIAL AND BACTERIAL DISEASES

Tuesday, November 2 English Suite
8:00 - 9:45 AM
Chairpersons: D. Kelly and P. O'Hanley

8:00 110 CULTURAL, MOLECULAR AND ANTIGENIC CHARACTERIZATION OF THE ATYPICAL CANINE EHRLICHIOSIS (TROPICAL CANINE PANCYTOPENIA) AGENT. Kakoma I*, Hansen RD, Anderson BE, Hanley TE, Sims KG, Liu L, Bellamy C, Long MT, and Baek BK. University of Illinois, Urbana, IL; Center for Disease Control, Atlanta, GA; Bramer Animal Hospital, Evanston, IL; and Chonbuk National University, Chonju, Korea.

8:15 111 WHITE-TAILED DEER AS POTENTIAL RESERVOIRS OF *EHRLICHIA CHAFFEENSIS*, CAUSATIVE AGENT OF HUMAN EHRLICHIOSIS. Dawson JE*, Stallknecht D, Davidson R, Lockhart M, Nettles V, Biggie K, Olson JG, and Childs JE. Viral and Rickettsial Zoonoses Branch, CDC, Atlanta, GA; and Southeastern Cooperative Wildlife Disease Study, UGA, Athens, GA.

8:30 112 EPIDEMIOLOGY OF *ROCHALIMAEA* INFECTIONS IN CATS. Childs JE*, Olson JG, Fakile Y, Rooney JA, McGinnis R, Cooper JL, and Regnery R. Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA; Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA ; and College of Veterinary Medicine, University of Florida, Gainesville, FL.

8:45 113 COMMUNITY-BASED PROFILES OF RICKETTSIAL INFECTIONS IN THE NILE RIVER DELTA OF EGYPT. Corwin AL*, Olson JG, Habib MA, Dasch G, Kelly D, Richards A, Darwish MA, Botros BB, Watts DM, and Arthur RR. U.S. NAMRU-3, Cairo, Egypt; CDC, Atlanta, Georgia; CFAR, MOH, Cairo, Egypt; NMRI, Bethesda, MD; Ain Shams University, Cairo, Egypt; and NAMRID, Peru.

9:00 114 UPDATE ON THE EPIDEMIOLOGY OF CHOLERA IN NORTH JAKARTA, INDONESIA: BASIS TO CONDUCT A LARGE-SCALE CHOLERA VACCINE TRIAL. Richie E*, Simanjuntak CH, Punjabi NH, Sukri N, Hisham MA, Pulungsih SP, Rifai AR, Supriharyanto E, Harahap DE, Rampengan TH, Sumual-Memah, and O'Hanley P. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; National Institutes of Health Research and Development, Jakarta, Indonesia; Infectious Diseases Hospital, North Jakarta, Indonesia; and Stanford University, Stanford, CA.

9:15 115 SAFETY, IMMUNOGENICITY AND TRANSMISSIBILITY OF SINGLE DOSE LIVE ORAL CHOLERA VACCINE CVD 103-HGR IN 2 TO 4 YEAR OLD INDONESIAN CHILDREN. Punjabi NH*, Simanjuntak CH, Suharyono, Hisham MA, Noriega F, Dykstra P, Pazzaglia G, Budiarso AD, Harun SR, Wasserman S, O'Hanley P, and Levine M. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; National Institute of Health Research and Development, Jakarta, Indonesia; Department of Pediatrics, University of Indonesia, Jakarta, Indonesia; Infectious Disease Hospital, Jakarta, Indonesia; Center for Vaccine Development, University of Maryland, Baltimore, MD; and Departments of Medicine and Microbiology, Stanford University, Stanford, CA.

9:30 116 STOOL TUMOR NECROSIS FACTOR α TNF IN HUMAN SHIGELLOSIS. Murphy JR*, Mourad AS, Stevens S, and MacDonald TT. U.S. Naval Medical Research Unit #3, Cairo, Egypt and Center for Infectious Diseases, University of Texas, Houston; Faculty of Medicine, Alexandria University, Alexandria, Egypt; Celltech Ltd; and Paediatric Gastroenterology, Saint Bartholomews Hospital, London, UK.

SCIENTIFIC SESSION K: MOLECULAR ENTOMOLOGY

Tuesday, November 2 York
8:00 - 11:15 AM
Chairpersons: B.M. Christensen and A.C. Morris

8:00 117 GROUP III DENSOVIRUSES (DNV'S) ARE WIDESPREAD IN INSECT DISEASE VECTORS AND POTENTIALLY USEFUL AS GENE EXPRESSION VECTORS. O'Neill SL*, Kittayapong P, Braig HR, Gonzalez JP, and Tesh RB. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand; and Institut Franais de Recherche Scientifique pour le Developpement en Cooperation, Paris, France.

8:15 118 TRANSFECTION OF SALIVARY GLANDS FROM THE MOSQUITO, *AEDES AEGYPTI*. Morris AC* and James AA. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA.

8:30 119 FEMALE-SPECIFIC ARYLESTERASE ISOLATED FROM *AEDES AEGYPTI* SALIVARY GLANDS. Argentine JA* and James AA. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA.

8:45 120 VASODILATORY TACHYKININS FROM SALIVARY GLANDS OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*. Champagne DE* and Ribeiro JM. Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ.

9:00 121 THE SALIVARY APYRASE OF THE MOSQUITO *AEDES AEGYPTI* IS A MEMBER OF THE 5'-NUCLEOTIDASE FAMILY. Smartt CT, Champagne DE*, Ribeiro JM, and James AA. Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ; Department of Molecular Biology and Biochemistry, University of California, Irvine, CA.

9:15 122 ENZYMES AND SUBSTRATES INVOLVED IN MELANOTIC ENCAPSULATION REACTIONS BY MOSQUITOES. Li J*, Zhao XL, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

9:30 123 SEQUENCE ANALYSIS OF A HEMOLYMPH POLYPEPTIDE PREFERENTIALLY EXPRESSED IN IMMUNE REACTIVE MOSQUITOES. Beerntsen BT* and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

9:45 124 REPRODUCTIVE COSTS ASSOCIATED WITH RESISTANCE IN A MOSQUITO-FILARIAL WORM SYSTEM. Ferdig MT, Beerntsen BT, Spray FJ, Li J, and Christensen BM*. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

10:00 Coffee Break

10:30 125 ROLE OF DOPA DECARBOXYLASE IN MOSQUITO DEFENSE REACTIONS. Ferdig MT*, Li J, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

10:45 126 CHARACTERIZATION OF AN *AEDES AEGYPTI* YAC LIBRARY. Cook GA*, Christensen BM, and Severson DW. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

11:00 127 EFFECT OF *ONCHOCERCA* INFECTION ON BLACKFLY REPRODUCTIVE PHYSIOLOGY. Hurd H* and Renshaw M. Centre for Applied Entomology and Parasitology, Department of Biological Sciences, Keele University, Keele, Staffordshire, UK.

SCIENTIFIC SESSION L: MALARIA IMMUNOLOGY - I

Tuesday, November 2 Stuart
8:00 - 11:30 AM
Chairpersons: G.S Hui and I.A. Quakyi

8:00 128 ANALYSIS OF BINDING OF MONOCLONAL ANTIBODY TO A MALARIAL PEPTIDE BY SURFACE-PLASMON RESONANCE BIOSENSOR AND INTEGRATED RATE EQUATIONS. Wohlhueter RM*, Parek K, Udhayakumar V, Fang S, and Lal AA. Scientific Resources Program, National Center of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Division of Parasitic Diseases, National Center of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

8:15 129 EFFECTOR MECHANISMS OF HUMAN T CELL CLONES SPECIFIC FOR THE *PLASMODIUM FALCIPARUM* CS TH/TC EPITOPE. Moreno A*, Xu SG, Levi A, and Nardin E. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY.

8:30 130 MONOCLONAL ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITE SURFACE PROTEIN 2 IDENTIFY THREE DISTINCT B CELL EPITOPES. Charoenvit Y*, Rogers WO, Fallarme V, Paul C, Yuan L, Kaur M, Aguiar CJ, de la Vega P, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

8:45 131 INDUCTION OF MURINE CYTOTOXIC T LYMPHOCYTES AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITE SURFACE PROTEIN 2. Wizel B*, Houghten RA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Johns Hopkins University, Baltimore, MD; and Torrey Pines Institute for Molecular Studies, San Diego, CA.

9:00 132 ROLE OF γδ T CELLS IN IMMUNITY AGAINST THE LIVER STAGES OF MALARIA. Tsuji M*, Mombaerts P, Lefrancois L, Nussenzweig RS, Tonegawa S, and Zavala F. Department of Medical and Molecular Parasitology, New York University, New York, NY; Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; and Department of Medicine, University of Connecticut Health Center, Farmington, CT.

9:15 133 HUMAN B AND T CELL RESPONSES TO SYNTHETIC PEPTIDES REPRESENTING CONSERVED BLOCK 17 IN THE MEROZOITE SURFACE PROTEIN-1 (MSP-1) OF *PLASMODIUM FALCIPARUM*. Lal AA*, Kern M, Shi Y, Anyona D, Nahlen B, Weiss W, Oloo AJ, Bloland P, Ruebush TK, Udhayakumar V, Campbell CC, AND McCormick J. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious

Diseases, Centers for Disease; Kenya Institute of Medical Research, Kenya; and NAMRI-Detachment, Kenya.

9:30 134 IDENTIFICATION OF T AND B CELL EPITOPES ON *PLASMODIUM FALCIPARUM* MSP-1. Parra ME*, Roberts T, Quakyi IA, Berzofsky JA, and Taylor DW. Department of Biology, Georgetown University, Washington, DC; and Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, NCI, NIH, Bethesda, MD.

9:45 135 LOCATION OF A CONSERVED SUB-DOMINANT T-CELL EPITOPE ON *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 (MSP-1), THAT INDUCES PARASITICIDAL T-CELLS. Quakyi IA*, Currier J, Fell A, Taylor DW, Roberts T, Houghten RA, England RD, Berzofsky JA, Miller LH, and Good MF. Georgetown University, Washington, DC; Queensland Institute of Medical Research, Queensland, Australia; Torrey Pines Institute for Molecular Studies, San Diego, CA.; Metabolism Branch, NCI, and Laboratory of Malaria Research, NIAID, NIH, Bethesda, MD.

10:00 Coffee Break

10:30 136 INDUCTION OF ANTIBODY RESPONSE BY IN VIVO CROSS-PRIMING USING HETEROLOGOUS *PLASMODIUM FALCIPARUM* MSP1 ALLELES. Hui GS*, Nikaido C, Hashimoto A, and Chang SP. Department of Tropical Medicine, University of Hawaii, Honolulu, HI.

10:45 137 CRYSTALLOGRAPHIC STUDY OF TRANSMISSION BLOCKING ANTI-MALARIA FAB 4B7 WITH CYCLIC AND LINEAR PEPTIDES FROM THE PFS25 PROTEIN OF *PLASMODIUM FALCIPARUM*. Stura EA*, Kang AS, Stefanko RS, Calvo J, Gaardner KL, Kaslow DC, and Satterthwait AC. Department of Molecular Biology, The Scripps Research Intitute, La Jolla, CA; and Laboratory of Malaria Research, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

11:00 138 MODULATION OF THE TRANSMISSION OF *PLASMODIUM VIVAX* TO MOSQUITOES BY ANTI-MOSQUITO ANTIBODIES. Ramasamy R*, Ramasamy MS and Srikrishnaraj KA. Division of Life Sciences, Institute of Fundamental Studies, Kandy, Sri Lanka.

SCIENTIFIC SESSION M:
ASP STUDENT PRESENTATIONS - II

Tuesday, November 2 Lancaster E
8:00 AM- 12:00 N
Chairpersons: G.D. Cain and L.A. Hertel

8:00 139 THE LIFE HISTORY AND ULTRASTRUCTURAL FEATURES OF A SPECIES OF *HEPATOZOON* (APICOMPLEXA: ADELEINA) OF THE NORTHERN WATER SNAKE. Smith TG* and Desser SS. Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

8:15 140 ULTRASTRUCTURE OF *CARYOSPORA BIGENETICA* GROWN IN VIPER SPLEEN CELLS. Van Houten R* and Sundermann CA. Department of Zoology & Wildlife Science, Auburn University, AL.

8:30 141 SCREENING GAMONT-SPECIFIC MONOCLONAL ANTIBODIES USING A CELL CULTURE-ADAPTED STRAIN OF *EIMERIA TENELLA*. Wilson E*, Zhang J, Yang S, and Healey MC. Department of Animal Dairy and Veterinary Science, Utah State University, Logan, UT.

8:45 142 ROLE OF NITRIC OXIDE (NO) IN SUPPRESSION OF LYMPHOCYTE PROLIFERATION DURING *PLASMODIUM CHABAUDI* AS INFECTION IN C57BL/6 MICE. Ahvazi B* and Stevenson MM. Institute of Parasitology and Centre for the Study of Host Resistance, Montreal General Hospital, Montreal, Quebec; and Research Institute, McGill University, Montreal, Quebec.

9:00 143 DETECTION OF BIOGENIC AMINES IN *BIOMPHALARIA GLABRATA* INFECTED WITH *SCHISTOSOMA MANSONI* USING HPLC-ED. Manger PM*, Li J, Christensen BM, and Yoshino TP. University of Wisconsin-Madison, Madison, Wisconsin.

9:15 144 PERSISTENCE OF IRRADIATED *PLASMODIUM BERGHEI* PARASITES IN THE HOST LIVER AND THEIR POSSIBLE ROLE IN THE INDUCTION OF PROTECTIVE IMMUNITY. Scheller LF* and Azad AF. Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD.

9:30 145 MECHANISMS OF PARASITE-INDUCED IMMUNOSUPPRESSION IN AN INVERTEBRATE HOST. DeGaffe GH* and Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM.

9:45 146 PLASMA INTERACTIONS OF SUSCEPTIBLE AND RESISTANT *BIOMPHALARIA GLABRATA* WITH SCHISTOSOME EXCRETORY-SECRETORY PRODUCTS. Davids BJ* and Yoshino TP. University of Wisconsin-Madison, Madison, WI.

10:00 Coffee Break

10:30 147 ROLE OF γδ T CELLS IN INTESTINAL INFLAMMATION INDUCED BY *NIPPOSTRONGYLUS BRASILIENSIS*. Barratt RA* and Scott AL. Department of Immunology and Infectious Diseases, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

10:45 148 MODULATION OF COMPLEMENT RESISTANCE AND VIRULENCE OF *NAEGLERIA FOWLERI* AMOEBAE BY ALTERATIONS IN GROWTH MEDIUM. Toney DM* and Marciano-Cabral FM. Virginia Commonwealth University/Medical College of Virginia, Richmond, VA.

11:00 149 THE ROLE OF $CD4^+$ T CELLS IN THE EXPANSION OF THE SPLENIC γδ T CELL SUBSET DURING MALARIA. van der Heyde HC*, Manning DD, and Weidanz WP. University of Wisconsin-Madison, Department of Medical Microbiology and Immunology, Madison, WI; and University of Alabama at Birmingham, Department of Medicine, Birmingham, AL.

11:15 150 INHIBITION OF *PLASMODIUM FALCIPARUM* GROWTH *IN VITRO* BY HUMAN γδ T CELLS. Elloso MM*, van der Heyde HC, vande Waa JA, and Weidanz WP. University of Wisconsin-Madison, Department of Medical Microbiology and Immunology, Madison, WI; University of Alabama at Birmingham, Department of Medicine, Birmingham, AL.

11:30 151 GENETIC VARIATION AND RESISTANCE TO PARASITES: AN EMPIRICAL TEST OF THE CORRELATION. Meagher S*. Mammal Division, Museum of Zoology, University of Michigan, Ann Arbor, MI.

11:45 152 ZINC DEFICIENCY AFFECTS *HELIGMOSOMOIDES POLYGYRUS* DURING BOTH PRIMARY AND CHALLENGE INFECTIONS IN MICE. Shi HN*, Scott ME, Stevenson M, and Koski K. Institute of Parasitology, McGill University, Quebec, Canada; Center for Host Resistance, Montreal General Hospital, Montreal, Quebec, Canada; and School of Human Nutrition and Dietetics, McGill University, Quebec, Canada.

SCIENTIFIC SESSION N:
ARBOVIRUS EPIDEMIOLOGY

Tuesday, November 2 Tudor
8:00 - 9:45 AM
Chairpersons: J. LeDuc and L. Wilson

8:00 153 WILD RATS: A VECTOR OF HANTAVIRUS DISEASE (HVD) IN N. IRELAND?. McKenna P*, Clement J, McCaughey C, and Coyle P. The Belgian Zoonosis Workgroup, Queen Astrid Military Hospital, Brussels, Belgium; and The Department of Microbiology and Immunology, Regional Virus Laboratory, The Royal Victoria Hospital, Belfast, N. Ireland.

8:15 154 PREVALENCE OF ANTIBODY TO JUNIN VIRUS IN SMALL MAMMALS OF THE ARGENTINE PAMPA. Mills JN*, Ellis BE, Childs JE, McKee KT, Maiztegui JI, Peters CJ, Ksiazek TG, and Jahrling PB. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD; Centers for Disease Control, Atlanta, GA; Preventive Medicine Service, Fort Bragg, NC; and Instituto Nacional de Enfermes Virales Humanes, Pergamino, Argentina.

8:30 155 LASSA FEVER EPIDEMIC IN PLATEAU STATE, NIGERIA - 1993. Rollin P*, Wilson L, Childs J, Peters C, Tomori O, Nasidi A, and Ksiazek T. Centers for Disease Control, Atlanta, GA

8:45 156 YELLOW FEVER IN NIGERIA, 1986-1993: CONSIDERATIONS ON EPIDEMIC PREPAREDNESS AND CONTROL. Tomori O*, Nasidi A, and Spiegel R. Department of Virology, University College Hospital, Ibadan, Nigeria; Federal Ministry of Health, Epidemiology Division, Lagos, Nigeria; and CCCD-USAID, Lagos, Nigeria.

9:00 157 EPIDEMIOLOGIC ASPECTS OF A YELLOW FEVER OUTBREAK IN NORTHWEST KENYA, 1992-93. Marfin AA*, Tukei PM, Agata NN, Sanders EG, den Boer JW, Reiter IP, McLean RG, Cropp CB, Moore PS, and Gubler DJ. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado; Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya; and Kenya Ministry of Health, Nairobi, Kenya; World Health Organization, Nairobi, Kenya.

9:15 158 YELLOW FEVER IN THE KERIO VALLEY, RIFT VALLEY PROVINCE, KENYA, 1992-93: ENTOMOLOGICAL INVESTIGATIONS. Reiter P*, Cordellier R, Ouma J, Tukei PM, Okelo GB, Agata N, Cherogony SC, Marfin AA, Cropp CB, Savage HM, McLean RG, and Gubler DJ. CDC Dengue Laboratories, Division of Vector-borne Infectious Diseases, San Juan, Puerto Rico; Institut Français de Recherche Scientifique pour le Développement en Coopération; Ministry of Health, Division of Vector-borne Diseases,

Nairobi, Kenya; Kenya Medical Research Institute, Nairobi, Kenya; Headquarters, Ministry of Health, Nairobi, Kenya; Provincial Medical Headquarters, Kabarnet, Kenya; CDC, and Division of Vector-borne Infectious Diseases, Fort Collins, CO.

9:30 159 NATURAL VERTEBRATE HOSTS IN THE TICK-BORNE ENCEPHALITIS VIRUS TRANSMISSION CYCLE: AMPLIFICATION OF INFECTION PREVALENCE BY NONVIREMIC TRANSMISSION. Labuda M*, Kozuch O, Eleckova E, Zuffova E, and Nuttall PA. Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia; and NERC Institute of Virology and Environmental Microbiology, Oxford, U.K.

SCIENTIFIC SESSION O: DEVELOPMENT, LIFE HISTORY, AND SYSTEMATICS OF PARASITES

Tuesday, November 2 Austrian Suite
8:00 AM - 12:00 N
Chairpersons: C.M. Bartlett and A.K. Prestwood

8:00 160 HOST-PARASITE BIODIVERSITY: THE INTERFACE OF FIELD PARASITOLOGY, SYSTEMATICS, AND MAMMALOGY. Gardner SL*. Department of Nematology, University of California, Davis, CA.

8:15 161 NEMATODE DIVERSITY OF NATIVE GRAPE IN CALIFORNIA. Al-Banna L*.

8:30 162 MICRODISTRIBUTION OF SELECTED GILL PARASITES OF THE SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*. Riekerk G* and Runey M. Marine Resources Research Institute, South Carolina Wildlife and Marine Resources Division, Charleston, SC; and College of Charleston, Charleston, SC.

8:45 163 TWO-HOST LIFE CYCLE IN THE MYXOSPOREA AND CONSEQUENT TAXONOMIC IMPLICATIONS IN THE MYXOZOA. Kent ML*, Margolis L, and Whitaker DJ. Department of Fisheries & Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada.

9:00 164 A NEW SPECIES OF GYMNOPHALLOIDES (TREMATODA: GYMNOPHALLIDAE) FROM HUMANS IN KOREA. Lee SH*, Chai JY, Hong ST, and Choi MH. Department of Parasitology, Seoul National University College of Medicine, Seoul, Korea.

9:15 165 A NEW SPECIES OF CESTODE IN *UROTRYGON CHILENSIS* FROM THE GULF OF NICOYA, COSTA RICA. Berman RL* and Brooks DR. University of Toronto, Department of Zoology, Toronto, Ontario.

9:30 166 SPECIFICITY IN THE GREGARINE ASSEMBLAGE PARASITIZING *TENEBRIO MOLITOR*. Clopton RE*. School of Biological Sciences, University of Nebraska, Lincoln, NE.

9:45 167 OBSERVATIONS ON THE LIFE CYCLE STAGES OF A NUMBER OF *DIPLOSTOMUM* SPECIES MAINTAINED IN THE LABORATORY. Irwin SW*, McKeown CA, and Field JS. Department of Biological and Biomedical Sciences, University of Ulster, Northern Ireland.

10:00 Coffee Break

10:30 168 THE EFFECTS OF CONCURRENT *NIPPOSTRONGYLUS BRASILIENSIS* AND *ECHINOSTOMA CAPRONI* INFECTIONS IN GOLDEN HAMSTERS. Huffman JE*, Holben DM, and Fried B. Department of Biological Sciences, East Stroudsburg, University, East Stroudsburg, PA; Department of Biology, Lafayette College, Easton, PA.

10:45 169 PARASITES OF THE ORECTILOBIFORM SHARK FAMILIES OTHER THAN RHINCODONTIDAE: WHAT HAPPENED TO *PEDIBOTHRIUM*? Caira JN*. Department of Ecology & Environmental Biology, University of Connecticut, Storrs, CT.

11:00 170 THE TAXONOMY AND SYSTEMATICS OF NON-HOOKED TETRAPHYLLIDEANS: A SPECULATIVE JOURNEY INTO THE REALM OF THE UNKNOWN. Ruhnke TR* and Jacob BA. Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs CT.

11:15 171 LIFE CYCLE OF *GNATHOSTOMA BINUCLEATUM* ALMEYDA-ARTIGAS, 1991 (NEMATODA), ONLY KNOWN CAUSAL AGENT OF HUMAN GNATHOSTOMIASIS IN MEXICO. Almeyda-Artigas RJ*, Alcolea-Herrera E, Mosqueda-Cabrera MA, and Saldana-Martinez G. Laboratorio de Sanidad Acuicola, Department El Hombre y su Ambiente, Universidad Autonoma Metropolitana-Xochimilco, Mexico.

11:30 172 HIRUDINEAN PHYLOGENY: IMPLICATIONS FOR THE EVOLUTION OF ECTOPARASITISM AND VECTOROLOGY. Siddall ME*, Burreson EM, and Desser SS. Department of Zoology, University of Toronto, Toronto, Ontario, Canada; and Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA.

11:45 173 DIFFERENTIATION OF STRONGYLE EGGS FROM CATTLE FECES USING GENUS SPECIFIC DNA PROBES. Christensen CM*, Gasbarre LC, Zarlenga DS. Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Biosystematics Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

SYMPOSIUM: TRANSFECTION

Sponsored by The Burroughs Wellcome Fund

Tuesday, November 2 Essex A/B
9:00 - 12:00 N
Chair: M. Parsons

9:00 S56 USE OF TRANSFECTION TECHNIQUES TO STUDY ORGANELLE BIOGENESIS ON *TRYPANOSOMA BRUCEI*. Parsons M. Seattle Biomedical Research Institute, Seattle, WA.

9:20 S57 ESTABLISHMENT OF A TRANSFECTION SYSTEM FOR *PLASMODIUM*. Wirth D. Harvard Public Health, Boston, MA.

9:40 S58 TRANSFECTION AS A TOOL TO STUDY THE GENETICS AND VIRULENCE OF *LEISHMANIA*. Beverly S. Department of Biological Chemistry, Harvard University, Boston, MA.

10:00 S59 FORWARD AND REVERSE GENETICS IN THE STUDY OF *TOXOPLASMA* CELL BIOLOGY AND DRUG RESISTANCE. Boothroyd J. Stanford, CA.

10:40 S60 GP72 OF *T.RYPANSOMA CRUZI* STUDIED BY GENOME MANIPULATION. Nozaki T. The Rockefeller University, NY, NY.

11:00 S61 APPLICATIONS OF MOLECULAR TECHNIQUES TO ANALYZE DRUG RESISTANCE IN MYCOBACTERIA. Crawford J. CDC, Atlanta.

11:20 S62 THE *LEISHMANIA* GENOME PROJECT. A TOOL FOR MOLECULAR BIOLOGISTS. Cruz A. Universidade de Sao Paolo, Faculdade de Odontologia de Ribeirao, Ribeirao Preto-SP, Sao Paolo, Brasil.

SCIENTIFIC SESSION P:
LYME DISEASE AND VECTORS

Tuesday, November 2 English Suite
10:30 AM - 12:00 N
Chairpersons: G. Korch and L. Durden

10:30 174 LYME BORRELIOSIS SURVEILLANCE IN THE UNITED STATES. Dennis DT*, Ettestad PJ, Campbell GL, and Craven RB. Centers for Disease Control, NCID, Division of Vector-Borne Infectious Diseases, Fort Collins, CO.

10:45 175 FIELD AND LABORATORY STUDIES OF *BORRELIA BURGDORFERI* IN TICKS USING AN OSPA ANTIGEN CAPTURE ELISA. Burkot TR*, Wirtz RA, Patrican LA, and Piesman J. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado; Department of Entomology, Walter Reed Army Institute of Research, Washington DC; and Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY.

11:00 176 ENHANCED TRANSMISSION OF LYME DISEASE SPIROCHETES BY PARTIALLY REPLETE VECTOR TICKS. Shih CM* and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:15 177 ECOLOGICAL STUDIES OF I*XODES SCAPULARIS* IN GEORGIA: IMPLICATIONS FOR LYME DISEASE EPIDEMIOLOGY IN THE SOUTHEAST. Durden LA* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

11:30 178 NIDICOLOUS TRANSMISSION OF THE LYME DISEASE SPIROCHETE. Pollack RJ*, Katavolos P, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:45 179 SPECIES CONCEPTS AND *IXODES* TICKS. Telford SR*. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

SCIENTIFIC SESSION Q:
ARBOVIRUS PATHOGENESIS AND MOLECULAR VIROLOGY

Tuesday, November 2 Tudor
10:30 AM - 12:30 PM
Chairpersons: G.V. Ludwig and J.T Roehrig

10:30 180 PHYLOGENY OF TWO EMERGING HUMAN PATHOGENIC ARENAVIRUSES FROM SOUTH AMERICA. Gonzalez JP*, Thayu M, and Rico-Hesse R. Institut Franais de Recherche Scientifique pour le Développement en Coopération, Paris, France; and Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT.

10:45 181 STUDY OF MOLECULAR EPIDEMIOLOGY OF HANTAVIRUS INFECTION IN SMALL MAMMALS BY POLYMERASE CHAIN REACTION. Avsic-Zupanc T*, Poljak M, Lavrencak J, Krystufek B, and Trilar T. Institute of Microbiology, Medical Faculty, Ljubljiana, Slovenia; Natural History Museum of Slovenia, Ljubljana, Slovenia.

11:00 182 EMERGENCE OF A NEW EPIDEMIC/EPIZOOTIC VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN SOUTH AMERICA. Rico-Hesse R*, de Siger J, and Salas R. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Instituto de Investigaciones Veterinarias, Centro Nacional de Invest. Agropecuarias, Maracay, Venezuela; and Instituto Nacional de Higiene "Rafael Rangel", Ministerio de Salud, Caracas, Venezuela.

11:15 183 EXPRESSION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS PROTEINS BY RECOMBINANT BACULOVIRUSES. Hodgson LA*, Ludwig GV, Lind CM, and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD.

11:30 184 BIOLOGICAL STUDIES ON WEE COMPLEX VIRUSES. Sabattini MS*, Bianchi TI, Aviles G, Daffner J, and Monath TP. Virology Institute "Dr. J.M. Vanella" and SENASA, Argentina; INEVH, Pergamino, Argentina; and OraVax, Inc., Cambridge, MA.

11:45 185 THE MODE OF NEUROINVASION BY JAPANESE ENCEPHALITIS VIRUS FOLLOWING INTRAPERITONEAL INOCULATION IN MICE. Dubois DR*, Hase T, Summers PL, and Eckels KH. Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

12:00 186 NONVASCULAR DELIVERY OF ST. LOUIS ENCEPHALITIS AND VENEZUELAN EQUINE ENCEPHALITIS VIRUS BY INFECTED MOSQUITOS DURING FEEDING ON A VERTEBRATE HOST. Turell MJ* and Tammarielo RF. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

12:15 187 AN IMMUNOCYTOCHEMICAL STUDY OF THE DISTRIBUTION OF DENGUE VIRUSES IN *AEDES AEGYPTI*. Linthicum KJ*, Platt K, Myint KS, and Lerdthusnee K. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok Thailand; Departent of Microbiology, Iowa State University, Ames,Iowa; and Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand.

ASTMH PRESIDENTIAL ADDRESS

Tuesday, November 2
1:30 - 2:30 PM

Regency Ballroom
(Falcon/Condor)

"TARGETED RESEARCH":
AN OXYMORON THAT NEEDS TO BE DISCUSSED

D.G. Colley
Parasitic Diseases Branch
Centers For Disease Control
Atlanta, GA

ASTMH AWARDS CEREMONY

Tuesday, November 2
4:00-4:30 PM
Chairperson: D.G. Colley

Regency Ballroom
(Falcon/Condor)

ANNUAL BUSINESS MEETING

Tuesday, November 2
2:30 - 4:30 PM
Chairperson: D.G. Colley

Regency Ballroom
(Falcon/Condor)

DISCUSSION OF DIPLOMA PROGRAM

Tuesday, November 2
4:30-5:30 PM
Chairperson: D. Krogstad

Regency Ballroom
(Falcon/Condor)

SYMPOSIUM
THE APPLICATION OF MOLECULAR BIOLOGY TO CLASSIC PROBLEMS IN PARASITOLOGY

Tuesday, November 2, 1993 Lancaster A/B
1:30 - 3:30 PM
Chair: T Geary

1:30 S63 REGULATION AND FUNCTION OF PARASITE PROTEASES. McKerrow J. University of California--San Francisco, San Francisco, CA.

2:10 S64 EXPRESSION CLONING OF AN AVERMECTIN AND GLUTAMATE-SENSITIVE CHLORIDE CHANNEL FROM *CAENORHABDITIS ELEGANS*. Cully D. Merck, Sharp & Dohme Research Laboratories, Rahway, NJ.

2:50 S65 THE COURSE OF DISEASE IN AFRICAN TRYPANOSOMIASIS IS DETERMINED BY HOST AND PARASITE GENE EXPRESSION. Mansfield J. University of Wisconsin, Madison, WI.

3:30 Coffee Break

SCIENTIFIC SESSION R:
ASP STUDENT PRESENTATION - III

Tuesday, November 2 Lancaster A/B
3:45 - 5:45 PM
Chairpersons: P.J. Hotez and J.M. Hawdon

3:45 188 CYTOCHROME OXIDASE ACTIVITY AND CYANIDE-INSENSITIVITY IN *BLASTOCYSTIS HOMINIS* MITOCHONDRIA. Hollebeke NL* and Mayberry LF. Department of Biological Sciences, University of Texas at El Paso.

4:00 189 OVEMB-1: A GENE EXPRESSED BY EMBRYOES OF IMPORTANCE FOR EMBRYOGENESIS IN *ONCHOCERCA VOLVULUS*. Triteeraprapab S*, Richie TR, Neubert T, and Scott AL. Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD; NAMRU-2, Jarkata, Indonesia; and Howard Hughes Research Institution, University of Washington School of Medicine, Seattle, WA.

4:15 190 PURIFICATION AND CLONING STRATEGIES FOR CYTOCHROME C PEROXIDASE IN *SCHISTOSOMA MANSONI*. Campos EG*, Smith JM, and Prichard RK. Institute of Parasitology, MacDonald College, McGill University, Quebec, Canada.

4:30 191 MOLECULAR CHARACTERIZATION AND COMPLETE SEQUENCE ANALYSIS OF THE EXTRACHROMOSOMAL DNA ELEMENT IN *NAEGLERIA GRUBERI*. Mullican JC* and Tracy SM. Pathology and Microbiology Department, University of Nebraska Medical Center, Omaha, NE.

4:45 192 INCREASED SURFACE EXPRESSION OF MHC CLASS I ON *TRYPANOSOMA CRUZI*-INFECTED MURINE CELLS. Stryker GA* and Nickell SP. Department of Immunology & Infectious Diseases, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD.

5:00 193 CYCLIN DEPENDENT KINASES IN *PLASMODIUM FALCIPARUM*. Wang H*, and Mikkelsen RB. Departments of Radiation Oncology and Microbiology/Immunology, Medical College of Virginia, Richmond, VA.

5:15 194 MYXOZOAN PHYLOGENY DETERMINED BY DNA SEQUENCE ANALYSIS OF THE 18S RIBOSOMAL RNA GENE. Smothers JF*, Smith LH, and Spall RD. Department of Biological Sciences, Idaho State University, Pocatello, ID.

5:30 195 EPITOPE SPECIFICITY OF A MOUSE MONOCLONAL ANTIBODY AGAINST A 50 KDA MAURER'S CLEFT-ASSOCIATED ANTIGEN OF *PLASMODIUM FALCIPARUM* MALARIA. Cohen SJ*, Lindler LE, Stoute JA, and Klotz FW. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; and Department of Microbiology & Immunology, The George Washington University, Washington, DC.

POSTER SESSION I

Wednesday, November 2 Regency Ballroom
7:30 - 10:00 (Falcon/Condor)

TEACHING / EDUCATION

196 IMMUNITY TO *HYMENOLEPIS DIMINUTA* AS A LABORATORY EXERCISE FOR UNDERGRADUATE PARASITOLOGY STUDENTS. Woodmansee DB*. Department of Biology, Wilmington College, Wilmington, OH.

197 *ANCYLOSTOMA CEYLANICUM* IN THE HAMSTER: A LABORATORY EXERCISE TO DEMONSTRATE THE RELATIONSHIP BETWEEN INFECTION AND DISEASE. Nolan TJ* and Schad GA. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

198 STUDENT MEMBERSHIP IN ASP: STABLE OR WORRISOME?. Siddall ME*. Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

SCHISTOSOMIASIS IMMUNOLOGY

199 THE RELATIONSHIP BETWEEN HUMORAL RECOGNITION OF SCHISTOSOMAL ANTIGENS, CLASS II HLA TYPE AND *SCHISTOSOMA JAPONICUM* INFECTION IN A CHINESE VILLAGE POPULATION. Wasley AM*, Yuan HC, Zhang SJ, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Department of Epidemiology, Shanghai Medical University, Shanghai, PRC; and Jiangxi Provincial Institute of Parasitic Diseases, Nanchang, PRC.

200 LYMPHOID-STIMULATORY IDIOTYPE EXPRESSION ON ANTIBODIES TO *SCHISTOSOMA HAEMATOBIUM* EGG AND WORM ANTIGENS. Abdel-Salam E, Fouad SA, Abdel-Meguid IE, Mansour MM, and Kamal KA*. Faculties of Medicine and Science, Cairo University, Cairo, Egypt; and U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

201 EOSINOPHILIA, ANTI-EGG RESPONSES, AND SUSCEPTIBILITY IN *SCHISTOSOMA JAPONICUM* INFECTION OF MICE EXPRESSING AN IL-5 TRANSGENE. Kuriyama T*, Amano T, Tominaga A, Takatsu K, Colley DG, and Minami M. Yokohama City University School of Medicine, Yokohama, Japan; Kumamoto Univ. Med. Sch., Kumamoto, Japan; Faculty of Medicine, University of Tokyo, Tokyo, Japan; and Parasitic Diseases Branch, DPD/NCID/Centers for Disease Control, Atlanta, GA.

202 PHENOTYPIC SPLEEN CELL DIFFERENCES IN DISTINCT CHRONIC CLINICOPATHOLOGIC SYNDROMES IN MICE WITH SCHISTOSOMIASIS MANSONI. Freeman, Jr. GL*, Nix NA, Colley TA, and Colley DG. Parasitic Diseases Branch, DPD/NCID/Centers for Disease Control, Atlanta, GA; Division of Oncology, Department of Medicine, Vanderbilt University, Nashville, TN; and Oberlin College, Oberlin, OH.

203 SUPPRESSION OF THE IMMUNE RESPONSE TO DIPHTHERIA TOXOID IN MURINE SCHISTOSOMIASIS. Craig JP* and Haseeb MA. Department of Microbiology & Immunology, State University of New York Health Science Center, Brooklyn, New York, NY.

204 *SCHISTOSOMA MANSONI*-INDUCED THROMBOCYTOPAENIA IN MICE : AN IMMUNOLOGICAL BASIS?. Wambayi EJ*, Ngaiza J, and Doenhoff MJ. Kenya Medical Research Institute, Nairobi, Kenya.; The New York Hospital, Cornell Medical Center, Division of Hematology-Oncology, New York, NY; and University of Wales, School of Biological Sciences, Bangor, Gwynedd, UK.

205 EFFECT OF CASTRATION AND TESTOSTERONE TREATMENT ON SCHISTOSOMIASIS MANSONI IN MALE MICE. Nakazawa M*, Eloi-Santos S, Olsen NJ, Kovacs WJ, and Colley DG. Parasitic Diseases Branch, DPD/NCID/Centers for Disease Control, Atlanta, GA; Faculty of Medicine, University of Federal Minas Gerais, Belo Horizonte, Brazil; and Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.

206 CHARACTERIZATION OF CLONED TH0 AND TH2 LYMPHOCYTES OBTAINED FROM LIVER GRANULOMAS OF *SCHISTOSOMA MANSONI* INFECTED MICE. Boros DL*, Zhu Y, and Lukacs NW. Imunology/Microbiology Department, Wayne State University School of Medicine, Detroit, MI.

207 IDENTIFICATION AND CHARACTERIZATION OF A SECOND FORM OF TROPOMYOSIN FROM *SCHISTOSOMA MANSONI*. Osman A*, Karim A, Abdel Fattah M, Thakur A, and LoVerde P. Department of Microbiology, State University of New York, Buffalo, NY; and Department of Biochemistry, Ain Shams University, Cairo, Egypt.

208 ANTI-CARBOHYDRATE ANTIBODIES MAY BE PROTECTIVE AGAINST *SCHISTOSOMIASIS MANSONI* IN HUMANS. Zimon AE*, Secor WE, Reis MG, Ramos EA, Carmo TM, Reis EA, Mattos EP, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, Bahia, Brazil.

209 MHC CLASS II HAPLOTYPE EFECTS ON CLINICAL FORM AND IMMUNOREACTIVITY TO *SCHISTOSOMA MANSONI* ANTIGENS IN AN ENDEMIC POPULATION IN NORTHEASTERN BRAZIL. del Corral E*, Secor WE, Reis MG, Ramos EA, Carmo TM, Reis EA, Mattos EP, Zimon AE, AND Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, Bahia, Brazil.

210 IL-10 INHIBITS SCHISTOSOMAL EGG ANTIGEN-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTIONS AS WELL AS EGG GRANULOMA FORMATION IN VIVO. Flores-Villanueva PO*, Reiser H, and Stadecker DE. Tufts University School of Medicine, Boston, MA; Harvard Medical Schools/Dana-Farber Cancer Institute, Boston, MA.

211 ANALYSIS OF FRACTIONATED SCHISTOSOMAL EGG ANTIGENS USING T CELL HYBRIDOMAS. Hernandez HJ*, Brodeur PH, and Stadecker MJ. Tufts University School of Medicine, Boston, MA.

212 CHARACTERISATION OF *SCHISTOSOMA MANSONI* ANTIGEN SM480. Cooper RO*, Miller CM, Fallon PG, Probert AJ, Doenhoff MJ. University of Wales Bangor, School of Biological Sciences, Brambell Bld, Deniol Road, Bangor, Gwynedd, UK.

213 INVESTIGATIONS INTO THE REASONS FOR OBSERVED INFLAMMATION CAUSED BY EXTRACTS OF *SCHISTOSOMA MANSONI* CERCARIAE. McNeice C*, Hellewell PG, Williams TJ, Doenhoff MJ, and Teixeira MM. School Of Biological Sciences, University of Wales, Bangor, Deniol Road, Gwynedd, UK.; and Department of Applied Pharmacology, National Heart and Lung Institute, Dovehouse Street, London, UK.

214 AN ALBUMIN-LIKE MOLECULE ASSOCIATED WITH *SCHISTOSOMA MANSONI*. Riley SL*, Fallon PG, Doenhoff MJ. School of Biological Sciences, University of Wales, Bangor, Gwynedd, UK.

215 GRANULOMA FIBROBLASTS ARE THE MAJOR SITE OF VIRAL REPLICATION IN *SCHISTOSOMA MANSONI* INFECTED MICE CHALLENGED WITH RECOMBINANT VACCINIA VIRUS. Actor JK*, Eltoum IA, Pimenta P, Buller RM, Berzofsky JA, and Sher A. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, MD; Metabolism Branch, NCI, National Institutes of Health, Bethesda, MD.

FILARIASIS

216 IMMUNOLOGIC CHARACTERIZATION OF INDIVIDUALS WITH LOW AND HIGH LEVELS OF ANTIFILARIAL IgG4. Marley SE*, Eberhard ML, and Lammie PJ. Department of Zoology, University of Georgia, Athens, GA; and Division of Parasitic Diseases, Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA.

217 ANALYSIS OF SPECIFIC IgG SUBCLASSES IN A POPULATION NATURALLY EXPOSED TO *LOA LOA*. Akue JP*, Devaney E, Egwang TG, Vincent J, and Hommel M. Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK; Centre International de Recherches Medicales de Franceville, Gabon; Department of Veterinary Parasitology, Unversity of Glasgow, Glasgow, Scotland; and Malaria Branch, NCID, Centers for Disease Control, Atlanta, GA.

218 DIROFILARIASIS IN THE ARABIAN GULF: AUTOCHTHONOUS HUMAN INFECTION IN KUWAIT? Hira PR*, Madda JP, Al-Shamali MA, and Eberhard ML. Faculty of Medicine, Kuwait University, Kuwait; and Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

219 GERBIL-*ACANTHOCHEILONEMA VITEAE* MODEL: SUPPRESSION OF MICROFILARIA LEVELS ASSOCIATED WITH PREGNANCY. Dickerson JW*, Walker EM, and Eberhard ML. Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

220 EFFECTS OF REPEATED CHALLENGE INFECTIONS OF RHESUS MONKEYS WITH INFECTIVE LARVAE OF *BRUGIA MALAYI* ON LYMPHATIC PATHOLOGY, AND IMMUNE RESPONSES. Lasater BL, Dennis VA*, Lowrie, Jr. RC, and Frantz RC. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

221 *BRUGIA MALAYI* EXCRETORY/SECRETORY ANTIGENS: SUPPRESSION OF CON A-INDUCED BLASTOGENIC RESPONSES OF T-CELLS FROM UNINFECTED RHESUS MONKEYS. Bakeer MK*, Dennis VA, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

222 DIAGNOSIS OF ONCHOCERCIASIS: DEVELOPMENT AND FIELD EVALUATION OF A SIMPLE DOT BLOT ASSAY ADAPTABLE FOR FIELD USE. Lavebratt C*, Dalhammar G, and Akuffo HO. Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden; and Department of Infectious Diseases, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden.

223 EVALUATION OF A PILOT VILLAGE BASED EPIDEMIOLOGICAL SURVEILLANCE SYSTEM FOR DRACUNCULIASIS ELIMINATION IN BURKINA FASO. Hutin YJ*, Ouedraogo JB, Fabre-Teste B, Soula G, Hien R, Guigemde TR. Service d'Epidemiologie, de Statistique et d'Information Sanitaire du Secretariat General de l'O.C.C.G.E., Bobo-Dioulasso, Burkina Faso; Centre Muraz (O.C.C.G.E.), Bobo-Dioulasso, Burkina Faso; and Direction de la Medecine Preventive, Ministere de la Sante, Ouagadougou, Burkina Faso.

224 THE PREVALENCE OF ONCHOCERCIASIS IN THE NORTHWESTERN PROVINCE OF CAMEROON. Siegel JA*, Nguefeu CN, and McKerrow J. Department of Anatomic Pathology, University of California, San Francisco, CA; and C.U.S.S. Department of Immunology and Biotechnology, University of Yaounde, Republic of Cameroon.

225 PROGRESS IN THE GHANA GUINEA WORM ERADICATION PROGRAM. Bugri SZ*. Medical Director, Northern Region, Ministry of Health, Ghana.

226 RAPID ASSESSMENT OF ONCHOCERCIASIS IN CAMEROON: A STUDY IN SAVANNA AND IN FOREST-SAVANNA MOSAIC. Boussinesq M, Prod'hon J, and Chippaux JP*. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon.

227 SUPPRESSION OF CELLULAR IMMUNE RESPONSIVENESS IN RHESUS MONKEYS EXPERIMENTALLY INFECTED WITH *LOA LOA*. Osae-Addo GA*, Dennis VA, and Lasater BL. Department of Parasitology, Tulane Regional Primate Research Center, and Covington, LA; and Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

228 ROLE OF SUBSETS OF T LYMPHOCYTES IN MURINE RESISTANCE TO THE HUMAN FILARIAL PARASITE, *BRUGIA MALAYI*. Rajan TV*, Greiner DL, Killeen NL, Littman DV, Shuiltz LD, and Yates J. University of Connecticut Health Center, CT; and Jackson Laboratory, Bar Harbor, ME; University of Massachusetts Medical Center, Worcester, MA; University of California San Francisco, San Francisco, CA; and Oakland University, Rochester, MI.

229 HISTOPATHOLOGICAL CHANGES IN THE SKIN OF SUDANESE ONCHOCERCAL PATIENTS UNDERGOING TREATMENT WITH IVERMECTIN. Barouka O, Mackenzie CD*, Mahmoud B, Magdi M, Williams J, and Campbell K. NIH Sudan Medical Parasitology Project, Khartoum, Sudan; Department of Pathology, Michigan State University, East Lansing, MI; and Department of Microbiology, Michigan State University, East Lansing, MI.

230 WESTERN BLOT ANALYSIS OF *ONCHOCERCA VOLVULUS* USING SERUM FROM MICE IMMUNE TO INFECTIVE STAGE LARVAE. Brigandi RA*, Lange AM, Yutanawiboonchai W, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

231 ABSENCE OF PROTECTIVE RESISTANCE TO HOMOLOGOUS CHALLENGE INFECTION IN JIRDS WITH CHRONIC, AMICROFILAREMIC INFECTIONS OF *BRUGIA PAHANGI*: Lin DS, Coleman SU*, Petit TA, Jones KS, Weil GJ, and Klei TR. Department of Veterinary Microbiology & Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA; and Washington University School of Medicine, St. Louis, MO.

232 IDENTIFICATION OF IMMUNOGENIC EXCRETORY-SECRETORY PROTEINS OF *ONCHOCERCA VOLVULUS* LARVAE. Irvine M*, Brotman B, Prince AM, and Lustigman S. The Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY; and Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia.

233 IDENTIFICATION AND CHARACTERIZATION OF TWO RECOMBINANT FILARIAL ANTIGENS RECOGNIZED BY IgE OF *WUCHERERIA BANCROFTI*-INFECTED INDIVIDUALS. Mollis SN*, Raghavan N, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD.

234 MOLECULAR CLONING AND SEROLOGICAL CHARACTERIZATION OF A *BRUGIA MALAYI* PEPSIN INHIBITOR HOMOLOG. Xu M *, Dissanayake S, Petralanda I, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; CAICET, Puerto Ayacucho, Venezuela.

235 MOLECULAR CLONING AND PARTIAL CHARACTERIZATION OF A *LOA LOA* ALLERGEN. Akue JP*, Egwang TG, and Ajuh PM. Centre Internationale de Recherches Medicales de Franceville (CIRMF), Franceville, Gabon.

236 CHARACTERIZATION OF A 39 KILODALTON *DIROFILARIA IMMITIS* LARVAL SPECIFIC PROTEIN WITH IMMUNOPROPHYLACTIC POTENTIAL. Tripp CA*, Frank RS, Frank GR, Mika-Grieve M, and Grieve RB. Paravax, Inc., Fort Collins, CO.

237 RELATIONSHIP BETWEEN *IN VITRO* KILLING OF MICROFILARIAE, PATENCY, AND HUMORAL RESPONSE TO *ONCHOCERCA VOLVULUS* ANTIGENS. Johnson EH*, Kass PH, Irvine M, Prince AM, and Lustigman S. Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia; School of Veterinary Medicine, University of California, Davis, CA; and The Lindsley F. Kimball Research Institute, The New York Blood Center, New York, NY.

238 FIELD EVALUATION OF A RECOMBINANT ANTIGEN-BASED ANTIBODY ASSAY FOR DIAGNOSIS OF BANCROFTIAN FILARIASIS IN EGYPT. Ramzy RM*, Helmy H, Chandrashekar R, Faris R, Gad AM, and Weil GJ. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

RICKETTSIAL DISEASES

239 CANINE EHRLICHIOSIS IN EGYPT: SEROEPIDEMIOLOGICAL SURVEY. Botros BA*, Elmolla MS, Salib AW, Calamaio CA, Dasch GA, and Arthur RR. NAMRU-3, Cairo, Egypt; Police Academy, Cairo, Egypt; and Naval Medical Research Institute , Bethesda, MD.

240 SEROLOGIC DIAGNOSIS OF LOUSEBORNE TYPHUS IN ETHIOPIA. Messele T, Tzianabos T*, and Olson J. National Institute of Health, Addis Ababa, Ethiopia; and Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

241 PROTECTION OF GUINEA PIGS FROM EXPERIMENTAL ROCKY MOUNTAIN SPOTTED FEVER WITH BACULOVIRUS EXPRESSED R. RICKETTSII ROMPA PROTEIN. Sumner JW*, Sims KG, Jones DC, Olson JG, and Anderson BE. Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

242 WESTERN BLOTTING ANALYSIS OF SERA FROM MILITARY PERSONNEL EXHIBITING SEROLOGICAL REACTIVITY TO SPOTTED FEVER GROUP RICKETTSIAE. Dasch GA, Kelly DJ*, Richards AL, Sanchez JL, and Rives CC. Naval Medical Research Institute, Bethesda, MD;

Walter Reed Army Institute of Research, Washington, DC; and Centers for Disease Control and Prevention, Atlanta, GA.

243 ANTIBIOTIC SUSCEPTIBILITY OF *RICKETTSIA TSUTSUGAMUSHI* FROM PATIENTS WITH SEVERE SCRUB TYPHUS IN NORTHERN THAILAND. Strickman D, Bodhidatta D, Kelly D*, Dasch G, Chouriyagune C, Watt G. Walter Reed Army Institute of Research, Washington, DC; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Naval Medical Research Institute, Bethesda, MD; and Prachanuchoa Hospital, Chiangrai, Thailand.

BACTERIAL DISEASE

244 VALUE OF TRANSAMINASE AND PLATELET EVALUATIONS IN ACUTE TYPHOID FEVER PATIENTS. El-Masry NA*, Bassily S, Farid Z, Sultan Y, Abu-Elyazeed R, and Hibbs RG. U.S. Naval Medical Research Unit No. Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

245 LYMPHOCYTE PROFILES AMONG INDONESIAN WITH TYPHOID FEVER. Pudjoprawoto N*, McGladdery S, Punjabi NH, Pulungsih S, and O'Hanley P. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Infectious Diseases Hospital, North Jakarta, Indonesia; and Stanford University, Stanford, CA.

246 EFFECT OF DEXAMETHASONE ON ACUTE INFLAMMATORY CYTOKINE LEVELS FROM CULTURED MONOCYTES OF TYPHOID FEVER PATIENTS. McGladdery S, Larasati R, Silitonga N, Punjabi NH, Lesmana M, Pulungsih SP, and O'Hanley P. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Infectious Diseases Hospital, North Jakarta, Indonesia; and Stanford University, Stanford, CA.

247 PRELIMINARY EVALUATION OF *VIBRIO CHOLERAE* NON-01 AGGLUTINATING STRAINS ASSOCIATED WITH ACUTE DIARRHEAL DISEASE IN NORTH JAKARTA. O'Hanley P*, Manurung N, Richie E, Peetosutan K, Punjabi N, Pulungsih SP, Masbar T, Widjaja D, Wangsasaputra F, Hanurawati W, McGladdery S, and Simanjuntak CH. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.; Stanford University, Stanford, CA; Infectious Disease Hospital, North Jakarta, Indonesia; and National Institutes of Health Research and Development, Jakarta, Indonesia.

VIROLOGY

248 DOSE-RESPONSE STUDY OF PASTEUR MERIEUX ' INACTIVATED HEPATITIS A VACCINE. Vidor E*, Garin D, Fanget B, Brasseur P, Delolme H, Caron F, Humbert G, Mojon M, Wallon M, Gravey D, Flehmig B, and Peyron F. P.M. sv., Marnes La Coquette, France; HIA Desgenettes, Medical Biology, Lyon, France; P.M sv., Lyon, France; CHR Charles Nicolle, Rouen, France; HIA Desgenettes, Lyon, France; CHU Croix Rousse, Lyon, France; and Hygiene Institute, Tubingen, Germany.

249 COMPARATIVE CONTROLLED STUDY OF THE IMMUNOGENICITY AND SAFETY OF TWO DOSING SCHEDULES OF HB VAX II HEPATITIS B VACCINE IN NEONATES. Bassily S*, Kotkat A, Gray GC, Hassan N, Imam Z, and Hibbs RG. U.S. Naval Medical Research Unit No. Three, Cairo, Egypt; High Institute of Public Health, Alexandria, Egypt; and Clinical Epidemiology Naval Health Research Center, San Diego, CA.

250 ACUTE HEV INFECTIONS IN DJIBOUTI AND SENEGAL. Rodier GR*, Polycarpe D, Michel P, El-Zimaity DT, Arthur RR, Carl M, and Hyams KC. U.S. NAMRU-3, Cairo, Egypt; Service

Medical Interentreprises, Djibouti; Institut Pasteur, Dakar, Senegal; and Naval Medical Research Institute, Bethesda, MD.

251 EVALUATION OF POXVIRUS-HANTAAN VACINES IN HAMSTERS. McClain DJ*, Summers PL, Henchel E, Dalrymple JM, and Schmaljohn CS. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

251 AN EVALUATION OF A MEASURE OF RODENT DENSITY AS AN INDICATOR OF RISK FOR ARGENTINE HEMORRHAGIC FEVER. Calderon GE*, Sabattini MS, Mills JN, Feuillade MR, and Maiztegui JI. Instituto Nacional de Enfermedades Virales Humanas, Pergamino, Argentina; and Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

253 ELEVATED LEVELS OF TOTAL AND SPECIFIC IgE IN SCANDINAVIAN TYPE OF HEMORRHAGIC FEVER WITH RENAL SYNDROME. Alexeyev OA*, Ahlm C, Billheden J, Settergren B, Wadell G, and Juto P. Department of Virology, Department of Infectious Diseases, University Hospital of Northern Sweden, Umea, Sweden.

254 HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS): PRESENT SITUATION IN RUSSIA. Tkachenko EA* and Drozdov SG. Institute of Poliomyelitis and Viral Encephalitides, Russian, Academy of Medical Sciences, Moscow, Russia.

255 MOLECULAR CHARACTERIZATION OF DEN-1 VACCINE CANDIDATE 45 AZ5. Puri B*, Nelson WM, Howland DF, Henchal EA, and Hayes CG. Viral and Rickettsial Disease Program, Infectious Disease Division, Naval Medical Research Institute, Bethesda, MD; Department of Molecular Virology, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Geo-Centers, Inc., Fort Washington, MD; and Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

256 GENETIC STUDY OF DENGUE-3 VIRUSES. Chungue E*, Deubel V, Cassar O, Laille M, and Martin PM. Institut Territorial de Recherches Medicales Louis Malarde, BP 30, Papeete, French Polynesia; Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex, France; and Institut Pasteur de Noumea, Noumea, New Caledonia.

257 DEVELOPMENT OF ELISAS TO DETECT FLAVIVIRUSES: GENUS SEROGROUP AND COMPLEX SPECIFIC ANTIGENS. Lewis TE*, Rossi CA, Montoya RR, Korch G, Hile J, and Mangiafico JA. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

258 ENZYME IMMUNOSORBENT ASSAY FOR DENGUE 2 VIRUS USING HUMAN IMMUNOGLOBULINS. Teruel-Lopez E, Costa-Leon L, Valero-Fuenmayor N, Fuenmayor D, Gutierrez L, and Martinez M. Instituto de Investigaciones Clinicas and Laboratorio Regional de Referencia Virologica, Universidad del Zulia, Maracaibo, Venezuela.

259 DEVELOPMENT OF A GENOMIC TYPING ASSAY UNIVERSAL FOR MOSQUITO-BORNE FLAVIVIRUSES: APPLICATION TO THE GROUPING OF AFRICAN WEST NILE VIRUS ISOLATES. Berthet FX*, Zeller HG*, Pierre V, Digoutte JP, and Deubel V. Arbovirus Research Unit, Institut Pasteur, Dakar, Senegal; and Department of Virology, Institut Pasteur, Paris, France.

260 EXPERIMENTAL TRANSMISSION OF POWASSAN VIRUS BY *IXODES DAMMINI* TICKS. Costero A*and Grayson MA. Department of Entomology, McGill University, Montreal, Canada; and New York State Department of Health, Wadsworth Center for Labs. and Research, Arbovirus Laboratory, Albany, N.Y.

261 EVALUATION OF IMMUNOASSAYS TO DETECT ANTIBODIES TO VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS AND CHIKUNGUNYA VIRUS IN SERUM SAMPLES. Rossi CA*, Mangiafico JA, McClain D, Danner DK, Lewis TE, and Korch G. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

262 EVALUATION OF IMMUNOCAPTURE PROCEDURES FOR PREPARING VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS SAMPLES FOR IDENTIFICATION BY RT-PCR. Knauert FK*, Parrish BA, Ibrahim MS, Kondig JP, and Korch GW. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

263 DEVELOPMENT OF AN IN SITU ELISA FOR THE DETECTION AND IDENTIFICATION OF SANDFLY VIRUSES. Summers PL*, Silverstein JA, and McClain DJ. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

264 PHLEBOTOMINE SANDFLIES AND ISOLATIONS OF ARBOVIRUSES FROM A SAHELIAN REGION IN SENEGAL. Fontenille D*, Traore-Lamizana M, Zeller HG, Trouillet J, Leclerc A, Mondo M, Ba Y, and Digoutte JP. ORSTOM, BP 1386, Dakar, Senegal; Institut Pasteur, BP 220, Dakar, Senegal; and Departement de Biologie Animale, Universite CAD, Dakar, Senegal.

265 VIRAL SURVEY OF TICKS IN SAUDI ARABIA. Tantawy TA, Al-Khalifa MS, Elyan DE, Diab FM, Al-Asgah NA, Hussein HH, Botros BA, and Arthur RR. Virology Division, Naval Medical Research Unit No. 3, Cairo, Egypt; and Department of Zoology, College of Science, King Saud University, Saudi Arabia.

266 SUSCEPTIBILITY OF THREE BREEDS OF INDIGENOUS SHEEP IN NIGERIA TO EXPERIMENTAL INFECTION WITH THE ZINGA STRAIN OF RIFT VALLEY FEVER VIRUS. Olaleye OD* and Tomori O. Department of Virology, College of Medicine, University College Hospital, Ibadan, Nigeria.

267 IMMUNO-ELECTRON MICROSCOPY OF INFLUENZA HAEMAGGLUTININ (HA1 & HA2) AND M2 PROTEIN MOLECULES AND THEIR INTRACELLULAR TRANSPORT. Ciampor F*, Vareckova E, Mucha V, Betakova T, Cmarko D, Hanincova J, and Zavodska E. Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia.

MALARIA

268 A DIFFERENTIAL SEROLOGICAL SCREEN IDENTIFIES NOVEL AND PUTATIVE PROTECTIVE MALARIAL EPITOPES. Lobo CA* and Sharma S. Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India.

269 MEFLOQUINE PROPHYLAXIS FAILURES IN DUTCH UNITED NATIONS TRANSITIONAL AUTHORITY IN CAMBODIA (UNTAC) TROOPS. Hopperus Buma AP*, Ohrt C, van Thiel PPAM, Tendeloo CH, and Kyle DK. Royal Netherlands Navy, Medical Service, The Hague, Netherlands; US Army Medical Component, Armed Forces Research Institute of Medical Science, Bangkok, Thailand; and Unit of Infectious Disease and Tropical Medicine, Academic Medical Center, Amsterdam, Netherlands.

270 MALARIA IN DUTCH MARINES DURING AND AFTER DEPLOYMENT IN CAMBODIA. van Thiel PPAM*, Hopperus Buma AP, Tendeloo CH, van Gool T, Kager PA. Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, Amsterdam, The

Netherlands; Royal Netherlands Navy, Medical Service, The Hague, The Netherlands; and Department of Medical Microbiology, Academic Medical Centre, Amsterdam, The Netherlands.

271 DOXYCYLINE AND CHLOROQUINE COMBINATION FOR MALARIA CHEMOPROPHYLAXIS IN AUSTRALIAN SOLDIERS DEPLOYED TO CAMBODIA. Roessler PM, Travers T, Barnett AK, Edstein MD, Shanks GD*, and Rieckmann KH. Army Malaria Research Unit, Ingleburn, Australia.

272 CHLOROQUINE CHEMOPROPHYLAXIS IN INDONESIAN SOLDIERS DEPLOYED TO CENTRAL CAMBODIA AS PART OF UNTAC PEACE-KEEPING OPERATIONS. Widodo S*, Mustadjab A, Purnomo, Richards AR, Shanks GD, and Corwin AL. Central Army and Gatot Soebroto Hospitals, Jakarta, Indonesia; U.S. NAMRU No. 2 Jakarta, Indonesia; and Army Malaria Research Unit, Australia.

273 ANTIMALARIAL EFFECTS OF NEEM: GAMETOCIDAL ACTIVITY. Udeinya IJ*, Quakyi I, Brown N, and Ajayi FO. Howard University College of Medicine, Washington, DC; Department of Biology, Georgetown University, Washington, DC; and Walter Reed Army Institute of Research, Washington, DC.

274 PRE-TRAVEL HEALTH ADVICE TO INTERNATIONAL TRAVELERS BY PRIMARY CARE PHYSICIANS. Lobel HO*, Kozarsky PE, Barber AM, Blass M, Waterman SH, and Campbell CC. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA.; Emory University School of Medicine, Atlanta, GA; and San Diego County Health Department, San Diego, CA.

275 COMPLIANCE WITH ANTIMALARIAL CHEMOPROPHYLAXIS - A MATCHED CASE - CONTROL STUDY. Gyrokos TW, Svenson JE, and MacLean JD. Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada; and McGill University Centre for Tropical Diseases, Montreal, Quebec, Canada.

276 QUANTITATIVE PCR TO PREDICT *PLASMODIUM FALCIPARUM* TREATMENT FAILURE. Kain KC*, Kyle DE, Brown AE, Mirabelli L, Webster HK, and Looareesuwan S. Tropical Disease Unit, Division of Infectious Disease, The Toronto Hospital, Canada; Department of Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

277 HALOFANTRINE PHARMACOKINETICS OF AN EXTENDED DOSE REGIMEN IN PATIENTS WITH ACUTE FALCIPARUM MALARIA AND HEALTHY VOLUNTEERS. Ohrt C *, Watt G, Teja-Isavadharm P, Loesuttiviboon L, Webster HK, Schuster B, and Fleckenstein L. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Surasinghanat Army Hospital, Aranyaprathet, Thailand; College of Pharmacy, University of Iowa, Iowa City, IA; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

278 ENHANCEMENT OF VIRUS REPLICATION BY ANTIMALARIAL TREATMENT IN CD-1 MICE. Sklarsh JW*, Sidhu GS, and Maheshwari RK. Uniformed Services University of the Health Sciences, Bethesda, MD.

279 AN INVESTIGATION INTO THE ANTIMALARIAL ACTIVITY OF CYMBOPOGUN CITRATUS ON *PLASMODIUM BERGHEI BERGHEI IN VITRO*. Obih P*, Makinde J, and Ojo J. College of Pharmacy, Xavier University of Louisiana, New Orleans, LA; and University of Ibadan, Nigeria.

280 RAPID DEVELOPMENT OF PYRIMETHAMINE RESISTANCE (PYR^R) *IN VITRO*. Krogstad FM* and Krogstad DJ. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

281 PHARMACOKINETICS OF ARTEMISININ AFTER ORAL ADMINISTRATION IN HEALTHY VOLUNTEERS. Loutan L*, Paris M, Plessas C, Benakis A, and and Andrial M. Parasitic Disease Division, Geneva University Hospital, Geneva, Switzerland; Department of Pharmacology, University Medical Centre, Geneva, Switzerland; and Medical Department Mepha Ltd., Basel, Switzerland.

282 RELATIONSHIP OF THE 3-DIMENSIONAL STRUCTURE OF HALOFANTRINE TO ANTIMALARIAL ACTIVITY. Karle JM*. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC.

283 EFFECT OF POLY ICLC AGAINST A MALARIAL INFECTION IN RHESUS MONKEYS. Maheshwari RK*, Levy HB, Dutta GP, Puri SK, and Kamboj VP. Uniformed Servies University of the Health Sciences, Bethesda, MD; NIAID, National Institutes of Health, Bethesda, MD; and Central Drug Research Institute, Lucknow, India.

284 MOLECULAR ANALYSIS OF *PFMDR1* IN *PLASMODIUM FALCIPARUM* ISOLATES FROM SUB-SAHARAN AFRICA. Basco L, Le Bras J, Rhoades Z, and Wilson CM*. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Laboratoire de Parasitologie, Hopital Bichat-Claude Bernard, Paris, France.

285 THE *IN VITRO* ACTIVITY OF CIPROFLOXACIN AND CHLORAMPHENICOL AGAINST *PLASMODIUM FALCIPARUM*. Yeo AE* and Rieckmann KH. Army Malaria Research Unit, Ingleburn, Australia.

286 MEPACRINE AND PYRONARIDINE UPTAKE BY *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES. Elueze EI*, Wu LJ, Croft SL, and Warhurst DC. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, UK; and Institute of Parasitic Diseases, Shanghai, China.

287 MOLECULAR EPIDEMIOLOGY OF ANTIFOLATE RESISTANT *PLASMODIUM FALCIPARUM* IN MALI. Plowe CV*, Boare M, Wellems TE, Peterson DS, and Doumbo 0. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD; and Malaria Research & Training Center, National School of Medicine & Pharmacy, Bamako, Mali.

288 DEVELOPMENT OF AN *IN VITRO* PHARMACODYNAMIC MODEL TO ASSESS IBI ARTEETHER ANTIMALARIAL ACTIVITY. Li X*, Brewer TG, Miller RE, Figueroa L, Gerena L, Oduola AJ, Nuzum EO, and Milhous WM. Division of Experimental Therapeutics, Walter Reed Army Institute of Research Washington DC; USA Medical Reserach Unit, Brazil, Rio de Janiero, Brazil; and Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.

289 ANTIMALARIAL AND CYTOTOXIC ACTIVITY OF NATURAL BISBENZYLISOQUINOLINE ALKALOIDS. Angerhofer CK*, Guinaudeau H, Lin LZ, Likhitwitayawuid K, Wongpanich V, Pezzuto JM, Ruangrungsi N, and Cordell GA. Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, College of Pharmacy, Chicago , IL; and Department of Pharmacognosy, Faculty of Pharmacy, University of Angers, France.

290 PRELIMINARY STUDY OF INTRA-RECTAL QUININE ADMINISTERED TO *PLASMODIUM FALCIPARUM* MALARIA CHILDREN IN NIAMEY, NIGER. Barennes H*, Kahistani F, Clavier F, Meynard D, Ndinfountawoo S, and Verdier F. French Cooperation, Ministry of Public Health, Niamey, Niger; Hôpital National de Niamey, Niger; INSERM, Unité 13, IMEA, Paris, France; and Faculté des Sciences de la Santé, Niamey, Niger.

291 USE OF A SERUM-FREE MEDIUM IN THE DETERMINATION OF *PLASMODIUM FALCIPARUM* DRUG SENSITIVITY. Ofulla AV, Orago AS, Githure JI, Burans JP, Aleman GM, Johnson AJ, and Martin SK*. Kenya Medical Research Institute, Nairobi, Kenya; Kenyatta University, Nairobi, Kenya; Naval Medical Research Institute, Bethesda, MD; and United States Army Medical Research Unit, Nairobi, Kenya.

292 SITE PREPARATION FOR MALARIA DRUG AND VACCINE TRIALS IN BRAZIL. Pang LW*, Milstrey EG, Martins OR, Arias JR, Milhous WK. USA Research Unit-Brazil, American Consulate Rio de Janeiro, Brazil; Municipal Secretary of Health, Peixoto, Mato Grosso, Brazil; and PAHO, WHO, Brasilia, Brazil.

293 INTRINSIC ACTIVITY OF SULFONAMIDES/SULFONES AGAINST FALCIPARUM MALARIA. Lucia L*, Miller RE, Pang LW, Schuster BG, and Milhous WK. USA Research Unit-Brazil, American Consulate Rio de Janeiro, Brazil; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

294 IN VITRO DRUG SENSITIVITY OF *PLASMODIUM FALCIPARUM* STRAINS FROM 3 ENDEMIC CONTINENTS TESTED AGAINST 4 CLASSIC ANTIMALARIALS AND 4 QINGHAOSU DERIVATIVES. Gay F*, Bustos DG, Venturin C, Ciceron L, Counali JB, Sampang J, Nadire M, Saniel MC, and Gentilini M. Department de Maladies Tropicales et Sante Publique, Groupe Hospitaliere Pitie-Salpetriere, Paris, France; Research Institute for Tropical Medicine, Alabang, Metro Manila, Philippines; and Service Departemental de Desinfection de la Guyane, Cayenne, Guyane.

295 CARDIAC EFFECTS OF STANDARD DOSE HALOFANTRINE THERAPY. Matson PA*, Luby SP, Redd SC, and Meriwether RA. Division of Field Epidemiology, Centers for Disease Control and Prevention, A; Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; and Communicable Disease Section, North Carolina Department of Health, Raleigh, NC.

296 TWO NOVEL APPROACHES FOR DETERMINING THERAPEUTIC DRUG LEVELS OF ARTEMISININES IN BIOLOGICAL FLUIDS. Mount DL*, Green MD, and Todd GD. Malaria Branch, Centers for Disease Control. Atlanta, GA.

297 CLINICAL EFFICACY OF MICRONIZED HALOFANTRINE FOR ACUTE UNCOMPLICATED FALCIPARUM MALARIA IN NONIMMUNE PATIENTS. Bouchaud O*, Basco LK, Gillotin C, Gimenez F, Genissel B, Farinotti R, LeBras J, and Coulaud JP. Service de Maladies Infectieuses et Tropicales, Hopital Bichat-Claude Bernard, Paris, France; Service de Parasitologie, Hopital Bichat-Claude Bernard, Paris, France; and Service de Pharmacie, Hopital Pitie-Salpatriere, Paris, France.

298 COMPARISON OF PHARMACOKINETICS, BIOAVAILABILITY AND HYDROLYSIS OF DIHYDROARTEMISININ, ARTEETHER, ARTESUNATE AND ARTELINATE IN RATS. Li Q*, Peggins JO, Masonic K, and Brewer TG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

299 PHARMACOKINETICS AND METABOLISM OF A NEW 8-AMINOQUINOLINE PRIMAQUINE ANALOG, WR242511. Marino MT*, Peggins JO, Brown LD, Idowu OR,

Urquhart M, and Brewer TG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

300 EARLY COMPARISON OF PERMETHRIN IMPREGNATED-BED NETS AND CURTAINS AND LAMDACYHALOTHRIN HOUSE SPRAYING FOR MALARIA CONTROL IN EASTERN NIGERIA. Sexton JD*, Breman JG, Ekanem OJ, Ezike VI, Roberts JM, Onyido AE, and Herrington JE. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Federal Ministry of Health, Nigeria; Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; International Health Program Office, Centers for Disease Control and Prevention, Atlanta, GA; and Federal Ministry of Health, Nigeria.

301 THE PUBLIC HEALTH IMPACT OF THE RAPID RESETTLEMENT OF A GROUP OF SOUTHEAST ASIAN REFUGEES. Paxton LA*, Schultz LJ, Luby SP, Meriwether RZ, and Slutsker LM. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta, Georgia; and North Carolina Department of Environment, Health, and Natural Resources, Raleigh, NC.

302 RAPID DIAGNOSIS OF MALARIA BY USE OF FLUORESCENT PROBES. Caramello P*, Negro C, Lucchini A, Dal Conte I, Pollono AM, Tanpradist S, and Gioannini P. Institute of Infectious Diseases, University of Turin, Italy; and Malaria Division, Ministry of Public Health, Bangkok, Thailand.

303 USING PCR TO DETECT MALARIA DIRECTLY FROM BLOOD SAMPLES IN THE VENEZUELAN AMAZON. Laserson KF, Petralanda I, Hamlin DM, Almera R, Fuentes M, Carrasquel A, and Barker, Jr. R*. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro American Amazonico Para la Investigacion y Control de Enfermedes Tropicales "Simon Bolivar", Venezuela.

304 MICROSCOPIC DIAGNOSIS OF MALARIA FOLLOWING A CYTOCONCENTRATION TECHNIQUE AFTER SAPONINE TREATMENT TO CAUSE HEMOLYSIS. Petithory JC*, Dufour M, Garnier R, Ardoin F, and Brumpt E. Controle de Qualite National en Parasitologie, Laboratoire Centre Hospitalier, Gonesse, France.

305 ASSESSMENT OF A RAPID MANUAL TEST FOR THE DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA IN *AOTUS* MONKEYS. Millet PG, Grady KK*, Maret SM, Sullivan JS, and Collins WE. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta GA; and Becton Dickinson Advanced Diagnostics, Baltimore MD.

306 EVALUATION OF CLINICAL DETERMINANTS IN PREDICTING MALARIA INFECTION. Svenson JE*, MacLean JD, and Gyorkos TW. Department of Epidemiology and Biostatistics, McGill University; McGill University Centre for Tropical DiseasesMontreal, Quebec, Canada; and Division of Clinical Epidemiology, Montreal General Hospital, Montreal, Quebec, Canada.

307 CLINICAL RECOGNITION OF ANEMIA IN MALAWI. Kazembe PN*, Redd SC, Luby SP, Ziba C, Nwanyanwu OC, Franco C, Chitsulu L, Wirima JJ, and Olivar MA. Kamuzu Central Hospital, Lilongwe, Malawi; Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; Child Health Science Unit, Ministry of Health, Malawi; and University of Malawi, College of Medicine, Blantyre, Malawi.

308 CLINICAL RECOGNITION OF MALARIA, MALAWI. Luby SP*, Redd SC, Ziba C, Nwanyanwu OC, Franco C, Kazembe P, Olivar MA, Cullinan T, Chitsulu L, and Wirima JJ. Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; Child Health Science Unit, Ministry of Health, Lilongwe, Malawi; Kamuzu Central Hospital, Lilongwe, Malawi; and University of Malawi, College of Medicine, Blantyre, Malawi.

309 A COMMUNICATION STRATEGY FOR THE VILLAGE-LEVEL PROMOTION OF INSECTICIDE-IMPREGNATED MOSQUITO NETS IN BAGAMOYO DISTRICT, TANZANIA. Winch PJ*, Makemba AM, Kamazima SR, Premji Z, Minjas JN, and Shiff CJ. Department of International Health, Johns Hopkins University, School of Hygiene & Public Health, Baltimore.; Bagamoyo Bed Net Project, Dar es Salaam, Tanzania; and Department of Parasitology & Medical Entomology, Muhimbili Medical Centre, Dar es Salaam, Tanzania.

310 MALARIA EPIDEMICS IN AMAZONAS, VENEZUELA: 1989 -1992. Almera R*, Fuentes M, Felicita S, Lopez A, Garcia M, Hung S, and Petralanda I. CAICET, Puerto Ayacucho, Amazonas, Venezuela.

311 EVALUATION OF MALARIA CONTROL IN AFRICA: CHANGING THE RULES. Ntahobari S*, Roungou JB, Nguyen-Dinh P, Bryce J, Naimoli JF, and Hersh B. Ministry of Public Health, Burundi; Ministry of Health, Central African Republic; Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; and International Health Program Office, Centers for Disease Control and Prevention, Atlanta, GA.

312 AREA-SPECIFIC RISKS OF MALARIA IN THAILAND. Thimasarn K, Suvannadabba S, Jatapadma S, Sirichaisinthop J, and Wongsrichanalai C*. Ministry of Public Health, Bangkok, Thailand; and Department of Immunology and Parasitology, Armed Forces Institute for Medical Sciences, Bangkok, Thailand.

313 A LONGITUDINAL STUDY OF ANTIBODY LEVELS IN AN AREA OF LOW MALARIA ENDEMICITY USING THE INDIRECT FLUORESCENT ANTIBODY (IFA) TECHNIQUE. Pasay CJ, Bustos DG*, Belizario VY, Lansang MA, and Saul AJ. Research Institute for Tropical Medicine, Department of Health, Alabang, Metro Manila, Philippines; and Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Australia.

314 GEOGRAPHIC VARIATION IN THE INCIDENCE OF CEREBRAL MALARIA IN A ZAMBIAN COMMUNITY. Thuma PE*, Njungu M, Thuma EN, Biemba G, and Gordeuk VR. Department of Pediatrics, Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA; Macha Mission Hospital, Choma, Zambia; and Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.

315 AN IMPORTED FOCUS IN NORTH CAROLINA OF 402 MONTAGNARD REFUGEES INFECTED WITH FOUR *PLASMODIUM* SPECIES: DIAGNOSTIC EVALUATION. Sulzer AJ*, Long EG, Gracia LS, Millet PG, Grady KK, Schultz LJ, Paxton LA, Slutsker LM, Luby SP, Robertson GC, and Turner LS. Malaria Branch, DPD, National Center for Infectious Disease, Centers for Disease Control, Atlanta, GA; Division of Bacterial Diseases, NCID, Centers for Disease Control, Atlanta, GA; UCLA Medical Center, Los Angeles, CA; and Division of Health Services, Dept of Environment, Health, and Natural Resources, Raleigh, NC.

KINETOPLASTIDAE IMMUNOLOGY AND PATHOLOGY

316 CHARACTERIZATION OF THE CELLULAR RESPONSES IN PATIENTS WITH TEGUMENTARY LEISHMANIASIS USING T-CELL IMMUNOBLOTTING. Ortiz-Ordonez JC* and Saravia NG. Fundacion Centro International de Entrenamiento e Investigaciones Medicas, Cali, Colombia.

317 ELEVATED SERUM ARGINASE LEVELS IN EXPERIMENTAL ANIMALS AND IN PATIENTS WITH VISCERAL LEISHMANIASIS. Evans TG, Fratkin M*, Hibbs JB, and Vasconcelos WA. Infectious Diseases Section, Salem Veterans Administration Medical

Center, Salem, VA; Division of Infectious Diseases, University of Utah, Salt Lake City, UT; and Nucleo de Medicina Tropical, Universidade Federal do Ceara, Fortaleza, Ceara, Brazil.

318 T CELL RESPONSES AND CYTOKINE PRODUCTION IN *LEISHMANIA MAJOR*-INFECTED MICE TREATED WITH PENTOSTAM. Nabors GS* and Farrell JP. Department of Pathobiology, University of Pennsylvania, Philadelphia, PA.

319 INFECTION OF HUMAN BONE MARROW DERIVED STROMA WITH VICEROTROPIC *LEISHMANIA TROPICA* PROMASTIGOTES. Rowton ED*, Leiby DA, Toro-Lopez L, and La Russa VF. Division of Communicable Diseases and Immuniology, Walter Reed Army Institute of Research, Washington, DC; and Division of Medicine, Walter Reed Army Institute of Research, Washington, DC.

320 IN VITRO INFECTION CHARACTERISTICS OF TWO STRAINS OF *TRYPANOSOMA CRUZI*. Luo H* and Rowland E. Department of Biological Sciences, Ohio University, Athens, OH.

321 AMERICAN CUTANEOUS LEISHMANIASIS IN COLUMBIA: CELLULAR AND VASCULAR PROFILE OF CUTANEOUS LESIONS AND MONTENEGRO SKIN TESTS. Palma G*, Mackenzie C, Salinas G, Guarin N, and Saravia A. CIDEIM & Department of Microbiology, Universitas de Valle, Cali, Columbia; and Department of Pathology, Michigan State University, East Lansing, MI.

322 TRYPANOSOMES AND MICROFILARIAE IN FERAL OWL AND SQUIRREL MONKEYS MAINTAINED IN RESEARCH COLONIES. Steurer F, Sullivan JJ*, Benavides G, Tarleton RL, Eberhard ML, and Landry S. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Department of Zoology, University of Georgia, Athens, GA; and Malaria Vaccine Development Program, Office of Health, U.S.A.I.D., Washington, DC.

323 DEGRADATION OF RABBIT IMMUNOGLOBULIN INGESTED BY *GLOSSINA MORSITANS MORSITANS* (WESTWOOD). Hampton RW*, Preston L, Narcisi EM, and Honigberg BM. Department of Pathology, University of South Alabama College of Medicine, Mobile, AL; and Department of Zoology, University of Massachusetts, Amherst, MA.

SCIENTIFIC SESSION S:
FILARIASIS IMMUNOLOGY AND PROTECTION

Wednesday, November 3 York
9:30 AM - 12:30 PM
Chairpersons: R. Maizels and E. Pearlman

9:30 324 THE IMMUNE RESPONSE OF DOGS IMMUNIZED WITH IRRADIATED L3 LARVAE OF *DIROFILARIA IMMITIS*: IDENTIFICATION OF POTENTIALLY PROTECTIVE ANTIGENS. Mejia JS* and Carlow CK. Molecular Parasitology, New England Biolabs, Beverly, MA.

9:45 325 ANTIBODY RESPONSES IN MICE IMMUNE TO THE LARVAL STAGES OF *ONCHOCERCA VOLVULUS*. Yutanawiboonchai W*, Lange AM, Haberstroh FH, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

10:00 326 EARLY DOWN REGULATION OF PARASITE ANTIGEN ASSOCIATED GRANULOMATOUS INFLAMMATORY RESPONSES IN *BRUGIA*-INFECTED JIRDS.

Klei TR*, Horohov DW, Coleman SU, Nguyen C, Philpott MS, and Nasarre C. Veterinary Microbiology & Parasitology, School of Vet Medicine, Louisiana State University, Baton Rouge, LA.

10:15 327 IMMUNE REACTIVITY TO THE GP15/400 MOLECULE OF *BRUGIA MALAYI*. Allen JE*, Lawrence RA, and Maizels RM. Wellcome Research Centre for Parasitic Infections, Department of Biology, Imperial College, London, UK.

10:30 328 IMMUNITY TO ONCHOCERCIASIS: IDENTIFICATION OF A PUTATIVELY IMMUNE POPULATION IN A HYPERENDEMIC AREA OF ECUADOR. Elson LH*, Guderian RH, Calvopina M, Araujo E, Parredes W, Bradley JE, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Department of Clinical Investigation, Hospital Vozandes, Quito, Ecuador; and Department of Biology, Imperial College of Science and Technology, London, UK.

10:45 329 HLA-DQ ALLELES ASSOCIATED WITH SUSCEPTIBILITY AND RESISTANCE TO ONCHOCERCIASIS. Meyer CG*, Gallin M, Erttmann KD, Brattig N, Schnittger L, Begovich AB, Erlich HA, and Horstmann RD. Bernhard Nocht Instute for Tropical Medicine, Hamburg, F.R. Germany; Department of Human Genetics, Roche Molecular Systems, Alameda, CA.

11:00 330 MHC CLASS II ASSOCIATIONS WITH CLINICAL MANIFESTATIONS IN LYMPHATIC FILARIASIS OBSERVED IN SOUTH INDIANS. Zimmerman PA*, Phadke P, Kumaraswami V, Vijayan V, Naryanan PR, Ottesen EO, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD; and Tuberculosis Research Center, Madras, India.

11:15 331 ANTIBODY RESPONSES TO EXTRACELLULAR MATRIX PROTEINS IN PATIENTS WITH ONCHOCERCIASIS AND LYMPHATIC FILARIASIS. Petralanda I*, Camico C, Carrasquel A, and Piessens WF. CAICET, Puerto Ayacucho, Amazonas, Venezuela; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:30 332 SCLEROSING KERATITIS INDUCED BY *ONCHOCERCA VOLVULUS* ANTIGENS CORRELATES WITH PRODUCTION OF TH2-ASSOCIATED CYTOKINES. Pearlman E*, Lass JH, Diaconu E, Hazlett, Jr FE, Bardenstein DS, and Kazura JW. Division of Geographic Medicine, Department of Medicine; and Department of Ophthalmology, Case Western Reserve University, Cleveland, OH.

11:45 333 REGULATORY CYTOKINES IN THE LYMPHATIC PATHOLOGY OF ATHYMIC MICE INFECTED WITH *BRUGIA MALAYI*. Rao UR*, Zometa CS, Vickery AC, Kwa BH, Nayar JK, and Sutton ET. College of Public Health; College of Medicine, University of South Florida, Tampa, FL; and Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL.

12:00 334 LOCAL TNF, PGE-2 AND HISTAMINE PRODUCTION DURING LIMB EDEMA IN *BRUGIA PAHANGI* INFECTED DOGS. Orton S, Schreuer D, and Hammerberg B*. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

12:15 335 UP-REGULATION OF ENDOTHELIAL CELL ICAM-1 AND E-SELECTIN EXPRESSION BY LOCAL CYTOKINES IN PATIENTS WITH LYMPHATIC FILARIASIS. Freedman DO*, Parker SB, De Almeida AB, Maciel MA, Braga C, Silva MC, and Furtado AF. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Centro des Pesquisas Aggeu Magalhaes, Recife, Pernambuco, Brazil.

SYMPOSIUM: EMERGING VIRUSES

Wednesday, November 3 Tudor Room
9:30 AM - 12:00 Noon
Chairpersons: C.J. Peters and R. Shope.

9:30 S66 RULES FOR VIRAL TRAFFIC: WHERE DO EMERGING VIRUSES COME FROM AND HOW DO WE RECOGNIZE THEM? Morse S. Rockefeller University, NY, NY.

9:50 S67 EVOLUTION AND EPIDEMIOLOGY OF MORBILLIVIRUSES: VIRUSES WITH ONE MAJOR HOST SPECIES. Bellini WJ. CDC, Atlanta.

10:10 S68 INFECTIOUS AGENTS AS GUARDIANS OF HUMAN DIVERSITY: INTERACTIONS OF THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX AND VIRAL PATHOGENS. Black, F. Yale University, New Haven, CT.

10:30 S69 POTENTIAL OF ANIMAL RESERVOIRS FOR GENERATION OF NEW HUMAN PATHOGENS. Mahy B. CDC, Atlanta.

10:50 S70 ECOLOGICAL DISTURBANCES AND EMERGENCE OF HUMAN PATHOGENS. Tesh R. Yale University, New Haven, CT.

11:10 S71 MEDICAL RESPONSE TO EMERGING VIRUSES. Jahrling PB, USAMRIID, Frederick, MD.

11:30 S72 VACCINES FOR EMERGING VIRAL DISEASES. Smith J. USAMRIID, Frederick, MD.

SYMPOSIUM
INTEGRATED CASE MANAGEMENT OF PEDIATRIC ILLNESS IN AFRICA

Wednesday, November 3, 1993 English
9:30 - 12:00 N
Chair: C. Campbell and D. Carroll

9:30 S73 PEDIATRIC MORTALITY AND MORBIDITY IN SUB-SAHARA AFRICA: BURDEN OF DISEASE AND DEMOGRAPHIC TRENDS. Mosley H. Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

9:45 S74 EXPERIENCE WITH DISEASE-SPECIFIC CONTROL PROGRAMS -RESPIRATORY DISEASES. Fagbule D. University of Ogun, Nigeria.

10:00 S75 EXPERIENCE WITH DISEASE-SPECIFIC CONTROL PROGRAMS -MALARIA: Steketee R. Malaria Branch, CDC, and Wirima J, University of Malawi, Blantyre, Malawi

10:15 S76 DISEASE SYNDROME OVERLAP: IMPLICATIONS FOR PROGRAMS. Kazembe P. Kamuzu Central Hospital, Lilongwe, Malawi.

10:30 DISCUSSION

10:40 COFFEE BREAK

11:00 S77 THE SICK CHILD INITIATIVE: PUTTING SUBSTANCE TO PHC. Tulloch J. Division of Control of Diarrheal and Respiratory Diseases, World Health Organization.

11:15 S78 INITIAL FIELD EVALUATIONS OF A "SICK CHILD ALGORITHM." KENYA: Zucker J. Malaria Branch, CDC, Atlanta. Otieno J. CDC/Kenya Medical Research Institute. THE GAMBIA: Mulholland K. Medical Research Council, The Gambia.

11:40 S79 THE CHALLENGES OF INTEGRATED DISEASE MANAGEMENT IN CHILD SURVIVAL PROGRAMS. Foster S. International Health Program Office, CDC, Atlanta.

11:50 DISCUSSION

SCIENTIFIC SESSION T:
SCHISTOSOMIASIS AND OTHER TREMATODES:
MOLECULAR BIOLOGY AND BIOCHEMISTRY

Wednesday, November 3 Stuart
9:30 - 11:45 AM
Chairpersons: R. Blanton and P. Knopf

9:30 336 INDUCTION OF A *SCHISTOSOMA MANSONI* SERPIN GENE FOLLOWING TRANSFORMATION. Ogundipe JO*, Aman RA, and Blanton RE. Division of Geographic Medicine, Department of Medicine, Case Western Reserve Universitiy, Cleveland OH; and National Museums of Kenya, Institute of Primate Research, Karen, Nairobi, Kenya.

9:45 337 THE *SCHISTOSOMA HAEMATOBIUM* SERINE PROTEASE INHIBITOR AT THE HOST-PARASITE INTERFACE. Blanton RE*, Fujioka H, Maeno Y, and Aikawa M. Case Western Reserve University, Department of Medicine and Department of Pathology, Cleveland, OH.

10:00 338 A RECOMBINANT *SCHISTOSOMA HAEMATOBIUM*-SPECIFIC ANTIGEN IS PREFERENTIALLY RECOGNIZED BY IgE AND IgG4 IN PATIENTS WITH URINARY SCHISTOSOMIASIS. Li Z*, King CL, Ogundipe JO, and Blanton RE. Case Western Reserve University, Division of Geographic Medicine, Department of Medicine, Cleveland, OH.

10:15 339 EXPRESSION AND PURIFICATION OF *SCHISTOSOMA MANSONI* CANDIDATE VACCINE SURFACE ANTIGEN GP22. Suri P*, Madikizela M, Lee J, Goldberg M, McCray JW, and Knopf PM. Division of Biology and Medicine, Brown University, Providence, RI; and Department of Biology, Morehouse College, Atlanta, GA.

10:30 340 CONSTRUCTION OF A VACCINE AGAINST *SCHISTOSOMA MANSONI* UTILIZING A TANDEMLY-REPEATED PEPTIDE DEMONSTRATING CROSS REACTIVITY WITH THREE PROTECTIVE WORM ANTIGENS. Petzke MM*, McCray, Jr. JW, and Knopf PM. Division of Biology and Medicine, Brown University, Providence, RI

10:45 341 CHARACTERIZATION OF A *SCHISTOSOMA MANSONI* cDNA CLONE THAT ENCODES PHOSPHOGLYCERATE KINASE, A POTENTIAL VACCINE CANDIDATE. Lee K, Karim A, Shalaby K, and LoVerde P*. Department of Microbiology, State University of New York, Buffalo, NY; and Department of Biochemistry, Ain Shams University, Cairo, Egypt.

11:00 342 IMMUNOLOGICAL CHARACTERIZATION OF LEUCINE AMINOPEPITIDASE FROM SCHISTOSOME EGGS. Kastens WA*, Secor WE, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

11:15 343 PARASITE CYTOCHROME c IS IMMUNOGENIC IN FASCIOLIASIS. Del Valle AI* and Hillyer GV. Laboratory of Parasite Immunology and Pathology, Department of Pathology, University of Puerto Rico School of Medicine, San Juan, PR.

11:30 344 A HUMAN SERUM FACTOR ACTIVATES PHOSPHORYLATION OF PUTATIVE SURFACE RECEPTORS IN *SCHISTOSOMA MANSONI*. Wiest PM* and Brautigan DL. International Health Institute, Brown University, Providence, RI; and Division of Biology and Medicine, Brown University, Providence, RI.

SCIENTIFIC SESSION U: CLINICAL TROPICAL MEDICINE I

Wednesday, November 3 Lancaster A/B
9:30 AM - 12:00 N
Chairpersons: L. Marcus and F.J. Bia

9:30 345 HUMAN STRONGYLOIDIASIS: AN AUTOPSY STUDY WITH QUANTITATIVE PARASITOLOGY. Haque AK, Schnadig V, Rubin SA, and Smith JH*. Department of Pathology, University of Texas Medical Branch, Galveston, TX; and Department of Radiology, University of Texas Medical Branch, Galveston, TX.

9:45 346 IVERMECTIN VS ALBENDAZOLE IN THE TREATMENT OF STRONGYLOIDIASIS IN ITALIAN PATIENTS. Scaglia M*, Gatti S, Bruno A, Bernuzzi AM, Cevini C, and Gaxotte P. Lab. Clinical Parasitology, Inst.Infectious Diseases, University-IRCCS S. Matteo, Pavia, Italy; and MSD-Chibret, Paris, France.

10:00 347 INTRAOCULAR INFECTION WITH *ALARIA* SP. MESOCERCARIAE: TWO CASES FROM CALIFORNIA]. McDonald HR, Kazacos KR*, Schatz H, and Johnson RN. One Daniel Burnham Court, Suite 210C, San Francisco, CA; and Department of Veterinary Pathobiology, Purdue University, West Lafayette, IN.

10:15 348 *BAYLISASCARIS PROCYONIS* CAUSING NEURAL LARVA MIGRANS IN A CHILD AND DUSN/OCULAR LARVA MIGRANS IN A MAN. Kazacos KR*, Cunningham CK, McMillan JA, Weiner LB, Goldberg MA, Katz B, Boyce WM, and Wozniak EJ. Department Veterinary Pathobiology, Purdue Univ, W. Lafayette, IN; Department Pediatrics, SUNY Health Science Center, Syracuse, NY; Department Neuro-Ophthalmology, California Pacific Med Center, San Francisco, CA; and Department Veterinary Microbiology & Immunology, University of California, Davis, CA.

10:30 349 CANINE HOOKWORMS IN THE HUMAN GUT. Prociv P*, Croese J, Loukas A, Opdebeeck J, and Fairley S. Department of Parsitology, The University of Queensland, Brisbane, Australia; and Townsville General Hospital, Queensland, Australia.

10:45 350 SNAKE BITES BY TAIPANS IN PAPUA NEW GUINEA: SEVERE NEUROTOXICITY AND HAEMOSTATIC DYSFUNCTION; LIMITED EFFICACY OF SPECIFIC ANTIVENOM. Warrell DA*, Lalloo DG, Trevett AJ, Black J, Paul M, Naraqi S, Hutton RA, and Theakston RD. Centre for Tropical Medicine, University of Oxford, UK; University of Papua New Guinea; Royal Free Hospital, London, UK; and Liverpool School of Tropical Medicine, Liverpool, UK.

11:00 351 PATTERN OF ANTI-HEV BY ELISA IN AN EPIDEMIC OF HEPATITIS IN PAKISTAN. Bryan JP*, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH, and Legters LJ. Department of Preventive Medicine, Uniformed Services University of the Health Science, Bethesda, MD; Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD; and Pakistan U.S. Lab for Seroepidemiology, Rawal, Pakistan.

11:15 352 ACUTE FEBRILE ILLNESS IN SOMALIA DURING OPERATION RESTORE HOPE. Magill AJ* and Smoak BL. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; and Department of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC.

11:30 353 THE UTILITY OF MAGGOT THERAPY FOR TREATING CHRONIC WOUNDS. Sherman RA*, Wyle F, Vulpe M, Levsen L, and Castillo L. Veterans Affairs Medical Center, Long Beach, CA; and University of California, Irvine, CA.

11:45 354 DOES BCG WORK? A META-ANALYSIS OF BCG EFFICACY IN PREVENTION OF TUBERCULOSIS. Colditz GA, Brewer TF, Berkey CS, Wilson ME*, Burdick E, Fineberg HV, and Mosteller F. Harvard School of Public Health, Boston MA; and Harvard Medical School, Boston MA.

SCIENTIFIC SESSION V: OPPORTUNISTIC PROTOZOA

Wednesday, November 3 Lancaster C
9:30 - 11:45 AM
Chairpersons: J. Schwartzman and E. Didier

9:30 355 MACROPHAGE-MEDIATED KILLING OF MICROSPORIDIA *IN VITRO*. Didier ES* and Aldras AM. Tulane Regional Primate Research Center, Covington, LA.

9:45 356 HUMAN CYTOTOXIC T-CELL CLONES SPECIFIC FOR *TOXOPLASMA GONDII* LYSE TACHYZOITE-INFECTED TARGET CELLS. Curiel TJ*, Purner MB, Krug EC, Xiong C, and Berens RL. Division of Infectious Disease, University of Colorado Health Sciences Center, Denver, CO; and Paravax, Inc., Ft. Collins, CO.

10:00 357 CHARACTERIZATION AND PURIFICATION OF *TOXOPLASMA GONDII* EXCRETED-SECRETED ANTIGENS. Frachette MJ, Autheman JM, and Rizvi FS*. Department of Parasite Immunology, Pasteur Mérieux Sérums & Vaccins, Marcy l'Etoile, France.

10:15 358 KT5926: AN AGENT WHICH MAY INTERFERE WITH MYOSIN FUNCTION IN *TOXOPLASMA GONDII*. Schwartzman JD* and Wellehan J. Department of Pathology, Dartmouth Medical School, Hanover, NH.

10:30 359 COMPARISON OF THE RIBOSOMAL DNA OF VIRULENT AND AVIRULENT STRAINS OF *TOXOPLASMA GONDII*. Nimmo KA and Brindley PJ*. Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

10:45 360 EFFICACY TRIAL IN CATS WITH EXPERIMENTAL VACCINE CONTAINING MODIFIED LIVE *TOXOPLASMA GONDII* T-263 STRAIN. Choromanski L*, Freyre A, Fishback J, and Popiel I. Miles Inc., Animal Health Products, Shawnee Mission, KS; University of Kansas Medical Center, Kansas City, KS; University of Kansas Medical Center, Kansas City, KS; and Paravax, Inc., Ft. Collins, CO.

11:00 361 DETECTION OF PATHOGENIC PROTOZOA IN FECAL SPECIMENS FROM URBAN DWELLING DOGS. Jafri HS*, Moorhead AR, Reedy T, Dickerson JW, Wahlquist SP, Schantz PM, and Bryan RT. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA.

11:15 362 BROAD SPECTRUM PCR-BASED STRATEGY FOR DETECTING *BABESIA* AND *THEILERIA* IN TICK AND VERTEBRATE HOSTS. McLaughlin GL*, Gordon VR, Vodkin MH, Ssenyonga GS, Nanteza E, Rubaire-Akiiki R, Wafula O, Hansen RD, and Kakoma I. Purdue University, West Lafayette, IN; University of Illinois, Urbana, IL; and Makerere University, Kampala, Uganda.

11:30 363 DIAGNOSIS OF *NEOSPORUM CANINUM* USING RECOMBINANT TACHYZOITE ANTIGEN. Jenkins MC*, Bjerkas I, and Dubey JP. Protozoan Diseases Laboratory, ARS, USDA, Beltsville, MD; Norwegian College of Veterinary Medicine, Oslo, Norway; Zoonotic Diseases Laboratory, ARS, USDA, Beltsville, MD.

SCIENTIFIC SESSION W: KINETOPLASTIDAE EPIDEMIOLOGY AND CHEMOTHERAPY

Wednesday, November 3 Lancaster D
9:30 AM - 12:00 N
Chairpersons: T.R. Navin and K.A. Weigle

9:30 364 POST KALA-AZAR DERMAL LEISHMANIASIS IN THE SUDAN: A POPULATION BASED STUDY. El-Hassan AM, Zijlstra EE*, Meredith SEO, Ismael A, and Ghalib HW. Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Sudan; Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, The Netherlands; NH Swellengrebel Laboratory of Tropical Hygiene, Royal Tropical Institute, Amsterdam, The Netherlands; Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Sudan; and Department of Microbiology, University of Juba, Sudan.

9:45 365 PLACENTAL PATHOLOGY OF CONGENITAL CHAGAS DISEASE FROM INFECTED NEONATES IN COCHABAMBA, BOLIVIA. Lora J*, Schwartz DA, Torrico F, Balderrama F, Moore AC, and Bryan RT. Facultad de Medicina-UMSS, Cochabamba, Bolivia; Programma Nacional de Control de la Enfermedad de Chagas, Cochabamba, Bolivia; Emory University, Atlanta, GA; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

10:00 366 ENDEMIC KALA-AZAR IN THE SUDAN: DOES PREVIOUS EXPOSURE TO *LEISHMANIA MAJOR* PROTECT AGAINST CHALLENGE WITH *LEISHMANIA DONOVANI*? Zijlstra EE*, El-Hassan AM, Ismael A and Ghalib HW. Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, The Netherlands; Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Sudan; and Department of Microbiology, University of Juba, Sudan.

10:15 367 APPLICATION OF THE PCR TO DETECTION OF *LEISHMANIA* PARASITES IN FINGER PRICK BLOOD SPOTS ON FILTER PAPER. Meredith SE*, Schoons GJ, Kroon N, Zijlstra EE, Van Eyes GJ, and El-Hassan AM. Department of Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands; Department of Infectious Diseases, Tropical Medicine and AIDS, AMC, Amsterdam, The Netherlands; Department of Molecular Cell Biology, University of Limburg, Maastricht, The

Netherlands; and Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Khartuom, Sudan.

10:30 368 DEVELOPMENT AND EVALUATION OF A CLINICAL PREDICTION RULE FOR AMERICAN CUTANEOUS LEISHMANIASIS IN COLOMBIA. Weigle KA*, Escobar MA, Arias AL, Martinez FR, and Rojas C. Department of Epidemiology, University of North Carolina, Chapel Hill, NC; and Fundacion Centro Internacional de Investigaciones Medicas (CIDEIM), Cali, Colombia.

10:45 369 LACK OF SYNERGY BETWEEN INTERFERON-γ AND GLUCANTIME IN TREATING CUTANEOUS LEISHMANIASIS IN GUATEMALA. Arana BA, Navin TR*, Arana FE, Berman JD, and Rosenkaimer F. Universidad del Valle de Guatemala, Guatemala City, Guatemala; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Walter Reed Army Institute of Research, Washington, DC; and Boehringer Ingelheim, Ingelheim am Rhein, Germany.

11:00 370 TREATMENT OF CUTANEOUS LEISHMANIASIS WITH A SHORT COURSE OF PENTAMIDINE. Soto J*, Grogl M, and Berman J. Bogota Military Hospital, Bogota, Colombia; and Walter Reed Army Institute of Research, Washington, DC.

11:15 371 EFFICACY OF 28-DAY AND 40-DAY REGIMENS OF SODIUM STIBOGLUCONATE (PENTOSTAM) IN THE TREATMENT OF MUCOSAL LEISHMANIASIS. Franke ED*, Llanos A, Echevarria J, Cruz ME, Campos P, Tovar AA, Lucas CM, and Berman JD. Naval Medical Research Institute Detachment, Lima, Peru; "Alexander von Humboldt" Institute of Tropical Medicine, Cayetano Heredia University, Lima, Peru; Hospital Regional del Cusco, Cusco, Peru; and Walter Reed Army Institute of Research, Washington, DC.

11:30 372 OPEN FIELD TRIAL OF SHORT COURSE LIPOSOMAL AMPHOTERICIN B IN COMPLICATED VISCERAL LEISHMANIASIS. Seaman J, Wilkinson R*, Boer C, de Wilde E, Sondorp HE, and Davidson RN. MSF (Holland), Nairobi, Kenya; and Department of Tropical Medicine, St. Mary's Hospital Medical School, Northwick Park Hospital, Harrow, UK.

11:45 373 SHORT COURSE LIPOSOMAL AMPHOTERICIN B ('AMBISOME') IN PATIENTS WITH MEDITERRANEAN VISCERAL LEISHMANIASIS. Davidson RN*, DiMartino L, Gradoni L, and Giacchino R. Infection and Tropical Medicine, St Marys Hospital Medical School, Northwick Park Hospital, Harrow United Kingdom; Division Pediatrics, Ospedale Pausilipon, Naples, Italy; Istitute Superiore Sanita, Laboratori di Parasitologica, Rome, Italy; and Department of Infectious Diseases, Istituto Gaslini, Genoa, Italy.

SCIENTIFIC SESSION X: SEVERE MALARIA

Wednesday, November 3 Essex A/B
9:30 AM - 12:00 N
Chairpersons: V.R. Gordeuk and G. Watt

9:30 374 AN INVESTIGATION OF THE POSSIBLE ROLE OF BACTEREMIA IN CEREBRAL MALARIA IN CHILDREN IN LAGOS, NIGERIA. Alabi SA*, Prada JJ, Ajayi-Obe, Prieto I, Omonigbehin EA, Sodeinde O, Lehman L, and Kremsner PG. National Institute for Medical Research, Lagos, Nigeria; Landesinstitut fur Tropenmedizin, Berlin, Germany;

Lagos University Teaching Hospital, Lagos, Nigeria; and Universidad Complutense de Madrid, Spain.

9:45 375 INTRACRANIAL PRESSURE MONITORING IN KENYAN CHILDREN WITH CEREBRAL MALARIA. Newton CR*, Kirkham FJ, Sowume A, Waruiru C, Mwangi I, Murphy S, and Marsh K. Kenya Medical Research Institute, Kilfi Kenya; and Department of Neurosciences, Institute of Child Health, London, UK.

10:00 376 MEASUREMENT OF CEREBRAL BLOOD FLOW IN AFRICAN CHILDREN WITH CEREBRAL MALARIA. Marsh K, Newton CR*, Edwards DA, Sowume A, Cope M, and Kirkham FJ. Kenya Medical Research Institute, Kilfi Kenya; Department of Paediatrics & Neonatal Medicine, Royal Postgraduate Medical School, London, UK; Department of Medical Physics & Bioengineering, University College, London, UK; Department of Neurosciences, Institute of Child Health, London, UK.

10:15 377 ELEVATED TRANSFERRIN SATURATIONS ARE ASSOCIATED WITH DEEP AND PROLONGED COMA IN CHILDREN WITH CEREBRAL MALARIA. Fernandes NF, Thuma PE, Biemba G, Zulu S, Parry D, Brittenham GM, and Gordeuk VR*. Department of Medicine, MetroHealth Medical Center, Case Western Reserve University School of Medicine, Cleveland, OH; Department of Pediatrics, Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA; and Macha Mission Hospital, Choma, Zambia.

10:30 378 ACUTE INFLAMMATORY CYTOKINE RESPONSES IN SERUM AND CEREBROSPINAL FLUID (CSF) AMONG INDONESIANS WITH CEREBRAL MALARIA OR SEVERE COMPLICATED MALARIA. Richie TL*, Harianto PN, Tjitra E, Solihin A, Basri H, Punjabi NH, Marwoto H, Alwi-Datau E, Larasati RP, Pudjoprawoto N, Hoffman SL, and O'Hanley PD. Naval Medical Research Unit #2 Jakarta, Indonesia; Department of Internal Medicine, University of Sam Ratulangi, North Sulawesi, Indonesia; National Institute of Health Research and Development, Jakarta, Indonesia; Subdivision of Immunology, Medical Faculty, University of Sam Ratulangi, North Sulawesi, Indonesia; Naval Medical Research Institute, Bethesda, Maryland MD; and Departments of Medicine and Microbiology, Stanford University, Stanford, CA.

10:45 379 METABOLIC DISORDERS, TNF-α, IFN-γ AND SOLUBLE ELAM AND ICAM-1 RECEPTORS IN SEVERE AND CEREBRAL MALARIA IN AFRICAN ADULTS. Deloron P*, Niyongabo T, Dumont N, Astagneau P, Ndarugirire F, Muhirwa G, Ndayiragije A, Brelivet JC, Aubry P, and Peyron F. INSERM Unité 13, Paris, France; Kamenge Hospital, Bujumbura, Burundi; and Fac of Medicine, Lyon, France.

11:00 380 HYPERBILIRUBINAEMIA IN PATIENTS WITH SEVERE MALARIA IN NORTHERN SULAWESI, INDONESIA. Harianto PN*, Tenda-Moeis E, and Thomas TL. Department of Internal Medicine, University of Sam Ratulangi, Gunung Wenang Hospital, Manado, Indonesia; and Departmente of Tropical Medicine, U.S. Naval Medical Research Unit #2, Jakarta, Indonesia.

11:15 381 SUPPORTIVE THERAPY OF FALCIPARUM MALARIA WITH PENTOXIFYLLINE: A PROSPECTIVE RANDOMIZED STUDY. Hemmer CJ*, Hort G, Chiwakata C, Kern P, Nawroth PP, and Dietrich M. Department of Medicine, Bernhard-Nocht-Institute for Tropical Medicine, Hamberg, Germany; and Heidelberg University Medical School, Heidelberg, Germany.

11:30 382 IN-HOSPITAL MORTALITY AND MORBIDITY DUE TO MALARIA-ASSOCIATED SEVERE ANEMIA IN TWO AREAS IN MALAWI WITH DIFFERENT MALARIA TRANSMISSION PATTERNS. Slutsker L*, Taylor TE, Wirima JJ, and Steketee RW. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; College of Osteopathic Medicine, Michigan State University, East Lansing, MI; and University of Malawi, College of Medicine, Blantyre, Malawi.

11:45 383 HEALTH IMPACT OF *PLASMODIUM FALCIPARUM* AMONG HOSPITALIZED PEDIATRIC PATIENTS. Zucker JR*, Ruebush TK, Olango C, Were JB, and Campbell CC. Malaria Branch, Centers for Disease Control, Atlanta, GA; Siaya District Hospital, Siaya, Kenya; and Kenya Medical Research Institute, Kenya.

SCIENTIFIC SESSION Y:
PARASITE BIOCHEMISTRY AND PHYSIOLOGY

Wednesday, November 3 Lancaster E
9:30 AM - 12:00 N
Chairpersons: J.R. Bristol and M.C. Jenkins

9:30 384 A COMPARATIVE STUDY OF ECDYSTEROID TITERS AND METABOLISM IN EMBRYOS OF TWO TICK SPECIES. Dotson EM*, Connat JL, and Diehl PA. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, Georgia; Departement de Zoologie et de Biologie Animale, Universite de Geneve, Geneve, Switzerland; and Institute de Zoologie, Universite de Neuchatel, Neuchatel, Switzerland.

9:45 385 THE PYRUVATE DEHYDROGENASE COMPLEX FROM ANAEROBIC PARASITIC HELMINTHS. Klingbeil M*, Summers M, Sidawy E, and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

10:00 386 THE PYRUVATE DEHYDROGENASE COMPLEX FROM ANAEROBIC PARASITIC HELMINTHS. Diaz F* and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

10:15 387 PROTEASE RELEASE COINCIDES WITH RE-ACTIVATION OF INFECTIVE HOOKWORM LARVAE. Hawdon JM, Perregaux MA, and Hotez PJ. Medical Helminthology Laboratory, Yale University School of Medicine, New Haven, CT.

10:30 388 PRELIMINARY CHARACTERIZATION OF NEMATODE BOMBESIN/GASTRIN-RELEASING PEPTIDE BINDING SITES. Huntington MK*, Thompson DP, Geary TG, Mackenzie CD, and Williams JF. Department of Microbiology, Michigan State University, East Lansing, MI; Animal Health Discovery Research, The Upjohn Company, Kalamazoo, MI; and Department of Pathology, Michigan State University, East Lansing, MI.

10:45 389 VOLATILE FATTY ACID EFFLUX FROM ISOLATED SEGMENTS OF *ASCARIS SUUM* BODY WALL. Blair KL*, Ho NF, Barsuhn CL, Geary TG, and Thompson DP. Animal Health Therapeutics, The Upjohn Company, Kalamazoo, MI; and Drug Delivery Systems Research, The Upjohn Company, Kalamazoo, MI.

11:00 390 APPARENT INVOLVEMENT OF A PROTON GRADIENT IN THE MITOCHONDRIAL ENERGY-LINKED TRANSHYDROGENATION OF ADULT *HYMENOLEPIS*

DIMINUTA. Park JP* and Fioravanti CF. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH.

11:15 391 CATALYSIS OF PYRIDINE NUCLEOTIDE TRANSHYDROGENASE ACTIVITY BY ADULT *HYMENOLEPIS DIMINUTA* SUBMITOCHONDRIAL PARTICLES. Whitmore MM* and Fioravanti CF. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH.

11:30 392 MITOCHONDRIAL NADH:NAD TRANSHYDROGENATION AND THE LIPOAMIDE DEHYDROGENASE OF ADULT *HYMENOLEPIS DIMINUTA*. Walker DJ* and Fioravanti CF. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH.

11:45 393 MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE OF cDNA CLONES ENCODING THE 2-METHYL BRANCHED CHAIN ENOYL CoA REDUCTASE FROM *ASCARIS SUUM*. Duran E*, Johnson K, Wheelock M, Komuniecki PR, and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

SYMPOSIUM
PARASITOLOGY AND BIODIVERSITY

Wednesday, November 3 — Italian Suite
10:00 AM - 12:00 N
Co-Organizers: D.R. Brooks and E.P. Hoberg
Chairperson: J.R. Lichtenfels

10:00 S80 THE ROLES OF PARASITOLOGY IN BIODIVERSITY STUDIES. Hoberg EP. USDA Biosystematic Parasitology Laboratory and US National Helminth Collection, Beltsville, MD.

10:25 S81 THE ROLE AND FUTURE OF COLLECTIONS IN BIODIVERSITY STUDIES. Hoagland E. Association of Systematics Collections, Washington, DC.

10:50 S82 SYSTEMATICS AGENDA 2000 AND BIODIVERSITY STUDIES. Davis G. Academy of Natural Sciences of Philadelphia, Philadelphia, PA.

11:15 S83 BIODIVERSITY OPPORTUNITIES THROUGH NSF. Yates T. Department of Biology, University of New Mexico, Albuquerque, NM.

11:40 S84 A MOBILIZATION PLAN FOR PARASITOLOGY IN ALL TAXON BIODIVERSITY INVENTORIES. Brooks D. Department of Zoology, University of Toronto, Toronto.

FRED L. SOPER MEMORIAL LECTURE

Wednesday, November 3 — Regency Ballroom (Condor)
1:30 - 2:15 PM
Chair: R. Tesh

NEW DISEASES AND UNLIKELY EVENTS

R. E. Shope
Yale Arbovirus Research Unit

ASP PRESIDENTIAL ADDRESS

Wednesday, November 3
1:30 - 2:30 PM
Chair: J. Lichtenfels

Regency Ballroom
(Falcon)

DR. STOLL'S WORMY WORLD REVISITED: THE NEGLECTED ANIMAL DISEASES

K. D. Murrell
Beltsville Agricultural Research Center, USDA, ARS
Beltsville, MD

PRESENTATION OF THE HENRY BALDWIN WARD MEDAL AND ASP STUDENT AWARDS

Wednesday, November 3, 1993
2:30-3:30
Chair: T. Yoshino

Regency Ballroom
(Falcon)

The Recipient of the 1993 Henry Baldwin Ward Medal is

MARTIN L. ADAMSON
Department of Zoology
University of British Columbia
Vancouver, British Columbia

ASP BUSINESS MEETING

Wednesday, November 3
3:30 - 4:30 PM
Chairperson: K. Murrell

Regency Ballroom
(Falcon)

R. BARCLAY MCGHEE MEMORIAL LECTURE

Wednesday, November 3, 1993
4:30 - 5:30 PM
Chair: P. Loverde

Regency Ballroom
(Falcon)

PARASITISM, DEVELOPMENT AND REPRODUCTION OF TICKS

J. H. Oliver, Jr.
Institute of Anthropodology and Parasitism
Georgia Southern University, Statesboro, GA

AMERICAN COMMITTEE ON ARBOVIROLOGY

HANTAVIRUS ACUTE RESPIRATORY DISTRESS SYNDROME (HARDS) IN NORTH AMERICA: AN EXPLOSIVE EMERGENCE

Wednesday, November 3 — Regency Ballroom
2:30 - 6:30 PM — (Condor)
Chair: K.M. Johnson

I. BACKGROUND

3:30 S85 WORLD-WIDE STATUS OF HANTAVIRAL DISEASE: THE PATH FROM DISCOVERY TO TREATMENT AND PREVENTION. Lee HW. Institute for Viral Diseases, Korea University, Seoul, Korea.

3:50 S86 SEROLOGICAL AND PHYLOGENETIC ANALYSIS OF HANTAVIRUSES. Schmaljohn C, Xiao S-Y, Chu Y-K, and Dalrymple JM. US Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, MD.

II. THE NEW HANTAVIRUS

4:05 S87 IMMUNOLOGICAL AND DIAGNOSTIC CHARACYERIZATION OF INFECTION: FROM A HETEROLOGOUS TOE-HOLD TO A SEARCH FOR HOMOGENEOUS DEFINITION. Ksiazek T, and Colleagues. Special Pathogens Branch, CID, Centers for Disease Control, Atlanta, GA.

4:25 S88 GENOTYPE IDENTIFICATION AND CLASSIFICATION USING EXISTENT HANTAVIRUS SEQUENCES AND THOSE FROM THE NEW AGENT. Nichol S. and Colleagues, Special Pathogens Branch, CID, Centers for Disease Control, Atlanta, GA.

4:40 S89 IMMUNOHISTOCHEMICAL LOCALIZATION OF VIRAL REPLICATION IN HUMANS AND CORRELATIONS AMONG THREE METHODS FOR IDENTIFICATION OF INFECTION IN HUMANS AND RODENTS. Zaki S. and Colleagues, DVRD, CID, Centers for Disease Control, Atlanta, GA.

4:50 Coffee Break

III. THE NEW DISEASE

5:00 S90 CASE DEFINITION AND EPIDEMIOLOGY. Butler JC. DBMD, CID, Centers for Disease Control, Atlanta, GA.

5:20 S91 CLINICAL FEATURES OF THE HARDS SYNDROME. Mertz G. and Colleagues. Department of Medicine and Infectious Diseases, University of New Mexico School of Medicine, Albuquerque, NM.

5:40 S92 PATHOLOGY OF HARDS. Nolte K, Fedderson R, Foucar K, Umland E, and Zumwalt R. Ofice of the Medical Investigator and Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM.

5:55 S93 RODENT RESERVOIR/VECTORS OF INFECTION. Childs J. and Many Colleagues. Viral and Rickettsial Zoonoses Branch, CID, Centers for Disease Control, Atlanta, GA.

IV. NOW WHAT?

6:15 S94 NATIONAL GOALS AND PLANS. Peters CJ. Chief, Special Pathogens Branch, CID, Centers for Disease Control, Atlanta, GA.

AMERICAN COMMITTEE ON CLINICAL TROPICAL MEDICINE AND TRAVELERS' HEALTH

Wednesday, November 3 — Lancaster A/B
2:30 - 6:00 PM
Chairs: C. Panosian and F. Bia

2:30 S95

VINCENZO MARCOLONGO MEMORIAL LECTURE

HUMAN RABIES: CLINICAL FEATURES, PATHOGENESIS, AND POTENTIAL TREATMENT

Thiravat Hemachuda
Chulalongkorn University
Bangkok, Thailand

3:30 Coffee Break

3:45 S96 CLINICAL TROPICAL NEUROLOGY CASES FROM THAILAND. Hemachuda T. Chulalongkorn University, Bangkok, Thailand.

4:00 S97 EPIDEMIC OF OPTIC AND PERIPHERAL NEUROPATHY IN CUBA. Mas P. Instituto Medicina Tropical "Pedro Kouri", Havana, Cuba.

4:30 S98 MALARIA UPDATE. Campbell C. CDC.

5:00 Business meeting.

SYMPOSIUM MOLECULAR BASIS OF PROTOZOAN-HOST CELL INTERACTIONS

Wednesday, November 3 — Lancaster D
2:30 - 5:00 PM
Chairs: M. Hollingdale and M. Aikawa

2:30 S99 CELL-CELL INTERACTIONS, AN INTRODUCTION. Bevalaqua M. Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA.

3:00 S100 PROPERTIES OF A SPOROZOITE LIGAND FOR HEPATOCYTES. Sinnis P. New York University, NY.

3:20 S101 ROLES OF HEAT SHOCK PROTEINS IN *TOXOPLASMA*. Himeno K. Tokushima University School of Medicine, Tokushima, Japan.

3:40 Coffee Break

4:00 S102 MOLECULAR BASIS OF *LEISHMANIA* VIRULENCE. Chang KP. Chicago Medical School, Chicago, IL.

4:20 S103 ROLE OF SIALIC ACID AND GLYCOSAMINOGLYCANS IN *TRYPANOSOMA CRUZI* - HOST CELL INTERACTIONS. Perreira M. Tufts University School of Medicine, Boston, MA.

4:40 S104 MECHANISMS OF INVASION OF *ENTAMOEBA HISTOLYTICA*. Petri W. University of Virginia, Charlottesville, VA.

SCIENTIFIC SESSION Z: GIARDIASIS AND CRYPTOSPORIDIOSIS

Wednesday, November 3 Lancaster C
2:30 - 4:45 PM
Chairpersons: H. Ward and C. Petersen

2:30 394 NEUTRALIZING ANTIBODIES INDUCE A CIRCUMSPOROZOITE-LIKE REACTION WITH *CRYPTOSPORIDIUM PARVUM* SPOROZOITES. Riggs MW*, Sterling CR, Stone AL, Westhof NC and Bentley DL. Department of Veterinary Science, University of Arizona, Tucson, AZ.

2:45 395 CLONING OF THE GENE FOR P68, A *CRYPTOSPORIDIUM PARVUM* SPOROZOITE PROTEIN THAT IS THE TARGET OF PROTECTIVE ANTIBODY *IN VITRO*. Petersen C*, Barnes DA, Lewis S, and Doyle PS. University of California, San Francisco, San Francisco General Hospital, San Francisco, CA; and ImmuCell Corp., Portland, ME.

3:00 396 *CRYPTOSPORIDIUM* INFECTION AND ATTEMPTED CONTROL OF TRANSMISSION IN A DAIRY HERD. Zajac AM*, Holland RJ, and Moore GA. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg VA; and College of Veterinary Medicine, Michigan State University, East Lansing MI.

3:15 397 EXPRESSION OF GLCNAC/GALNAC TRANSFERASE ACTIVITIES DURING ENCYSTATION OF *GIARDIA LAMBLIA*. Das S* and Gillin FD. Department of Pathology, UCSD-Medical Center, San Diego, CA.

3:30 398 DEFECTIVE CYST WALL ANTIGEN EXPRESSION AND TRANSPORT AND BILE SALT UPTAKE BY AN ENCYSTATION DEFICIENT SUBLINE OF *GIARDIA LAMBLIA*. Reiner DS*, Hetsko ML, Das S, Ward HD, McCaffery M, and Gillin FD. Department of Pathology, UCSD Medical Center, San Diego, CA; Division of Geographic Medicine and Infectious Diseases, Tufts University School of Medicine, Boston, MA; and Division of Cellular and Molecular Medicine, UCSD, San Diego, CA.

3:45 399 THE CYSTEINE PROTEASE OF *GIARDIA LAMBLIA* IS A TARGET FOR STRUCTURE-BASED DRUG DESIGN. Ward WL* and McKerrow JH. Department of Anatomic Pathology, University of California, VAMC, San Francisco, CA.

4:00 400 AN ADULT MOUSE MODEL FOR *GIARDIA LAMBLIA*. Byrd LG, Conrad JT, and Nash TE*. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

4:15 401 ISOLATION OF ADHESION DEFICIENT *GIARDIA LAMBLIA* CLONES WITH A REDUCED ABILITY TO ESTABLISH INFECTION IN MONGOLIAN GERBILS. Hernandez-Sanchez J and Ortega-Pierres MG. Department of Genetics and Molecular Biology, Center for Research and Advanced Studies IPN, Mexico, DF, Mexico.

4:30 402 QUANTITATION OF *GIARDIA* CYSTS AND *CRYPTOSPORIDIUM* OOCYSTS IN FECAL SAMPLES BY A DIRECT IMMUNOFLUORESCENCE ASSAY. Xiao L* and Herd RP. Department of Veterinary Preventive Medicine, The Ohio State Uinversity, Columbus, OH.

SCIENTIFIC SESSION AA: KINETOPLASTIDAE: IMMUNOLOGY AND PATHOLOGY

Wednesday, November 3 York
2:30 - 4:15 PM
Chairpersons: R.L. Tarleton and T.G. Evans

2:30 403 IL-12 PRODUCTION IN RESPONSE TO *LEISHMANIA MAJOR* BY RESISTANT AND SUSCEPTIBLE STRAINS OF MICE. Vieira LQ*, Wysocka M, Scharton TM, Afonso LC, Trinchieri G, and Scott P. Department of Pathobiology, University of Pennsylvania, Philadelphia, PA; and The Wistar Institute, Philadelphia, PA.

2:45 404 LEISHMANIA INFECTION OF HUMAN MONOCYTES IN VITRO INDUCES PRODUCTION OF TGFβ. Kanesa-thasan N*, Danielpour D, and Nacy C. Walter Reed Army Institute of Research, Washington, DC; and National Cancer Institute, National Institutes of Health, Bethesda, MD.

3:00 405 FAB FRAGMENTS OF THE MONOCLONAL ANTIBODY 4A4 TO *TRYPANOSOMA CRUZI* GP 83 NEUTRALIZE TRYPOMASTIGOTE BINDING AND ENTRY AND CONFER IMMUNOPROTECTION. Villalta F*, Smith C, Ruiz-Ruano A, Johnston D, and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

3:15 406 *TRYPANOSOMA CRUZI* INFECTION AND IMMUNUNIZATION IN CLASS I AND CLASS II MHC DEFICIENT MICE. Tarleton RL*, Postan M, Grusby M, and Glimcher L. Department of Zoology, University of Georgia, Athens, GA; and Department of Cancer Biology, Harvard School of Public Health, Boston, MA.

3:30 407 DIFFERENTIAL INFECTIVITY OF HUMAN MONOCYTES BY AMASTIGOTES AND PROMASTIGOTES OF *LEISHMANIA MAJOR* BASED ON FLOW CYTOMETRIC ANLAYSIS. Leiby DA*, McMahon-Pratt D, and Toro LA. Transmissible Diseases, American Red Cross, Rockville, MD; Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT; and Bacterial Diseases, Walter Reed Army Institute of Research, Washington, DC.

3:45 408 THE ROLE OF THE *LEISHMANIA* SURFACE PROTEASE-GP63 IN COMPLEMENT FIXATION AND CELL ADHESION. Brittingham A*, Morrison CJ, McMaster RM, and Mosser DM. Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA; and Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

4:00 409 T HELPER CELL CYTOKINE PROFILES AND B CELL IG ISOTYPE RESPONSES TO THE TRYPANOSOME VSG MOLECULE SUGGEST ALTERNATIVE CLASS SWITCH REGULATORY MECHANISMS. Schopf LR*, Schieifer KW, and Mansfield JM. Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

SCIENTIFIC SESSION BB: MALARIA CHEMOTHERAPY

Wednesday, November 3 Tudor
2:30 - 5:00 PM
Chairpersons: T.G. Brewer and S.L. Anderson

2:30 410 COMPARATIVE COMPLEMENT SELECTION IN BACTERIA CAN BE USED TO SCREEN FOR SELECTIVE INHIBITORS OF A PURINE SALVAGE ENZYME OF *PLASMODIUM FALCIPARUM*. Eakin AE*, Serrano AE, and Craig SP. Department of Biochemistry, University of Puerto Rico School of Medicine, San Juan, PR; and Department of Microbiology, University of Puerto Rico School of Medicine, San Juan, PR.

2:45 411 EVALUATION OF THE ANTIMALARIAL ACTIVITY OF WR250417 (PS-15) USING THE *IN VITRO - IN VIVO* MODEL. Rieckmann KH*, Yeo AE, Edstein MD, Jacobus DP, and Canfield CJ. Army Malaria Research Unit, Ingleburn, Australia; Jacobus Pharmaceutical Company, Inc, Princeton, NJ; and Pharmaceutical Systems Inc, Gaithersburg, MD.

3:00 412 NEW, ANTIMALARIAL TRICYCLIC 1,2,4-TRIOXANES; PRECLINICAL *IN VIVO* EVALUATIONS. Posner GH*, Oh CH, Webster HK, and Rossan RN. Department of Chemistry, The Johns Hopkins University, Baltimore, MD; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Gorgas Memorial Laboratory, Panama, Panama.

3:15 413 DIFFERENTIAL NEUROTOXICITY OF ARTEMISININ ANALOGS IN AN *IN VIVO* MODEL. Brewer TG*, Petras JM, Peggins JO, Li Q, Lin AJ, Sperry M, Figueroa L, Aguilar A, and Schuster BG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research Washington, DC; and Division of Neuroscience, Walter Reed Army Institute of Research, Washington, DC.

3:30 414 IN VITRO NEUROTOXICITY OF ARTEMISININ ANALOGS. Wesche DL*, DeCoster M, Tortella F, and Brewer TG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Division of Neuroscience, Walter Reed Army Institute of Research, Washington, DC.

3:45 415 PHARMACOKINETICS OF SODIUM ARTESUNATE AFTER IM AND IV ADMINISTRATION. Benakis A*, Paris M, Plessas C, Hien TT, Waller D, and White NJ. Department of Pharmacology, University Medical Center, Geneva, Switzerland; Center for Tropical Diseases, Cho Quan Hospital, Ho Chi Minh City, Viet Nam; and Wellcome-Mahidol University of Oxford Tropical Medicine Research Programme, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

4:00 416 PRIMAQUINE ADJUNCT TO 28 DAY EVALUATION OF HALOFANTRINE VS. CHLOROQUINE FOR THERAPY OF MALARIA IN PEOPLE REMAINING EXPOSED TO INFECTION IN IRIAN JAYA. Fryauff DJ*, Baird JK, Basri H, Bangs MJ, Wiady I, Purnomo, Masbar S, Tjitra E, and Hoffman SL. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Institute of Research of Infectious Diseases, Jakarta, Indonesia; and Naval Medical Research Institute, Bethesda, MD.

4:15 417 CAUSAL PROPHYLAXIS USING PRIMAQUINE IN NON-IMMUNE TRANSMIGRANTS IN THE ARSO REGION OF IRIAN JAYA, INDONESIA. Baird JK*, Fryauff DJ, Basri H, Bangs MJ, Wiady I, Purnomo, Masbar S, Richie T, Tjitra E,

Subianto B, Jones TR, and Hoffman SL. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Institute of Research of Infectious Diseases, Jakarta, Indonesia; Provincial Health Service Jayapura, Irian Jaya, Indonesia; and Naval Medical Research Institute, Bethesda, MD.

4:30 418 EXTENDED DOSE HALOFANTRINE FOR DRUG-RESISTANT FALCIPARUM MALARIA. Watt G *, Loesuttiviboon L, Jongsakul K, Shanks GD, Ohrt C, Karnasuta C, Schuster B, Fleckenstein L. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Surasinghanat Army Hospital, Aranyaprathet, Thailand; Army Malaria Research Unit, Ingleburn, Australia; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

4:45 419 MALARIA IN U.S. ARMY SOLDIERS DURING AND AFTER OPERATION RESTORE HOPE. Smoak BL*, Defraites RF, Magill AJ, and Wellde BT. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington DC; and Department of Immunology, Walter Reed Army Institute of Research, Washington DC.

SCIENTIFIC SESSION CC: MALARIA BIOLOGY

Wednesday, November 3 Essex A/B
2:30 - 5:00 PM
Chairpersons: D.J. Krogstad and R.S Nussenzweig

2:30 420 MALARIA CIRCUMSPOROZOITE PROTEIN BINDS TO HEPARAN SULFATE PROTEOGLYCANS ON THE SURFACE OF HEPATOCYTES. Frevert U*, Sinnis P, Cerami C, Shreffler W, Takacs B, and Nussenzweig V. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY; Michael Heidelberger Division of Immunology, Department of Pathology, New York University Medical Center, New York, NY; and F. Hoffmann-La Roche Ltd., Basel, Switzerland.

2:45 421 THE IN VITRO DEVELOPMENT OF *PLASMODIUM YOELII* EXOERYTHROCYTIC FORMS IN HUMAN HEPATOMA CELL LINES. Calvo Calle JM*, Moreno A, Frevert U, and Nardin E. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

3:00 422 CEREBRAL MALARIA IN MICE: VASCULAR ADHESION OF RBC AND MICRORHEOLOGIC CHANGES. Kaul DK, Nagel RL, and Shear HL*. Division of Hematology, Albert Einstein College of Medicine-Montefiore Hospital, New York, NY.

3:15 423 IN VITRO CYTOADHERENCE OF *PLASMODIUM FRAGILE* TO BRAIN RHESUS ENDOTHELIAL CELLS. Krogstad DJ*, Krogstad FM, Didier PJ, Malmstrom SL, Collins WE, and Aikawa M. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; Tulane Regional Primate Research Center, Covington, LA; Centers for Disease Control, Atlanta, GA; and Case Western Reserve University School of Medicine, Cleveland, OH.

3:30 424 MAINTENANCE OF *PLASMODIUM FALCIPARUM* IN THE PERITONEAL CAVITY OF NOD SCID MICE. Moore JM*, Shultz LD, and Rajan TV. Departments of Pathology and Microbiology, University of Connecticut Health Center, CT; and Jackson Laboratory, Bar Harbor, ME.

3:45 425 EFFECT OF ERYTHROCYTE MEMBRANE ON THE EXTRACELLULAR DEVELOPMENT OF THE ERYTHROCYTIC CYCLE OF *PLASMODIUM FALCIPARUM*. Williams JH, Gill GS, and Trager W. The Rockefeller University New York, NY.

4:00 426 CA^{2+} AND H^{+} HOMEOSTASIS IN *PLASMODIUM FALCIPARUM*. Mikkelsen RB* and Dyer M. Departments of Radiation Oncology and Microbiology/Immunology, Medical College of Virginia, Richmond, VA.

SCIENTIFIC SESSION DD:
CLINICAL TROPICAL MEDICINE II - CESTODES

Thursday, November 4 Lancaster D
8:00 AM - 12:00 N
Chairpersons: R.S. Goldsmith and B. Gottstein

8:00 427 HIGH PREVALENCE OF CYSTICERCOSIS IN BALI. Theis J *, Goldsmith RS, Flisser A, Koss J , Chioino C , Plancarte A, Segura A, Widjana D , and Sutisna P. University of California, San Francisco, CA; Universidad Nacional Automa, Mexico City, Mexico; Arizona State University, Scottsdale, AZ; and Universitas Udayana, Bali, Indonesia.

8:15 428 AN EPIDEMIOLOGICAL STUDY OF *TAENIA SOLIUM* TAENIASIS AND CYSTICERCOSIS IN TWO GUATEMALAN COMMUNITIES. Garcia-Noval J, Fletes C, Moreno E, Mencos F, de Mata F, Torres R, Allan JC*, and Craig PS. Facultad de Medicina, Universidad de San Carlos, Guatemala; Hospital General, Instituto Guatemalteco de Seguridad Social, Guatemala; Hospital San Juan de Dios, Guatemala; and Department of Biological Sciences, Salford University, Salford, UK.

8:30 429 *INVITED PAPER.* IMMUNODIAGNOSIS OF ALVEOLAR AND CYSTIC HYDATID DISEASE (ECHINOCOCCUS). Gottstein B.

9:00 430 LONG-TERM FOLLOWUP OF ALBENDAZOLE TREATMENT OF HYDATID DISEASE. Nahmias J*, Goldsmith RS, Soibelman M, El-On J. University of California, San Francisco, CA; and Ben Gurion University of the Negev, Israel.

9:15 431 EVALUATION OF AN IMMUNOASSAY FOR THE SEROLOGICAL DIAGNOSIS OF CYSTICERCOSIS IN HUMANS. Rosenblatt JE*, Sloan LM, and Schneider SK. Division of Clinical Microbiology, The Mayo Clinic, Rochester, MN.

9:30 432 IMMUNOBLOT SURVEYS SHOW *TAENIA SOLIUM* CYSTICERCOSIS TO BE A SIGNIFICANT GLOBAL PUBLIC HEALTH PROBLEM. Tsang VC*, Pilcher JB, Singhal BS, Gilman R, Wei GZ, Garcia F, Roman G, Geerts S, Asch H, Lee RV, Schantz PM, and Bryan RT. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

9:45 433 FAMILY CLUSTERING OF NEUROCYSTICERCOSIS, SAN PABLO DEL LAGO, ECUADOR. Cruz I*, Cruz M, Canelos P, Schantz PM, and Roman G. Ecuadorean Academy of Neurosciences, Quito, Ecuador; Parasitic Diseases Branch, National Center For Infectious Diseases, CDC, Atlanta, GA; and Neuroepidemiology Branch, National Institute of Neurologic Diseases and Stroke, NIH, Bethesda, MD.

10:00 434 CYSTICERCOSIS SEROPREVALENCE IN AN ORTHODOX JEWISH COMMUNITY IN NEW YORK CITY. Moore AC*, Lutwick LT, Schantz PM, Pilcher J, Wilson M, Fried J, Ware D, Haichou X, Hyon S, Chapnick EK, Abter EI, and Grossman JR. Division of

Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Maimonides Medical Center, Brooklyn, NY.

10:15 435 A TAENIACIDAL DOSE OF PRAZIQUANTEL PROVOKED NEUROLOGICAL SYMPTOMS IN A PERSON WITH PREVIOUSLY UNDIAGNOSED NEUROCYSTICERCOSIS. Flisser A*, Madrazo I, Plancarte A, Schantz P, Allan J, Craig P, and Sarti E. Facultad de Medicina, UNAM, Mexico D.F., Mexico; Hospital de Especialidades, CMN Siglo XXI, IMSS, Mexico D.F., Mexico; Parasitic Diseases Branch, DPD, NCID, CDC, Atlanta, GA; Department of Biological Sciences, University of Salford, United Kingdom; and Direccion de Epidemiologia, Secretariat of Health, Mexico D.F., Mexico.

SYMPOSIUM: A COMMEMORATION OF THE 100TH ANNIVERSARY OF THE WALTER REED ARMY INSTITUTE OF RESEARCH

Thursday, November 4, 1993 Essex A/B
8:00 - 12:00 Noon

8:00 S105 OPENING REMARKS. Russell PK. Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

8:10 S106 THE WALTER REED ARMY INSTITUTE OF RESEARCH: A ONE HUNDRED YEAR LEGACY. Kelley PW. Walter Reed Army Institute of Research, Washington, DC.

8:40 S107 THE ARMY BOARD FOR THE STUDY OF TROPICAL DISEASE IN THE PHILIPPINES, 1900-1926. Joy RJT. Uniformed Services University of the Health Sciences, Bethesda, MD.

9:10 S108 WALTER REED'S LEGACY: THE GLOBAL IMPACT OF VIROLOGY RESEARCH AT THE WALTER REED ARMY INSTITUTE OF RESEARCH. Hoke CH. Walter Reed Army Institute of Research, Washington, DC.

9:30 Coffee Break

10:00 S109 THE WRAIR'S CONTRIBUTIONS TO THE STUDY OF ENTERIC DISEASES. Sadoff JC. Walter Reed Army Institute of Research, Washington DC.

10:20 S110 THE WRAIR MALARIA VACCINE DEVELOPMENT PROGRAM. Ballou WR. Walter Reed Army Institute of Research, Washington, DC.

10:40 S111 ANTIMALARIAL DRUG DEVELOPMENT--THEN AND NOW. Schuster BG. Walter Reed Army Institute of Research, Washington, DC.

11:00 S112 A TALE OF TWO VIRUSES: HEPATITIS A VIRUS, HEPATITIS E VIRUS, AND THE FIGHT TO CONTROL CAMPAIGN JAUNDICE. Innis BL. Walter Reed Army Institute of Research, Washington, DC.

11:20 S113 WRAIR TROPICAL DISEASE EPIDEMIOLOGY IN THE FIELD: VIETNAM 1966-8, SOMALIA 1992-3. DeFraites RF. Walter Reed Army Institute of Research, Washington, DC.

11:40 CONCLUDING REMARKS. Russell PK.

SYMPOSIUM
CYTOKINES AND INFECTION

Fourth Annual Merck, Sharpe and Dohme Symposium

Thursday, November 4, 1993 Lancaster A/B
8:30 - 12:00 Noon
Chairs: S. Reed and A. Sher

8:30 INTRODUCTION. Reed S. Seattle Biomedical Research Institute.

8:35 S114 ROLE OF IL-12 IN INFECTION AND ITS POTENTIAL USE AS A VACCINE ADJUVANT. Trinchieri G. Wistar Institute.

9:15 S115 CLONING AND BIOLOGICAL ACTIVITY OF A NEW T CELL GROWTH FACTOR. Grabstein K. Immunex Corporation.

9:55 S116 REGULATION OF *LEISHMANIA* INFECTION BY IL-12. Sypek J. Genetics Institute.

10:15 S117 IL-12 POTENTIATES T-INDEPENDENT RESISTANCE AGAINST *TOXOPLASMA GONDII*. Gazzinelli R. National Institutes of Health.

10:35 S118 CYTOKINE EXPRESSION DURING NEMATODE INFECTION. Gause B. Uniformed Services University of Health Sciences.

10:55 S119 T CELL RECEPTOR USAGE IN *LEISHMANIA* INFECTION. Reiner S. University of California, San Francisco.

11:15 S120 REGULATORY FUNCTIONS OF IL-13 ON PARASITIC INFECTION. Doherty M. DNAX Research Institute.

11:35 S121 CYTOKINES AND THE LIVER STAGE OF MALARIA. Hoffman S. Naval Medical Research Institute.

SCIENTIFIC SESSION EE:
LATE BREAKERS IN BIOLOGY AND MOLECULAR BIOLOGY

Thursday, November 4 Tudor
8:00 AM - 12:00 N
Chair: B.L.K. Sim

AMERICAN COMMITTEE ON MEDICAL MALACOLOGY

Thursday, November 4 Lancaster C
8:00 AM - 12:00 N
Chair: W.O. Granath and C. Adema

8:00 436 INTERLEUKIN-1 IN *BIOMPHALARIA GLABRATA*: INCREASED HEMOCYTE SUPEROXIDE PRODUCTION AND DECREASED OUTPUT OF *SCHISTOSOMA MANSONI* CERCARIAE. Connors VA, De Buron IC, and Granath, Jr. WO*. Division of Biological Sciences, University of Montana, Missoula, MT.

8:15 437 *FASCIOLOIDES MAGNA* INTERMEDIATE SNAIL HOSTS: HABITAT PREFERENCES AND INFECTION PARAMETERS. Laursen JR* and Stromberg BE. University of Wisconsin-Madison, Madison, Wisconsin; University of Minnesota, Saint Paul, MI.

8:30 438 DETECTION OF *FASCIOLA HEPATICA*-INFECTED INTERMEDIATE HOSTS WITH AN OLIGONUCLEOTIDE-BASED ASSAY. Rognlie MC*, Maika KL, and Knapp SE. Veterinary Molecular Biology, Montana State University, Bozeman, MT.

8:45 439 RECOMMENDATIONS FOR THE SELECTION OF CONTROL AGENTS FOR THE BIOLOGICAL CONTROL OF AQUATIC VECTOR ORGANISMS. Hofkin BV*. Department of Biology, University of New Mexico, Albuquerque, NM.

9:00 440 SATELLITE THERMAL INFRARED IMAGERY AND THE DISTRIBUTION OF SCHISTOSOMIASIS IN THE NILE DELTA OF EGYPT. Malone JB*, Huh OK, Fehler DP, Wilson PA, and Elmagdoub AI. Veterinary Microbiology & Parasitology, Louisiana State University, Baton Rouge, LA; Coastal Studies Institute and Landscape Architecture, Louisiana State University, Baton Rouge, LA; Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

9:15 441 GASTROPOD INTERNAL DEFENSE AND TREMATODE PARASITE SURVIVAL. Adema CM*. Department Biology, University of New Mexico, Albuquerque, NM.

9:30 442 PARASITIC CASTRATION BY *SCHISTOSOMA MANSONI* DOES NOT PREVENT INFECTED *BIOMPHALARIA GLABRATA* SNAILS FROM ACTING AS MALES. Cooper LA* and Lewis FA. Biomedical Research Institute, Rockville, MD.

9:45 443 *SCHISTOSOMA MANSONI*: INFLUENCE OF LARVAL INFECTION ON POLYPEPTIDE SYNTHESIS AND TRANSLATABLE RNA POOLS IN SNAIL CEREBRAL GANGLIA. Bai G* and Yoshino TP. University of Wisconsin-Madison, Madison, Wisconsin.

10:00 Coffee Break

10:30 444 FEASIBILITY OF COMMUNITY FINANCING FOR MOLLUSCICIDING: SOME RESULTS FROM A SCHISTOSOMIASIS ENDEMIC REGION OF NORTHERN CAMEROON. Khan MM*, Greer GJ, Hewett BS, and Cline BL. Department of Health Systems Management and International Health and Development, Tulane University, New Orleans, LA; Department of Tropical Medicine, Tulane University, New Orleans, LA; and Department of Anthropology, Washington State University, Pullman, WA.

10:45 445 AN UNUSUAL HUMORAL RESPONSE OF *BIOMPHALARIA GLABRATA* TO INFECTION WITH *ECHINOSTOMA PARAENSEI*: CHARACTERIZATION AND FUNCTIONAL STUDIES. Hertel LA* and Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM.

11:00 Business Meeting

SCIENTIFIC SESSION FF:
FILARIASIS EPIDEMIOLOGY AND DIAGNOSIS

Thursday, November 4 English
8:00 - 11:45 AM
Chairpersons: T. Unnasch and D. Addiss

8:00 446 IMPACT OF A PILOT DRACUNCULIASIS ELIMINATION PROGRAM AFTER THREE YEARS OF INTERVENTION IN TWO PROVINCES OF BURKINA FASO. Ouedraogo JB*, Hutin YJ, Yameogo G, Soula G, Fabre-Teste B, Hien R, and Guigemde TR. Centre Muraz (O.C.C.G.E.),Bobo-Dioulasso, Burkina Faso; Service d'Epidemiologie, de Statistique et d'Information Sanitaire du Secretariat General de l'O.C.C.G.E., Bobo-Dioulasso, Burkina Faso; and Direction de la Medecine Preventive, Ministere de la Sante, Ouagadougou, Burkina Faso.

8:15 447 USE OF GEOGRAPHIC POSITIONING TECHNOLOGY IN IVERMECTIN DISTRIBUTION ACTIVITIES IN GUATEMALA. Richards F*, Bennett P, Lujan R, Zea R, Castro J, Gonzalez C, and Klein R. Division of Parasitic Diseases, Centers for Disease Control and Prevention; Universidad del Valle de Guatemala; and Guatemalan Ministry of Public Health and Social Welfare; The River Blindness Foundation.

8:30 448 EVALUATION OF ALTERNATE METHODS OF RAPID ASSESSMENT OF EPIDEMICITY OF *ONCHOCERCA VOLVULUS* IN COMUNITIES IN SOUTHERN CAMEROON. Kollo B*, Mather FJ, and Cline BL. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; and Department of Biostatistics and Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

8:45 449 LARGE SCALE APPLICATION OF *ONCHOCERCA VOLVULUS* DNA PROBE BASED TECHNOLOGY BY THE ONCHOCERCIASIS CONTROL PROGRAMME OF WEST AFRICA. Toe L, Merriweather A* and Unnasch TR. Insecticide Research Unit, Onchocerciasis Control Programme, Bouake 01, Cote d'Ivoire; and Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

9:00 450 ***INVITED PAPER.*** SERODIAGNOSIS INITIATIVE UPDATE. Weiss N. Swiss Tropical Institute, TDR/Filariasis.

9:30 451 THE CULTURAL CONTEXT AND CONTROL OF "FOREST" ONCHOCERCIASIS IN SOUTH PROVINCE, CAMEROON. Hewlett B*, Cline BL, and Kollo B. Department of Anthropology, Washington State University, Pullman, WA; and Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

9:45 452 POLYMERASE CHAIN REACTION BASED DIAGNOSIS OF *ONCHOCERCA VOLVULUS* INFECTION: IMPROVED DETECTION OF ACTIVE INFECTION. Nutman TB*, Zimmerman PA, Aruajo E, Elson LH, Phadke P, Kubofcik J, and Guderian RH. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and National Center of Tropical Medicine-Quito Extension, Hospital Vozandes, Quito, Ecuador.

10:00 Coffee Break

10:30 453 A SURVEY OF KNOWLEDGE, ATTITUDES, AND PERCEPTIONS (KAPS) OF LYMPHATIC FILARIASIS AMONG RESIDENTS IN LEOGANE, HAITI. Eberhard ML*, Walker EM, Addiss DG, and Lammie PJ. Division of Parasitic Diseases, National Center for Infectious Diseases, Center for Diseaase Control, Atlanta, GA.

10:45 454 ENVIRONMENTAL FACTORS INFLUENCING THE DISTRIBUTION OF LYMPHATIC FILARIASIS IN THE NILE DELTA. Faris R*, Ramzy RM, Emira HA, and Gad AM. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt.

11:00 455 AGE-SPECIFIC PREVALENCE OF *WUCHERERIA BANCROFTI* ANTIGENEMIA IN A HAITIAN POPULATION. Lammie PJ*, Eberhard ML, Dickerson JW, Walker EM, and Hightower AW. Division of Parasitic Diseases, Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA.

11:15 456 *WUCHERERIA BANCROFTI* PCR FOR THE DETECTION OF INFECTIVE L_3 LARVAE IN POOLS OF MOSQUITO HEADS. Chanteau S*, Luquiaud P, Failloux AB, Plichart C, Ung A, Lardeux F, and Williams SA. Institut Louis Malarde, Tahiti; Centre ORSTOM, Tahiti; and Smith College, Northampton, MA.

11:30 457 DETECTION OF *WUCHERERIA BANCROFTI* CIRCULATING ("FREE") DNA IN BLOOD AND PLASMA USING THE *SSP* I PCR SYSTEM. Zhong M*, Williams SA, McCarthy J,and Ottesen E. Department of Biological Sciences, Smith College, Northampton, MA; and Laboratory of Parasite Diseases, National Institutes Health, Bethesda, MD.

SCIENTIFIC SESSION GG: IMMUNOLOGY AND CONTROL OF PARASITES

Thursday, November 4 York
8:00 - 11:30 AM
Chairpersons: G.A. Conder and R.B. Grieve

8:00 458 SURFACE COAT AND EPICUTICLE ANTIGENS FROM LARVAE OF THE PARASITIC NEMATODE TOXOCARA CANIS. Gems DH and Maizels RM*. Wellcome Research Centre for Parasitic Infections, Department of Biology, Imperial College, London, UK.

8:15 459 BRONCHOALVEOLAR LAVAGE EOSINOPHILS FROM MICE INFECTED WITH *TOXOCARA CANIS* DO NOT EXPRESS Fc RECEPTORS FOR IgE. Kayes SG*, Hester RB, Finkelman FD, and Jones RE. Department of Structural and Cellular Biology, University of South Alabama College of Medicine, Mobile, AL; Department of Microbiology and Immunology, University of South Alabama College of Medicine, Mobile, AL; and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.

8:30 460 ALLATOSTATIN-IMMUNOREACTIVE NEUROSECRETORY CELLS IN THE SYNGANGLION OF THE TICK *DERMACENTOR VARIABILIS* (ACARI: IXODIDAE). Zhu XX* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

8:45 461 IDENTIFICATION AND CHARACTERIZATION OF MENINGEAL WORM ANTIGENS. Neumann NF*, Samuel WM, and Belosevic M. Departments of Zoology and Immunology, University of Alberta, Edmonton, Alberta, Canada.

9:00 462 *TRICHURIS SUIS* (WHIPWORM) INFECTION IN PIGS EXACERBATES PRE-EXISTING PORCINE INTESTINAL ADENOMATOSIS. Mansfield LS, Urban JF, and Hill DE. Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Biosystematics Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

9:15 463 CHARACTERIZATION OF A NOVEL 3,6-DIDEOXYHEXOSE AND ITS ROLE IN DEFINING THE ANTIGENICITY OF IMMUNODOMINANT *TRICHINELLA SPIRALIS* GLYCOPROTEINS. Wisnewski N *, Zeidner NS, McNeil M, Grieve RB, and Wassom DL. Paravax, Inc., Fort Collins, CO; and Departments of Pathology and Microbiology, Colorado State University, Fort Collins, CO.

9:30 464 IL4 REGULATES PROTECTIVE IMMUITY TO *TRICHINELLA SPIRALIS*. Urban JF*, Gamble HR, Madden KB, Katona IM, and Finkelman FD. Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Departments. of Pediatrics and Medicine, Uniformed Services Univ. of the Health Sciences, Bethesda, MD.

9:45 465 THE HOOKWORM ANTICOAGULANT: A NOVEL TISSUE FACTOR PATHWAY INHIBITOR. Cappello M, Vlasuk GP, Hawdon JM, and Hotez PJ. Departments of Pediatrics and Epidemiology & Public Health, Yale University School of Medicine; and Corvas International, Inc.

10:00 Coffee Break

10:30 466 CUTICULAR ANTIGENS OF THE EQUINE NEMATODE *STRONGYLUS VULGARIS*. Philpott MS and Klei TR*. Department of Veterinary Microbiology & Parasitology, School of Vet Medicine, Louisiana State University, Baton Rouge, LA.

10:45 467 AN ULTRASTRUCTURAL STUDY OF *EIMERIA* DEVELOPMENT IN UNIMMUNIZED AND FOREIGN HOST IMMUNIZED CHICKENS. Danforth HD* and Augustine PC. Protozoan Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

11:00 468 QUANTITATIVE GENETIC CHANGES AT THE β-TUBULIN GENES IN *HAEMONCHUS CONTORTUS* ASSOCIATED WITH RESISTANCE TO BENZIMIDAZOLES. Beech RN*, Prichard RK, and Scott ME. Institute of Parasitology, McGill University, Quebec, Canada.

11:15 469 CONTROL OF PERINATAL TRANSMISSION OF *TOXOCARA CANIS* WITH MILBEMYCIN OXIME IN DOGS. Stewart VA*, Hepler DI, and Grieve RB. Department of Pathology, Colorado State University, Fort Collins, CO; and CIBA-GEIGY Animal Health, CIBA-GEIGY Corporation, Greensboro, NC.

SCIENTIFIC SESSION HH:
MALARIA IMMUNOLOGY II: VACCINES

Thursday, November 4 Stuart
8:00 - 11:30 AM
Chairpersons: S. Herrera and S. Chang

8:00 470 IMMUNOGENICITY OF MULTIPLE ANTIGEN PEPTIDES (MAPS) CONTAINING *PLASMODIUM VIVAX* CS EPITOPES. de Herrera MA*, Escobar P, De Plata C, Corradin G, and and Herrera S. School of Health, Universidada del Valle, Cali, Colombia; and Institute of Biochemistry, University of Lausanne, Lausanne, Switzerland.

8:15 471 IMMUNOGENICITY OF A MAP SYSTEM CONTAINING B AND T HELPER REPEAT EPITOPES OF *PLASMODIUM FALCIPARUM* CS PROTEIN IN ALUM: POSSIBLE VACCINE APLICATION. De Oliveira GA*, Clavijo P, Nussenzweig RS, and Nardin EH. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

8:30 472 IMMUNOGENICITY OF A MULTIPLE ANTIGEN PEPTIDE CIRCUMSPOROZOITE PROTEIN MALARIA VACCINE. Church P*, Corradin G, Hunter RL, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Rockville, MD; Department of Biochemistry, University of Lausanne, Epalinges, Switzerland; and Department of Pathology, Emory University , Atlanta, GA.

8:45 473 PROTECTION AGAINST *PLASMODIUM YOELII* BY CS PROTEIN MULTIPLE ANTIGEN PEPTIDE-INDUCED POLYCLONAL ANTIBODIES. Wang R*, Charoenvit Y, Porrozzi R, Mellouk S, Sedegah M, Corradin GP, Hunter RL, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Dpto. Ultraestrutura e Biol. Cel. IOC-FIOCRUZ, Rio de Janeiro, Brazil; Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland; and Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA.

9:00 474 EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF A FALCIPARUM SPOROZOITE VACCINE. Migasena S, Kyle DE*, Khusmith S, Singhasivanon P, Suntharasamai P, Srisuriya P, Pavanand K, Wongsrichanalai C, Viravan C, Cohen J, Ballou WR, Webster HK, Chongsuphajaisiddhi T, and Gordon DM. Department of Immunology and Parasitology, AFRIMS, Bangkok, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Immunology, WRAIR, Washington, DC; and SmithKline Beecham Biologicals, Rixensart, Belgium.

9:15 475 SAFETY, IMMUNOGENICITY AND EFFICACY OF LIPOSOME-ENCAPSULATED *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE ANTIGEN ADJUVANTED WITH ALUM AND LIPID A. Heppner DG*, Gordon DM, Gross M, Trofa AF, McKinney DA, Alving CR, Owens R, Sylvester DR, Porter TG, Theisen T, Sadoff JM, and Ballou WR. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC; SmithKline Beecham Pharmaceuticals, King of Prussia, PA; and Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC.

9:30 476 COMBINED USE OF RECOMBINANT INFLUENZA VIRUS AND RECOMBINANT VACCINIA VIRUS INDUCES CD8+ T CELL-MEDIATED PROTECTIVE IMMUNITY AGAINST MALARIA. Rodrigues M*, Li S, Rodriguez D, Rodriguez JR, Esteban M, Palese P, Nussenzweig RS, and Zavala F. Department of Medical and Molecular

Parasitology, New York University, New York, NY; Department of Microbiology, Mount Sinai School of Medicine, New York, NY; and Department of Biochemistry, State University of New York, New York, NY.

9:45 477 SAFETY AND IMMUNOGENICITY OF ALUM-ADJUVANTED SPf66 PRODUCED IN THE UNITED STATES UNDER CGMP STANDARDS. Gordon DM*, Sadoff JC, Heppner DG, Klotz FW, Seguin MC, Duffy PE, Krzych U, and Ballou WR. Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

10:00 Coffee Break

10:30 478 PROTECTION IN *PLASMODIUM YOELII* MALARIA IS ASSOCIATED WITH AN ANAMNESTIC IgG2a ANTIBODY RESPONSE. Kidd MR*, Lal AA, and Hunter RL. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease, Atlanta, GA; and Department of Pathology, Emory University, Atlanta GA.

10:45 479 A 12 KDA FRAGMENT OF THE *PLASMODIUM YOELII YOELII* 17XL MEROZOITE SURFACE PROTEIN MSP-1, EXPRESSED IN *ESCHERICHIA COLI*, PRODUCES PROTECTIVE IMMUNITY IN MICE. Daly TM* and Long CA. Department of Microbiology and Immunology, Hahnemann University, Philadelphia, PA.

11:00 480 EXPOSURE TO*PLASMODIUM FALCIPARUM* HIGHLY INCREASES THE PROTECTIVE CAPACITY OF MALARIA RECOMBINANT PROTEINS. Herrera S, De Herrera MA, Zapata C, Renjifo G, Gonzalez M, and Schoenfeld HJ*. Certa U. School of Health, Universidad del Valle, Cali, Colombia; and Pharma Research Technology, F. Hoffman-LaRoche Ltd, Basel, Switzerland.

11:15 481 REGULATION OF *PLASMODIUM FALCIPARUM* MSP-1 T CELL EPITOPE RECOGNITION BY THE MAJOR HISTOCOMPABILITY COMPLEX. Chang SP*, Hashimoto AK, Kanda P, and Hui GS. Department of Tropical Medicine & Medical Microbiology, John A. Burns School of Medicine, Honolulu, HI; and Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX.

SCIENTIFIC SESSION II: VIRAL DISEASES

Thursday, November 4 Grecian Suite
8:00 - 10:15 AM
Chairpersons: B.L. Innis and R. Yanagihara

8:00 482 NATURAL HISTORY OF HIV-1 INFECTION IN A COHORT OF FIFTY-FOUR SEROPOSITIVE, FILIPINO PROSTITUTES. Perrault JG*, Manaloto CR, Caringal LT, Santiago EG, Basaca-Sevilla V, Hayes CG, and Anthony RL. U.S. Naval Medical Research Unit No. 2, Manila Detachment, Republic of the Philippines.

8:15 483 LONGITUDINAL STUDIES OF BABIES BORN TO HIV-1 SEROPOSITIVE FILIPINO PROSTITUTES; DIAGNOSTIC DILEMMAS. Manaloto CR*, Caringal LT, Hayes CG, Perrault JG, Santiago EV, Gonzales VL, and Anthony RL. U.S. Naval Medical Research Unit No. 2 Detachment Republic of the Philippines; and San Lazaro Hospital, Manila, Republic of the Philippines.

8:30 484 HIV-2 IN INDIA. Banerjee K*. National Institute of Virology, Pune, India.

8:45 485 GENETIC ANALYSIS AND MOLECULAR PHYLOGENY OF PRIMATE T-CELL LYMPHOTROPIC VIRUS TYPE I. Song KJ*, Nerurkar VR, Saitou N, and Yanagihara R. Laboratory of Central Nervous System Studies, NINDS, National Institutes of Health, Bethesda, MD; and Laboratory of Evolutionary Genetics, NIG, Mishima, Japan.

9:00 486 HTLV-I FROM IRANIAN-BORN MASHHADI JEWS WITH SPASTIC MYELOPATHY: GENETIC AND PHYLOGENETIC LINK WITH VIRUS STRAINS FROM JAPAN AND INDIA. Nerurkar VR*, Song KJ, Achiron A, Melland RR, Hamiel O, Shohat B, Melamed E, and Yanagihara R. Laboratory of Central Nervous System Studies, NINDS, NIH, Bethesda, MD, U.S.A.; Beilinson Medical Center, Petah-Tiqva, Israel; Sheba Medical Center, Tel-Hashomer, Israel; and Weizmann Institute of Science, Rehovot, Israel.

9:15 487 PREVALANCE OF HEPATITIS E AMONG NON-A, NON-B PATIENTS IN THE KATHMANDU VALLEY, NEPAL. Innis BL, Myint KS, Clayson ET*, Narupiti S, Mongkolsirichaikul D, Manomuth C, and Shrestha MP. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Teku Hospital, Kathmandu, Nepal.

9:30 488 MOLECULAR CHARACTERIZATION OF CARRIER RABIES VIRUSES. Warner CK* and Fekadu M. Viral & Rickettsial Zoonoses Branch, CDC, Atlanta, GA.

9:45 489 IDENTIFICATION OF GLOBAL RESERVOIRS OF DOG RABIES. Smith JS*, Orciari LA and Yager PA. Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

10:00 490 EPIZOOTIC RACCOON RABIES IN NEW ENGLAND: ENVIRONMENTAL ASSOCIATIONS OF EXPANDING DISTRIBUTION AND HUMAN RISK. Wilson ML*, Cartter ML, and Cooper GH. Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT; and Epidemiology Section, Department of Health Services, State of Connecticut, Hartford, CT.

SCIENTIFIC SESSION JJ:
SCHISTOSOMIASIS: EPIDEMIOLOGY AND CLINICAL ASPECTS

Thursday, November 4 Lancaster E
8:00 - 10:15 AM
Chairpersons: C. Chappell and V. Tsang

8:00 491 FAILURE OF PRAZIQUANTEL THERAPY IN CHRONICALLY INFECTED PATIENTS WITH *SCHISTOSOMA HAEMATOBIUM* IS ASSOCIATED WITH ELEVATED PARASITE-SPECIFIC IgG4. Medhat A*, Nafeh M, Shata T, Mohamed S, Shehata M, Helmy A, Saad M, Strickland GT, and King CL. Department of Microbiology, Assiut University, Assiut, Egypt; Department of Tropical Medicine, Assiut University, Assiut, Egypt; Department of Epidemiology and Preventive Medicine, University pf Maryland, Baltimore, MD; and Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH.

8:15 492 SEROLOGIC DETECTION OF *SCHISTOSOMA MANSONI* USING CP1, AN ADULT WORM ANTIGEN. Chappell CL* and Newman PT. Department of Family Medicine, Baylor College of Medicine, Houston, Texas.

8:30 493 SCHISTOMIASIS OF EXPATRIATES AND VISITORS TO MALAWI: SEROLOGIC DIAGNOSIS AND SPECIATION. Pilcher JB*, Tsang VC, Noh J, Al-Sherbiny MM, Wilson M, Ware DA, Cetron MS, Addis DG, and Chitsulo L. PDB, NCID, Centers for Disease Control and Prevention, Atlanta, GA; Ministry of Health, Malawi; and Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

8:45 494 PRESENCE OF *BULINUS GLOBOSUS*, INTERMEDIATE HOST OF *SCHISTOSOMA HAEMATOBIUM*, IN LAKE MALAWI, AFRICA: A CHANGING PERSPECTIVE ON TRANSMISSION. Sullivan JJ*, Cetron MS, Chitsulo L, Nakhate W, and Addiss DG. Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Community Health Sciences Unit, Ministry of Health, Lilongwe, Malawi.

9:00 495 SCHISTOSOMIASIS TRANSMISSION ALONG THE SHORES OF LAKE MALAWI: AN EPIDEMIOLOGICAL INVESTIGATION OF RESIDENT EXPATRIATES AND VISITORS OF MALAWI. Cetron MS*, Chitsulo L, Addiss DG, Sullivan JJ, Pilcher JB, and Tsang VC. Division of Parasitic Diseases, NCID, Centers for Disease Control, Atlanta, GA; Community Health Sciences Unit, Ministry of Health, Lilongwe, Malawi.

9:15 496 SERODIAGNOSIS OF SCHISTOSOMIASIS IN KENYA USING SCHISTOSOME EGG ANTIGENS IN ELISA. Doenhoff MJ*, Butterworth AE, Hayes RJ, Tricker K, Ouma J, and Koech D. School of Biological Sciences, University of Wales, Bangor, UK.; Department of Pathology, University of Cambridge, Cambridge, UK.; Department of Epidemiology & Population Sciences, London School of Hygiene & Tropical Medicine, London, UK.; Department of Epidemiology and Social Research, Christie Hospital, Manchester, UK; Division of Vector-Borne Diseases, Nairobi, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

9:30 497 THE IMPACT OF SCHISTOSOMIASIS ON NUTRITIONAL STATUS OF BRAZILIAN CHILDREN. Parraga IM*, Assis AO, Reis MG, Prado MS, King CH, Barreto ML, and Blanton RE. Case Western Reserve University, Cleveland, OH; Federal University of Bahia, Salvador, Brazil; and Oswaldo Cruz Foundation, Salvador, Brazil.

9:45 498 LOCAL PERCEPTIONS OF THE CLINICAL FEATURES OF SCHISTOSOMIASIS IN EGYPT AND THEIR IMPACT ON TREATMENT SEEKING BEHAVIOR. Mehanna S*, Winch PJ, El-Katsha S, and Watts S. Social Research Center, American University in Cairo, Cairo, Egypt; and Department of International Health, The Johns Hopkins University, School of Hygiene & Public Health, Baltimore, MD.

10:00 499 WORKING WITH LOCAL PEOPLE AND HEALTH UNIT STAFF TO IMPROVE SCHISTOSOMIASIS CONTROL: A PARTICIPATORY RESEARCH PROJECT IN MENOUFIA GOVERNORATE, EGYPT. El-Katsha S*, Watts S, Khairy A, and El-Sebaie O. Social Research Center, American University in Cairo, Cairo, Egypt; and High Institute of Public Health, Alexandria, Egypt.

POSTER SESSION II

Thursday, November 4 Regency Ballroom
12:00 - 2:00

FILARIASIS II

500 DETECTION OF *BRUGIA MALAYI* MICROFILARIAL DNA IN INDONESIAN BLOOD SAMPLES USING THE POLYMERASE CHAIN REACTION. Lizotte MR and Williams SA*. Department of Biological Sciences, Smith College, Northampton, MA.

501 EFFECT OF IVERMECTIN PROPHYLAXIS ON ANTIBODY RESPONSES TO *ONCHOCERCA VOLVULUS* RECOMBINANT ANTIGENS IN EXPERIMENTALLY INFECTED CHIMPANZEES. Chandrashekar R, van Swinderen B, Taylor HR, and Weil GJ. Washington University School of Medicine, St. Louis, MO; and Victoria Eye and Ear Hospital, East Melbourne, Australia.

502 ANALYSIS OF GENETIC DIVERSITY IN *DIROFILARIA IMMITIS* BY PCR WITH ARBITRARY PRIMERS. van Swinderin B*, Berg D, and Weil GJ. Washington University School of Medicine, St. Louis, MO.

503 DEVELOPMENT OF A POLYMERASE CHAIN REACTION (PCR) TEST TO SURVEY FOR VECTORS OF DOG HEARTWORM, *DIROFILARIA IMMITIS*. Scoles GA*. Vector Biology Laboratory, Department of Biological Science, University of Notre Dame, Notre Dame, IN.

504 LOCALIZATION OF LECTINE BINDING ON EXTRACELLULARLY MELANIZED MICROFILARIAE OF *BRUGIA MALAYI* (NEMATODA:FILARIOIDEA) IN *ANOPHELES QUADRIMACULATUS*. Nayar JK*, Chikilian ML, Mikarts LL, Knight JW, and Bradley TJ. Florida Medical Entomology Laboratory, IFAS-University of Florida, Vero Beach, FL; and Department of Ecology and Evolutionary Biology, University of California, Irvine, CA.

505 ORIENTATION OF *ONCHOCERCA LIENALIS* MICROFILARIA WITHIN *SIMULIUM VITTATUM*. Lehmann T*, Cupp EW, Cupp MS. Department of Entomology, University of Arizona, Tucson, AZ.

506 INFECTIVITY AND NORMAL DEVELOPMENT OF THIRD STAGE *BRUGIA MALAYI* MAINTAINED *IN VITRO*. Yates JA*, Schmitz KA, Nelson FK, and Rajan TV. Oakland University, Department of Biological Sciences, Rochester, MI; and University of Connecticut Health Center, Farmington, CT.

507 VISUALIZATION OF *WUCHERERIA* AND *BRUGIA* LARVAL STAGES IN INTACT MOSQUITOS. Green DF* and Yates JA. Oakland University, Department of Biological Sciences, Rochester, MI.

508 CHRONIC HEARTWORM INFECTION DEPRESSES ENDOTHELIUM-DEPENDENT RELAXATION OF THE IN VITRO CANINE PULMONARY ARTERY. Mupanomunda M*, Tithof PK, Williams JF, and Kaiser L. Department of Physiology Michigan State University, East Lansing MI; and Department of Microbiology and Public Health Michigan State University, East Lansing MI.

509 CHARACTERIZATION OF THE CYTOCHROME B GENE AND MITOCHONDRIAL GENOME OF *ONCHOCERCA VOLVULUS*. Keddie EM*, and Unnasch TR. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL; and Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL.

510 IMMUNE RESPONSE TO OV 18 A RECOMBINANT MOLECULE OF *ONCHOCERCA VOLVULUS*. Bradley JE*, Tree TI, Gillespie AJ, Elson L, Guderian RH, and Nutman TB. Department of Biology, Imperial College of Science and Technology, London, UK; Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; and Department of Clinical Investigation, Hospital Vozandes, Quito, Ecuador.

511 ROLE OF THE FILARIAL RAN/TC4/SPI1 HOMOLOG IN THE DEVELOPMENTAL MATURATION OF *BRUGIA MALAYI* MICROFILARIAE. Dissanayake S*, Xu M, Chen GH, Wang SH, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Guizhou Provincial Institute of Parasitic Diseases, Guiyang, The People's Republic of China.

512 CLONING OF THE *ONCHOCERCA VOLVULUS* HOMOLOGUE OF PROLYL-4-HYDROXYLASE, AN EXZYME INVOLVED IN THE BIOSYNTHESIS OF COLLAGEN. Wilson W*, Unnasch TR. Division of Geographic Medicine, Department of Medicine, University of Alabamsa at Birmingham, Birmingham, AL.

513 CLONING AND CHARACTERIZATION OF CDNA CLONES ENCODING MEMBERS OF THE STEROID RECEPTOR SUPERFAMILY FROM *ONCHOCERCA VOLVULUS*. Yates R* and Unnasch TR. Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

514 CLONING AND CHARACTERIZATION OF THE *BRUGIA MALAYI* HOMOLOG OF RIBOSOMAL PROTEIN S15. Chen GH*, Wang SH, Xu M, Dissanayake S, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Guizhou Provincial Institute of Parasitic Diseases, Guiyang, The People's Republic of China.

515 *BRUGIA MALAYI* CONTAINS A TRANSLATION PRODUCT DERIVED FROM DNA SEQUENCES HOMOLOGOUS TO MAMMALIAN LINES AND RETROVIRAL REVERSE TRANSCRIPTASES. Wang SH*, Chen GH, Xu M, Dissanayake S, Piessens WF, Araunje AC, and de Souza W. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Guizhou Provincial Institute of Parasitic Diseases, Guiyang, The People's Republic of China; and Instituto de Biofisica Carlos Chagas Filho, Rio de Janeiro, Brazil.

516 IMMUNOHISTOCHEMICAL LOCALIZATION OF ANTIGENIC PROTEINS IN THE TISSUES OF *BRUGIA MALAYI* LARVAE AND ADULTS. Hale TJ*, Rajan TV, Peralta ME, and Yates JA. Oakland University, Department of Biological Sciences, Rochester, MI; and University of Connecticut Health Center, Farmington, CT.

517 CLONING OF AN EARLY IMMUNODOMINANT FILARIAL ANTIGEN: A NOVEL MEMBER OF THE *BRUGIA MALAYI* MYOSIN HEAVY CHAIN FAMILY. Li BW*, Hoppe PE, and Weil GJ. Washington University School of Medicine, St. Louis, MO.

518 DEVELOPMENT OF NOVEL COMPOUNDS FOR THE TREATMENT OF ONCHOCERCIASIS. Strote G*, Bonow I, v. Stenglin E, Wywiol A, and Attah S. Bernhard Nocht Institute for Tropical Medicine, Hamburg, F.R.G.; and Onchocerciasis Chemotherapy Research Centre, Hohoe, Ghana.

519 IMIDAZOPYRIDINES: A NEW CLASS OF POTENTIAL ANTIFILARIAL DRUGS IDENTIFIED. Kinnamon KE*, Engle RR, Sundberg RJ, McCall JW, and Dzimianski MT. Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Department of Chemistry, University of Virginia,

Charlottesville, VA; and Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA.

520 LONG TERM EFFECT OF TWO SINGLE DOSES OF IVERMECTIN ON MICROFILAREMIA (MF) OF BANCROFTIAN FILARIASIS IN EGYPT. Youssef FG*, Hassanein SH, Safwat M, Rida M, Fouad R, and Cummings CE. Basic Science Division, U.S. Naval Medical Research Unit No.3, Cairo, Egypt; Health Units, Qalyubia Governorate, Egypt.

521 MASS CHEMOPROPHYLAXIS OF LYMPHATIC FILARIASIS WITH SINGLE DOSES OF IVERMECTIN IN A POLYNESIAN VILLAGE. EFFICACY AND ADVERSE REACTIONS. Nguyen NL, Moulia-Pelat JP, Glaziou P*, Plichart R, Lardeux F, Martin PM, and Cartel JL. Institut Territorial de Recherches Medicales Louis Malarde, Tahiti, French Polynesia.

SCHISTOSOMIASIS AND OTHER TREMATODES: BIOLOGY

522 EVALUATION OF A TWICE WEEKLY APPLICATION OF 1% NICLOSAMIDE LOTION IN PREVENTING *SCHISTOSOMA HAEMATOBIUM* RE-INFECTION. Abu-Elyazeed RR*, Podgore JK, Mansour NS, Youssef FG, Gere JA, and Hibbs RG. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and U.S. Army Medical Material Development Activity, Ft. Detrick, MD.

523 FIELD TRIAL OF A 1% NICLOSAMIDE LOTION, TOPICALLY APPLIED FOR THE PREVENTION OF *SCHISTOSOMA MANSONI* INFECTION IN BRAZILIAN SCHOOL CHILDREN. Dietze R, Alencar F, Cerutti C, Bendet I, Pang L, Miller R *, and Gere J. USA Medical Research Unit-Brazil, American Consulate Rio de Janeiro, Brazil; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC; and US Army Medical Development Activity, Ft. Detrick, Frederick, MD.

524 HEPATOSPLENIC *SCHISTOSOMA MANSONI* INFECTION IS INFREQUENTLY DIAGNOSED IN NORTHERN NIGERIA. Newsome F *. Columbia University College of Physicians and Surgeons, Harlem Hospital Center, New York, NY.

525 RECOMBINANT ANTIGENS FOR THE PREPATENT IMMUNODIAGNOSTIC OF SCHISTOSOMIASIS MANSONI. Oliveira GC* and Kemp WM. Department of Biology, Texas A&M University, College Station, TX.

526 EXPRESSION OF THE *SCHISTOSOMA MANSONI* EGF RECEPTOR HOMOLOGUE AND ALTERNATIVELY SPLICED VARIANTS IN INSECT CELLS. Ramachandran H* and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

527 CLONING AND CHARACTERIZATION OF *CLONORCHIS SINENSIS* TROPOMYOSIN cDNA. Sohnn EJ, Choi WS, and Hong SJ*. Departments of Parasitology and Anatomy, Gyeong-Sang National University College of Medicine, Chinju, Korea.

528 IN VITRO CULTURING OF ADULT *SCHISTOSOMA MANSONI* USING SERUM-FREE MEDIA AND DIALYSIS BAGS. Hancock K, Tsang VC, and Call JL*. Parasitic Disease Branch, NCID, Centers for Disease Control and Prevention, Atlanta GA.

529 CHEMOKINETIC BEHAVIOR IN CERCARIAE OF *SCHISTOSOMA MANSONI*. THE ROLE OF LINOLEIC ACID IN BOTH ATTRACTION AND PENETRATION RESPONSE. Shiff CJ* and Graczyk TK. Department of Immunology and Infectious Diseases, The Johns Hopkins University, Baltimore, MD.

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541 FURTHER CHARACTERIZATION OF *TAENIA SOLIUM* (CYSTICERCOSIS) GLYCOPROTEIN ANTIGEN. Hyon SS, Pilcher JB*, and Tsang VC. Parasitic Diseases Branch, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

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MALARIA

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598 REDUCED SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* TO PERMETHRIN ASSOCIATED WITH THE USE OF PERMETHRIN-IMPREGNATED BED NETS AND CURTAINS IN KENYA. Vulule JM*, Beach RF, Atieli FK, and Roberts JM.

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KINETOPLASTIDAE: MOLECULAR BIOLOGY AND BIOCHEMISTRY

608 DEVELOPMENT OF POLYMERASE CHAIN REACTION TO IDENTIFY *LEISHMANIA MAJOR* ISOLATES FROM THE SINAI PENINSULA. Francies WM*, Alwen A, Galloway DR, and Hedstrom R. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

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610 *TRYPANOSOMA (DUTTONELLA VIVAX)*: PURIFICATION, CHARACTERIZATION AND IMMUNOLOCALIZATION OF A LEUCINE AMINOPEPTIDASE. Ibitayo AL*, Olorunsogo OO, Wells CW, and Lonsdale-Eccles JD. Department of Biochemistry, University of Ibadan, Nigeria.

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KINETOPLASTIDAE: CHEMOTHERAPY AND EPIDEMIOLOGY

615 CHAGAS' DISEASE CONTROL IN BOLIVIA: A MODEL FOR COMMUNITY DEVELOPMENT AND VECTOR CONTROL. Balderrama F*, Bermudez H, Torrcio F, Bryan RT, Kuritsky J, Tonn RJ, and Arata A. Programa de Control de Chagas - CCH/USAID; Universidad Mayor de San Simon, Cochabamba, Bolivia; CDC, Atlanta, GA; Community & Child Health Project, USAID/Bolivia; Vector Biology & Control Project, Arlington, VA.

616 CHAGAS' DISEASE IN BOLIVIA: EFFECTS OF MATERNAL *TRYPANOSOMA CRUZI* INFECTION ON NONINFECTED NEWBORNS. Torrico F, Moore A, Balderrama F, Castro M, Dorado C, Arandia R, and Bryan RT. CUMETROP, Facultad de Medicina-UMSS and Programa de Control de Chagas-CCH, Cochabamba, Bolivia; and Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA

617 BLOOD DONORS IN A VECTOR FREE ZONE OF ECUADOR ARE POTENTIALLY INFECTED WITH *TRYPANOSOMA CRUZI*. Escalante L*, Grijalva MJ, Rowland EC, Powell MR, and McCormick TS. Pathology Department, Instituto Nacional de Higiene y Medicina Tropical, Quito, Ecuador; and Department of Biological Sciences & Tropical & Geographic Disease Institute, Ohio University, Athens, OH.

618 PREVENTION OF TRANSFUSION-ACQUIRED LEISHMANIASIS: A COMPARISON OF THREE METHODS AVAILABLE TO BLOOD BANKS. Daugirda JL* and Grogl M. Department of Pathology, Walter Reed Army Medical Center, Washington, DC; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

619 CUTANEOUS LEISHMANIASIS IN U.S. RANGERS AND MARINES ASSOCIATED WITH JUNGLE WARFARE TRAINING IN FRENCH GUIANA DURING 1992-1993. Grogl M*, Gasser Jr. RA, Magill AJ, Johnson SC, and Oster CN. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.; Infectious Disease Section, Walter Reed Army Medical Center, Washington, DC; and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

620 INCIDENCE OF *TRYPANOSOMA CRUZI* INFECTION AMONG OPOSSUMS AND RACCOONS IN SOUTHEAST GEORGIA. Banks CW, Durden L, Krissinger MW, and Pung OJ*. Department of Biology, Georgia Southern University, Statesboro, GA; Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, GA.

621 EVALUATION OF DOT ELISA EMPLOYING NATIVE gp63 FROM *LEISHMANIA MAJOR* FOR LEISHMANIASIS DIAGNOSIS. Mohareb EW*, Youssef FG, and Galloway DR. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

622 IDENTIFICATION OF A MISLEADING TRYPANOSOMATID PARASITE FROM *GERBILLUS PYRAMIDUM* AND *G. ANDERSONI* IN A *LEISHMANIA MAJOR* ENDEMIC AREA IN NORTH SINAI. Mikhail EM*, Mansour NS, Mohareb EW, Francies WM, Galloway DR, Fryauff DJ, and Modi GB. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

623 COMBINATION CHEMOTHERAPY OF DRUG RESISTANT *TRYPANOSOMA BRUCEI RHODESIENSE* INFECTIONS IN MICE USING DL-α-DIFLUOROMETHYLORNITHINE AND SURAMIN. Bacchi CJ*, Yarlett N, McCann PP, Sjoerdsma A, Saric M, and Clarkson, Jr. AB. Haskins Laboratories and Biology Dept., Pace University, New York, NY; Marion Merrell Dow Research Institute, Cincinnati, OH; and Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY.

624 CHANGING PATTERN OF AMERICAN VISCERAL LEISHMANIASIS: FURTHER OBSERVATIONS FROM THE URBAN OUTBREAK IN NATAL, BRAZIL. Jeronimo SB*, Oliveira RO, Mackay S, Costa RM, Sweet J, Nascimento ET, Luz KG, Fernandes MZ, Jernigan JA, and Pearson RD. Department of Biochemistry, Universidade Federal do Rio Grande do Norte; Fundaão Nacional de Saúde; Hospital Gizelda Trigueiro, Natal, RN, Brazil; and Department of Medicine, University of Virginia, Charlottesville, VA.

625 CHARACTERIZATION OF PARASITES CAUSING CUTANEOUS AND VISCERAL LEISHMANIASES IN PAKISTAN. Masoom Yasinzai M* and Chang KP. Institute of Biochemistry, University of Balochistan, Quetta, Pakistan; and Department of Microbiology/Immunology, UHS/Chicago Medical School, North Chicago, IL.

626 HIGH SEROPREVALENCE OF CHAGAS ANTIBODY POSITIVE SPECIMENS FOUND IN THE U.S. SHOULD A DIAGNOSTIC TEST FOR *TRYPANOSOMA CRUZI* BE IMPLEMENTED ?. Pan AA*, Schur JD, Brashear RJ, Winkler MA, Cantrell L, Rivera D, Shih J, and Holzer T. Diagnostic Biology Research, Abbott Laboratories, North Chicago, IL; and Quality Scientific Support, Abbott Laboratories, North Chicago, IL.

OPPORTUNISTIC PROTOZOA

627 *PNEUMOCYSTIS* PREPARATIONS OF HIGH PURITY AND VIABILITY. Kaneshiro ES*, Ellis JE, Wyder MA, Zhou LH, Langreth SG, and Voelker DR. Department of Biological Sciences, University of Cincinnati, Cincinnati, OH; Department of Microbiology, Uniformed Services University of Health Sciences, Bethesda, MD; and Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

628 A NOVEL *IN SITU* MODEL TO STUDY *PNEUMOCYSTIS CARINII* ADHESION TO LUNG ALVEOLAR EPITHELIAL CELLS. Pavia-Ruz N*, Ortega-Barria E, Alroy J, and Pereira ME. Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospitals, Boston, MA; and Department of Pathology, Tufts University School of Medicine and Veterinary Medicine, Boston, MA.

629 ALBENDAZOLE INHIBITS *PNEUMOCYSTIS CARINII* IN MOUSE MODELS. Bartlett MS*, Edlind TD, Shaw MM, Smith JW. Department of Pathology, Indiana University School of Medicine, Indianapolis, IN; and Department of Microbiology, Medical College of Pennsylvania, Philadelphia, PA.

630 ENTEROPATHOGEN PARASITES IN STOOLS OF HIV-POSITIVE PATIENTS WITH DIARRHEA. Houze-Savage S*, Van Gool T, Lemann F, Bouchaud O, Verdon R, Leport C, Ruggeri C, and LeBras J. Service de Parasitologie, Hospital Bichat-Claude Bernard, Paris, France; Service de Maladies Infectieuses et Tropicales, Hospital Bichat-Claude Bernard, Paris, France; and Medical Microbiology, Academic Medicla Center, Amsterdam, The Netherlands.

631 WILL POOLING THREE SPECIMENS INCREASE THE DIAGNOSTIC YIELD COMPARED TO ROUTINE EXAMINATION OF THREE SAF PRESERVED STOOLS?. MacPherson DW and Stephenson BJ*. Regional Parasitology Lab, St. Joseph's Hospital, Hamilton, Ontario; Infectious Diseases and Tropical Medicine Clinic, Chedoke-McMaster Hospitals; and Dept. of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Canada.

632 STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF LIGAND BINDING TO URACIL PHOSPHORYBOSYLTRANSFERASE FROM *TOXOPLASMA GONDII*. Tankersley KO* and Iltzsch MH. Department of Biological Sciences, University of Cincinnati, Cincinnati, OH.

633 DIFFERENTIAL MODES OF ACTION OF DICLAZURIL AGAINST THE RELATED PROTOZOANS *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* IN VITRO. Lindsay DS*, Toivio-Kinnucan MA, and Blagburn BL. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

634 EXPERIMENTALLY INDUCED OCULAR TOXOPLASMOSIS IN NURSING PIGS. Pinckney RD*, Lindsay DS, McLaughlin SA, Boosinger TR, and Blagburn BL. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL; and Department of Small Animal Surgery and Medicine, College of Veterinary Medicine, Auburn University, AL.

635 TRANSMISSION OF TOXOPLASMA IN PANAMA. Frenkel, JK*, Quintero R, Hassanein KM, Hassanein RS, Brown EF, and Thulliez, PH. Department Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS; Gorgas Memorial Laboratory, Panama City, Rep. of Panama; Instit. de Seguro Social, Panama City, Rep. of Panama; Department Biometry, University of Kansas Medical Center, Kansas City, KS; and Lab. de Serologie Neo-natale, Inst. de Puericulture, Paris, France.

636 BOVINE *NEOSPORA* ISOLATES: CULTIVATION, CHARACTERIZATION AND EXPERIMENTAL INFECTIVITY. Conrad PA*, Barr BC, Sverlow K, Rowe J, Tarantal A, Marsh A, BonDurant R, Ho M, and Hendrickx AG. School of Veterinary Medicine, University of California, Davis, CA; and California Regional Primate Research Center, Davis, CA.

637 IMMUNOGENICITY OF *TRICHOMONAS VAGINALIS* HEAT SHOCK PROTEINS IN HUMAN INFECTIONS. Davis SR*, Finley RW, and Lushbaugh WB. Parasitology Division, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson MS; and Infectious Diseases Division, Department of Medicine, University of Mississippi Medical Center, Jackson MS.

638 USE OF VERO-CELL CULTURES TO ASSESS CYTOPATHOGENICITY AND VIRULENCE OF *NAEGLERIA* SPECIES. John DT* and John RA. Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK; and Symex Corp., Tulsa, OK.

639 EPIDEMIOLOGICAL CHARACTERISTICS OF *ACANTHAMOEBA* KERATITIS IN SUB-SAHARAN AFRICA. Resnikoff S*, Le Flohic AM, Traore L, Huguet P, and Peyramaure F. Institute of African Tropical Ophthalmology, OCCGE, Bamako, Mali; and Department of Parasitology, Faculte de Medecine de Brest, France.

640 CHARACTERIZATION OF IMMUNODOMINANT PEPTIDES OF *THEILERIA SERGENTI* MEROZOITE ANTIGEN. Baek BK*, Rhim BM, Kim BS, Park KH, Rhim TE, Hansen RD, Vodkin MH, McLaughlin GL, and Kakoma I. Chonbuk National University, Chonju, Korea; University of Illinois, Urbana, IL; and Purdue University, West Lafeyette, IN.

641 *TETRATRICHOMONAS GALLINARUM* ASSOCIATED ENCEPHALITIS IN A MOCKINGBIRD (*MIMUS POLYGLOTTOS*). Patton S* and Patton CS. Department of Environmental Practice, University of Tennessee College of Veterinary Medicine, Knoxville, TN; and Department of Pathobiology, University of Tennessee College of Veterinary Medicine, Knoxville, TN.

642 *CARYOSPORA BIGENETICA* DEVELOPMENT AT LOW TEMPERATURE. Sundermann CA*. Department of Zoology & Wildlife Science, Auburn University, AL.

INTESTINAL PROTOZOA

643 PARTIAL SEQUENCING AND ISOLATION OF DNA POLYMERASE δ FROM *CRYPTOSPORIDIUM PARVUM*. Mead JR*, Lloyd RM, You XD, Arrowood MJ, Slemenda SB, Pieniazek NJ, and Schinazi RF. Emory University, Atlanta, GA; VA Medical Center, Decatur, GA; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

644 *CRYPTOSPORIDIUM PARVUM* SURFACE GLYCOPROTEINS ARE THE TARGET OF PROTECTIVE ANTIBODY. Doyle PS*, Lewis S, Barnes DA, and Petersen C. University of California, San Francisco, CA; San Francisco General Hospital, San Francisco, CA; and ImmuCell Corp., Portland, ME.

645 CLINICAL EFFICACY OF AMINOSIDINE SULPHATE IN THE TREATMENT OF AIDS-RELATED CRYPTOSPORIDIOSIS. Scaglia M, Atzori C*, Marchetti G, Olliaro P, Malfitano A, and Maserati R. Inst. Infectious Diseases, University-IRCCS S. Matteo, Pavia; Department Infectious Diseases, Hospital, Busto Arsizio (VA); and Farmitalia-C.Erba, Milano, Italy.

646 TESTING ANTI-CRYPTOSPORIDIUM AGENTS IN A CHRONICALLY INFECTED IMMUNODEFICIENT MOUSE MODEL. Leitch GJ* and He Q. Department of Physiology, Morehouse School of Medicine, Atlanta, GA.

647 FURTHER EFFICACY EVALUATION OF DICATIONIC MOLECULES AGAINST *CRYPTOSPORIDIUM PARVUM* IN HsD/ICR SWISS MICE. Blagburn BL*, Lindsay DS, Parsons LC, and Rippey NS. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

648 *CRYPTOSPORIDIUM* INFECTION INDUCES A DOSE AND TIME DEPENDENT DECREASE IN RESISTANCE ACROSS CACO-2 CELL MONOLAYERS. Griffiths JK*, Moore R, Keusch GT, and Tzipori S. Department of Comparative Medicine, Tufts University Schools of Medicine and Veterinary Medicine; Division of Infectious Diseases, St. Elizabeth's Hospital; Division of Geographic Medine and Infectious Diseases, Tufts-New England Medical Center, Boston, MA; and Department of Pathology, Tufts University Schools of Medicine and Veterinary Medicine, N. Grafton, MA.

649 RISK FACTORS FOR CRYPTOSPORIDIOSIS AMONG PERSONS WITH AIDS IN LOS ANGELES COUNTY. Sorvillo FJ, Lieb LE, Ash LR, and Kerndt P*. HIV Epidemiology Program, Department of Health Services, Los Angeles County, CA; and Department of Epidemiology, School of Public Health, University of California at Los Angeles, CA.

650 *CRYPTOSPORIDIUM* INFECTIONS IN A SUBURBAN COMMUNITY IN MARACAIBO, VENEZUELA. Chacin-Bonilla L*, de Young MM, Cano G, Guanipa N, Estevez J, and Bonilla E. Instituto de Investigacions Clinicas, Universidad del Zulia, Maracaibo, Venezuela.

651 IMPROVED METHODS FOR ASSESSING CRYPTOSPORIDIAL PARASITEMIA USING FLOW CYTOMETRY. Arrowood MJ*, Hurd MR, Brandt FB, and Mead JR. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Emory University, Atlanta, GA; and VA Medical Center, Decatur, GA.

652 CHRONIC DYSENTERY, THE OLD NAME OF AMEBIC DYSENTERY. Acuna-Soto R*. Division of Infectious Diseases, Beth Israel Hospital, Harvard Medical School, Boston, MA; Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

653 STUDY OF THE SERUM IgM ANTIBODY RESPONSE TO THE GALACTOSE-INHIBITABLE ADHERENCE PROTEIN OF *ENTAMOEBA HISTOLYTICA*. Abd-Alla MD, Jackson TF, El-Hawey AM, and Ravdin JI*. Case Western Reserve University and the Cleveland VA,

Cleveland Ohio; El-Hussain University Hospital, Cairo, Egypt; and Medical Research Council (Natal), South Africa.

654 TRANSFORMING GROWTH FACTOR-β_1 PRIME MACROPHAGES FOR AN ENHANCED NITRIC OXIDE-DEPENDENT CYTOTOXICITY AGAINST *ENTAMOEBA HISTOLYTICA*. Lin JY*, Seguin R, Keller K, and Chadee K. Institute of Parasitology, McGill University, Ste Anne de Bellevue, PQ. Canada.

655 CLONING, STRUCTURE AND EXPRESSION OF A MYOSIN LIGHT CHAIN KINASE GENE OF *ENTAMOEBA HISTOLYTICA*. Que X* and Reed SL. Department of Pathology, UCSD Medical Center, San Diego, CA.

656 IN VITRO ACTIVITY OF ATOVAQUONE AGAINST *ENTAMOEBA HISTOLYTICA* AND *E. DISPAR*. Ashley LS*, Ragland BD, Rogers MD, Petri WA. University of Virginia, Charlottesville, VA; and Burroughs Wellcome, Research Triangle Park, NC.

657 MONOCLONAL ANTIBODY-BASED ELISAS TO DETECT *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* INFECTION IN STOOL. Haque R, Kress KD, Lyerly D, Wilkins T, and Petri, Jr. WA*. Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh; TechLab Inc., Blacksburg, VA; and University of Virginia, Charlottesville, VA.

658 AMEBICIDAL AND TRICHOMONICIDAL ACTIVITY OF NALIDIXIC ACID. Anaya-Velazquez F*, Lopez-Godinez J, and Moreno-Rodriguez M. I.I.B.E., Fac. de Quimica, University de Guanajuato, Guanajuato, Gto. Mexico.

659 ISOLATION FROM *GIARDIA* OF A GENE ENCODING FIBRILLARIN, A PROTEIN REQUIRED FOR PRE-RIBOSOMAL RNA PROCESSING. Narcisi EM*, Glover CV, and Fechheimer M. Department of Zoology, University of Georgia, Athens, GA; and Department of Biochemistry, University of Georgia, Athens, GA.

660 PRODUCTION AND SECRETION OF PROTEASES BY *GIARDIA LAMBLIA* TROPHOZOITES. Alvarado L*, Cedillo-Rivera R*, Munoz O, Ortega-Pierres MG. U.I.M.E.I.P., Hospital de Pediatria, CMN SXXI, IMSS., Mexico, D.F., Mexico; Department of Genetics and Molecular Biology, CINVESTAV-IPN, Mexico, D.F., Mexico.

661 RISK FACTORS FOR DEVELOPMENT OF FIRST SYMPTOMATIC *GIARDIA* INFECTIONS AMONG INFANTS OF A BIRTH COHORT IN RURAL EGYPT. Mahmud MA*, Chapell C, Hossain M, Habib M, and DuPont HL.

662 DETECTION OF *GIARDIA* CYSTS IN FOODS USING DIRECT IMMUNOFLUORESCENCE. Dixon BR*. Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada.

663 DETECTION AND DIFFERENTIATION OF *GIARDIA LAMBLIA* AND *GIARDIA MURIS* CYSTS IN SURFACE WATER BY IMMUNOFLUORESCENCE FLOW CYTOMETRY. Stibbs HH*. Department of Cell and Molecular Biology, Tulane University, New Orleans, LA.

664 *BLASTOCYSTIS HOMINIS* AND *CRYPTOSPORIDIUM* IN PATIENTS WITH DIARRHOEA IN SLOVENIA. Logar J*, Andlovic A, Poljsak-Prijatelj M, and Golog V. Institute of Microbiology, Medical Faculty, University of Ljubljana, Zalska, Slovenia.

665 *BLASTOCYSTIS HOMINIS*: A CAUSE OF DIARRHEA IN PRE-SCHOOL CHILDREN. Nimri LF*. Department of Biological Sciences, Jordan University of Science and Technology, Irbed, Jordan.

666 TAXONOMIC UNCERTAINTY OF *BLASTOCYSTIS* (PROTOZOA:SARCODINA). Hollebeke NL* and Mayberry LF. Department of Biological Sciences, University of Texas at El Paso.

LYME DISEASE

667 LYME DISEASE IN THE RHESUS MONKEY, I: A MODEL FOR THE INFECTION IN HUMANS. Philipp M*, Aydintug MK, Bohm, Jr. RP, Cogswell FB, Dennis VA, Gu Y, Lanners HN, Lowrie, Jr. RC, Roberts ED, Conway MD, Gubler DJ, Johnson BJ, and Piesman J. Departments of Parasitology, Veterinary Sciences and Pathology, Tulane Primate Center, Covington, LA; Centers for Disease Control and Prevention, Fort Collins, CO; and Louisiana State University Eye Center, New Orleans, LA.

668 LYME DISEASE IN THE RHESUS MONKEY, II: LONGITUDINAL ASSESSMENT OF THE IgM AND IgG ANTIBODY RESPONSES TO *BORRELIA BURGDORFERI*. Aydintug MK and Philipp M*. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

669 LYME DISEASE IN THE RHESUS MONKEY, III: LONGITUDINAL ASSESSMENT OF ANTIBODY-DEPENDENT COMPLEMENT-MEDIATED KILLING OF *BORRELIA BURGDORFERI*. Gu Y*, Aydintug K, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

670 LYME DISEASE IN THE RHESUS MONKEY, IV: TISSUE LOCALIZATION OF SPIROCHETES BY PCR. Cogswell FB*, Roberts ED, Lowrie, Jr. RC, Lanners HN, and Philipp M. Departments of Parasitology and Pathology, Tulane Regional Primate Research Center, Covington, LA.

671 LYME DISEASE IN THE RHESUS MONKEY, V: LONGITUDINAL ASSESSMENT OF LYMPHOPROLIFERATIVE RESPONSES TO *BORRELIA BURGDORFERI* ANTIGENS. Dennis VA, Lasater BL*, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

672 POTENTIAL DIAGNOSTIC ANTIGENS FOR LYME DISEASE RECOGNIZED BY HUMANS AND RHESUS MONKEYS. Povinelli L*, Burkot T, Johnson B, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA; and Centers for Disease Control and Prevention, Fort Collins, CO.

673 PREDICTING *IXODES SCAPULARIS* ABUNDANCE ON WHITE-TAILED DEER USING GIS. Glass GE*, Amerasinghe FP, Morgan JM, Scott TW. Department of Immunology & Infectious Diseases, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD; Department of Entomology and Center for Agricultural Biotechnology, University of Maryland, College Park, MD; Department of Geography and Environmental Planning, Towson State University, Towson, MD.

674 DETACHMENT PERIODICITY OF JUVENILE *IXODES PACIFICUS* TICKS FROM COMMON VERTEBRATE HOSTS. Vredevoe LK*, Richter PJ, and Kimsey RB. Department of Entomology, University of California, Davis, CA; and Department of Comparative Pathology, University of California, Davis, CA.

675 DIFFERENTIATION OF *BORRELIA* SPECIES AND STRAINS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS. Nicholson WL* and Glass GE. Department of Immunology & Infectious Diseases, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD.

676 PHENOTYPIC AND GENOTYPIC VARIATION IN *BORRELIA BURGDOFERI* CULTURED FROM CHRONICALLY INFECTED *PEROMYSCUS LEUCOPUS* CAPTURED LONGITUDINALLY AT A LYME DISEASE ENZOOTIC SITE IN MARYLAND. Hofmeister E* and Childs J. The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD; and Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

CLINICAL TROPICAL MEDICINE

677 INCIDENCE OF SEVERE ANEMIA DURING THE FIRST YEAR OF LIFE IN INFANTS IN TANZANIA. Redding-Lallinger R*, Ting DY, Mmari MP, Kalokola F, Wilkinson WE, Lillinger G, and Durack DT. Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania; and Duke University Medical Center, Durham, NC.

678 SAFETY AND PHARMACOKINETICS OF A HUMAN HYPERIMMUNE BOTULINUM ANTITOXIN IN VOLUNTEERS. Hack DC*, Sjogren MH, and Crabbs C. Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD.

679 VARIATION IN BCG EFFICACY BY GEOGRAPHIC LATITUDE: SOME PLAUSIBLE HYPOTHESES. Wilson ME* and Fineberg HV. Division of Infectious Disease, Department of Medicine, Mt Auburn Hospital, Cambridge MA; and Harvard School of Public Health, Harvard Medical School, Boston MA.

680 MORE THAN MICROBES: THE SOCIAL AND CULTURAL BASES OF INFECTIOUS DISEASE. Etkin NL*. Department of Anthropology, University of Hawaii, Honolulu, HI.

681 CASE-CONTROL STUDY OF ENDEMIC DIARRHEAL DISEASE IN EGYPTIAN CHILDREN. Mortagy AK*, Mourad AS, Bourgeois AL, Kilpatrick ME, Kleinosky M, and Murphy JR. US NAMRU-3 & Faculty of Medicine, Ain Shams University, Egypt; Faculty of Medicine, Alexandria University, Egypt; Naval Medical Research Institute, Bethesda, MD; Naval Hospital, Orlando, FL; and Center for Infectious Diseases, University of Texas, Houston, TX.

682 MODULATION OF CELL PROLIFERATION IN BACTERIAL MENINGITIS BY SOLUBLE IL-2 RECEPTORS. El Ghorab NM*, Mansour MM, Girgis NI, Salah LA, and Abu-Elyazeed RR. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt.

683 A COMPARISON OF REDUCED DOSAGE CIPROFLOXACIN WITH THE STANDARD DOSAGE AS EMPIRIC THERAPY FOR TRAVELERS' DIARRHEA. Ferguson DD, Cimino AL, Panza N, Basnyat B, and Bia FJ*. Department of Internal Medicine, Yale University School of Medicine; Department of Laboratory Medicine, Yale University School of Medicine; and Nepal International Clinic, Kathmandu, Nepal.

684 CANINE HOOKWORM INFECTIONS: A LEADING CAUSE OF HUMAN EOSINOPHILIC ENTERITIS IN AUSTRALIA. Loukas A*, Croese J, Opdebeeck J, and Prociv P. Department of Parasitology, The University of Queensland, Queensland, Australia; and Townsville General Hospital, Townsville, Queensland, Australia.

685 CANINE HOOKWORMS: A CAUSE OF HUMAN INTESTINAL DISEASE. Croese J, Prociv P*, Loukas A, Opdebeeck J, and Fairley S. Department of Parasitology, The University of Queensland, Brisbane, Queensland, Australia; and Townsville General Hospital, Queensland, Australia.

ABSTRACTS

1 CRITICAL PARAMETERS OF CULTURED *PLASMODIUM FALCIPARUM* GAMETOCYTES THAT AFFECT INFECTIONS IN *ANOPHELES GAMBIAE* MOSQUITOES. Noden BH, Beadle P, Vaughan JA, and Beier JC. Department of Immunology and Infectious Diseases, The Johns Hopkins University, Baltimore, MD.

Anopheles gambiae mosquitoes were infected with *Plasmodium falciparum* gametocytes from *in vitro* cultures to identify key determinants of infection. A retrospective study analyzing 90 experimental infections indicated that gametocytemia and exflagellation intensity were the most important characteristics for successful *An. gambiae* infections. However, a wide range of oocyst infection rates, and inconsistent relationships between several characteristics of gametocytes and mosquito infections prompted an intense short-term study. Over twelve weeks, one culture flask was used each week for daily feeds (4-5 feeds per culture per week). Twenty-six parameters were recorded each day. The experimental infections were then evaluated by multivariate analysis using oocyst infection rate as the dependent variable. We have identified several characteristics of gametocyte cultures that can be used to predict whether an *in vitro* culture of *P. falciparum* will successfully infect *An. gambiae*.

2 SPOROGONIC DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN SIX SPECIES OF LABORATORY-INFECTED *ANOPHELES* MOSQUITOES. Vaughan JA*, Noden BH, and Beier JC. Immunology & Infectious Diseases, School of Hygiene & Public Health, Johns Hopkins University, Baltimore, MD.

Sporogonic development of *Plasmodium falciparum* NF54 was compared in 6 *Anopheles* spp. Absolute densities were determined for each sporogonic lifestage. Four aspects of parasite population dynamics were quantified: 1) successive loss in parasite abundance from gametocyte to oocyst stage; 2) oocyst production of sporozoites; 3) correlation between various lifestage parameters; 4) parasite distribution. Comparative susceptibilities were; *A. freeborni* >> *A. gambiae, A. arabiensis, A. dirus* > *A. stephensi, A. albimanus*. The key lifestage transition determining overall susceptibility differed among species. Despite species differences in oocyst densities and infection rates, sporozoite production per oocyst (ca. 640) were the same among species. The most consistent association between lifestage parameters was the positive correlation between densities and infection rates of homologous lifestages. The curvilinear relationship between ookinete and oocyst densities in *A. gambiae* indicated that a threshold density (ca. 30 ookinetes per mosquito) was required for ookinete conversion to oocysts. The same relationship in *A. freeborni* was linear, with no distinct threshold. Ookinete and oocyst populations in all mosquito species were skewed to the left. Indices of heterogeneity in mosquito susceptibility to infection indicated that gene frequencies determining susceptibility fluctuated over time in all species, except *A. freeborni* where susceptibility remained homogeneous throughout the study. This approach provides a framework for identifying mechanisms of susceptibility and for evaluating *Plasmodium* sporogony of wild parasites in coindigenous vector species.

3 FACTORS AFFECTING THE INFECTIVITY OF MALARIA SPOROZOITES. Beier JC*, Pumpuni CB, and Mendis C. Department of Immunology and Infectious Diseases, The Johns Hopkins Univeristy, Baltimore MD.

Is the dose of malaria sporozoites delivered by a blood-feeding mosquito a key factor affecting host infection? We have conducted several experiments to quantify *in vitro* the dose of *Plasmodium falciparum* sporozoites inoculated by "infective" mosquitoes. Consistently, most mosquitoes transmit few or no sporozoites, and there is no convincing relationship between the number of sporozoites transmitted and sporozoite loads. Experiments are being done with *Anopheles stephensi* infected with *P. yoelii* to evaluate factors affecting sporozoite transmission. Individual mosquitoes that transmitted sporozoites to 13 of 30 mice had higher sporozoite loads than non-transmitters. To

test whether the probability of transmission is dependent strictly upon sporozoite dose or whether sporozoites simply vary in their infectiousness, mice are being challenged intravenously with either 25 or 250 sporozoites from individual mosquitoes. At the low dose of only 25 sporozoites, the probability of host infection increases as a function of the number of sporozoites in the salivary glands. These preliminary findings that sporozoites from individual mosquitoes vary in their infectivity relative sporozoite loads raise the possibility that sporozoite "quality" may be an important determinant affecting sporozoite transmission.

4 LONG-TERM SURVIVAL OF *PLASMODIUM* SPOROZOITES *IN VITRO*. Pumpuni CB* and Beier JC. Department of Immunology and Infectious Diseases, The Johns Hopkins Univeristy, Baltimore MD.

Malaria sporozoites are adapted for long-term survival in the salivary glands of the mosquito. However, once removed from the mosquito,sporozoites quickly lose their infectivity and die. Simple methods wereused for keeping sporozoites alive, outside the mosquito, for up to 21 days. Salivary glands of *Anopheles stephensi* mosquitoes infected with either *Plasmodium falciparum* or *Plasmodium yoelii* were triturated, and counted sporozoites were incubated in media containing various combinations of antibiotics, glucose, saline, sera, and salivary gland extracts. With most combinations, sporozoites were eliminated by bacteria within 48 hours. Sporozoite survival for >14 days was observed only for samples incubated in 1:1 saline:glucose plus antibiotics. In this media, sporozoites of both *P. falciparum* and *P. yoelii* survivedfor 21 days without significant losses in the numbers of sporozoites. These sporozoites were normal in appearance, motile, and elicited circumsporozoite precipitin (CSP) reactions when mixed with immune sera. However, corresponding infection experiments with *P. yoelii* indicatedthat the sporozoites held under these conditions were not infective to mice beyond 24 hours. The ability to maintain sporozoites alive, outside the mosquito, provides a starting point for evaluating the biologic basis of sporozoite infectivity.

5 PERITROPHIC MATRIX (PM) AS A DETERMINANT OF MALARIA PARASITE SPECIFICITY FOR MOSQUITO VECTOR. Shahabuddin M* and Kaslow DC. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; and National Institutes of Health, Bethesda, MD.

Transmission of each *Plasmodium* spp. is normally associated with a restricted number of mosquito species. Thus *P. falciparum* parasites are transmitted preferentially by *Anophelene* mosquitoes and *P. gallinaceum* is preferably transmitted by *Aedes* spp. In an incompatible mosquito the parasite fails to develop succesfully and dies. Within a few hours post bloodmeal mosquitoes develop a chitinous tube-like peritrophic matrix (PM), that gradually surrounds the food bolus. Because this PM may form before *Plasmodium* ookinetes mature, the matrix is thought to be a determinant of the malaria parasite-mosquito vector specificity. To study the role of the mosquito PM on specificity, we disrupted PM formation by inhibiting chitin synthase with polyoxin D or by digesting the PM's chitin with a fungal chitinase *in vivo*. Polyoxin D was found to be non-toxic to mosquitoes at the concentrations used. At a conc. of 100 uM or more, Polyoxin D completely blocked PM formation. When *P. gallinaceum* were fed to a compatible vector, *Aedes aegypti*, no significant effect was observed on the sporogonic development of the parasite either in Polyoxin D- or chitinase-fed mosquitoes as compared to untreated mosquitoes. When the same parasite was fed to a partially compatible vector, *An. gambiae*, and two completely refractory vectors, *An. freeborni* and *An. stefensi*, no reversal in incompatibility was observed in the absence of the PM. These data demonstrate that PM is not the primary determinant of *Plasmodium*-mosquito specificity.

6 GENETIC ASPECTS OF SUSCEPTIBILITY OF *AEDES AEGYPTI* TO *PLASMODIUM GALLINACEUM*. Thathy V*, Severson DW, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Several studies have demonstrated a genetic basis for variation in susceptibility of *Aedes aegypti* to *Plasmodium gallinaceum*. Although *P. gallinaceum* susceptibility in *A. aegypti* is determined primarily by a single autosomal dominant gene, it has been suggested that other genetic factors also are involved. Three sublines, one refractory, one of intermediate susceptibility and one of increased susceptibility to *P. gallinaceum* have been isolated from the Moyo-In-Dry strain (Moyo) of *A. aegypti* by selectively breeding for the required characteristic. Mosquitoes were designated as refractory when no viable oocysts were found. Prior to selection, the Moyo population was 20.2% refractory. Susceptible individuals had considerable intrapopulation variability in oocyst distribution with a range of 1-55 oocysts per mosquito. The refractory and intermediate susceptibility sublines were derived from the same pedigree after two generations of isofemale line selection with pairwise matings, followed by a generation of mass selection. The high susceptibility subline was isolated after five generations of selection using a mixture of isofemale line and mass selection systems. The highly susceptible Rockefeller strain (RKF) of *A. aegypti* was interposed as a control throughout the selection procedure. The RKF strain and the selected refractory and intermediate susceptibility sublines of the Moyo strain were utilized in genetic studies to further clarify the mode of inheritance of *Plasmodium* susceptibility in *A. aegypti*.

7 CHROMOSOMAL MAPPING OF GENETIC LOCI ASSOCIATED WITH *PLASMODIUM GALLINACEUM* SUSCEPTIBILITY IN *AEDES AEGYPTI*. Severson DW*, Thathy V, Mori A, Zhang Y, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Susceptibility of the mosquito *Aedes aegypti* to the avian malarial parasite *Plasmodium gallinaceum* is determined primarily by genetic factors. A single dominant gene for susceptibility (*pls*) has been described using classical genetics. Isolation and characterization of genes conferring susceptibility could identify novel approaches for interrupting parasite transmission. We are using restriction fragment length polymorphism (RFLP) markers to identify quantitative trait loci (QTL) for *P. gallinaceum* susceptibility. QTL identify regions of the mosquito genome that carry genes associated with the susceptible phenotype. We produced F_2 segregating populations of mosquitoes from crosses between the highly susceptible Red strain and a recently selected highly refractory strain derived from the Moyo-In-Dry strain. Adult F_2 females were exposed to parasite infected bloodmeals and subsequently dissected to determine the status of their susceptibility. Two independent trials involving 196 and 124 females, respectively, have been conducted. The mean oocyst count per female for the first trial was 62 with a range of 0 to 520 and that for the second trial was 46 with a range of 0 to 320. Southern blots carrying genome digests of each of these females were produced and screened with RFLP markers that define 10-20 map unit distances across the *A. aegypti* genome. QTL analyses of the RFLP data indicate that at least two independently segregating loci are associated with the susceptible phenotype. One of these loci corresponds to the general genome location reported for the *pls* locus.

8 FIELD RELEASES TO INTRODUCE TROPICAL TRAITS INTO A TEMPERATE POPULATION OF *AEDES STEGOMYIA ALBOPICTUS*. Mutebi JP*, Craig Jr. GB, and Novak RJ. Vector Biology Lab., Department. of Biological Science, University of Notre Dame, Notre Dame, IN; and Illinois Natural History Survey, Champaign, IL.

Males of a tropical, selected, genetically marked strain of *Aedes albopictus* (SP2) were released into a temperate population in East St. Louis IL. The aim was to reduce cold hardiness and photoperiod sensitivity of the field population, making it vulnerable to winter conditions. Tropical strains are not cold hardy and are not sensitive to photoperiod. The hybrids are intermediate. Earlier field trials by Hanson et al. (1993) showed that the hybrids do not survive the winter. The diagnostic genetic marker is a-glycerol-3-phosphate dehydrogenase (a-GPD). SP2 is homozygous for the fast-migrating

allele (rf 1.15) and the East St. Louis populations are homozygous for the slow-migrating one (rf 1.00). Field releases were started on June 8th 1992 and went on biweekly until the 12th of October 1992. A total of 160,100 SP2 males were released. Hybridization levels were determined by assaying for heterozygotes of the slow and fast-migrating alleles of a-GPD using polyacrylamide gel electrophoresis. At the end of September 53.2% of the field eggs sampled were heterozygotes. Relative population estimates were similar at both the experimental and the control sites. The population will be monitored in the summer of 1993 for the fast-migrating allele of a-GPD, and for population size. We presume the fast-migrating (tropical) allele of a-GPD would have disappeared from the field population.

9 VECTOR COMPETENCE OF *AEDES ALBOPICTUS* FROM PINE BLUFF, ARKANSAS, FOR A ST. LOUIS ENCEPHALITIS VIRUS STRAIN ISOLATED DURING THE 1991 EPIDEMIC. Savage HM*, Smith GC, and Mitchell CJ. Medical Entomology-Ecology Branch, DVBID, NCID, Centers for Disease Control and Prevention, Fort Collins, CO.

In 1991, an epidemic of St. Louis encephalitis (SLE) occurred in Pine Bluff, AR, involving 25 human cases. Studies conducted in 1991 resulted in 12 isolates of SLE virus: 11 from 6,768 *Culex pipiens quinquefasciatus*, and 1 from a pool of *Cx. (Culex)* spp. No SLE virus isolates were obtained from 1,234 specimens of *Ae. albopictus* tested. However, *Ae. albopictus* was the fourth most commonly collected species in adult collections, and the most frequently encountered species in larval surveys with an average of 1.0 *Ae. albopictus* positive habitat per residence. The catholic bloodfeeding pattern of *Ae. albopictus*, combined with its common occurrence in residential neighborhoods suggest a possible role for *Ae. albopictus* in SLE transmission to humans. To better assess the role of *Ae. albopictus* as a potential vector, we conducted vector competence studies using *Ae. albopictus* and a SLE strain from Pine Bluff. Low-passage SLE virus seed was prepared by intrathoracic inoculations into *Cx. p. pipiens*, which were sacrificed after 7 days of incubation. A virus profile for the SLE seed virus in hamsters was determined by subcutaneously inoculating 3-6 week-old hamsters with 1,000 PFU of virus and bleeding hamsters at 24, 48, 72, 96 and 120 hrs. post-inoculation. Groups of 50, 3-5 day-old *Ae. albopictus* were fed at regular intervals on a new group of hamsters inoculated as above. Five freshly-fed specimens from each group were frozen immediately. At 7 and 14 days post-infection, transmission trials were conducted using the remaining *Ae. albopictus* and 1-3 day-old suckling mice. Individual mosquitoes and suspensions from dead and sick mice were tested in cell culture to verify the presence of virus. Results will be presented and the potential role of *Ae. albopictus* in SLE transmission in the southern U.S. discussed.

10 APPARENT CHANGES IN THE DISTRIBUTION AND ABUNDANCE OF MALARIA VECTORS IN GRENADA. Manguin S*, Peyton EL, James AC, and Roberts DR. Preventive Medicine & Biometrics, Uniformed Services University of Health Sciences, Bethesda, MD; Walter Reed Biosystematics Unit, Department of Entomology, Walter Reed Army Institute of Research, Washington DC; and Ministry of Health, St George's, Grenada.

Results of a survey of three malaria vectors, *Anopheles (Nyssorhynchus) aquasalis, An. (Nys.) argyritarsis* and *An. (Anopheles) pseudopunctipennis*, on the island of Grenada in April 1992 are contrasted with the results of the only other published survey of these species on Grenada in 1938. Results suggest that a significant change in the distribution and abundance of each species has occurred over this period. Physical characteristics of the aquatic habitats for each species are described, compared and discussed. We considered the potential effects of these changes on human health if malaria were reintroduced.

11 THE USE OF REMOTE SENSING AND LANDSCAPE FEATURES TO ACCURATELY PREDICT THE PRESENCE AND ABUNDANCE OF TWO MALARIA VECTORS IN FOOTHILL AREAS OF

BELIZE. Roberts DR*, Paris JF, Manguin S, Harbach RE, Woodruff R, Rejmankova E, Polanco J, and Legters LJ. Uniformed Services University of the Health Sciences, Bethesda, MD; Department of Biology, California State University, Fresno, CA; Smithsonian Institution, Washington, DC; University of California, Davis, CA; and Ministry of Health, Belize City, Belize.

Remote sensing and cartographic data were empoyed to predict localities of high, medium and low malaria vector densities during the dry season along the Hummingbird Highway in Belize. Predictions based on four criteria were developed by persons with no prior experience in Belize and without benefit of ground truth information. Criteria for predictions related to presence of rivers, elevation, amount of forest between houses and riverine areas, and presence of humans. Once predictions were developed, field surveys were conducted in April and May, 1993 to verify presence and abundance of vectors and accuracy assessments of remotely-sensed data interpretations. Houses in areas predicted to have high densities of vectors were consistently (100%) positive for *Anopheles darlingi*, and 50% were positive for *An. pseudopunctipennis*. Houses at all sites predicted to have medium to low densities of malaria vectors were negative for *Anopheles* mosquitoes. These results reflect the extreme localization of both *An. darlingi* and *An. pseudopunctipennis* populations to riverine localities. Although the SPOT satellite data employed in this test were from 1990, still the data interpretations relating to ground cover and sites of houses and human activities were generally accurate. While single houses with thatch roofs cannot be detected, there were other characters indicative of human activities, and thus presence of human populations. This test demonstrates the potential for using remotely-sensed data and geographic information systems for managing and targeting vector and disease control measures.

12 PROPOSAL FOR EXPERIMENTAL FIELD EVALUATION OF ENVIRONMENTAL METHOD TO CONTROL VECTOR-BORNE DISEASES AROUND MANANTALI RESERVOIR IN WESTERN MALI. Jobin WR*. Director of Blue Nile Associates, Foxboro, MA.

Fluctuations in the water level of Manantali Reservoir on the Senegal River in Mali are proposed as a method to assist in preventing nearby transmission of bilharzia, malaria, river blindness and Rift Valley Fever. A range of fluctuation schedules are proposed for field evaluation, based on experience in the Tennessee River Valley and Puerto Rico, and modified for maximum impact on insects and snails found in Mali. Repeated fluctuations will be possible in wet years as excess flow continues to enter the full reservoir during the flood season. Manantali Reservoir started to fill in July 1987, and several health problems erupted. By October, Rift Valley Fever epidemics had occurred in three places in the river valley. An outbreak of severe intestinal bilharzia began in the lower valley in 1988. The reservoir filled to spillway elevation in 1991 and villages around the reservoir shoreline were suffering from year-round transmission of bilharzia and malaria by 1992. Downstream of the dam, habitats of the blackflies which transmit river blindness no longer suffered from seasonal flow interruption. These myriad problems created by the dam are offset by an unusual opportunity for field experimentation, due to several years of delay expected before installation of the hydroelectric turbines. It is proposed that the experimental evaluation be conducted by the Senegal River Basin Authority with assistance from the Ministry of Health of Mali and the WHO Onchocerciasis Control Program of West Africa. It is further proposed that the experimental work be directed and monitored by a panel of experts (PEEM) from WHO.

13 *ENTAMOEBA HISTOLYTICA* STIMULATES TNF-α AND C-FOS GENE EXPRESSION IN MACROPHAGES THROUGH PROTEIN KINASE C SIGNAL TRANSDUCTION. Seguin RM*, Keller K, and Chadee K. Institute of Parasitology, McGill University, Quebec, Canada.

Entamoeba histolytica infections are associated with a state of transient suppression of cell-mediated immunity through an unknown mechanism. The cytokine TNF-α is involved in stimulating macrophages for host defence against amoebiasis. In this study we determine whether soluble

amoebic lysates (HM1-IMSS) can modulate TNF-α gene expression and the signal transduction mechanism involved in murine bone marrow-derived macrophages (BMM). Pretreatment of BMM (0.5-3hr) with amoebic lysates (50 μg/ml) stimulated TNF-α and the proto-oncogene c-fos gene expression as determined by Northern analysis. The Protein Kinase C (PKC) inhibitor, H7 abrogated amoebae induced TNF-α gene expression and reduced c-fos gene expression by 65%. *E. histolytica* induced the translocation of the PKC enzyme from the cytoplasm to the membrane fraction with increased enzyme activity. Analysis of the mRNA stability after transcriptional blockage by actinomycin D showed that the *E. histolytica* induced TNF-α mRNA had a reduced half-life (1 hr vs. 6 hr in LPS stimulated cells); c-fos mRNA was less stable when induced by *E. histolytica* but only by a 0.5 hr difference as compared to LPS stimulation. These results demonstrate that *E. histolytica* stimulates a transient and unstable TNF-α gene expression through PKC signal transduction and suggest that the parasite may suppress macrophage functions at the transcriptional level.

14 THREE ISOFORMS OF AMOEBAPORE, THE PORE-FORMING PEPTIDE OF PATHOGENIC *ENTAMOEBA HISTOLYTICA*. Leippe M*, Andrä J, Tannich E, and Müller-Eberhard HJ. Department of Molecular Biology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, F.R. Germany.

The pore-forming peptide amoebapore is considered part of the cytolytic armament of pathogenic *Entamoeba histolytica*. The active peptide has been localized to cytoplasmic vesicles of the amoebae. During purification of amoebapore from these granules it became evident that two minor active pore-forming components exist in pathogenic *E. histolytica*. Further purification on HPLC and protein sequencing revealed that these activities are due to two additional isoforms of the pore-forming peptide. These peptides were named amoebapore B and C to differentiate them from the major isoform termed amoebapore A, the primary and secondary structure of which has previously been solved. Cloning and DNA sequencing resolved the primary structure of the two other peptides which were found to have the same size, to be identical in amino acid sequence with isoform A to 57% and 47%, respectively, and to have a sequence homology to each other of 35%. Among the residues conserved are all of the six cysteine residues and the histidine at position 75. Activities of the three isoforms toward artificial and natural targets were studied suggesting that the amoebapore family is instrumental in the killing of bacteria and host cells.

15 CLONING OF AN *ENTAMOEBA HISTOLYTICA* GENE (EHVMA3) ENCODING THE PUTATIVE PROTEOLIPID OF A VACUOLAR MEMBRANE-ATPASE. Descoteaux S, Yu Y, Lohia A, and Samuelson J *. Department. of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department. of Biochemistry, Bose Institute, Calcutta, India.

We are interested in mechanisms of phagocytosis and bacterial killing by *Entamoeba histolytica* parasites. One important factor may be vacuolar acidification, which is mediated by vacuolar proton-translocating ATPases (V-type H^+-ATPases) that are inhibited by bafilomycin A and DCCD. V-type H^+-ATPases are composed of at least three peptides, including a 67-kDa ATPase (VMA1), a 55-kDa regulatory peptide (VMA2), and a 16-kDa proteolipid (VMA3), which is the hydrogen ion transporter. Here we report the cloning an ameba gene (EhVMA3) encoding a putative VMA3 proteolipid. EVMA3 contains a 175 amino acid ORF, encoding an 18-kDa protein with a 55 % positional identity and with the proteolipid of *Neurospora crassa*. The EhVMA3 ORF is very hydrophobic as is the case with other VMA proteolipids and contains the conserved glutamate, which is covalently modified by DCCD. We plan to test the function of EhVMA3 in yeast mutants and to localize the native amebae using anti-EhVMA3 antibodies.

16 EXPRESSION OF AN ACTIVE, RECOMBINANT CYSTEINE PROTEINASE OF *ENTAMOEBA HISTOLYTICA*. Reed SL*, Que X, Herdman DS, Hirata KK, Torian BE, and McKerrow JH. Departments of Pathology and Medicine, University of California, San Diego, CA; Department of Pharmaceutical Sciences, Idaho State University, Pocatello, Idaho; and Department of Pathology, VA Medical Center, San Francisco, CA.

Cysteine proteinases (ACP) are an important virulence factor of *E. histolytica*. At least three genes encoding cysteine proteinases have been identified, one of which is unique to pathogenic strains. To further characterize these enzymes, we have expressed active recombinant ACP 2, a proteinase present in both pathogenic and nonpathogenic amebae. We had previously isolated a genomic clone encoding 932 bp of the mature enzyme by screening a genomic library with a probe encoding the active site of the enzyme. The remaining prepro- and carboxyterminal nucleotide sequences were obtained by PCR amplification of DNA purified from a λ gt11 cDNA library. The complete coding sequence was then amplified by PCR to add a 5'*Sac*II and 3' *Xba*I site and ligated into the pCheY expression vector with a enteropeptidase cleavage recognition site. Following induction with IPTG, at least three bands with apparent Mr of 65, 58, and 54 kD could be detected in both the soluble and insoluble fractions of *E. coli* DH5a by Coomassie blue staining and by immunoblots, suggesting that the fusion protein underwent autoproteolysis. The soluble fusion protein(s) had enzymatic activity as demonstrated by the cleavage of synthetic cathepsin substrates, but only one species (~58 kD) appeared to be active by gelatin substrate gels. Further characterization of the recombinant enzyme will provide important information on the expression and processing of these proteinases and may potentially suggest novel approaches for the design of inhibitors.

17 IN VITRO GALACTOSE SPECIFIC BINDING OF RECOMBINANT *ENTAMOEBA HISTOLYTICA* ADHESION LECTIN MAPS TO THE CYSTEINE-RICH REGION OF THE 170 KDA HEAVY SUBUNIT. Wan P *, Ravdin JI, and Kain KC. Tropical Disease Unit, Division of Infectious Diseases, The Toronto Hospital, Toronto, Canada; and Department of Medicine, Case Western Reserve University School of Medicine and VA Medical Center, Cleveland, OH.

Adherence of *Entamoeba histolytica* to colonic mucins and epithelial cells is essential to its pathogenicity. Adherence is mediated by a galactose-binding lectin composed of 170 kDa and 35 kDa subunits. The present study was undertaken to identify the galactose-binding domain of the adherence lectin. The gene encoding the 170 kDa subunit was amplified from HM1:IMSS strain genomic DNA by PCR and cloned into an *in vitro* expression vector. Full length and overlapping polypeptides of the 170 kDa subunit were synthesized by Expression-PCR and *in vitro* translation and refolded post-translationally with protein disulfide isomerase. Proper conformation was confirmed by immunoprecipitation with monoclonal antibodies that inhibit galactose-specific adhesion by the native lectin. Neutralizing monoclonal antibody epitopes were mapped to the cysteine-rich domain of the adherence lectin extending recently reported data. *In vitro* synthesized and refolded 170 kDa subunit bound to CHO cells. Binding to CHO cells was inhibited by galactose and a monoclonal antibody against an epitope that maps to the cysteine-rich domain, but was not inhibited by mannose or dextrose. Galactose-dependent binding of the 170 kDa subunit was lost when portions of the cysteine-rich extracellular domain were deleted. The cysteine-rich domain of the 170 kDa subunit may represent a novel carbohydrate binding region and could be a potential candidate for a subunit vaccine.

18 RECOMBINANT AND NATIVE *ENTAMOEBA HISTOLYTICA* 29KDA ANTIGEN DEMONSTRATES SPECIFICITY FOR AMEBIC LIVER ABSCESS. Soong G*, Abd-Alla MD, Kain KC, Jackson TF, Torian BE, and Ravdin JI. Case Western Reserve University and the Cleveland VA, Cleveland, OH; University of Toronto and The Toronto Hospital, Toronto, Canada; Medical Research Council (Natal), Natal, South Africa; and Idaho State University, Pocatello, ID.

ABSTRACTS

A cDNA clone of a 29kDa *Entamoeba histolytica* antigen was obtained by screening a UniZap cDNA library with human immune sera. The ~830 bp insert was subcloned into pRsetA, expressed as a fusion protein with 6 histidine residues added, and the r29kDa protein was purified by sequential metal chelate and immunoaffinity chromatography. Anti-r29kDa serum abs. were found by ELISA in 80% of 100 subjects from Egypt and South Africa with amebic liver abscess (ALA) compared to documented infection with nonpathogenic zymodemes ($p<0.01$, ALA compared to each group). Purified native 29kDa provided similar results in all groups. Peripheral blood lymphocytes from 8 ALA subjects demonstrated blastogenic responses to both recombinant and native 29kDa antigen (1-10 μg/ml) in a dose dependent manner ($p<0.01$). The r29kDa antigen was immunogenic in gerbils as determined by serum anti-r29kDa IgG ab. Immunoblotting gerbil immune sera to total *E. histolytica* protein demonstrated exclusive recognition of a 29kDa antigen. In summary, the 29kDa antigen was specific for amebic liver abscess by ELISA serology and lymphocyte blastogenesis, is immunogenic in gerbils and is being evaluated as a protective antigen for experimental amebic liver abscess.

19 ANTIBODIES TO THE SERINE RICH *ENTAMOEBA HISTOLYTICA* PROTEIN (SREHP) PREVENT AMEBIC LIVER ABSCESS IN SCID MICE. Tonghai Z, Cieslak PR, Foster L, Kunz-Jenkins C, and Stanley, Jr. SL*. Department of Medicine, Washington University School of Medicine; Departments of Medicine and Molecular Microbiology, Washington University School of Medicine.

Amebic liver abscess caused by the intestinal protozoan parasite *Entamoeba histolytica* is a major cause of morbidity and mortality worldwide. We used mice with severe combined immunodeficiency (SCID mice) to study the role of antibody in protection from amebic liver abscess, and to identify protective antigens of *E.histolytica*. Antisera to recombinant versions of two major surface antigens of *E. histolytica*, the serine rich *E. histolytica* protein (SREHP) and the 170 kDa adhesin were used in this study. We found that of 10 scid mice passively immunized with antiserum to the recombinant SREHP molecule all 10 were completely protected from developing amebic liver abscess after intrahepatic challenge with virulent *E. histolytica* trophozoites. In contrast only 2/9 scid mice immunized with antiserum to a portion of the 170 kDa adhesin were protected. Since one possible mechanism by which antibody may mediate protection is by blocking amebic adhesion to hepatic cells we examined the ability of each of the antisera to inhibit *E. histolytica* trophozoite binding to a mammalian cell line. Antiserum to the recombinant SREHP molecule inhibited amebic adhesion to 25% of control levels; this was significantly different from antiserum to the 170 kDa adhesin (82% of control levels). Our study demonstrates that antibodies to a recombinant version of the amebic SREHP molecule can protect against amebic liver abscess, and that the protective efficacy of a given antibody may correlate with its ability to block amebic adhesion to mammalian cells.

20 EPIDEMIOLOGY OF INVASIVE AMEBIASIS IN A FAVALA IN FORTALZA, BRAZIL. Braga LL*, Mann BJ, Sears C, Wuhib T, Newman R, Lima A, Guerrant R, and Petri WA. Universidade Federal do Ceara, Fortaleza, Brazil; Johns Hopkins University School of Medicine, Baltimore, MD; and University of Virginia, Charlottesville, VA.

Goncalves Dias is a community of 1900 inhabitants occupying 405 dwellings in an urban slum (favala) in Fortaleza, Brazil. Sera collected from 29 families from Goncalves Dias was tested for antibodies against the galactose-specific adherence lectin of *Entamoeba histolytica*. Serum anti-lectin antibodies are a marker for current or prior invasive amebiasis, and *in vitro* neutralize amebic adherence, contact-dependent cell killing and resistance to the membrane attack complex. Sera from a total of 164 individuals from 29 families were tested for anti-lectin antibodies at a 1:1000 dilution; overall 23% (38/164) of the sera were positive, including 17% (14/84) of the males and 30% (24/80) of the females. Anti-lectin antibodies were most common for ages 6-14, where 41% of all children were seropositive. No children less than age 1 were seropositive, while 22% of children age 1-5 years old and 20% of individuals over age 14 were seropositive. Seropositive patients clustered in families, with 84% of the cases occurring in 8 of the 29 families. There was no apparent relationship between family size

and the presence of anti-lectin antibodies in family members. These initial studies have demonstrated that invasive amebiasis, as measured by serum anti-lectin antibodies, is a common infection in children living in Goncalves Dias, and that seropositivity is more frequent in females and clusters in families. The favala offers an opportunity to understand acquired immunity to amebiasis, transmission of infection within families, and the role of anti-lectin antibodies in immunity.

21 HOMOLOGUES OF SMALL HEAT SHOCK PROTEINS THAT ARE CONSTITUTIVELY EXPRESSED IN FILARIAE. Lillibridge D* and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

Two cDNA fragments encoding a 27 ± 1.6 kDa homologue of members of the small heat shock protein (sHsp) family were isolated from expression libraries prepared from mRNA of both *Brugia malayi* (cDNA = 624 bp) and *Dirofilaria immitis* (cDNA = 627 bp) adult male filariids. The sHsp homologues' derived amino acid sequences show near identity to one another and significant similarity (48%) to *Drosophila* sHsp, the mammalian alpha-crystallin lens proteins and the p40 egg antigen of *Schistosoma mansoni*. One of the truncated cDNA fragments was used as a probe to identify the complete gene coding-sequence of the other, in a polymerase chain reaction-based technique developed *ad hoc*. This technique allows for the swift isolation of complete gene coding-sequences from cDNA libraries initially screened with antibody. In contrast to other sHsp, whose expression is induced or augmented by stress stimuli and is often developmentally regulated, the expression of the 27 kDa protein was uniform throughout the third molt *in vitro* and was not significantly enhanced when worms were cultured at 43°C. The sHsp homologues had been localized to the cuticle and hypodermal layers of 3rd and 4th-stage larvae (unpublished). Although their function there is unknown, heat shock proteins have been shown to act as molecular chaperones in the folding and translocation of polypeptides. Third-stage *D. immitis* larvae were grown *in vitro* and pulse-labeled with [^{35}S] methionine on 4 consecutive days. Larval PBS extracts were collected each day, and equal TCA-precipitable counts were immune-precipitated with a monospecific antibody raised against recombinant Hsp27. Autoradiographs of these gels show a 33.5 kDa protein co-precipitating with the Hsp27 homologue. The intensity of the 33.5 kDa band peaked by 28 h in culture, a time which precedes L_3 ecdysis *in vitro* by one day and therefore coincides with the synthesis of the 4th larval cuticle. One interpretation of this result is that the constitutively expressed sHsp homologues we identified function as molecular chaperones in the transport of cuticular components to the nascent cuticle.

22 SUPPRESSION OF REPRODUCTIVE CAPACITY IN MALE *DIROFILARIA IMMITIS* BY PROPHYLACTIC TREATMENT WITH MILBEMYCIN OXIME. Lok JB*, Knight DH, Selavka CM, and Bergman RN. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; and Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

In dogs with preexisting heartworm (HW, *Dirofilaria immitis*) infections, prophylactic treatment with milbemycin oxime consisting of six consecutive monthly doses of 500 µg/kg body weight, causes clearance of circulating microfilariae (MF) and infertility in female HWs which persists for at least nine months after suspension of treatment. We asked whether infertility in milbemycin treated female HWs is associated with reduced reproductive capacity in male HWs. Reciprocal crosses between HWs from MF-, milbemycin treated dogs and HWs from MF+, nontreated dogs were established by surgically implanting the appropriate combinations of males and females into naive recipient dogs.The dog receiving 4 treated female and 15 normal male HWs became MF+ 11 weeks post-op and at necropsy 8.5 months later yielded one fertile and two nonfertile females. The dog receiving 15 treated males and 15 normal females immediately became MF+ and remained so until necropsy. However, all 13 female HWs recovered were nonfertile. The control dog receiving 15

normal male and 4 normal female HWs was also immediately MF+, and all three females recovered were fertile. Unoperated controls with either treated or nontreated HWs remained MF- or MF+ respectively. We conclude that the effect of milbemycin oxime on fecundity in female HWs is due at least partly to suppressed reproductive capacity of the male HWs.

23 MOLECULAR PHYLOGENETIC STUDIES ON FILARIAL PARASITES. Xie H*, Bain O, and Williams SA. Program in Molecular and Cellular Biology, University of Massachusetts at Amherst, MA; Laboratoire de Zoologie-Vers, Museum National d'Histoire Naturelle, Paris, France; and Department of Biological Sciences, Smith College, Northampton, MA.

This project is the first large-scale molecular phylogenetic study on filarial parasites (family Onchocercidae) and includes 16 species of 6 genera: *Brugia malayi, B. pahangi, B. timori, B. patei, B. beaveri, B. buckleyi, Wuchereria bancrofti, W. kalimantani, Mansonella perstans, Loa loa, Onchocerca volvulus, O. ochengi, O. gutturosa, Dirofilaria immitis, Acanthocheilonema viteae* and *Litomosoides sigmodontis*. Two sets of sequence data (5S rDNA spacer and *Hha* I repeat) were collected by PCR, cloning and dideoxy sequencing. The 5S rDNA gene spacer region sequences were aligned and analyzed by various methods to construct phylogenetic trees. Bootstrap analysis was used to test the robustness of the different phylogenetic reconstructions. The various tree-building methods gave very similar results. The study of ss rDNA identified four clades which are strongly supported by bootstrap analysis: the *Brugia* clade; the *Wuchereria* clade; the *Brugia-Wuchereria* clade and the *Onchocerca* clade. The results of this study do not support the classification which places *Loa loa* and *D. immitis* into the Dirofilariinae subfamily. *Hha* I repeat sequences are 322 nucleotides in length, are highly repeated are tandemly arranged, and are unique to the nuclear genomes of the genus *Brugia* . Phylogenetic analyses of the *Hha* I repeat sequence data set identified at least two clades in *Brugia* : the *B. pahangi-B. beaveri* clade and the *B. malayi-B.timori-B. buckleyi* clade. Results from the analyses of both data sets were combined and a hypothesis for the phylogeny of the sixteen species of filarial parasites was proposed.

24 MOLECULAR CLONING AND NUCLEOTIDE SEQUENCING OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT OF *ONCHOCERCA VOLULUS*. Egwang TG* and Ajuh PM. Centre Internationale de Recherches Medicales de Franceville (CIRMF), Franceville, Gabon.

Nematodes have well-developed nervous and muscluar systems and acetylcholine is an excitatory neurotransmitter in these organisms. The acetylcholine receptor (ACHR) may be a suitable target for drug development for the following reasons. Firstly, certain anthelmintic drugs cause spastic contraction of nematode muscle and appear to mediate their action at ACHRs. Secondly, levamisole binds specifically to the ACHR of the free-living nematode *Caenorhabditis elegans* and *C. elegans* mutant strains which are levamisole-resistant lack normal cholinergic receptors. Finally, unexplained pharmacologic differences exist between mammalian and helminth ACHRs. These differences could be exploited for the rational design of filaricides. As a first step towards this objective, we have decided to clone and characterise *O. volvulus* ACHR. Oligonucleotde probes corresponding to highly conserved regions of ACHRs were used to screen an *O. volvulus* λ gt11 cDNA library. One phage clone λ OVR11, was isolated and contained an Eco RI insert of 1700 bp which hybridized to the oligonucleotides on a southern blot. The 1700-bp insert was subcloned into pUC 18 and both strands were sequenced. A truncated open reading frame of 1308 bp capable of encoding 436 amino acids with a calculated Mr of 51,209 was identified. Confirmation that the clone encodes ACHR was provided by homology searches at the DNA (GenBank and EMBL) and amino acid (NBRF-PIR and SWISS_PROT) sequence levels.

25 IDENTIFICATION OF POTENTIAL NUCLEAR HORMONE RECEPTORS IN *DIROFILARIA IMMITIS* AND *CAENORHABDITIS ELEGANS*. Richer JK*, Hough DM, and Maina CV. New England Biolabs, Beverly, MA.

The presence of ecdysone has been demonstrated in *Dirofilaria immitis* and *Caenorhabditis elegans*, as well other helminths. Ecdysone has been implicated in stimulation of the L3 to L4 molt in *D. immitis* and *Ascaris suum* and in the formation of microfilaria in *Brugia pahangi*. We are using the Drosophila ecdysone receptor (EcR), a member of the NHR superfamily, as a means to identify the potential nematode homolog, as well as to identify other hormone receptors. In Southern hybridization experiments, the DNA and hormone binding domains of the Drosophila EcR cDNA hybridize to a small number of *D. immitis* and *C. elegans* DNA fragments at high stringency and a larger number of fragments at lower stringency conditions. Polymerase chain reaction (PCR) experiments using degenerate primers to conserved regions in the DNA binding domain of the NHR superfamily amplified products from both *D. immitis* and *C. elegans* genomic DNA. To date we have identified 3 PCR products from *D. immitis* and 5 from *C. elegans* as new members of the NHR superfamily based on amino acid sequence. One of the PCR products from *D. immitis* shows 95% identity at the amino acid level and 76% identity at the DNA level to ultraspiracle (the Drosophila homolog of human retinoid X receptor), which heterodimerizes with ecdysone receptor to effect activity. We are currently screening libraries to obtain full length cDNA clones and northern and in situ hybridization experiments are underway to determine stage and tissue specificity. It is our hope that this approach will advance our understanding of developmental regulation in important life cycle events, such as molting, in the filarial parasites.

26 CYCLOPHILIN-LIKE PROTEIN OF THE FILARIAL NEMATODE *BRUGIA MALAYI*. Page AP* and Carlow CK. New England BioLabs, 32 Tozer Road, Beverly, MA.

A clone has been isolated from an adult male *Brugia malayi* cDNA library which has extensive similarity to cyclophilin. This clone, termed Bmcyp-1, contains an 1823 base pair (bp) insert with an ORF at bp 57, and is open throughout. This clone encodes a protein of 589 amino acids which has extensive similarities to cyclophilins, in particular the newly described cyclophilin-like proteins. This is the first reported isolation of a cyclophilin gene from a parasitic nematode. Cyclophilin is a ubiquitous protein known to bind avidly to the immunosuppressive agent cyclosporin A (CsA). Recently cyclophilin has been shown to possess peptidyl-prolyl cis-trans isomerase (PPIase) activity, and CsA has been demonstrated to actively inhibit this enzymatic activity. CsA has previously been shown to posses anti-parasitic effects, even against filarial nematodes. The Bmcyp-1 clone has therefore been expressed as an MBP fusion protein and analysis of this recombinant cyclophilin is being undertaken to determine possible functions of this interesting protein with regards to the host-parasite relationship.

27 CHARACTERIZATION OF THE GENE AND CDNA FOR *ONCHOCERCA VOLVULUS* EXTRACELLULAR SUPEROXIDE DISMUTASE. James ER* and McLean DC. Department of Ophthalmology, Medical University of South Carolina, Charleston, SC.

The purpose of this study was identify a cDNA responsible for the secreted extracellular superoxide dismutase (OvEcSOD) activity observed in *in vitro* microfilarial and adult worm culture supernatants and to determine if this cDNA is unique or alternatively spliced from the cytosolic SOD (OvCySOD) gene. An anti-sense primer to a region highly conserved in Cu-Zn SODs used with a spliced leader (SL1) primer in PCR with a cDNA library as template yielded products of 300 and 400 nt. The 300 nt sequence was identical to the OvCySOD 5'. The 400 nt sequence was also characteristic of a Cu-Zn SOD, but was only 67% similar over its 3' region to OvCySOD and had 126 nt 5' which translated as a highly hydrophobic signal peptide. A full length OvEcSOD clone was obtained from a cDNA library screened using the 400 nt product. This OvEcSOD cDNA (accession # L13778) is 628 nt long including

the 5' SL1. The portion 3' to the signal peptide is 68% similar to the OvCySOD cDNA and is 3 nt longer. PCR of a genomic library using internal primers identified 3 putative introns. The first intron of 226 nt begins 79 nt 5' to the start ATG. The second intron occurs >199 nt 5' to the start ATG, and contains an Eco R1 site since flanking primers generate no product with library DNA. The third intron occurs >326 nt 5' to the start ATG. Second and third intron sequencing is in progress. In conclusion, *O. volvulus* contains two distinct SOD genes for OvEcSOD and OvCySOD. The OvEcSOD is postulated to be central to the parasite's defense against host-generated reactive oxygen species, to be evolutionarily relatively recent in origin and is dissimilar to both human and schistosome ECSODs.

28 EFFECTIVENESS OF CGI 18041 AGAINST *BRUGIA PAHANGI* IN BEAGLES WITH INDUCED LYMPHATIC INFECTIONS. Dzimianski MT*, McCall JW, Supakorndej P, and Jun JJ. Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, GA.

Compound CGI 18041, a novel benzothiazole derivative, is undergoing development as a filaricide in a collaborative project between Ciba-Geigy Ltd. and the World Health Organization. In the study reported here, CGI 18041 was evaluated for effectiveness against the macrofilariae and the microfilariae of *Brugia pahangi* in dogs. Twenty-four beagles infected 5 months earlier with 200 infective larvae of *B. pahangi* were selected for use and randomly allocated to 6 groups of 4 dogs each. One group served as a control, while 3 groups were given CGI 18041 as a single oral dose of 100 mg/kg, 50 mg/kg, and 25 mg/kg, respectively, and 2 groups were given an oral daily dose of 50 mg/kg or 25 mg/kg for 2 consecutive days. The dogs were bled for microfilarial counts prior to treatment and on days 1, 2, 7, 14, 28, 42, 56, 85, 112, 145, and 168 after the start of treatment. All of the dogs were necropsied 24 weeks after treatment began. The number of adult *B. pahangi* recovered from the control dogs ranged from 7 to 94 with an average of 47.8 worms. Sixteen of the total of 20 dogs given CGI 18041 were cleared of macrofilariae, but one dog given a single dose of 100 mg/kg and 3 dogs given a single dose of 25 mg/kg had adult worms. In general, microfilariae were rapidly cleared after dosing with CGI 18041, with 12 of 20 treated dogs completely cleared and 4 other treated dogs having a reduction in microfilarial counts of at least 82% within the first 24 hours. These low microfilaremias persisted throughout the trial. The posttreatment microfilarial counts for the remaining 4 treated dogs not cleared of adult worms and the controls were similar to pretreatment levels.

29 MORPHOMETRICS OF THE LYME DISEASE VECTOR, *IXODES SCAPULARIS* SAY, 1821, INCLUDING THE JUNIOR SUBJECTIVE SYNONYM, *I. DAMMINI* SPIELMAN *ET AL*, 1979. Hutcheson HJ*, Oliver JH. Inst. of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

Ixodes scapularis Say, 1821 (Acari: Ixodidae), a member of the *I. ricinus* species complex, is a three-host tick found in the eastern US, and is the principal vector of the Lyme borreliosis and human babesiosis agents, *Borrelia burgdorferi* and *Babesia microti*, respectively. *Ixodes dammini* Spielman et al, 1979, found in the northeastern US, was described based on morphological comparisons with *I. scapularis*. However, *I. dammini* has been recently designated a junior subjective synonym of *I. scapularis*, based on studies of hybridization, assortative mating, host preference, karyotypes, isozymes, DNA, morphometrics, and vector competency of *B. burgdorferi*. Recognizable differences among nymphs and other stages from MA and GA are quantitative rather than qualitative, therefore, the purpose of this study was to examine interpopulational morphological variation in *I. scapularis*. *I. pacificus*, a member of the *I. ricinus* complex that does not produce fertile hybrids with I. scapularis from MA or GA, was included for comparison. Lab-reared offspring were used, to minimize short-term environmental effects. Uni- and multivariate analyses suggest a latitudinal cline. Conventional and size-free (sheared) discriminant analyses included 34, 24, 17 and 25 morphological characters of larvae, nymphs, females and males, respectively. Scatterplots suggest ticks from NC and MO are similar to *I. scapularis* from GA; those from MD and MN appear similar to ticks from MA. F1 laboratory hybrids

(GA females X MA males and MA females X GA males) were intermediate. All groups were distinctly separate from *I. pacificus*.

30 A COMPARISON OF YOLK PROTEINS FROM THE EGGS OF A HARD TICK, *AMBLYOMMA AMERICANUM* AND A SOFT TICK, *ORNITHODOROS PARKERI*. Dudley CK*, James AM, and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

Vitellin, the major egg yolk protein in ticks, was purified from the eggs of the metastriate tick, *Amblyomma americanum* and the argasid tick, *Ornithodoros parkeri*. The composition of each vitellin purified, from *A. americanum* and *O. parkeri* appear to be similar. Vitellins from both species were hemoglycolipoproteins as shown by specific staining of polyacrylamide gels, carbohydrate analyses and lipid analyses. The molecular weight was 360 kDa for *A. americanum* and 600 kDa for *O. parkeri* as shown on PAGE. Under reducing conditions (SDS-PAGE), *A. americanum* had 7 major polypeptides from 245-45 kDa and *O. parkeri* had 6 major polypeptides from 135-66 kDa. The absorption maxima for both vitellins was 280 and 400 nm which is indicative of heme containing proteins. The predominant carbohydrates detected in both proteins were mannose and N-acetylglucosamine as determined by gas chromatography. *A. americanum* had 6.9% carbohydrates and *O. parkeri* had only 13.5% carbohydrates associated with each vitellin. The neutral lipids detected in both proteins were cholesterol, cholesteryl esters, fatty acids, triacylglycerides and diacylglycerides.

31 ABILITY OF THE TICKS *IXODES SCAPULARIS, AMBLYOMMA AMERICANUM* AND *DERMACENTOR VARIABILIS* TO TRANSMIT *BORRELIA BURGDORFERI* FROM FLORIDA. Sanders FH* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, Georgia.

Controversy exists concerning the ability of metastriate ticks to transmit the causative agent of Lyme disease, *Borrelia burgdorferi*. Although the lone star tick, *Amblyomma americanum*, has been implicated in some Lyme disease cases, and *B. burgdorferi* has been detected in the American dog tick, *Dermacentor variabilis*, laboratory studies have shown these tick species are incompetent vectors of *B. burgdorferi* and do not appear to maintain *B. burgdorferi* infections transstadially. In this study the ability of black-legged tick, *Ixodes scapularis, A. americanum*, and *D. variabilis* to maintain and transmit *B. burgdorferi* from Florida is compared. Twelve hamsters were inoculated with an isolate (MI-6) from *Sigmodon hispidus* (Rodentia: Cricetidae) from Merritt Island, Florida, and four hamsters were inoculated with an isolate (SH2-82) from Shelter Island, New York. Larval ticks were allowed to feed to repletion on infected hamsters and molt. Resulting nymphs were fed on naive laboratory mice (*Mus musculus*); these mice were tested for the presence of *B. burgdorferi. I. scapularis* transmitted the MI-6 and SH2-82 isolates. Preliminary results indicate that *A. americanum* and *D. variabilis* do not transmit or maintain either isolste transstadially.

32 CHARACTERIZATION AND LOCALIZATION OF VITELLIN FROM THE LYME DISEASE VECTOR, *IXODES SCAPULARIS* SAY (ACARI: IXODIDAE). James AM* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, Georgia.

In most temperate regions, *Ixodes* species transmit agents causing babesioses and Lyme disease to humans and animals. Therefore, understanding the reproductive events in *Ixodes* species is of prime importance. Vitellin from the eggs of the black-legged tick, *I. scapularis* was characterized using electrophoretic and chromatographic techniques. Immunocytochemistry and organ cultures were used to determine the sites of vitellin production. Characterization of vitellin from *I. scapularis* eggs indicated that its molecular weight was 430 Kd, with subunits from 45-110 Kd. Mannose and n-acetylglucosamine were the predominant carbohydrates associated with this yolk protein. Additionally, mannose is terminally linked to vitellin. The amino acid composition of vitellin was

high in leucine, but low in methionine. Two of six lipids associated with this yolk protein were triacylglycerides and phosphatidycholine. A polyclonal antibody produced to vitellin recognized the native form as well as all seven subunits. The anti-vitellin cross reacts with vitellins from other *Ixodes* species, but does not recognize vitellins from metastriates or argasids. Preliminary results indicate that vitellin is primarily synthesized by the fat body. These results will be discussed in relation to other tick species.

33 FACTORS INFLUENCING THE EIMERIAN COMMUNITY IN A FREE-LIVING POPULATION OF TOWNSEND'S GROUND SQUIRRELS (*SPERMOPHILUS TOWNSENDII*). Wilber PG*, Hanelt B, and Duszynski DW. Department of Biology, The University of New Mexico, Albuquerque, NM.

Over 1,300 fecal samples were collected from Townsend's ground squirrels (*Spermophilus townsendii*) during a mark-recapture study at the Snake River Birds of Prey Area (SRBOPA) in 1992 and 1993. Seven species of coccidia (Apicomplexa: Eimeriidae) were observed. Temporal changes in eimerian prevalence in adult ground squirrels for all 7 eimerians combined showed significant decline (r^2=0.79, P<0.001) during the host's non-torpid period (February-June) in 1992, probably in response to severe drought conditions that year. Drought also resulted in essentially no 1992-1993 overwinter survival of juveniles born in 1992 (<1%) and also reduced adult survival. Since juveniles seem to be important over-hibernation hosts for coccidia, the changed host population age structure in combination with wetter weather and decreased host density on most sites probably contributed to the lower, but more stable prevalence of eimerians in 1993.

34 DEVELOPMENT OF IMMUNITY TO *EIMERIA ARIZONENSIS* BY DEERMICE (*PEROMYSCUS MANICULATUS*): A COMPARISON OF LABORATORY AND FIELD RESULTS. Fuller CA*. Department of Zoology, Oregon State University, Corvallis, OR.

Hosts which develop immunity to a parasite may have an impact on the parasite's population dynamics. However, little is known about the ability of free-living hosts to develop immunity to their parasites under natural field conditions. I found that laboratory reared deermice (*Peromyscus maniculatus*) became immune to *Eimeria arizonensis* after inoculation with > 1000 oocysts. Based on this observation, I tested three predictions of the hypothesis that free-living deermice would also develop immunity to *E. arizonensis*. However, these predictions were falsified. First, deermice observed using mark-recapture techniques over three field seasons became reinfected repeatedly with *E. arizonensis*. Second only 33% of free-living deermice developed immunity to experimental infections. Third, there was no inverse correlation between the age of free-living animals and oocyst output either for observational or experimental infections. The third prediction is based on the assumption that older animals have been exposed to *E. arizonensis* more than younger animals. Thus, a significantly greater proportion of deermice developed immunity to *E. arizonensis* under laboratory than under field conditions. Because laboratory strains of both the host and the parasite originated from the field population, these data suggest that environmental factors in the field may limit an animals ability to develop immunity.

35 TRANSPLACENTAL TRANSMISSION OF *NEOSPORA CANINUM* IN BITCHES INFECTED DURING EARLY PREGNANCY. Cole RA*, Lindsay DS, Dubey JP, Sorjonen DC, and Blagburn BL. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL; AL and ARS, USDA, Beltsville, MD.

Neospora caninum initially described from dogs, infects and may cause disease in various mammals. This protozoan can cause rigid hind limb paralysis in transplacentally infected pups. Herein we present data on experimental infections of six mixed-breed bitches infected subcutaneously with 5 x 10^6 tachyzoites 21 days after insemination (a.i.). Bitch 1 aborted after day 28 a.i., with 1 mummified

pup and 3 partially resorbed pups recovered. Histological, immunohistochemical (IH) and cell culture assays of tissues from bitch and pups were negative for *N. caninum*. Bitch 2 aborted between day 35 and 43 a.i. Six macerated pups were recovered. Cell culture assays of uterine tissues and IH staining of tissues from 1 pup were positive for *N. caninum*. Bitch 3 delivered 3 live pups 65 days a.i. *N. caninum* was isolated from placental tissues. Pups exhibited slight signs of proprioception deficits and hind limb tonicity. Bitch 4 died 38 days a.i. Various tissues from the bitch were positive. No tissues from pups were recoverable. Bitch 5 was euthanized day 54 a.i. No tissues from the bitch were positive; no tissues were recovered from the pups. Bitch 6 was euthanized on day 39 a.i. Tissues from the bitch and 9 of 12 pups were positive. Results indicate *N. caninum* may be transmitted to pups when bitches are inoculated early in pregnancy, and may induce abortion with subsequent resorption of pups.

36 *GIARDIA LAMBLIA* INFECTION IN CHILDREN FROM THE STATE OF TAMAULIPAS, MEXICO: A PRELIMINARY STUDY. Faulkner CT* and Patton S. Department of Environmental Practice, University of Tennessee College of Veterinary Medicine, Knoxville, TN.

As part of a continuing study of the health status of indigent children in Tamaulipas, Mexico, fecal samples were obtained from 17 children attending a free public health clinic and examined for endoparasitic helminth eggs and larvae, intestinal protozoan cysts and oocysts. The age of the children ranged from 3 months to 13 years. Eight families were represented; 12 samples were from males, and 5 were from females. Suspected parasitic infection was the primary clinical complaint for 9 children with some or all of the following symptoms: anorexia, weight loss, fatigue, and visual recognition of worms passed in the stools. *Giardia lamblia* cysts were identified in 7/9 samples, and 2 cases of infection with the parasite were identified by an enzyme immunoassay for *Giardia Stool Antigen 65* (Alexon, Mountain View CA). Helminth eggs and larvae were conspicuously absent. Fecal samples from asymptomatic siblings were also solicited, and 2/6 samples were positive for *G. lamblia* cysts. Fecal samples from the other 4 children were negative for *Giardia* antigen by the EIA. Helminth eggs or larvae were not identified in any of the samples from the asymptomatic children. The distribution of positive cases in the sample population indicated that 6 families had at least 1 infected child. Consumption of the municipal water supply was noted for 3 positive families, and may be a potential source of infection for the rest of the population. This preliminary study underscores the importance of protozoal agents in illness attributed to parasitic infection.

37 THE EFFECTS OF ODONATE INTERMEDIATE HOST ECOLOGY ON THE LEVELS OF LARVAL TREMATODE INFECTION: IN THE WRONG PLACE AT THE WRONG TIME? Wetzel EJ* and Esch GW. Biology Department, Wake Forest University, Winston-Salem, NC.

The variation in life history traits among odonate intermediate hosts of larval trematodes may determine the apparent patterns of parasite infection for these host species. In the present study, naiads of 3 species of odonates, each representing a different life history pattern, were compared for the mean intensity and prevalence of infection by the metacercariae of 2 congeneric trematodes, *Haematoloechus longiplexus* and *H. complexus*. Naiads were sampled from June 1992 - May 1993 from 4 sites within Charlie's Pond, a 2-ha. pond in the Piedmont area of North Carolina. The insects were brought to the lab, isolated in separate jars filled with pond water and subsequently necropsied. Since infection levels did not differ significantly between the 4 locations, results from individual sites were lumped. *Libellula cyanea* (a sprawler) had significantly greater mean intensities of both *H. longiplexus* and *H. longiplexus*, with the greatest intensities in August and July, respectively; *L. cyanea* had the highest prevalences of *H. longiplexus* and *H. complexus*, except in August when prevalence of *H. complexus* was highest in *Ischnura posita* (a climber). Based on the life history characteristics of the 3 odonate species, it is reasoned that *L. cyanea* should have the greatest likelihood of contacting motile cercariae because of its location in the aquatic microhabitat. The results suggest that subtle variations in life history traits are sufficient to influence the potential for

larval odonates to serve as intermediate hosts, and require consideration when evaluating the transmission dynamics of digenetic trematodes.

38 MARKOVIAN CHAIN DYNAMICS OF PARASITE PREVALENCE IN THE KANGAROO RAT, *DIPODOMYS MERRIAMI*. Patrick MJ*. Department of Biology, The University of New Mexico, Albuquerque, NM.

In this study, the preliminary results of a Markovian Chain Analysis of the parasite community structure in the kangaroo rat (*Dipodomys merriami*) is presented. A total of 175 kangaroo rats have been captured 600 times, marked, and released within the Sevilleta National Wildlife Refuge since July 1992. The recapture success was high. In 9 trapping events 20 animals were caught 9 times; 2 were caught 8 times; 11 were caught 7 times; 16 were caught 6 times; 5 were caught 5 times; 7 were caught 4 times; 23 were caught 3 times; 22 were caught 2 times and 65 were caught one time. Fecal samples have been collected and analyzed by sugar floatation/sedimentation techniques, and fifteen different species of parasite have been identified. Five species of nematode: *Heteromoxyuris deserti, Mastophorus dipodomis, Pterygodermatites dipodomis, Trichuris dipodomis, Physaloptera* sp.; eight species of coccidia: *Eimeria arizonensis, E. balphae, E. chihuahuaensis, E. chobotari, E. dipodomysis, E. merriami, E. mohavensis, E. scholtysecki*; and two species of tapeworm: *Schizorchis dipodomis* and an unidentified species. The most common species were the nematodes *M. dipodomis*, and *P. dipodomis*; and the coccidian *E. chobotari*.

39 THE STRENGTH OF SPATIAL AND TEMPORAL HETEROGENEITY AS STRUCTURING FORCES OF THE PARASITE COMMUNITIES IN *HELISOMA ANCEPS* AND *PHYSA GYRINA*. Sapp KK* and Esch GW. Biology Department, Wake Forest University, Winston-Salem, NC.

A total of 1231 *Physa gyrina* and 1532 *Helisoma anceps* was collected over a 12 month period from Charlie's Pond in southern Stokes county, North Carolina. Similarity in the infra- and component communities in *Helisoma anceps* and *Physa gyrina*, combined with differences in the snail's life histories, provided an opportunity to compare the effects of various life history traits on the acquisition of larval trematodes in a number of different microhabitats. In order to assess the effects of microhabitat partitioning on the infection status of the two snail species, bi-monthly collections were made. Site location, water depth, snail depth at capture, distance at capture, distance from shore, type of substrata, infection status, and host size were recorded for each snail. Multivariate statistics were used to determine which variables had the greatest impact on determining a snail's infection status. Data analysis indicates that certain of the microhabitat variables are better predictors of a snail's infection status than are others. Manipulation of infracommunity structure, through the exclusion of parasites, allowed the examination of the influence of temporal heterogeneity, as compared to trematode antagonism, as a force in structuring the infra- and component communities. Snails were maintained in cages in the field. Enclosures were positioned a few centimeters above the substrata to prevent infection of the snail's via egg ingestion, effectively preventing the more prevalent parasite species from infecting the snails. Statistical analyses indicate that preventing snails from ingesting eggs of some parasites had no effect on the recruitment of other parasites into the snail.

40 SEASONAL RECRUITMENT AND MATURATION OF *BOTHRIOCEPHALUS ACHEILOGNATHI* IN LOUISIANA MOSQUITOFISH *GAMBUSIA AFFINIS*. Mars CL* and Font WF. Department. of Biological Sciences, Southeastern Louisiana University, Hammond, LA.

Population dynamics of the introduced Asian fish tapeworm *Bothriocephalus acheilognathi* Yamaguti, 1934 was studied in the mosquitofish *Gambusia affinis* from a man-made pond in Hammond, LA. Tapeworms were assigned to one of five developmental stages: stage I (plerocercoid

without bothria), II (plerocercoid with bothria), III (immature strobila), IV (mature), and V (gravid). Seasonal dynamics were monitored from May 1992 through April 1993 in the largest fish (males > 39mm; females > 59mm), medium females (40-49 mm), and the new cohort (< 30mm). Larger fish harbored significantly more worms than smaller fish. Abundance showed a seasonal pattern of bimodal peaks in spring and autumn, associated with periods of recruitment (stage I plerocercoids). Spring recruitment was followed by rapid maturation of worms (Stage IV and V) whose large size limited intestinal space available to smaller worms and contributed to the decline in abundance observed during summer. Autumn recruitment, in contrast, was not followed by worm maturation. Abundance declined in the winter. All fish sex and size classes showed similar seasonal patterns except the new cohort. These young of the year fish were born in June, too late for spring recruitment and acquired their first infections in autumn simultaneously with reinfection of adult fish.

41 PYLORIC CECA VS. ANTERIOR INTESTINE AS SUITABLE HABITAT FOR *LEPTORHYNCHOIDES THECATUS* (ACANTHOCEPHALA) IN GREEN SUNFISH (*LEPOMIS CYANELLUS*). Richardson DJ*. School of Biological Sciences, University of Nebraska, Lincoln, Nebraska.

Preferential site selection of helminths within the alimentary canal of their vertebrate definitive hosts is a well documented phenomenon. *Leptorhynchoides thecatus* and green sunfish offer an excellent system in which to investigate the mechanisms underlying such preferential site selection. Many juvenile *L. thecatus* initially attach in the anterior 1/4 of the intestine; however, by 2 weeks post-infection, all worms are restricted to the 7 pyloric ceca and between cecal region. Evidence indicates that worms must establish in the cecal region to survive and that remaining intestinal worms are passed from the host. Implicit in this observation is the conclusion that the intestine is unsuitable for long-term survival. Characterization and comparison of physiological aspects of the pyloric cecal region and anterior intestine of *L. cyanellus* are being undertaken to gain insight into factors influencing the preferential site selection exhibited by *L. thecatus*. It has been suggested that the ceca provide a sheltered region for helminths or that they serve as a "nutrient sequestering region." Results show that although a substantial amount of food enters the ceca, no food materials remain in the ceca subsequent to gastric evacuation. It appears then, that the microhabitat specificity exhibited by *L. thecatus* must be a result of differences in physiological or biochemical attributes of the two regions. Current examinations encompass the potential roles of concentrations and total amounts of carbohydrates, proteins, and bile salts, and of enzymatic activity and other physiological parameters as they pertain to the suitability of the ceca and anterior intestine as habitat for *L. thecatus*.

42 THE USE OF A GEOGRAPHIC INFORMATION SYSTEM (GIS) TO ANALYZE THE DISTRIBUTION OF THE HUMAN SCHISTOSOME-TRANSMITTING SNAILS IN KENYA. Boyce TG*, Rizor DD, and Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM; and University of New Mexico Technology Application Center, Albuquerque, NM.

To better understand the geographical distribution of the two primary snail taxa involved in transmitting human schistosomes in Kenya, *Biomphalaria pfeifferi* and the *Bulinus (=Physopsis africanus* group, a Geographic Information System (GIS) was applied to a recent survey of 218 freshwater sites in the southern half of the country know to contain snails. To evaluate the environmental parameters affecting snail distributions, a study area of 251,000 km^2 that enclosed all 218 sample sites was defined. In addition to snail data, elevation, rainfall and temperature data for Kenya were incorporated in the GIS and overlaid either singly or in combination with the sites occupied by each snail taxon. The primary goal of the study was to identify the environmental parameter(s) and the specific ranges within those parameters that encompassed 100% of the snail data points under the smallest area. Preliminary results for *Biomphalaria pfeifferi* indicated that areas delimited by particular elevation ranges most efficiently encompassed sites inhabited by this snail species. For example, the area obtained that encompassed all *B. pfeiffei* sites when using elevation

alone comprised 71% of the total study area, while rainfall and the combination of temperature and rainfall comprised 99.9 and 97% of the study area, respectively. Results for the *Bulinus (=Physopsis africanus)* group are still under evaluation.

43 TRANSMISSION OF *LEPTORHYNCHOIDES THECATUS* (ACANTHOCEPHALA) THROUGH BASS AND GREEN SUNFISH POPULATIONS. Olson PD*. School of Biological Sciences, University of Nebraska.

The ability of a generalist parasite to maintain its suprapopulation within its host community often relies on the differential utilization of host species. Relative flow of the acanthocephalan *Leptorhynchoides thecatus* through fishes in a natural community suggests that largemouth bass (*Micropterus salmoides*) are not capable of supporting infrapopulations of the parasite in the absence of green sunfish (*Lepomis cyanellus*). To test this hypothesis, 6 populations each of largemouth bass and green sunfish were held in 5,700-L mesocosms and allowed to feed naturally on *Hyallela azteca* (Amphipoda) experimentally infected with *L. thecatus* cystacanths. Recruitment, maturation, and transmission of the parasite were monitored by taking a census of the populations at 3-wk intervals. Success of mean recruitment was near or above estimated exposure for both host species, and by week 11, gravid worms were observed. By week 17, members of both host species harbored 2nd generation worms. Overall mean comparisons of prevalence, intensity, and abundance at the end of 17 wk were highly similar between host species, suggesting that differences observed in nature are indicative of the host community ecology, rather than intrinsic differences in the host/parasite associations.

44 HOST-SPECIFICITY AMONG SPECIES OF *HAEMATOLOECHUS*. Snyder SD*. School of Biological Sciences, University of Nebraska, Lincoln, NE.

Experimental infections in the laboratory indicate differences in host-specificity among two species of frog lung flukes that occur sympatrically in a pond in Keith County, Nebraska. *Haematoloechus complexus* and *Haematoloechus medioplexus* utilize different species of snails as first intermediate hosts. At the level of the second intermediate host, *H. medioplexus* has been found only in anisopteran odonates whereas a variety of odonate and non-odonate arthropods have been infected with metacercariae of *H. complexus*. These arthropods include amphipods, isopods, chironomid larvae, and ephemeropteran larvae. The disparity in second intermediate host utilization between these two fluke species indicates that anurans may become infected with *H. complexus* by ingesting a much wider range of infected invertebrates than previously believed. Independent laboratory infections of *H. complexus* and *H. medioplexus* have been maintained in the anuran definitive hosts *Rana pipiens* and *Bufo woodhousei*. Both fluke species remain gravid for a longer period of time in *R. pipiens* than in *Bufo woodhousei*. These data indicate that the *Haematoloechus* system provides a useful model for the experimental exploration of general concepts relating to host-specificity and the evolution of complex life cycles.

45 RETINOIDS IN NEMATODE DEVELOPMENT. Wolff KM*, Scott AL. Immunology and Infectious Diseases, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

Retinoids appear to play striking and diverse roles in a wide range of biological systems including embryonal development, cellular differentiation, and growth. A handful of studies suggest an interaction between retinoids and parasitic nematodes, and the vitamin A (VA) status of hosts does appear to be a factor in the development and growth of certain parasites. This suggests that VA is a nutritional requirement of proper differentiation and growth of the parasites. Steady-state levels of retinoids in adults of the filarial nematode *Brugia malayi* were determined by HPLC analysis of whole worm extracts. Retinol was identified in the worm extracts and also found to be released from the worms into the media. To determine if exogenous retinoic acid (RA) is taken up by the worms, adult

make and female *B. malayi* were incubated in 3H-RA. Extraction results indicated that the label is not simply adhering to the outer worm surface but is being taken up in a time-and temperature-dependent fashion. Tissue sections of worms which had been incubated in 3H-RA indicated a specific uptake of RA. The label was dispersed throughout all cells, but not in non-cellular structures. There appeared to be a higher density of label associated with embryos, a focus of high cellular differentiation and development. Understanding the role of retinoids in nematode growth and reproduction will not only increase the knowledge of the biology of the parasite, but may also offer new intervention strategies.

46 SEQUENCE VARIATION IN THE CS GENE OF *PLASMODIUM VIVAX*. Mann VH*, Huang TY, Cheng Q, Bustos D, Huang YM, and Saul A. Malaria and Arbovirus Unit, Queensland Institute of Medical Research, Brisbane, Qld. Australia; Guizhou Provincial Institute of Parasitic Diseases, Guiyang, Guizhou, P. R. China; and Research Institute for Tropical Medicine, Metro Minila, Philippines; and Guang Xi Institute of Parasitic Diseases Control, Nanning, Guang Xi, P. R. China.

There is noted sequence variation in the circumsporozoite gene from *Plasmodium vivax*. The natural polymorphisms in the target determinants of the CS protein may compromise the efficacy of a vaccine which is based on this protein. Using direct sequencing of PCR amplified material we have determined the sequences of the flanking regions and the central repeat domain of the CS gene from 18 isolates of *P. vivax* from the Philippines, China, the Solomon Islands and Papua New Guinea. All isolates had the type I (GDRADGQPA) repeat. All Chinese and one Philippino isolate contained 2 to 7 copies of an additional 4 amino acid repeat (GGNA) in the region 3' to the central repeats. In the 5' non-repetitive region, a single polymorphism was detected giving an N38 to G38 substitution. Immediately 3' to the repeats, sequences differed by the inclusion or deletion of blocks of sequence. No polymorphisms were found in other non-repetitive regions. Two important findings have come from this study. First, the additional 4 amino acid repeat seen in the Chinese samples, and in a previously described North Korean isolate, appears to be a regional marker, and may distinguish the temperate, long incubation form of *P. vivax* from tropical strains. Second, there appears to be little variation in non- repetitive regions. This contrasts with the finding from a previous report which analysed M13 clones of PCR amplified genes, which are known to have a high error rate. This lack of variation augurs well for a vaccine based on the *P. vivax* CS protein.

47 IMMNOCLONING OF A LIVER STAGE ANTIGEN OF *PLASMODIUM VIVAX* CROSS-REACTING WITH ANTI-*PLASMODIUM CYNOMOLGI* LIVER STAGE ANTIBODIES. Yang C*, Nelson C, Collins WE, Pieniazek NJ, David PH, and Millet P. Department of Pathology, Emory University School of Medicine, Atlanta, GA; Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Department of Immunology, Institut Pasteur, Paris, France.

Studies on rodent malarias and *Plasmodium falciparum* have indicated that liver stage antigens are involved in protective immunity. A rhesus monkey immunized with *in vitro* cultured liver stages of *P. cynomolgi* provided a unique opportunity to characterize immunogenic liver stage proteins of *P. vivax*. Previous experiments showed cross-reactivities between liver stages of *P. vivax* and *P. cynomolgi*. Sera from the monkey were used for screening a genomic library of *P. vivax*. Forty-eight positive clones were isolated from immunoscreenings. DNA sequencing of one clone showed that it contained a 156bp fragment (G + C content of 51.3%). Database similarity search revealed that this fragment had little homology to other known DNA sequences. Deduced amino acid sequence indicated that this 52-amino acid Polypeptide did not contain repeat sequences as seen in many plasmodial amino acid sequences. Immunologic analysis using synthetic polypeptides in FAST ELISA and fusion protein in Western blot showed that naturally infected human sera reacted with the polypeptides and fusion protein. In addition, anti-*P. cynomolgi* liver stage and anti-*P. vivax* blood-stage antibodies also recognized the synthetic polypeptides and fusion protein.

48 BINDING DOMAINS OF THE DUFFY ANTIGEN BINDING PROTEINS OF *PLASMODIUM VIVAX* AND *P. KNOWLESI*. Chitnis CC* and Miller LH. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; National Institutes of Health, Bethesda, MD.

The invasion of erythrocytes by malarial merozoites involves specific interactions between receptors on the erythrocyte surface and parasite ligands. The human malaria *Plasmodium vivax* and the related primate malaria *P. knowlesi* can invade Duffy positive human erythrocytes, that is, erythrocytes that carry the Duffy blood group antigen. Duffy negative human erythrocytes are refractiry to invasion by these parasites. Parasite ligands that specifically bind Duffy positive human erythrocytes have been identified from *P. vivax* and *P. knowlesi* and the genes for these Duffy antigen binding proteins have been cloned. We have determined the binding domains of these Duffy antigen binding proteins. We developed a mammalian cell expression system to express different regions of these proteins on the surface of monkey kidney (COS) cells. The transfected COS cells were tested for their ability to bind erythrocytes. We have identified the 5'-cysteine rich regions of the Duffy antigen binding proteins of P. vivax and P. knowlesi as the binding domains. COS cells expressing these regions on their surface specifically bind Duffy positive human erythrocytes. Identification of the binding domain of the *P. vivax* Duffy antigen binding protein should allow the development of an effective vaccine that blocks an important receptor-ligand interaction and prevents erythrocyte invasion by *P. vivax*.

49 THE MICRONEME PROTEIN-1 (DUFFY BINDING PROTEIN) OF *PLASMODIUM VIVAX* IS POLYMORPHIC IN CLINICAL ISOLATES FROM PAPUA NEW GUINEA. Tsuboi T, AL-Yaman F, Alpers MP, and Adams JH*. Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana; Papua New Guinea Insitute of Medical Research, Madang, Papua New Guinea; and Papua New Guinea Insitute of Medical Research, Goroka, Papua New Guinea.

Malaria merozoites require the presence of specific surface receptors to invade erythrocytes. The Microneme Protein-1 (MP-1; =Duffy Binding Protein) of the *Plasmodium vivax* merozoite interacts with the Duffy blood group antigens of human erythrocytes during invasion. Because of this essential biological function, the MP-1 is a malaria vaccine candidate. Extensive polymorphisms and recombination between gene types has been identified in the related gene family of *P. knowlesi*. Because polymorphisms in the *P. vivax* MP-1 may compromise its efficacy as a vaccine, we have characterized the MP-1 gene of *P. vivax* clinical isolates from Papua New Guinea (PNG) to determine the extent of polymorphism. Oligonucleotide primers were synthesized based on sequence from conserved regions of a *P. vivax* laboratory isolate (Salvador I) and were used to PCR-amplify the erythrocyte binding domain of MP-1 genes from 26 PNG isolates. Restriction fragment-length polymorphism analysis of the PCR-amplified MP-1 genes identified several different gene types. Nucleotide sequence of these clinical isolates identified two major gene types present in PNG. Our data for the PNG clinical isolates are similar to the types of polymorphisms identified in *P. knowlesi*.

50 BINDING DOMAIN OF EBA-175, A *PLASMODIUM FALCIPARUM* LIGAND FOR INVASION INTO ERYTHROCYTES. Sim KL*, Chitnis C, and Miller LH. Laboratory of Malaria Research, National Institute of Allegy & Infectious Diseases, National Institutes of Health, Bethesda, MD.

Plasmodium sp. merozoites released from rupturing erythrocytes must rapidly invade other erythrocytes to survive. Invasion of malaria merozoites into erythrocytes is a multi-step process involving parasite ligands recognizing and interacting with specific receptors on the erythrocyte surface. The 175 kD sialic acid binding protein of *P. falciparum* (EBA-175) has been thought to be a parasite invasion ligand because it binds to erythrocytes in a fashion that correlates with the ability of the erythrocyte to be invaded by merozoites. To identify the domains of EBA-175 that bind to erythrocytes, we transfected defined regions of EBA-175 into monkey COS cells using a vector that

allowed for the expression of EBA-175 fused to a portion of the herpes simplex glycoprotein D. We report the specific binding of human erythroctyes to COS cells expressing a 66 amino acid residue portion of EBA-175 (region II). This region of EBA-175 contains 27 cysteine residues and several aromatic residues which are conserved, spaced with regions which are non-homologous among *P. falciparum, P. vivax* and *P. knowlesi*. The binding pattern of region II on the surface of Cos cells to erythrocytes is identical to the binding pattern of authentic EBA-175 to erythrocytes in suspension; like authentic EBA-175, the binding of region II to erythrocytes is neuraminidase and trypsin sensitive, and is dependent on glycophorin A. This study defines the domain of EBA-175 responsible for binding to glycophorin A on erythrocytes, and identifies region II as the target for a malaria vaccine.

51 THE LARGEST SUBUNITS OF *PLASMODIUM FALCIPARUM* NUCLEAR RNA POLYMERASE HAVE UNIQUE FEATURES. Bzik DJ* and Fox BA. Department of Microbiology, Dartmouth Medical School, Hanover, NH.

Nuclear RNA polymerases (RNAP) are responsible for the synthesis of mRNA, tRNA, rRNA and other RNA species. Since little information is available on the *Plasmodium* nuclear transcription apparatus and it's regulation we are interested in determining whether components of the parasite transcription apparatus are significantly different from that of other eukaryotes. We characterized the largest subunits of *Plasmodium* nuclear RNA polymerase I, II, and III and compared them to the analogous protein(s) from other eukaryotes. We found significant differences in these three *Plasmodium* proteins. Our findings suggest that these proteins, or other parts of the transcription apparatus, might serve as targets for antimalarial drug development. Each *Plasmodium* subunit was much larger than any other eukaryotic subunit due to the presence of large amino acid inserions which were found only between conserved domains of the subunits. Insertions in RNAPI were in domains A', D', DE2', and F', while RNAPII and RNAPIII insertions were in domains DE1', E', F', and C', E, and F', respectively. Each subunit had a large insertion within domain F'. The serine-rich heptapeptide repeat in the C-terminal domain (CTD) of *Plasmodium* RNAPII was different from other CTD's. Domain E' of RNAPIII contained a 10 amino acid serine-rich repeat unique to *Plasmodium*. Domain F' of RNAPI contained a 6 amino acid serine-rich repeat unique to *Plasmodium*. The serine-rich repeats of *Plasmodium* RNAPI, II, and III shared a Ser-X-X-X-Ser motif. We speculate that the serine-rich repeat domains are sites for phosphorylation, or dephosphorylation, events that regulate RNA polymerase activity in *Plasmodium*.

52 ADENINE NUCLEOTIDE TRANSLOCATOR OF *PLASMODIUM FALCIPARUM*. Dyer M *, Wong H, Huynh P, Jackson M, and Mikkelsen RB. Departments of Radiation Oncology and Microbiology/Immunology, Medical College of Virginia, Richmond, VA.

Adenine nucleotide transport has been demonstrated to occur between *Plasmodium falciparum* and the host erythrocyte via a translocator with biochemical properties similar to a transporter which carries out this function in the inner membrane of mitochondria. Using two primers based on conserved sequences between mitochondrial ADP/ATP translocators, a single fragment of the predicted size was amplified from *P. falciparum* genomic DNA by PCR. The 200 base pair fragment was cloned, sequenced and found to contain a single open reading frame throughout. The translated sequence is highly homologous to the corresponding region of other ADP/ATP translocators. This DNA fragment was used as a probe to screen a λ gt11 cDNA library. A single hybridising clone was isolated and analysed. This clone contains an open reading frame of 903 bp which represents a full length cDNA encoding an adenine nucleotide translocator of *P. falciparum* This sequence translates into a peptide with a predicted MWt of 33.6 kDa. The translocator RNA was detected by Northern blot analyses in RNA prepared from early and late erythrocytic stages of the parasite. Its synthesis during gametocytogenesis is currently under investigation and its cellular location is being determined.

53 SEQUENCE ANALYSIS AND PHYLOGENETIC MAPPING OF A *PLASMODIUM VIVAX* PROTEIN KINASE. Dimayuga FO and Levitt A. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY.

The protein kinases are a large and well-conserved family ofproteins that mediate cellular responses to environmental stimuli. We have isolated a gene called PvPk-1 from the human malaria parasite*Plasmodium vivax* whose predicted amino acid sequence shares homology with protein kinases from higher organisms. The downstream portion ofPvPk-1 includes all eleven of the well-conserved regions found in the catalytic domains of protein kinases, including an ATP-binding site and a peptide binding region characteristic of serine/threonine kinases. Unusually, the catalytic domains also include a glycine/serine-rich repetitive sequence that could play a role in substrate binding or in establishing the enzyme's subcellular location. In addition, theupstream portion of PvPk-1 includes sequence elements that resemble the negative regulatory sites found in the non-catalytic regions of other protein kinases. We are performing a phylogenetic analysis of the PvPk-1 enzyme in relation to the protein kinases of vertebrate and invertebrate animals, plants, and other protozoan parasites. By grouping PvPk-1 with protein kinases of similar function, it will be possible to make predictions concerning the activity and regulation of PvPk-1 that can be tested biochemically using recombinant PvPk-1 enzyme.

54 IDENTIFICATION OF A NOVEL *PLASMODIUM FALCIPARUM* SEXUAL STAGE PROTEIN WITH HOMOLOGY TO Pgs28. Duffy PE and Kaslow DC. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD; and Department of Immunology, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

We previously described Pgs28, a 28 kD surface protein of *Plasmodium gallinaceum* ookinetes, to be a target of potent transmission-blocking antibodies. Using degenerate oligonucleotides based on the nucleotide sequences of Pgs28 and other EGF-like sexual stage antigens (Pfs25, Pgs25, and Pbs21), we PCR-amplified from *P. falciparum* genomic DNA a unique 300 bp fragment encoding the prototypical EGF-like motif. Probes based on this sequence were used to screen a *P. falciparum* genomic library and identified a clone (p6-1) with a 1.7 kB insert containing a 654 bp open reading frame. The deduced amino acid sequence predicts a protein (Pfs28) containing a signal sequence, 4 EGF-like domains, and a terminal hydrophobic region without a cytoplasmic tail. The spacing of cysteines within the EGF motifs indicates that there are at least 2 subfamilies of EGF-like sexual stage proteins: Pfs28 belongs to a group with Pgs28 and Pbs21, while Pfs25 and Pgs25 are in another distinct group. By PCR studies, Pfs28 is present in *P. falciparum* late stage gametocyte cDNA. We are currently studying the ability of recombinant forms of Pfs28 to elicit transmission-blocking antibodies.

55 CLUSTERED AMINO ACID VARIATION IN PFS230 FROM DIFFERENT ISOLATES OF *PLASMODIUM FALCIPARUM*. Williamson KA* and Kaslow DC. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; and National Institutes of Health, Bethesda, MD.

Pfs230 is a prime *Plasmodium falciparum* transmission-blocking target antigen in part because the presence of antibodies to Pfs230 correlate with transmission blocking activity in human sera from a malaria-endemic region. The gene for Pfs230 has been sequenced, as reported previously, and our effort is now directed toward producing a recombinant peptide that induces transmission blocking immunity. Regions of increased amino acid variation may signify immunological pressure and thus be B- or T-cell epitopes; therefore, regions of variation between different strains of *P. falciparum* where identified. There are 25 nucleotide differences out of a total of 9417 bp (14 bp 5' UTR and 9403 bp ORF) between 3D7 and 7G8 strains of *P. falciparum*. Only 3 bp changes are silent; the others change 19 aa. 13 of the nucleotide changes are clustered in two regions (A & B). Region A (87 bp) encodes the

4-amino acid repeat area and region B (285 bp) includes a 7-cysteine motif. The rest of the changes are scattered throughout the gene. Region A has 6 bp changes altering 4 aa, while region B has 7 bp changes altering 7 aa. One of the changes in region B ablates a restriction site in 7G8. Regions A and B were PCR amplified from five different *P. falciparum* isolates. In region A LF4, CAMP, and 3AD5 were all distinct and differed from 3D7 and 7G8. In region B LF4, Hb3, and 3AD5 all contained the restriction site seen in 3D7 but missing in 7G8. Direct sequencing of a PCR product containing region B from LF4 revealed that 4 of 7 variant bp were the same as 3D7 and the others were the same as 7G8. Currently, vectors are being constructed for expression of regions A and B in *E. coli* and yeast to determine whether they contain B- and/or T-cell epitopes, and more importantly, to test whether they induce transmission blocking immunity. To insure that important sites are not missed full length Pfs230 will also be expressed in the vaccinia virus system and tested for transmission blocking activity.

56 TRANSFECTION OF *PLASMODIUM GALLINACEUM* ZYGOTES AND EXPRESSION OF FIREFLY LUCIFERASE. Goonewardene R, Daily J*, Kaslow D, Sullivan TJ, Duffy P, Carter R, Mendis K, and Wirth D. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Malaria Research Unit, Department of Parasitology, University of Colombo, Colombo, Sri Lanka; National Institutes of Health, Malaria Section, Bethesda, Maryland; and Institute of Animal Genetics, Edinburgh, Scotland.

Malaria continues to be a major cause of morbidity and mortality in many tropical countries. The ability to analyze the function of important genes in the pathogenesis of malaria has been limited by the lack of a DNA transfection system. Here we report the successful development and expression of a transient transfection system. A chimeric gene was constructed in which the firefly luciferase gene was inserted in frame into the coding region of the pgs28 gene of *Plasmodium gallinaceum*. This gene codes for a surface protein known to be expressed during the ookinete stage of development. The plasmid DNA (pgs28.1-luc) was introduced into *P. gallinaceum* gametes and fertilized zygotes by electroporation and assays for luciferase activity were performed twenty four and forty eight hours later. Lysates of parasites electroporated in the presence of the pgs28.1-luc had significantly higher luciferase activity than the lysates of parasites electroporated in the presence of control vectors. This successful introduction and expression of a foreign gene in the malaria parasite demonstrate the feasibility of this approach to developing methods for functional analysis of genes responsible for drug resistance in malaria.

57 KINETICS OF MATERNAL ANTIBODIES TO MSP-1 OF *PLASMODIUM FALCIPARUM* IN INFANTS BORN IN A MALARIA ENDEMIC AREAS OF PAPUA NEW GUINEA. Kramer K, Sehgak V, Alpers M, Hui G, and Chang S.

The kinetics of passively transferred maternal antibody to the precursor to the major merozoite surface coat protein (MSP-1) of *Plasmodium falciparum*, a blood stage vaccine antigen candidate, was studied in infants born in villages outside of Madang, Papua New Guinea. Fifty infants were followed between August 1985 and July 1986 in a longitudinal study. At birth, maternal and cord blood were collected for serum and peripheral blood smears were made for the detection of malaria parasites. Blood smears and serum samples were collected from the infants during monthly follow-up visits. Anti-MSP-1 antibodies were detected by enzyme-linked immunosorbent assay using FUP parasite-derived MSP-1. The difference in mean MSP-1 titer values between maternal and cord blood was not statistically significant. The median age at which infants lost detectable maternal anti-MSP-1 antibodies was 20 weeks. The median age of infants who became infected with malaria was 17 weeks. The majority of these infants (68%) were infected with *P. falciparum*. These results parallel a previous study using the same sera and measuring antibodies against whole parasite antigens. It appears that the loss of malaria specific antibody including the loss of MSP-1-specific maternal antibody is associated with increased risk of infection in infants less than 3 months old.

58 ACQUISITION OF *PLASMODIUM FALCIPARUM* INFECTION AND DEVELOPMENT OF ANEMIA IN INFANTS IN WESTERN KENYA. Bloland PB*, Ruebush TK, Boriga DA, Nahlen BL, Oloo AJ, and McCormick JB. Malaria Branch, Division of Parasitic Disease, NCID, Centers for Disease Control, Atlanta, GA; and Vector Biology and Control Research Center, Kenya Medical Research Institute, Kisumu, Kenya.

Plasmodium falciparum is a major cause of morbidity and mortality among young children in western Kenya. To provide insight into the dynamics of the acquisition of *P. falciparum* malaria and its relationship to clinical disease and development of anemia, infants born in 15 villages in western Kenya were monitored every 2 weeks after birth. Clinical histories and axillary temperatures were obtained at each visit; blood smears for malaria and a capillary blood sample for measurement of hemoglobin concentration (Hb) were obtained once each month or any time the child was found to have a temperature ≥37.5°C. As of May 1, 1993, 227 (63.4%) of the 358 newborns enrolled in the study had acquired *P. falciparum* malaria. The mean (standard deviation) age at first positive blood smear was 84 (49.8) days, but this varied considerably with season of birth. Although the mean Hb of newborns was 19.0 (2.8) g/dl, the mean age when Hb was first recorded to be ≤11.0 g/dl or ≤8.0 g/dl was only 72 (39.5) and 123 (56.3) days, respectively. Forty- three percent of first infections were associated with fever (temperature >37.5°C), and parasitemia was associated with lower Hb ($p<0.001$) as early as the second month of life. Malaria parasitemia and anemia begin to manifest themselves very early in life in western Kenya. For interventions aimed at reducing the impact of these conditions to have maximal effect, they must include children in early infancy.

59 EVALUATION OF ILLNESS INDICATORS IN A PEDIATRIC POPULATION-KENYA, 1993. Paxton LA*, Zucker JR, Steketee RW, Nahlen BL, Olongo C, Oloo A, and Campbell CC. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta, Georgia; and Kenya Medical Research Institute; and Siaya District Hospital, Kenya.

Childhood mortality is extremely high in Africa. Rapid identification of young children at highest risk of death may allow health workers to optimize treatment and prevent mortality. We evaluated children ages 2 to 60 months seen in the pediatric out- patient (OPD) and inpatient departments (IPD) in a rural district hospital in Kenya to identify signs and symptoms associated with increased risk of death. Children presenting with severe dehydration, abnormal mental status, severe malnutrition, respiratory distress, or severe anemia were classified as having severe illness. Usual clinical care was provided and in-hospital survival was recorded. Of 320 inpatients (47% female, mean age 16 months), 17 died and 70 (22%) had severe illness. Of the 17 who died, only 10 had severe illness. Risk of death was higher for children with severe illness (10/70, 14%) than for those without severe illness (7/250, 3%) (RR=5.10, $p<.01$). Respiratory distress (7/17, 41%) and severe anemia (6/17, 35%) were most often associated with death. Of 1,280 ill children seen in the OPD, fewer than 5% had severe illness. Children meeting our definition of severe illness were at high risk of death; although few children with those symptoms present to the OPD, they should be targeted for immediate intervention.

60 COST-EFFECTIVENESS OF ANTIMALARIAL REGIMENS DELIVERED THROUGH ANTENATAL CLINICS TO PREVENTT MALARIA DURING PREGNANCY. Schultz LJ*, Steketee RW, Wirima JJ, Macheso A, and Chitsulo L. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta, GA; and Malawi Ministry of Health, Malawi.

Antenatal clinics(ANC) provide an opportunity to deliver interventions to promote maternal and infant health. In areas with hyperendemic *Plasmodium falciparum* malaria, infection during pregnancy contributes to low birth weight (LBW), the greatest risk factor for neonatal mortality. To

aid in policy decisions and promote wise use of resources, we examined the cost-effectiveness of antimalarials delivered as part of an antenatal care package in Malawi. A decision-analysis model was constructed to predict the number of LBW cases prevented in a setting of high prevalence of chloroquine (CQ)-resistant parasites, factoring in drug efficacy, number of ANC visits, compliance, prevalence of placental malaria, and LBW incidence. Regimens examined were 1) sulfadoxine-pyrimethamine (SP) treatment once during second trimester and repeated at the onset of third trimester (SP/SP); 2) SP treatment followed by weekly CQ prophylaxis (SP/CQ); and 3) CQ treatment followed by CQ prophylaxis (CQ/CQ). Using local costs of antimalarials, for 10,000 women attending ANC, SP/SP would prevent 212 LBW cases and cost $9.60 per LBW case prevented; SP/CQ would prevent 63 LBW cases at $59/case prevented; and CQ/CQ would prevent 20 LBW cases at $169/case prevented. In areas endemic for CQ- resistant *P. falciparum*, SP/SP is a cost-effective intervention to reduce LBW, which should be included in the antenatal care package.

61 PRESUMPTIVE VS DIRECTED TREATMENT OF UNCOMPLICATED MALARIA IN ADULT MALAWIANS: RELATIVE COSTS. Jonkman A, Chibwe RA, Khoromana CO, Liabunya UL, Chaponda ME, Kandiero SE, and Taylor TE*. Queen Elizabeth Central Hospital, Blantyre, Malawi; and College of Osteopathic Medicine, Michigan State University, East Lansing, MI.

In many malaria endemic areas, presumptive treatment of uncomplicated febrile illnesses with antimalarial drugs is the recommended policy, but results in over-administration of drugs. Treating only microscopically confirmed cases can decrease drug use but is assumed to be too costly for first-line use. We compared these two approaches in adult Malawian patients attending an urban outpatient clinic. Three one-week study phases were conducted in successive months during the peak malaria season. During Phase I, the number of prescriptions (Rxs) filled and the number of Rxs for antimalarials drugs were tallied. During Phases II-III, blood films were also examined on all patients diagnosed presumptively as malaria. In Phase III, antimalarial Rxs were restricted to parasitemic patients. The total number of Rxs filled was similar in each phase, but the proportion of antimalarial Rxs fell, from 39.9% (2883/7216) to 21.1% (1171/5556) to 6.6% (357/5377) between Phases I, II and III respectively. The percentage of persons with suspected malaria who were parasitemic was similar (30%) in phases II and III. Annual savings from microscopy-directed treatment in this setting are an estimated 56,000 Malawi kwacha (US $13,000), 3% of the annual drugs budget, enough to justify a change in policy.

62 MALARIA CONTROL AND INFANT AND CHILD MORTALITY: COMPARING THE IMPACT OF DECREASING TRANSMISSION VERSUS PREVENTION OF SERIOUS DISEASE. Courval JM* and Singer B. Program in Epidemiology, Michigan State University, East Lansing, MI; and School of Public Health, Yale University, New Haven, CT.

Malaria is a major killer of young children, especially in Africa. This study compares the impact on childhood mortality of reducing transmission versus treatment of fever cases in a hyper-endemic malaria area. Data are from the Garki project, which was carried out in Nigeria from 1970-1976. Villagers in intervention areas and untreated areas were followed before, during, and after the use of insecticide spraying and mass drug administration aimed at reducing malaria transmission. After the intervention phase ended, chemotherapeutic measures were used to prevent mortality as transmission returned. A Cox's proportional hazards regression model with time-varying covariates was used to estimate the impact of these malaria control measures on mortality. During the intervention phase, transmission in treated areas decreased to about 2% of previous levels. Mortality in these areas was reduced by 50% in infants and children ($p < 0.0001$). During the post-intervention phase, transmission returned almost to pre-intervention levels by the first wet season. During the post-intervention phase mortality was reduced by 25% (N.S.) in the previously treated areas compared to the untreated areas. In the Garki study decreasing transmission appeared to be more effective in reducing mortality than treatment of fever cases. This may be related to differences in

how fully each type of measure was implemented rather than inherent differences in efficacy between measures.

63 FIELD EVALUATION OF THE SENSITIVITY AND SPECIFICITY OF A RAPID DIPSTICK ANTIGEN-CAPTURE ASSAY FOR THE DETECTION OF *PLASMODIUM FALCIPARUM*. McElroy PD*, Long GW, Beadle C, Maret SM, Weiss WR, Oloo AJ, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Johns Hopkins University School of Hygiene and Public Health; Becton Dickinson Co., Baltimore, MD; Naval Medical Research Institute-Detachment, Kisumu, Kenya; U.S. Army Medical Research Unit-Kenya, Nairobi, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya

A blinded field study was conducted to assess the validity of an antigen-capture assay for the qualitative detection of *Plasmodium falciparum* histidine-rich protein II (HRPII) in peripheral blood. The assay was evaluated on two separate occasions among 170 children, 8-15 years of age, who participated in a malaria drug treatment trial at one of four primary schools in western Kenya. The specimen requirement was a 50 µl microhematocrit tube of whole blood. Thick and thin blood films were prepared at the same time. Each 50 µl specimen was expelled into a tube containing a RBC lysing reagent, then placed in an ice cooler and transported by vehicle to a rural health clinic where the HRPII assay was performed. A drop of lysed blood was allowed to wick up a dipstick containing an immobilized monoclonal antibody which captured the free HRPII antigen. Antigen was then visually detected as a colored line on the dipstick when a polyclonal antibody to HRPII, conjugated to dyed liposomes, was allowed to wick up the dipstick. Blinded positive and negative control specimens were included in the evaluation. Preliminary analysis indicated a sensitivity of 96% (95% CI 93-100%) among individuals with a parasite density >60/µl according to thick blood film examination (n=118). The sensitivity among specimens containing 60 or fewer parasites/µl is currently being evaluated. The observed specificity was 92% (95% CI 86-98%) among children whose thick blood films were negative upon examination of 2000 white blood cells (n=71). Overall concordance with expert microscopy was 94%. Analysis of 40 known negative specimens by both diagnostic methods showed no significant difference in the estimate of specificity. In this study, specimens were assayed in groups of ten within 30 minutes, but analysis of a single specimen may be completed within 20 minutes. The ease and rapidity of this antigen-capture assay, even under field conditions, makes it an excellent alternative to microscopic examination.

64 A SIMPLE METHOD FOR THE DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA IN RURAL SETTINGS. THE RAPID MANUAL PARASIGHT®-TEST. Premji J*, Minjas JN, and Shiff CJ. Muhimbli University College of Health Sciences. Dar es Salaam, Tanzania; and The Jonhs Hopkins University, Baltimore, MD.

Strategies for malaria control in rural regions of the world lack an important component, the ability to diagnose infection rapidly and to provide immediate treatment if indicated. With chloroquine resistant *Plasmodium falciparum* now prevalent, presumptive diagnosis and treatment is no longer feasible nor affordable if second line drugs are to be used. We have tested the Parasight®-F test in 281 persons in coastal Tanzania and in comparison with thick films and QBC Malaria test done on the same persons, we have shown the test to be very effective. The test was found to be 87% sensitive and 88% specific. We followed the levels of detectable antigenemia and parasitaemia in 40 individuals who attended the District Hospital outpatient clinic and showed that 10 days post treatment over 50% of patients were negative and after 14 days only 4 individuals remained positive. We have used the test in rural clinics and found it to perform satisfactorily in the hands of Village Health Workers. We conclude that the test can be used to undertake reliable diagnosis in the field.

65 CLINICAL TRIALS OF LIVE ATTENUATED DENGUE VACCINE CURRENT STATUS.
Bhamarapravati N*, Yoksan S, and Angsubhakorn S. Mahidol University, Bangkok, Thailand.

A Dengue Vaccine Development program has been pursued at Mahidol University in Bangkok, Thailand since 1980. The objective is to produce a Tetravalent, Live Attenuated Dengue Vaccine that is safe and confers strong and long lasting neutralising immunity against the four serotypes of Dengue Viruses. Attenuation has been achieved by serial passages of the viruses through primary dog kidney cellculture certified to be free from human canine infectious agents. A number of Biological markers were used to determine the level of attenuation; i.e. a loss in the ability to produce disease while retaining the ability to infect and producing neutralising antibodies. Three monovalent vaccines were developed this way, while DEN-3 candidate vaccine was adapted in Primary Green Monkey production seed and cadidate vaccine were produced in fetal rhesus lung cells. All the monovalent Dengue Vaccines went through safety tests including monkey neurovirulence test and passed phase I, human trail with safety and strong neutralising antibody responses. Minimal infectious doses 50 were determined for each monovalent vaccines in phase II studies. They showed no reversion after one human passage and one further mosquito passage. Poor infectivity, low dissemination and poor transmissibility were observed in mosquitoes as well. Dengue 2 monovalent candidate vaccine was tested outside Thailand in a phase I trail and was found to elicit bivalent, trivalent and finally in tetravalent forms, first in health adult volunteers in areas virtually free from *Aedes aegypti* in the North and Northeast of Thailand. The vaccines have been shown to be safe with minimal reaction; i.e. fever noted mostly with DEN-3 candidate vaccine, and to produce neutralizing antibodies against all 4 serotypes of Dengue Viruses. Trials of the tetravalent vaccine in several age groups of children have been conducted. The early result appears to be very promising and will be reported.

66 FEASIBILITY OF A DENGUE VACCINE EFFICACY TRIALS IN NORTHERN THAILAND.
Vaughn DW*, Nisalak A, Kozik CA, Snitbhan R, Kunasol P, Suntayakorn S, Pinyopornpanich S, Poopatanakul W, and Innis BL. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Department of Communicable Diseases, Ministry of Public Health, Bangkok, Thailand; and Kamphaeng Phet Provincial Hospital and Department of Health, Kamphaeng Phet.

To evaluate the suitability of testing tetravalent dengue vaccine efficacy in school children in northern Thailand, we determined the rates of dengue during May to September 1991 in a cohort of 40,119 and the rates of serologically defined flavivirus infections (dengue and Japanese encephalitis) over the same interval in a sample of the cohort (n = 3,667). School absences, hospitalizations, and deaths, identified by active surveillance, were evaluated with serology and virus isolation. Preliminary analysis revealed a 4 month incidence of flavivirus infection to be 8.5% (310/3667); 85% were secondary infections. Despite the high infection rate, the attack rate of dengue (clinical illness causing 2 or more days of school absence) was estimated to be only 0.6% and the rate of hospitalization for dengue estimated to be 0.2%. Estimates of attack rates for the full cohort are not yet available. Infections with 3 of the 4 dengue serotypes were documented (complete data pending). In conclusion: 1) analysis of vaccine protection against primary infection in this age group will be challenging due to low rates of primary infection; 2) hospital-based surveillance may be preferred because either dengue frequently leads to hospitalization, or out-patient surveillance is less reliable; and 3) although the rate of secondary dengue infection is relatively high, sample size calculations must be done with estimates of serotype-specific infection rates.

68 ANALYSIS OF A MINIMAL CTL EPITOPE ON DENGUE VIRUS NS3 PROTEIN RECOGNIZED BY MURINE DENGUE-SPECIFIC T CELLS.
Rothman AL*, Kurane I, Dai L, and Ennis FA. Division of Infectious Diseases & Immunology, University of Massachusetts Medical Center, Worcester, MA.

Dengue virus infections continue to be an important cause of morbidity worldwide, but the nature of the protective and potentially pathologic immune responses to dengue virus remains unclear. We are using murine cytotoxic T lymphocytes (CTL) to help identify the immunogenic epitopes of dengue virus. We previously identified a region of the dengue NS3 protein that is important for recognition by CD8+ CTL from H-2^d mice. We used dengue-specific CTL clones and lines to further define this response. Using progressively truncated synthetic peptides, we localized this CTL epitope to a 9-amino-acid segment (residues 298-306 of dengue-4 NS3). CTL recognition of this epitope was restricted by the K^d molecule. CTL clones generated from dengue-2- immunized mice by stimulation with dengue-2 virus recognized the dengue-2 and dengue-4 epitopes, which are identical in this region, but not the dengue-3 epitope, which differs by a single amino acid at position 305. CTL lines generated from NS3-immunized mice by stimulation with a peptide containing the dengue-3 epitope recognized the dengue-2, dengue-3, and dengue-4 epitopes. These data define an important dengue virus epitope for recognition by CTL from H-2^d mice, and indicate that CTL directed at this epitope can display several patterns of dengue serotype cross-reactivity. Further studies using these CTL clones and lines may help define the *in vivo* effects of dengue-specific CTL.

69 RECOGNITION OF DENGUE VIRUS ENVELOPE (E) PROTEIN BY SEROTYPE- SPECIFIC CD4+ CD8- CYTOTOXIC T LYMPHOCYTES. Livingston PG, Kurane I*, Lai CJ, and Ennis FA. Division of Infectious Diseases and Immunology, Department of Medicine, University of Massachusetts Medical Center, Worcester, MA; and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

We have been analyzing human T cell responses to dengue viruses in order to understand the role of dengue virus-specific T cells in recovery from dengue virus infections and in the pathogenesis of dengue hemorrhagic fever. In this study we analyzed dengue virus serotype-specific CD4+ CD8- cytotoxic T lymphocytes (CTL). Thirteen dengue virus type 4-specific CD4+ CTL clones were established from two healthy adults who had been immunized with an experimental live dengue 4 vaccine. Six clones which include five clones from donor 1 and one clone from donor 2 were further examined. All of these six clones recognized dengue 4 antigen in an HLA-DR7-restricted fashion. Four clones from donor 1 and one clone from donor 2 recognized envelope (E) protein. One clone from donor 1 did not recognize E or NS3 protein. These results indicate that E protein contains serotype- specific CD4+ CTL epitope(s). We have already reported that dengue virus-specific CD8+ CTL of donor 1 recognized E protein. Dengue virus protein is therefore important because it contains CD4+ and CD8+ CTL epitopes in addition to possessing neutralizing and enhancing epitopes.

70 AN ANALYSIS OF DENGUE VIRUS-SPECIFIC CD4+ CYTOTOXIC T CELL CLONES DERIVED FROM A RECIPIENT OF AN EXPERIMENTAL LIVE-ATTENUATED DENGUE 1 VACCINE. Green S*, Kurane I, Edelman R, Tacket CO, Zeng L, Brinton M, Pincus S, Paoletti E, and Ennis FA. Division of Infectious Disease and Immunology, University of Massachusetts Medical Center, Worcester, MA, Center for Vaccine Development, University of Maryland, Baltimore, MD, Department of Biology, Georgia State University, Atlanta, Georgia, Virogenetics Corporation, Troy, NY.

There are an estimated 100 million cases of infection by dengue viruses each year, occurring predominantly in tropical and subtropical regions of the world. The majority of infections are asymptomatic or cause a self-limited illness, but the more severe form of the disease known as dengue hemorrhagic fever/dengue shock syndrome, is characterized by plasma leakage and may be life-threatening. Crossreactive T lymphocytes have been implicated in the pathogenesis of this syndrome. In this study, T lymphocytes were cloned from a recipient of an experimental live-attenuated dengue 1 vaccine. Seventeen clones were studied. All were found to be CD4+. Thirteen clones were found to be dengue 1-specific. Four clones were dengue 1-dengue 3 crossreactive. Recognition of antigen by the three dengue 1- specific clones analyzed to date was restricted by HLA-DPw4, HLA- DPw3 and HLA-DRw52 respectively. We are determining the proteins recognized by

these CTL clones using purified proteins and recombinant vaccinia viruses which contain parts of the dengue 1 virus genome. NS3, NS5 and E proteins are not recognized by these clones. Preliminary data suggest that one clone recognizes an epitope on NS1 or NS2a. These results indicate that primary infection with one serotype of dengue viruses induces serotype- specific and serotype-crossreactive CD4+ memory T cells and that the HLA class II restriction of these specific T cells is heterogeneous.

71 CYTOKINE RESPONSES TO DENGUE INFECTION AMONG PUERTO RICAN PATIENTS. Kuno G* and Bailey RE. Division of Vector-Borne Infectious Diseases, CDC, San Juan, PR; Division of Vector-Borne Infectious Diseases, CDC, Ft. Collins, CO.

Elevated levels of cytokines in blood were recently found to be positively correlated with the development of the severe dengue syndromes (DHF/DSS) in patients from Asia and the South Pacific. WE have determined the concentrations of three cytokines in dengue patients in Puerto Rico and compared the values by age, hospitalization status, and development of hemorrhagic symptoms. The concentrations of interleukin-1 (IL-1 β) were not positively correlated with hospitalization or hemorrhagic manifestation. In contrast, the levels of interleukin 6 (IL-6) in hospitalized adults and children were significantly higher than in the corresponding outpatients. Tumor necrosis factor (TNF) levels were higher in hospitalized children than in outpatient children or hospitalized adults. While hemorrhagic manifestation was not correlated with higher level of cytokine concentration, need of hospitalization was. Thus, hospitalization, as a rough measure of the severity of dengue infection, is supported by the cytokine analysis.

72 A CANDIDATE VACCINE AGAINST ROSS RIVER VIRUS. Yu S, and Aaskov J*. WHO Colaborating Centre for Arbovirus Reference and Research, Centre for Molecular Biotechnology, School of Life Science, Queensland University of Technology, Brisbane, Queensland, Australia.

Ross River virus is an alphavirus which causes a disease known as epidemic polyarthritis. The virus is endemic in Australia causing 4,500 cases of arthritis in 1992 alone and in 1979-80 an epidemic of Ross River virus infection swept Fiji, Samoa, Tonga, and the Cook Islands as well as causing disease in New Caledonia. Epidemic polyarthritis patients may develop a rash, fever/chills and a range of other non-specific symptoms. The arthritis lasts 30-40 weeks with 25% of patients having residual athralgia a year after onset of symptoms. We have developed a protocol for the inactivation of Ross River virus using biethyimine with retains the antigenicity of the virus. Mice immunized with inactivated RRV with and without alhydrogel adjuvant switched from IgM to IgG anti-RRV antibody production and both groups of mice mounted strong anamnestic responses to a second i.m. immunization. No viremia could be detected in mice challenged i.v. with ten thousand infectious doses of RRV 3 months after a single i.m. injection with 20 μg inactivated RRV. Two i.m. injections of 0.2 μg inactivated virus provided similar levels of protection. While inactivated RRV administered with alhydrogel elicited a stronger antibody response than virus alone, better neutralization *in vitro* and protection *in vivo* was achieved with non-adjuvanted virus. This may have been due to different patterns of IgG subclass production in response to these two forms of the vaccine.

73 ALPHAVIRUS INTERACTIONS: SUPPRESSION OF IMMUNE RESPONSE TO VEE VACCINE IN PERSONS PREVIOUSLY VACCINATED WITH VEE OR WEE VACCINES. Pittman PR*, Makuch RS, Cannon T, and Gibbs P. Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Biometrics & Information Management Division, USAMRIID, Fort Detrick, Frederick, MD.

To assess the possibility of interactions between eastern (EEE) and western (WEE) equine encephalitis vaccines and the vaccine for Venezuelan equine encephalitis (VEE), we reviewed data in the Special Immunizations Clinic obtained from January 1976 to December 1990. Four hundred thirty-eight volunteers, who received VEE TC-83 live, attenuated vaccine, had antibody titers to VEE determined before receiving EEE or WEE vaccines. The geometric mean titer (GMT) for VEE for this group was 1:84. In another group, forty-eight volunteers received EEE and WEE vaccines before receiving VEE vaccine. These individuals had a GMT of 1:44.5 for VEE. The difference in VEE antibody titers between the 2 groups was significant (p= 0.0082). The plaque-reduction neutralization titer ($PRNT_{80}$) of non-responders in both groups (defined as a $PRNT_{80}$ <1:20) paralleled the GMT measurements. Those receiving only VEE vaccine had a non-responder rate of 18.3%. However, individuals who received EEE and WEE vaccines before the VEE vaccine had a non-responder rate of 33.33%. The difference between the two groups was statistically significant (p=0.013). These analyses suggest significantly suppressed antibody response to VEE in volunteers previously given an alphavirus vaccine (EEE,WEE).

74 DOSE-SEEKING STUDY WITH A LIVE, ATTENUATED JUNIN VIRUS VACCINE CANDID #1, LOT 2, IND 2257. Makuch RS*, Barrera-Oro J, Lewis T, Rossi C, Higgins Y, Mangiafico J, Schmaljohn A, and Sjogren MH. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD; and Salk Institute, Clearwater, PA.

At the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), 25 volunteers were vaccinated with live, attenuated Junin virus vaccine as part of a dose-seeking study. The 25 volunteers were randomized into 5 groups of 5 individuals. The first group received a 10-4 dilution of vaccine; the second group received a 10-3 dilution; the third group received a 10-2 dilution; the fourth group received a 10-1 dilution. The final group received the standard undiluted vaccine. All vaccines were administered intramuscularly. Plaque-reduction neutralization titer 80% (PRNT) and lymphocyte transformation (LT) assays were performed on days 0, 28, 84 and 182. Capture anti-IgG ELISA was performed on days 0, 7, 14, 21, 28, 42, 56, 84, and 182. Six-month results showed a persistent PRNT response in 100% of volunteers in the undiluted, 10-1, and 10-2 groups. Capture anti-IgG ELISA titers were detected in 100% of volunteers in the undiluted group, 75% of the 10-1 group, and 60% of the 10-2 group. Three-month LT were 100% positive in volunteers in the undiluted, 10-1, 10-2, and 10-3 groups. Clinical reaction rate during the study was unremarkable. Despite a definite need for expanded studies, our results show a strong potential for maximum efficacy at the 10-1 dilution. This would greatly expand current supplies of the vaccine and decrease cost.

75 RNA VIRUSES OF *LEISHMANIA BRAZILENSIS*: THE COMPLETE cDNA SEQUENCE OF LRV1-4. Scheffter SM*, Widmer G, and Patterson JL. Infectious Diseases, Children's Hospital, Boston MA; Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA; and Tufts University School of Veterinary Medicine, N. Grafton, MA.

RNA viruses of the protozoan parasite *Leishmania brazilensis* may provide a vehicle to study the molecular biology of *Leishmania* and may play a role in determining parasite virulence. The viruses also provide a model for studies on RNA-protein interactions. A complete cDNA sequence was obtained for Leishmania RNA virus LRV1-4 and compared to one reported for LRV1-1. Both viral genomes exhibit a similar molecular organization and encode closely related gene products, supporting their assignment to the same genus (LRV1). The comparison also identified several regions of potential secondary structure conserved through complementary base-pair substitutions in both isolates suggesting a functional importance. Among these structures are a pseudoknot located between ORF-2 and ORF-3 and a prominent stem-loop structure located at the 3'-terminus. The latter resembles the encapsidation signal recognized by the RNA-dependent RNA polymerase (RDRP) of LA virus. Interestingly, the RDRP motifs encoded in LRV1 polymerase most closely resemble those

present in LA virus. In vitro transcription and translation products will be used in a gel-shift assay to characterize RNA-protein interactions in LRV1-4.

76 HETEROGENEITY OF gp63 PROTEINS IN *LEISHMANIA CHAGASI.* Roberts SC, Donelson JE, Streit JA, and Wilson ME. Department of Biochemistry, The University of Iowa, Iowa City, IA; Department of Medicine, The University of Iowa, Iowa City, IA.

Expression of the surface protein gp63 correlates with virulence in*Leishmania chagasi. L. chagasi* has three classes of gp63 genes that are differentially regulated and encode slightly different proteins. Two of these have a glycan phosphatidylinositol (GPI) membrane anchor. Using anti-gp63 serum or sera against specific gp63 peptides we studied expression of gp63 during growth of *L. chagasi* promastigotes from log phase (low infectivity) to stationary phase (high infectivity). Western blots showed a 63 kDa protein in both log and stationary promastigotes. Stationary promastigotes also had a 59 kDa protein reacting to anti-gp63 serum and one of the anti-peptide sera, expressed only in virulent but not attenuated promastigotes. Cleavage with PI-specific phospholipase C showed both (the 63 kDa and 59 kDa) proteins possessed a GPI anchor. *L. chagasi* converted to amastigotes in U937cells expressed gp63 proteins of different M_r. We hypothesize that various gp63 proteins expressed by different *L. chagasi* life stages could have different functions in these stages contributing to parasite virulence.

77 *LEISHMANIA* AMASTIGOTES ADHERE TO HEPARAN SULFATE PROTEOGLYCANS ON MAMMALIAN CELLS. Love DC* and Mosser DM. Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA.

Leishmania are dimorphic intracellular parasites. The amastigote form is responsible for spreading the infection from cell to cell in the mammalian host. We report a heparin-binding activity on the surface of *Leishmania amazonensis* amastigotes which is involved in amastigote adhesion to mammalian cells. Amastigotes bound approximately 120,000 molecules of soluble heparin per cell with a binding constant of 8.8×10^{-8}M. This heparin-binding activity mediates amastigote binding to several adherent cell types, while amastigotes failed to bind to two mutant CHO cell-lines, each deficient in heparan sulfate proteoglycans. A CHO cell-line expressing undersulfated heparan sulfate bound amastigotes almost as well as did wild-type CHO cells. Pretreatment of amastigotes with soluble heparin decreased their binding to murine peritoneal macrophages by 60%. Failure to completely inhibit amastigote binding to macrophages suggests macrophages have more than one mechanism for recognizing amastigotes. We show that *L. amazonensis* amastigotes possess a heparin-binding activity on their surface and this heparin-binding activity is responsible for mediating amastigote adhesion to mammalian cell-surface proteoglycans containing heparan sulfate. We are currently analyzing the heparin-binding activity of other leishmania species. Ion-exchange and affinity chromatography are currently being used to isolate the leishmania amastigote heparin-binding activity.

78 EGF-SENSITIVE PHOSPHORYLATION OF THE 90 KD EGF-RECEPTOR HOMOLOGUE OF *TRYPANOSOMA CRUZI* AMASTIGOTES. Freeman-Junior P* and Lima MF. Division of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

In order to complete its life cycle, *Trypanosoma cruzi* must develop intracellularly within mammalian hosts. This requirement for intracellular localization suggests that amastigote multiplication may be influenced by the same set(s) of regulatory mechanisms that govern host cell proliferation. Previous reports from our laboratory indicated that the addition of EGF to cultured amastigotes stimulated [^{3}H]thymidine incorporation with a corresponding increase in parasite growth. In this study, we report that the binding of ^{125}I-EGF to amastigotes is concentration

dependent and saturable. Scatchard analysis of the binding data revealed that amastigotes present 1 x 10^3 receptors per parasite with a K_d of 0.19 nM. Furthermore, monoclonal antibodies against distinct domains of the human EGF receptor recognized a 90 kD protein on the surface of amastigotes by immunoprecipitation and Western blot analyses. This protein is distinct from the 170 kD EGF receptor present on A431 human carcinoma cells and Vero cell fibroblasts. We have also found that the 90 kD amastigote protein shows EGF-sensitive phosphorylation in the presence of ^{32}P-orthophosphate. These results suggest that the 90 kD amastigote protein is the putative receptor for EGF and may function to transduce growth signals in *T. cruzi*. Since disease pathology depends on exposure to parasite burden, an understanding of the mode of amastigote multiplication is essential to the development of novel strategies to interrupt the life cycle of the parasite and prevent disease progression in mammalian hosts.

79 DETECTION OF MINICIRCLE DNA FROM THE NATURALLY DYSKINETOPLASTIC *TRYPANOSOMA EVANSI* BY PCR. Lun ZR*, Lu LX, and Desser SS. Department of Biology, Zhongshan University, Guangzhou, P.R. China; and Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

Trypanosomiasis caused by *Trypanosoma evansi* is an economically important parasitic disease of domestic animals in Asia, Africa and South America. It has been demonstrated that kinetoplast DNA of *T. evansi* does not contain maxicircles. Detection of the minicircles from kinetoplastic and dyskinetoplastic forms will allow us to investigate the function of minicircles in the physiology of the parasite. Minicircles of cloned kinetoplastic and naturally dyskinetoplastic forms were detected by PCR using minicircle-specific primers for *T. evansi*. An amplification product of three hundred seventy-three base pairs was detected in the kinetoplastic forms, but minicircle DNA could not be amplified in the dyskinetoplastic forms. Experimental data indicated the loss of minicircles in naturally dyskinetoplastic forms. No detectable differences in pathogenicity to mice and *in vitro* were observed in either of the forms, suggesting that minicircles of this parasite do not influence important physiological functions.

80 CHARACTERIZATION OF *LEISHMANIA DONOVANI* CATION TRANSPORTING ATPASE TRANSCRIPTS. Meade JC*, Kong L, and Hicock PI. Division of Parasitology, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS.

Leishmania donovani contains a pair of differentially expressed cation transporting ATPase genes (1A & 1B). The two genes are tandemly linked in the genome and separated by 2 kb of intervening sequence. ATPase 1A transcripts are present in both the promastigote and amastigote forms of *Leishmania*; ATPase 1B transcripts are detected in amastigote RNA. Primer extension experiments with promastigote and amastigote RNAs have identified putative 5' splice sites for the mini-exon leader sequence appended to *Leishmania* transcripts. The results of RNase protection assays for both transcripts are in agreement with primer extension data. The 5' slice sites for ATPases 1A and 1B are not identical but are homologous to the eukaryotic consensus splice sequence and previously reported *Leishmania* min-exon splice sequences. PCR amplification of 5' cDNA ends (RACE) is being performed to determine the sequence of the mini-exon in both transcripts.

81 CLONING AND CHARACTERIZATION OF TWO DISTINCT CYSTEINE PROTEASE GENES IN *LEISHMANIA DONOVANI CHAGASI* . Omara-Opyene AL*, Ismail SO, Bhatia A, and Gedamu L. Department of Biological Science, University of Calgary, Canada.

The molecules that empower *Leishmania* to survive inside the macrophages remain unidentified. We believe cysteine protease is one such molecule. Accordingly, we have amplified a 500 bp cysteine protease gene fragment from *L. donovani* through PCR, utilizing conserved regions from closely

related organisms as primers. Using the 500 bp gene fragment as a probe, we have screened the *Leishmania donovani chagasi* cDNA library in λ-ZAP II. Sequencing of the isolated cDNA clones has revealed that we have isolated two species of cysteine protease cDNAs. In one we found an open reading frame encoding 471 amino acids and this is yet the longest sequence of cysteine protease reported from Trypanosomatids. This open reading frame contains a preregion followed by a propeptide region, a mature protease core and a c-terminal region with an extra 24 amino acid residues compared to *Trypanosoma cruzi*. Comparison of the deduced amino acid sequences of the protein with those of other cysteine protease showed that our cysteine protease contain the ERFNIN motif which is common to all cysteine proteases except the cathepsin B-like protease. The other cDNA has an open reading frame encoding 300 amino acids and differs from the first cDNA in that it lacks a proregion. Aside from the absence of a proregion in the shorter cDNA, both the two cDNA have a common 8 nucleatide sequence at beginning of the 5' end and the are 98% homologous at the amino acid level. Genomic Southern analysis indicates that cysteine protease in *L. donovani chagasi* may be encoded by more than one gene copy. We have expressed the two cDNAs in a bacterial expression system. The recombinants will be used for further characterization of *Leishmania* cysteine proteases.

82 THE CERCARIAL GLYCOCALYX OF *SCHISTOSOMA MANSONI* IS A LYMPHOCYTE MITOGEN. Xu XF*, Holm MJ, Devens BH, and Caulfield JP. Syntex Discovery Research, Palo Alto, CA.

The glycocalyx (GCX) that covers schistosomal cercariae is a complex immunogen that has epitopes recognized by monoclonal antibodies protective in passive transfer experiments. Here, we purified a large quantity of GCX in order to study its immunogenic properties. In a typical preparation, 10 million cercariae were extracted with phenol. The extract was dialyzed and chromatographed on a Sepharose 2B-CL column. Approximately 40 mg of carbohydrate eluted in void volume of which 32 mg was glycogen. The glycogen was separated from the GCX by affinity chromatography on Anguilla anguilla lectin and its identity established by sensitivity to amylase digestion. The GCX bound to the affinity column and was composed of 8 mg carbohydrate and 1.2 mg protein. Fucose was the major carbohydrate; glucose, galactose, glucosamine, and galactosamine were also found. About 30% of the amino acids were serine and threonine, which are potential attachment sites for O- link glycans. Splenocytes from naive mice exposed to 2 μg/ml to 100 μg/ml GCX incorporated 3H-thymidine in a concentration dependent manner. By phase microscopy, blasting was present in the splenocyte cultures exposed to GCX. Thus, the purified glycocalyx is a mitogen.

83 *SCHISTOSOMA MANSONI*: ACTIVE IMMUNIZATION WITH WORM MEMBRANE ANTIGENS ENHANCES PRAZIQUANTEL EFFICACY. Fallon PG*, Ripley BA, Riley SL, and Doenhoff MJ. School of Biological Sciences, University of Wales, Bangor, Gwynedd, U.K.

In passive transfer experiments antibodies reactive against antigens exposed by praziquantel (PZQ) on the worm surface of *Schistosoma mansoni* act synergistically with the drug in *S. mansoni*-infected mice. We have previously shown that polyspecific rabbit sera raised against crude adult worm membrane preparations (WS), and monospecific sera raised against a 25-27 kD antigen with esterolytic activity act synergistically with PZQ. This study examined whether active immunization of mice with WS or with the esterase affected the efficacy of PZQ against a subsequentially administered *S. mansoni* infection. The schistosomicidal activity of subcurative doses of PZQ (100mg/kg) was significantly increased in immunized animals compared to appropriate control animals ($P<0.001$). Sera from both sets of immunized animals had intense antibody reactivity to the 25-27 kD antigen in Western immunoblots at the time of *S. mansoni* infection. However, immunization with the crude worm membrane preparation did not confer any protection against the challenge with 200 *S. mansoni* cercariae, but the mice immunized more specifically with the esterase

were partially protected (20% reduction in adult worm number). These results will be discussed in the context of the chemotherapy and immunoprophylaxis of schistosomiasis.

84 DEVELOPMENTALLY REGULATED PHOSPHORYLATION OF SMIRV1, A 90 KDA *SCHISTOSOMA MANSONI* PROTEIN RECOGNIZED BY SERA OF MICE VACCINATED WITH IRRADIATED CERCARIA. Hawn TR* and Strand M. Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore MD.

Vaccination of mice with radiation-attenuated cercaria induces protective immunity against schistosomiasis which is targeted at the lung-stage schistosomula and can be partially transferred with sera to naive mice. The molecular targets of this immunity as well as the molecular mechanisms of the development of cercaria into schistosomula remain largely unknown. Two potential immunoprophylactic antigens, IrV1 and IrV5, were previously isolated based on their enhanced immunoreactivity with sera from immunized mice compared to infected, unprotected mice. IrV1 has been molecularly cloned and shown to be a 90 kDa protein with sequence similarity to calnexin, an ER phosphoprotein which has been hypothesized to serve as a molecular chaperone. Antibodies against recombinant IrV1 immunoprecipitated the native antigen from extracts of cercaria, schistosomula, and adult worms. In addition, preliminary studies indicated that IrV1 was immunoprecipitated from extracts of surface-biotinylated schistosomula. Based on its similarity to calnexin, we examined the potential role of phosphorylation in the function of IrV1. Furthermore, in order to examine developmental signaling events, we compared extracts from cercaria and schistosomula labeled *in vitro* with ^{32}P-orthophosphate. In detergent extracts of cercaria, several proteins were phosphorylated including two proteins with molecular masses of 18 and 10 kDa which were only labeled in this stage. In comparison, a number of proteins were specifically phosphorylated in schistosomula including those with molecular masses of 90, 50, 40, 25, and a doublet at 14 kDa. The 90 kDa protein was identified as IrV1 by immunoprecipitation experiments and found to be phosphorylated on serine and threonine residues. Future studies will include assessment of the immunoprophylactic potential of IrV1 as well as analysis of the signaling events regulating its phosphorylation.

85 ELEVATED TH1 CYTOKINE AND NO SYNTHASE RNA EXPRESSION IN THE LUNGS OF MICE VACCINATED WITH IRRADIATED CERCARIAE DURING CHALLENGE INFECTION. Wynn TA*, Oswald IP, Eltoum I, Lewis FA, James SL, and Sher A. Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; and Biomedical Research Institute, Rockville, MD.

Several studies have suggested that the lung is the primary site for immune killing of challenge *Schistosoma mansoni* larvae in mice previously immunized with radiation-attenuated cercariae. We have shown that both macrophages and endothelial cells become activated to produce toxic nitrogen oxides and to kill schistosomula following *in vitro* exposure to combinations of the cytokines, IFN-γ, TNF-α, and IL-1. In order to determine whether these mechanisms may be operating *in vivo*, we have analyzed by RT-PCR the cytokine mRNA response in the lungs of mice vaccinated and challenged with cercariae. In immunized and challenged mice, we observed a dramatic elevation in the Th1 cytokines (IFN and IL-2) in all animals on days 18-22, a period during which killing of schistosomula is believed to occur. These increases were associated with a similar increase in NO synthase mRNA. Interestingly, we also observed elevated Th2 cytokine mRNA expression in these mice, however, the increases in IL-4 and IL-5 were not significantly different from those seen in non-immunized and challenged control animals. By contrast, expression of IL-10 was substantially elevated. Neutralization of IFN and/or IL-2 by treatment with mAbs confirmed the importance of IFN in the rejection of challenge infection. Abs to IL-2 reduced the level of protection in immunized mice by 14%, while neutralization of IFN reduced immunitiy approximately 50%. This reduction was associated with a marked diminution of NO synthase mRNA expression. Together, these

observations support a role for macrophage/endothelial cell-mediated killing through production of NO. Furthermore, the observation that the down regulatory cytokines IL-4 and IL-10 are expressed even in immunized mice, may provide an explanation for the failure of vaccination to provide complete protection.

86 IDENTIFICATION AND CHARACTERIZATION OF *SCHISTOSOMA MANSONI* ANTIGENS RECOGNIZED BY T AND B LYMPHOCYTES OF HUMANS WITH EARLY ACTIVE SCHISTOSOMIASIS. Al-Sherbiny MM*, El Ridi RA, Guirguis NI, and Dean DA. Zoology Department, Faculty of Science, Cairo University; Research Department, VACSERA, Cairo; and Naval Medical Research Unit No. 3, Cairo, Egypt.

Schistosome antigens selected as vaccine candidates should induce in humans T and B cell-mediated immunity that results in protection against infection. As a first step towards the identification of such antigens, we attempted to define and characterize the soluble adult *Schistosoma mansoni* worm antigen (SAWA) bands that are recognized by serum antibodies and/or peripheral blood mononuclear cells of Egyptian children with early active *S. mansoni* and/or *S. haematobium* schistosomiasis. Considerable inter-subject variation was observed in the selection of bands recognized by both antibodies and lymphocytes, as demonstrated by Western blotting and T cell Western assays, respectively. The humoral response rate for electrophoretically resolved antigenic bands varied between 0% and 88% of infected subjects. Antigens with 153, 144, 38 and 32 kDa reacted with the sera of 60% to 88% of infected subjects but not with the sera of uninfected controls. The bands of molecular weights 144, 38, 32 and 18 elicited proliferative responses in the lympocytes of 42% to 63% of infected subjects. Therefore, SAWA bands of 144, 38 and 32 kDa are likely to carry both T and B cell epitopes that could stimulate immune responses in a majority of individuals. The selected bands (144, 38 and 32 kDa) were found to include glycoproteins containing D-mannopyranosyl or glycosyl residues, with 62.5%, 46% and 55% of amino acids by weight, respectively. The amino acid molar ratios of these bands were completely different, suggesting that they contain distinct molecules rather than degredation or aggregation products.

87 EFFICIENCY OF CLASS II MHC EXPRESSING EOSINOPHILS AS ANTIGEN PRESENTING CELLS TO CD4+ T CELLS. Mawhorter SD*, Kazura JW, and Boom WH. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; Department of Medicine, Case Western Reserve, OH.

Eosinophils (eos) are associated with CD4+T cells in immunologic lesions including *Schistosoma mansoni* egg granulomas. Tissue eos express class II MHC on their surface. To evaluate the possible immunoregulatory role of class II bearing eos on T cells we evaluated the antigen presenting capacity of eos to resting and activated CD4+ cells. Peripheral blood eos were purified to > 99% and cultured in GM-CSF for 1-7 days to allow upregulation of class II MHC (range 17-91%, n=5) before use in antigen presenting cell (APC) assays. We compared eos as APC to plastic adherence purified, irradiated, macrophages (Mϕ) as APC. Using the superantigen Staphylococcal enterotoxin A (SEA) ^{3}H-thymidine incorporation was measured in a proliferation assay with eos and Mϕ class II MHC expressing 87% and 84% respectively by FACS analysis.

	APC:T Ratio			
	1:100	1:10	1:2	1:1
T+Eos+SEA	-	654	2,856	9,833
T+Mø+SEA	578	7,674	36,311	55,730

cpm

Proliferative responses to PPD, Tetanus, or *Brugia malayi* Ag were observed in only 3 of 9 studies with eos as APC. Mϕ controls consistently showed a significantly greater response. Given the importance of B7 as a costimulatory molecule in T cell proliferation we measured GM-CSF cultured eos for B7 (Mab BB1) and found them consistently negative)(n=3). In contrast, EBV-transformed B cells expressed 51% B7. These data show that eos are inefficient APC to CD4+ T cells despite significant class II MHC expression, which may be explained in part by the lack of B7 expression on eos.

88 INCREASED TH2 CYTOKINE RESPONSES IN PATIENTS INFECTED WITH *SCHISTOSOMA MANSONI*. Williams ME, Wynn TA*, Montenegro S, Doningues AL, Teixeira K, Coutinho A, and Sher A. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and Centro de Pesquisas Aggeau Magalhaes, FIOCRUZ, Recife, PE, Brasil.

During murine schistosomiasis, Th2 (IL-4, IL-5, IL-10) cytokine expression is enhanced while Th1 (IL-2, IFNγ) cytokine responses appear to be down-regulated. In this study, we demonstrate that a similar increase in Th2 cytokine production occurs in humans infected with *Schistosoma mansoni*. The polyclonal and antigen specific responses of peripherial blood mononuclear cells from intestinal and hepatosplenic patients in Recife, PE, Brasil have been analyzed. Cytokine production was measured using the ELISPOT assay, ELISA, and RT PCR. ELISA data indicate that patients produce higher levels of IL-4 and IL-5 in response to mitogen than control patients. Similarly, by ELISPOT analysis patients showed a higher frequency of IL-4 producing cells in response to mitogen than uninfected individuals. RT PCR analysis demonstrated increased levels of mRNA for the Th2 cytokines IL-4, IL-5, and IL-10 in infected patients in response to soluble egg antigen and soluble adult worm antigen. With respect to Th1 cytokines, IFNγ production by patients in response to mitogen was not found to differ from controls using either ELISA or ELISPOT assays. By RT PCR, patients produced IL-2 and IFNγ in response to SWAP, but not to SEA. We observed no significant differences between intestinal and hepatosplenic patients in terms of their cytokine responses. These results indicate that humans infected with *S. mansoni* produce higher levels of Th2 cytokines in response to mitogens and antigen. In addition, we did not detect Th1 cytokine production in response to SEA in infected individuals; a situation similar to that seen in the mouse.

89 *SCHISTOSOMA MANSONI* EGG ATTACHMENT TO VASCULAR ENDOTHELIAL CELLS. Ngaiza JR*, Doenhoff MJ, and Jaffe EA. Division Hematology Oncology, Cornell University Medical College, New York, NY; and School of Biological Sciences, Bangor, Gwynedd, UK.

Adult schistosomes normally reside and lay eggs intravascularly. Critical to the continuation of the parasite's life cycle is the extravasation and excretion of its eggs from its mammalian host. The interactions of the eggs with host cells during these events may be involved in determining other outcomes of the infection such as granuloma-induced pathology and resistance to reinfection. In order to study the host-parasite interaction at this stage of the infection, we investigated the attachment of *Schistosoma mansoni* eggs to endothelial (EC) cells. *S. mansoni* eggs readily attached to human and bovine EC. While the interaction required that the eggs be intact, glutaraldehyde-fixed eggs and live eggs attached in a comparable manner. In contrast, eggs did not attach to glutaraldehyde-fixed EC and pretreatment of EC with cytochalasin B, colchicine or aspirin inhibited attachment of eggs. The attachment assay was conducted in medium alone, medium with serum or medium with plasma. Attachment was most efficient in medium containing serum suggesting that platelet products are involved. In the presence of platelet release products, attachment of eggs to EC was increased by approximately 75%. Similarly, SEA promoted or inhibited egg attachment depending on whether SEA is incubated with eggs or with EC before the attachment assay. Binding of I125-SEA to EC was direct and specific. The attachment of *S. mansoni* eggs to EC is therefore an active process which is modulated by platelet-derived factors and SEA components.

90 *SCHISTOSOMA MANSONI* EGG DEPOSITION RESULTS IN INCREASED ICAM-1EXPRESSION IN LIVER TISSUE. Ritter DM, Rosen S, Singer M, and McKerrow JH. University of California at San Francisco, San Francisco, CA, and Department of Veterans Affairs Medical Center, San Francisco, CA.

Previously our laboratory demonstrated that the presence of tumor necrosis factor (TNF)α was necessary for formation of granulomas around eggs in livers of *Schistosoma mansoni* infected mice. Our current interest in granuloma formation is to determine which component(s) in addition to TNFα is responsible for the localization of the granuloma around the egg. We examined the role of the endothelial derived adhesion molecules in promoting binding of immune cells to vascular endothelium around the deposited egg. During a variety of inflammatory reactions, intercellular adhesion molecule-1 (ICAM-1) expression on vascular endothelial cells is enhanced. It has also been demonstrated that ICAM-1 mRNA and surface expression of ICAM-1 can be upregulated by LPS, IL-1 and TNFα. In *S. mansoni* infection, ICAM-1 surface expression is significantly enhanced at 6 weeks of infection, at the time when eggs start to be deposited in the liver. ICAM-1 expression remains upregulated for at least 10 weeks post-infection as more eggs are deposited. In contrast, no surface expression of the L-selectin receptor, GlyCAM-1, was detected. ICAM-1 expression on vascular endothelium around the egg may mediate binding of activated memory leukocyte functional antigen-1 (LFA-1) positive cells to this area to initiate a granuloma. This hypothesis is supported by inhibition of binding of T cell clones to frozen liver section expressing ICAM-1, when preincubated with LFA-1 antibody.

91 SOLUBLE INTERCELLULAR ADHESION MOLECULES IN HUMAN SCHISTOSOMIASIS: CORRELATIONS WITH CLINICAL FORM OF DISEASE AND PROLIFERATIVE RESPONSES. Secor WE*, Reis MG, Ramos EA, Carno TM, Reis EA, Mattos EP, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, Salvador, Bahia, Brazil.

Granuloma formation, the major pathologic event in schistosomiasis produces a highly organized cellular lesion involving both immunologic and nonhematopoietic cells. A growing body of research indicates a strong interaction between cells of the immune system and cells of the vasculature and matrix. Integrins, selectins, and cytokines are important for cell-cell communication in each of these cell types and therefore provide a variety of mechanisms by which these cells can affect one another. The effect of cytokines in schistosomal infections continues to be an area of intense research; however, the role of cellular adhesion molecules in this diseases remains practically unexamined. To this end, groups of high egg excreting (HEE), low egg excreting (LEE), intestinal (INT), and hepatosplenic (HS) patients were examined for circulating intercellular adhesion molecule 1 (sICAM-1) and endothelial leukocyte adhesion molecule 1 (sELAM-1) in sera. All patient groups had significantly higher levels of sICAM-1 than never infected controls and the HEE group had significantly higher higher levels of sICAM-1 than age and sex matched patients in the INT group. Also, in the HEE and LEE groups, peripheral blood mononuclear cell proliferative responses to soluble egg antigen sELAM than noninfected individuals. The levels of sELAM in the HS group were not significantly different tan normals but were significantly lower than INT group patients.

92 OLIGOSACCHARIDE INTERACTIONS WITH MONONUCLEAR CELLS FROM MICE INFECTED WITH *SCHISTOSOMA MANSONI* OR *TRYPANOSOMA CRUZI* MAY LEAD TO $CD4^+$ T CELL SUBSET REGULATION. Velupillai P *, Pereira M, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department. of Geographic Medicine, Tufts Medical School, Boston, MA.

In experimental murine schistosomiasis there is a profound shift in CD4+ T cell subsets from Th1 to Th2 concomitant with the onset of egg deposition. A down-regulation of Th1 functions also is found in *Trypanosoma cruzi* infections. Both parasites possess large amounts of immunoreactive glycoproteins. Using a series of anti-egg MAbs each of which recognize distinct oligosaccharide epitopes we previously determined that one of the epitopes was the biologically interesting sugar Lacto-N-fucopentaose III (LNFPIII). LNFPIII and structurally related sugars were tested for biological activity in lymphocyte proliferation/cytokine production assays. We found that LNFPIII specifically induces B cells from schistosome or *T. cruzi* infected mice to proliferate and produce large amounts of IL-10, a cytokine known to down-regulate Th1 cell populations. The stimulated cells did not produce IL-4. We also examined the ability of these sugars to elicit peritoneal B-1 (Ly-1+, B220+) cells, which are known to produce large amounts of IL-10. We observed that Injection of LNFPIII into the peritoneal cavity caused the population of B-1 cells to increase 2-3 fold in CBA/J and C3H/HeJ but not C57Bl/6J mice. Specificity of the responses was examined by testing sugars which are nonfucosylated homologues of LNFPIII or otherwise structurally related to LNFPIII. We found that the related sugars were able to induceproliferation, however no IL-10 was produced. These sugars were also incapable of eliciting peritoneal B-1 cells. Thus, a specific-oligosacchardie ligand LNFPIII may be partially responsible for the Th1 to Th2 shift in schistosomiasis and *T. cruzi* infections.

93 PURIFICATION, CHARACTERIZATION, AND IDENTIFICATION OF A *SCHISTOSOMA MANSONI* EGG ANTIGEN RECOGNIZED BY A GRANULOMATOUS T CELL CLONE. Chikunguwo SM*, Secor WE, Stadecker MJ, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Department of Geographic Medicine, Tufts Medical School, Boston, MA.

Pathology related to schistosomal infection is largely a consequence of the host's T cell-mediated response to soluble antigens released from parasite eggs. Previously, we produced murein Th1 T cell clones to soluble egg antigens of *Schistosoma mansoni*. Several of these clones were shown to be granulomatogenic *in vivo* in naive mice, and were utilized in T cell Western blot experiments to identify granuloma-inducing egg antigens. Two egg antigens with Mrs of 64-68 kDa and 38-42 kDa were identified and purified. These two species of egg antigens were probed with anti-egg monoclonal antibodies (MAb) each of which recognize distinct oligosaccharide epitopes and with mannose-binding protein (MBP). Interestingly, we observed that while MBP bound strongly to both Mr species, indicating high mannose core, the nature of the complex oligosaccharides on the two antigens was distinct, as only the 564-68 kDa antigen bound MAb E.5, which recognized the interesting sugar lacto-N-fucopentaose III, Identification at the amino acid level was attained by sequencing tryptic peptides of electro-blotted purified antigen. For the 38-42 kDa antigen, 3 peptides were sequenced yielding 77 total unambiguous amino acid residues. These residues were scanned for known homology against the Swiss.Prot databank and were found to be 100% homologous to the previously described major egg antigen P40. We obtained recombinant P40 from Dr. John Cordingley and demonstrated that it contained the target epitope recognized by granulomatogenic T cell clone F1. We are now focusing on measuring *in vitro* and *in vivo* granuloma formation to native and recombinant P40, and sequencing the 64-68 kDa antigen.

94 STRATEGIES FOR THE DOWN-REGULATION OF GRANULOMATOUS INFLAMMATION IN SCHISTOSOMIASIS. Stadecker MJ*, Flores-Villanueva PO, Ricklan DE, and Harris TS. Tufts University School of Medicine, Boston, MA.

A critical requisite for the amelioration of severe schistosomal disease is the preventive down-regulation of granulomatous inflammation around parasite eggs. Based on our previous observation that macrophages isolated from egg granulomas (GM) induce anergy in cloned specific granulomagenic murine CD4+ Th-1-type lymphocytes, we tested two approaches to down-regulate the granulomatous inflammation associated with a challenge schistosomal infection. First, active

ectopic granuloma induction in response to *S. mansoni* eggs, but not to *A. lumbricoides* eggs, resulted in a significant reduction of granulomatous disease *in vivo*, as well as in the inhibition of specific proliferation of mesenteric lymph node cells *in vitro*. Second, passive administration of purified GM, but not of normal peritoneal cells (PC), resulted, similarly, in significant reduction of granuloma size *in vivo* and specific lympho-proliferation *in vitro*. Moreover, cytokine analysis in supernatants from stimulated mesenteric lymph node cells disclosed that IL-2 dropped to undetectable levels, at the same time as IL-4 and IL-10 increased, in animals receiving GM, in contrast to those receiving PC or no cells. These findings are consistent with the interpretation that adoptive transfer of GM induces unresponsiveness in specific Th-1 lymphocytes, which, in turn, results in the down-regulation of granulomatous disease and its *in vitro* correlates.

95 THE IMMUNOREGULATION OF GRANULOMA FORMATION AND FIBROSIS IN SCHISTOSOMIASIS BY ANTIGEN-SPECIFIC IMMUNOCONJUGATES. Ali MR*, Farid AG, Gabr NS, Shi S, and Phillips SM. Parasitology Department, Faculty of Medicine, Minia University, Minia, Egypt; and Allergy and Immunology Section, School of Medicine, University of Pennsylvania, Philadelphia, PA.

T lymphocytes, reactive to soluble egg antigen (SEA) are primarily responsible for the induction and regulation of immunopathology in schistosomiasis. These T cells are heterogeneous. Therefore, approaches to regulate pathology through immunologic means are complicated in man. T cell receptor: CD interactions limit the efficacy of T cell specific immunosuppression. Approaches which avoid genetic restriction by directly addressing Antigen: T cell interactions will avoid genetic limitations. We have conjugated SEA and other antigens to Daunomycin (DM), a cytotoxic rhodamycin. Antigen presenting cells and T cells with receptors for SEA bind, internalize, and activate the DM. Subsequently the cells are killed or inactivated. We have studied the ability of SEA-DM conjugate to regulate granuloma formation and fibrosis. SEA-DM can suppress antigen mediated blast transformation and *in vitro* granuloma formation. The suppression is antigenically specific but not genetically restricted. SEA-DM conjugate demonstrates less non-specific toxicity than does free DM. When administered by intravenous injection, SEA-DM specifically suppresses the DTH reaction against SEA and the *in vivo* granulomas formed by infected mice in response to *Schistosoma mansoni* eggs. In addition, SEA-DM reduces the deposition of hepatic matrix proteins such as Type I and III collagen and fibronectin. These *in vivo* effects are not genetically restricted and are immunologically specific. Thus target specific immunosuppressive compounds, containing low molecular weight cytocidal drugs, may have potential for the reduction of pathology due to schistosomiasis in man.

96 IDIOTYPIC REGULATION IN SCHISTOSOMIASIS: II. RELATIONSHIP BETWEEN LEVEL OF IDIOTYPES AND MORBIDITY OF DISEASE IN *SCHISTOSOMA HAEMATOBIUM*-INFECTED PATIENTS. Shata MT*, Helmy A, Badary MS, Deaf EA, Mohamed AM, Naser AM, Nafi MA, Napi AK, Elrehawy N, and Sercarz E. Faculty of Medicine, Assuit University, Assuit, Egypt; and Department of Microbiology, University of California, Los Angeles, CA.

The nature of the mechanisms regulating immune responses in schistosome infections is a subject of controversy. Regulation at both the B cell level (idiotypes) and T cell level (differentiatial lymphokine secretion) have been described. In this study, we examined the relationship between the expression of dominant idiotypes in the sera of 100 cases of *Schistosoma haematobium* infections and the morbidity of the disease. Affinity purified anti-SEA antibodies were prepared from pooled sera of *S. haematobium*-infected hamsters. Binding of these hamster anti-SEA antibodies to patients' sera infected with *S. haematobium* was significantly higher in comparison to the control sera. No correlation was seen between the level of anti-SEA antibodies during affinity purifications. Our data indicate the presence of Ab2 in schistosome-infected patients' sera which recognizes dominant idiotypes in Ab1 of the *S. haematobium*-infected hamsters. Additionally, there is a significant

realtionship between the level of Ab2 in patients' sera and the duration of infection, the age of the patients, the intensity of infections as well as the morbidity of the disease. The mechanism of this relationship is under investigation.

97 RADIONUCLIDE LYMPHOSCINTIGRAPHY IN 45 SYMPTOMATIC AND ASYMPTOMATIC SUBJECTS WITH BANCROFTIAN FILARIASIS. Almeida Filho P*, Besh S, Silva MC, Braga C, Maciel MA, Furtado AF, and Freedman DO. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; Nuclear Medicine Unit, Laboratorios Cerpe, Recife, Pernambuco, Brazil; and Centro des Pesquisas Aggeu Magalhaes, Recife, Pernambuco, Brazil.

In order to characterize quantitatively lymphatic function in individuals with filarial pathology as well as that in clinically asymptomatic but microfilaremic patients, bilateral lower limb lymphoscintigrams were performed in 45 individuals. Dynamic flow studies were performed over inguinal regions for the first 45 minutes, and static images of the legs, thighs, and pelvis were obtained at 4 hrs. Analysis of clearance from the injection site as well as time-activity curves and appearance times over the inguinal regions showed the expected patterns of stasis (in some cases obstruction) in patients with clinical pathology. In marked contrast, individuals with asymptomatic microfilaremia almost uniformly had a quantitatively enhanced pattern of flow with dilated vessels, a significantly sharper rise in the time-activity curves, and dramatically increased peak flows when compared to controls. Qualitative analysis of the delayed static images for visualization of lymph vessels, dilatation of lymph vessels, collateralization, dermal backflow, and visualization of pelvic lymph nodes was utilized to classify patients into discreet subgroups. Clinico-pathologic correlation was obtained by quantitating circulating antigen, cytokine production by ag stimulated lymphocytes, and degree of upregulation of adhesion molecules in our *in vitro* endothelial cell system. We conclude that in filariasis radionuclide lymphoscintigraphy can identify discreet and quantifiable defects in clinically asymptomatic individuals as well as more obvious structural defects in patients with clinical deformity. As the technique can be readily repeated, objective data regarding efficacy of therapeutic intervention is obtainable.

98 DIETHYLCARBAMAZINE VS IVERMECTIN FOR TREATMENT OF BANCROFTIAN FILARIASIS IN PAPUA NEW GUINEA. Kazura JW*, Greenberg J, Perry R, Weil G, Day K, and Alpers M. Case Western Reserve University, Cleveland, OH; Imperial College, London, England; Washington University, St Louis, MO; and Papua New Guinea Institute of Medical Research, Papua, New Guinea.

This double-blind study compared the clinical safety and parasitologic efficacy of "single dose" regimens of diethylcarbamazine (DEC) and ivermectin (IVR) for treatment of bancrofitan filariasis in Papua New Guinea. Five groups of 10 men each with mean levels of parasitemia ranging from 2985 to 5185 microfilariae (mf)/ml were given DEC (6 mg/kg/body weight one time or 1 mg/kg then 6 mg/kg 4 days later) or IVR (220 μg /kg; 20 μg/kg then 200 μg/kg 4 days later; or, 20 μg/kg then 400 μg/kg 4 days later). No significant side effects (eg, acute adenolymphangitis, fever lasting >8 hours, hypotension) were observed in any of the 5 treatment groups. The magnitude of reduction in microfilariemia was greater ($P<0.01$) for the 3 IVR groups vs. the 2 DEC groups in the first 30 days after drug administration (mf levels <1% of pre-treatment values vs 22.6 to 41.5%, respectively). At 90 and 180 days, mf levels continued to decrease in the DEC groups whereas they increased in the IVR groups given a total dose of 220 μg/kg. Eighteen months after drug administration, individuals given DEC or 420 μg/kg IVR had the greatest degree of reduction in microfilaremia (86-90% compared to the pre-treatment values). Decreases in parasite antigenemia measured by ELISA for a secreted 200kDa adult worm antigen were greatest for the single dose DEC group (39.7% decrease relative to the pre-treatment level vs 7.8-15.7% for the IVR groups). The acute microfilaricidal activity of IVR and long-term suppression of parasitemia and possible decrease in adult worm accumulation effected by single

dose DEC suggest that a combination of these drugs may be useful for mass chemotherapy to control bancroftian filariasis.

99 DRUG DOSAGE AND INTAKE PERIODICITY FOR MASS CHEMOPROPHYLAXIS OF BANCROFTIAN FILARIASIS. Moulia-Pelat JP, Glaziou P*, Nguyen NL, Chanteau S, Martin PM, and Cartel JL. Institut Territorial de Recherches Medicales Louis Malarde, Tahiti, French Polynesia.

Single doses of diethylcarbamazine 6 mg.kg-1 (DEC6) twice yearly is the treatment recommanded in 1993 for mass chemoprophylaxis of bancroftian filariasis in French Polynesia. Several trials were conducted between 1986 and 1993 to compare the efficacy of single doses of ivermectin (IVER) at different dosages and periodicities of intake to DEC. The efficacy of the treatment was estimated by the geometric mean microfilaremia (mf) recurrence percentage as compared to the pre-initial treatment mf level. The results can be summarized in 3 points; (i) in terms of immediate clearence or complete negativation of mf, IVER 400 µg.kg-1 (IVER400) was more effective than DEC; (ii) 6 months after treatment, the mf recurrence percentage were 27 19 12 and 5% in carriers treated respectively with IVER100 DEC3 DEC6 and IVER400 ; whereas with a single dose of IVER400, it was 20%.12 months after; (iii) in a recent trial with the combination IVER400 plus DEC the mf recurrence percentage was 3% 6 months after treatment. In conclusion, ivermectin could be an alternative for treatment of bancroftian filariasis; the dosage IVER400 is the most effective, but a yearly intake may be not sufficient. Longer follow-up and further studies are planned before considering a yearly intake with the combination for mass chemoprophylaxis treatment.

100 ADVERSE REACTIONS FOLLOWING IVERMECTIN TREATMENT IN HYPERENDEMIC LOIASIS AREA. Chippaux JP*, Garcia A, Ranque S, Schneider D, Boussinesq M, Cot S, Le Hesran JY, and Cot M. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon; and Antenne ORSTOM, OCEAC, Yaounde, Cameroun.

Mass treatment against onchocerciasis may occur in areas were loiasis is endemic. Side effects following ivermectin treatment in people infected with *Loa loa* are poorly documented. The main purpose of this study was to evaluate the risks of adverse reactions induced by mass treatment with ivermectin. A survey was conducted in a forest village of South Cameroon. Prevalence of *Loa loa* microfilaraemia was 31%. The entire population has been observed every day within the week after ivermectin treatment (200 mcg.kg^{-1}). 47 randomized couples formed each with one hyperfilaraemic (more than 100 microfilariae per ml of blood) and one a-filaraemic individuals, both with the same sex and age, were matched. A follow-up of temperature, blood (eosinophil count, microfilarial density) and urine (protein, glucose, blood, and microfilarial density) analysis was carried out on treatment day, then on days 3 and 7. In two patients (14%) with high levels of *Loa* parasitemia, we observed severe asthenia and conscience troubles during the week following treatment. The temperature increased significantly in hyperfilaremic individuals ($p < 10^{-3}$). Blood appeared or increased significantly in urine samples during the first few days after treatnment in people with high microfilaraemia ($p<10^{-3}$). Proteinuria and microfilaruria did not change after treatment. We conclude that fever and blood in urine could be considered as a good early indicators of severe adverse reaction.

101 ANTIFILARIAL IMMUNE RESPONSES IN INDIVIDUALS EVALUATED OVER A 17-YEAR PERIOD ON AN ISLAND ENDEMIC FOR BANCROFTIAN FILARIASIS. Ottesen EA*, Steel C, Guinea A, McCarthy J, and Poindexter RW. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD; and Health Department, Mauke, Cook Islands.

By reevaluating a population endemic for subperiodic bancroftian filariasis 17 years after it had been previously studied we were able to add a temporal component to the usual cross-sectional approach to categorizing patients and assessing their immune responses. Three age-matched groups of

individuals were defined for study: those who had remained free of infection (and circulating antigen-negative) despite continued exposure ('putatively immune' endemic normals; n=20), those with persistent microfilaremia (n=20), and those whose initial microfilaremias had completely resolved (n=22). Lymphocyte responses to filarial antigen (but not other antigens) were significantly higher in the endemic normals than in either of the other groups ($p<.05$-$.01$ for all comparisons) when assessed both by proliferation and by production of Th1-type (IL-2, IFN-γ), Th2-type (IL-5) or other (GM-CSF) cytokines. By contrast, antifilarial IgG4 antibodies (a possible marker of active infection) were significantly lower in endemic normals than in the other groups ($p<.05$). Indeed, while those with persistent microfilaremia maintained unchanged high levels of IgG4 antibodies over the 17 years between studies, those who cleared their microfilaremias had significant decreases in their IgG4 antibody levels; no corresponding differences were found for antibodies of other IgG subclasses. Such highly characterized study groups offer the means for identifying those mechanisms responsible for susceptibility and persistence of infection in bancroftian filariasis or, alternatively, for developing protective immune responses.

102 THE EFFECT OF PRENATAL EXPOSURE TO MATERNAL MICROFILAREMIA ON THE IMMUNE RESPONSIVENESS TO PARASITE ANTIGENS IN ADOLESCENTS. Steel C*, Guinea A, McCarthy JS, Zimmerman PA, and Ottesen EA. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD; and Mauke Hospital, Mauke Island, Cook Islands.

To identify long-term influences of prenatal exposure to maternal filarial infection on subsequent immune responsiveness to filarial antigens, we assessed lymphocyte responses in 21 Polynesian children born 17-19 years previously to mothers diagnosed in an earlier study as either microfilaremic (MIC) or amicrofilaremic (AMIC). All children lived on an island endemic for bancroftian filariasis but were infection-free when studied. Striking differences between the groups were seen in lymphocyte responses to microfilarial (Mf) Ag. Mf Ag induced significantly greater proliferation in the AMIC children than in the MIC children (S.I. = 8.2 vs. 2.37; $p<.03$) and also stimulated production of significantly higher levels of the cytokines IL-2 (605 vs 46 pg/ml; $p<.01$), IL-5 (151 vs 81.8 pg/ml; $p<.03$) and GMCSF (87 vs 31 pg/ml; $p<.03$) in the AMIC children. No such differences were found between these two groups in either proliferation or cytokine responses to non-parasite Ag. Interestingly, in contrast to their lower lymphocyte responses MIC children had significantly higher serum levels of anti-Mf IgG4 Ab (674 vs 25.8 u/ml; $p<.03$). As no differences in environmental exposure (location of houses), microfilaremic status of fathers or genetic predisposition (HLA DQA1 and DQB1) could be identified, we attribute the difference in Mf-specific immune responsiveness to prenatal "tolerization" of the children born to MIC mothers. In general, lymphocyte and IgG antibody responses to filarial Ags in the AMIC children appeared similar to those of endemic normal individuals while those of the MIC children resembled more closely those of patients with chronic filarial infections.

103 REACTIVITY OF HUMAN SERA WITH RECOMBINANT *BRUGIA MALAYI* MICROFILARIAL CHITINASE. Piessens WF*, Perler FB, Southworth MW, Dissanayake S, Xu M, Wang SH, Chen GH, Morin PM, Deng BJ, Watawana L, Zheng HJ, and Fuhrman JA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; New England Biolabs, Beverly, MA; Guizhou Provincial Institute of Parasitic Diseases, Guiyang, China; Shanghai Medical University, Shanghai, China; Faculty of Medicine, Peradeniya, Sri Lanka; and Department of Biology, Tufts University, Medford, MA.

Passive transfer of the monoclonal antibody (Mab) MF1 reduces microfilaremia in recipient jirds or nude mice infected with *Brugia malayi*. The epitope recognized by Mab MF1 resides on a developmentally regulated calcium-binding protein, which has been identified as a microfilarial chitinase. Previously reported pilot studies indicated that the MF1 epitope is preferentially recognized by sera from immigrants who remained amicrofilaremic 3 - 6 years after arrival in an area

where brugian filariasis is endemic. We now have examined >350 sera from donors with bancroftian filariasis for reactivity with recombinant microfilarial chitinase (by direct Elisa) and the MF1 epitope (by competitive Elisa). Depending on their geographic origin, 11 - 22 % of sera from amicrofilaremic donors with evidence of bancroftian filariasis and 0 - 21 % of sera from microfilaremic donors react with chitinase. Paired serum samples (N = 124) indicate that the prevalence of IgG antibodies to the MF1 epitope is higher in microfilaremic Sri Lankans who remain amicrofilaremic after DEC treatment (30 %) than in those with recurrent parasitemia (13 %); the prevalence of IgG antibodies to rec chitinase is the same in both groups (30 %). Only 51/74 sera reacting with the rec antigen contained antibodies to the MF1 epitope. Our findings indicate that microfilarial chitinase contains at least 2 distinct antigenic determinants. Recognition of the epitope defined by Mab MF1 is associated with resistance to recurrent microfilaremia following DEC treatment. Further, reactivity patterns of sera from donors with bancroftian filariasis differ from those we have previously observed in patients with brugian filariasis.

104 FILARIASIS WITHOUT MICROFILAREMIA. Weil GJ*, Liftis F, Chandrashekar R, Gad AM, Faris R, and Ramzy RM. Washington University School of Medicine, St. Louis, MO; and Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt.

Occult or amicrofilaremic filariasis is well described in animals, and it is often associated with stage-specific immunity to microfilariae (MF). Our studies have shown that sera from a significant proportion of amicrofilaremic and asymptomatic people in *Wuchereria bancrofti* endemic areas ("endemic normals") contain filarial antigens detectable by ELISA. This group accounted for 14% of the population above age 10 in a village in the Nile Delta which had a MF prevalence of 28%. Filarial antigenemia without microfilaremia was associated with age, female sex, and residence in portions of the village with high prevalence rates of MF and clinical filariasis. Immunoblot studies showed that sera from such people contained the same filarial antigens as sera from microfilaria carriers. Sera from antigen-positive endemic normals contained significantly higher levels of IgG4 antibodies to *Brugia* adult antigen and to a recombinant Brugia antigen (BmM14) than sera from antigen negative endemic normal controls matched for age and sex. Antibodies to *B. malayi* MF surface were detected by IFA in 13 of 14 sera from antigen positive endemic normals and 3 of 14 controls. These results suggest that amicrofilaremic filariasis is relatively common in endemic areas and that it may be due to stage-specific immunity to MF.

105 DIFFERENTIAL TH1 ACTIVATION IN FILARIAL ANTIGEN-NEGATIVE AND ANTIGEN-POSITIVE INDIVIDUALS. Dimock KA*, Lammie PJ, and Eberhard ML. Division of Parasitic Diseases, Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA.

The concept of the "endemic normal" has raised questions related to protective immunity in lymphatic filariasis. Are individuals with no detectable microfilaremia and no clinical evidence of infection immune or temporarily parasite-free? In an attempt to define immune responses which may be associated with protective immunity, we compared B and T cell responses in individuals who are microfilaria negative (Mf-) with those of Mf+ individuals. In clinic-based studies in Haiti, approximately 30% of Mf- individuals tested are positive for circulating *Wuchereria bancrofti* antigen (Ag+). In Ag- individuals (n=21) antifilarial IgG2 levels were elevated (56.2 μg/ml) and IgG4 levels were low (4.3 μg/ml), as compared with levels in Mf+ individuals (14.5 μg/ml and 18.8 μg/ml respectively; n=15). IgG4 levels were elevated in Mf-/Ag+ (43.0 μg/ml; n=11) and Mf+ individuals, however, IgG2 levels in Ag+ individuals (38.1 μg/ml) were more comparable to those of Ag- persons. Filarial antigen specific Th1 responses were monitored by assessing *in vitro* proliferation and IL-2 production. Both proliferation and IL-2 production induced by adult antigen were greater with PBMC from Ag- than from Mf+ individuals. Proliferative responses of PBMC from Ag+ individuals were intermediate between Ag- and Mf+ individuals, whereas IL-2 production was comparable to that of Mf+ individuals. No significant differences were observed in responses to larval antigens. Further

analysis of Th2 responses should help define the extent to which these responses are associated with Ag- and Ag+ states.

106 *BRUGIA MALAYI* MICROFILARIAE - COSTIMULATION AND MODULATION OF HUMAN T LYMPHOCYTE RESPONSES. Weller PF*, Liu LX, and Kim J. Beth Israel Hospital, Harvard Medical School, Boston, MA.

Since blood borne microfilariae will be in proximate contact with lymphocytes, we evaluated whether *Brugia malayi* microfilariae (Mf) can stimulate or inhibit activation responses of normal human T lymphocytes. Mf were isolated from infected jirds and rigorously depleted of jird peritoneal macrophages, as confirmed by anti-CD14 mAb flow cytometry. Human lymphocytes, isolated from normal donors by Hypaque-Ficoll with depletion of adherent monocytes, were cultured 24-48 hrs without stimulation or with Con A or immobilized anti-CD3 mAb stimulation, both without Mf and with Mf present at 1:1/16 and 1:1/4 lymphocyte:Mf ratios. Mf alone did not stimulate lymphocyte production of interleukin (IL)-2 or IL-4 or expression of IL-2 (CD25) or transferrin (CD71) receptors. Mf, however, did provide a powerful co-stimulatory signal to lymphocytes activated with Con A or anti-CD3 mAb, and quantities of IL-4 and especially IL-2 produced were significantly greater (up to 20-50 fold increased) with greater ratios of Mf. The co-stimulatory effect of Mf was abolished with heat-, microwave- or aldehyde-killed Mf, was not present in supernatant fluids of Mf, and required intimate contact of Mf with lymphocytes. In addition, Mf-derived prostanoids inhibited lymphocyte activation. Mf supernatant fluids inhibited IL-2 production, and the potent co-stimulatory effect of Mf for IL-2 and IL-4 release was further enhanced by treatment of Mf with indomethacin or ETYA, inhibitors of prostanoid synthesis. Thus, while viable Mf may modulate lymphocyte activation by producing inhibitory prostanoids, Mf also exert a potent co-stimulatory effect to augment lymphocyte activation

107 ELEVATED IL-10 PRODUCTION BY CIRCULATING MONONUCLEAR CELLS IN INDIVIDUALS WITH LYMPHATIC FILARIASIS MANIFESTED AS ASYMPTOMATIC MICROFILAREMIA. Mahanty S*, Mollis SN, Ravichandran M, Jayaraman K, Kumaraswami V, Abrams JS, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; Centre for Biotechnology, Anna University, Madras, India; Tuberculosis Research Centre, Madras, India; and DNAX Research Institute, Palo Alto, CA.

To determine if interleukin (IL)-10 plays a crucial role in the generation and maintenance of the immunologically hyporesponsive state characteristic of microfilaremia (MF), we compared the production of IL-10 by peripheral blood mononuclear cells (PBMC) from individuals with MF (n=10) or with chronic lymphatic obstruction (CP; n=11). Peak spontaneous IL-10 secretion by PBMC was significantly higher for MF (geometric mean (GM) [range] 560 pg/ml [118-4085]) than for CP (<20 pg/ml [<20-1107]; $p<0.005$). Filarial antigen (Ag)- driven production of IL-10 by PBMC was ~6-fold higher for MF (GM: 390 pg/ml [90-5870]) than for CP (GM: 60 pg/ml [<20-1960]; $p<0.05$). The non-filarial Ag, PPD inhibited spontaneous IL-10 secretion in both groups. Spontaneous and filarial Ag-stimulated IL-10 production by plastic-adherent monocytes was also higher in MF than in CP. A negative correlation was found between Ag-driven IL-10 production by PBMC and Ag-induced T cell proliferation in MF individuals ($p<0.05$). To determine the source of the IL-10 in MF individuals, reverse transcriptase PCR was employed to detect mRNA for IL-10 in unstimulated and filarial Ag-stimulated PBMC and immunomagnetically purified $CD4^+$, $CD8^+$, $CD19^+$ and plastic-adherent cells from both groups. The excess IL-10 appears to derive from multiple cell populations including CD4+ cells and macrophages. These data suggest that IL-10 may play an important role in the generation or maintenance of the hyporesponsive state seen in microfilaremic individuals.

108 IL-12 REGULATION OF PARASITE ANTIGEN-DRIVEN IGE PRODUCTION IN HUMAN HELMINTH INFECTIONS. King CL*, Stupi RJ, Shata T, Saad M, Nafeh M, and Medhat A. Division

of Geographic Medicine, Case Western Reserve University, Cleveland, OH; Department of Microbiology, Assiut University, Assiut, Egypt; and Department of Tropical Medicine, Assiut University, Assiut, Egypt.

IL-12 or natural killer cell stimulatory factor, a product of phagocytic cells and B cells, affects T cell lymphokine production and differentiation. To examine the role of IL-12 in the regulation of serum IgE levels in individuals with helminth infections, we studied antigen-driven polyclonal IgE production and corresponding cytokine synthesis by peripheral blood mononuclear cells (PBMC) from 8 patients with filariasis or schistosomiasis. Addition of neutralizing anti-IL-12 to PBMC cultures significantly augmented helminth Ag-driven IgE production by 88 to 390% demonstrating an inhibitory role for endogenously produced IL-12 in the IgE response. The presence of neutralizing anti-IL-12 in parallel cultures significantly enhanced parasite Ag-driven IL-4 production in all 8 individuals studied ($p<0.001$) and suppressed Ag-driven IFN-γ production in 6 of 8 subjects ($p<0.05$). Addition of 10-100 U/ml of human recombinant IL-12 to PBMC completely inhibited parasite Ag-driven IgE production, markedly enhanced parasite Ag-driven IFN-γ production by 9 to 35-fold and variably suppressed Ag-driven IL-4 synthesis that ranged from 11% ($p=0.6$) to >10-fold. An IL-4 and IFN-γ independent effect of IL-12 on Ag-driven IgE production was suggested by the ability of recombinant human IL-12 to suppress IgE synthesis by 27-52% (n=2) in the presence of neutralizing anti-IFN-γ and recombinant human IL-4 in culture supernatants. This study demonstrates that parasite Ag-driven IgE production in patients with helminth infections is modulated by IL-12 through its capacity to enhance IFN γ production while suppressing IL-4 production in response to parasite Ags.

109 ONCHOCERCIASIS IN ENDEMIC AND NONENDEMIC SUBJECTS: DIFFERENCES IN CLINICAL, LABORATORY, AND IMMUNOLOGICAL FINDINGS. McCarthy JS*, Ottesen EA, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

The skin and eye disease characteristic of onchocerciasis (OV) in residents of endemic areas (END) have been well described. However, disease manifestations in those who become infected as visitors (VIS) to endemic areas may differ significantly from END. To characterise the clinical and laboratory differences of OV in these 2 groups, and to explore underlying differences in immune responsiveness, 22 VIS were evaluated and compared with 21 END. All END had skin microfilariae (MF), 6/21 (30%) had palpable nodules (NOD), and 17/21 (85%) had ocular disease(OD), whilst only 8/22 (36%) VIS had skin MF, and none had NOD or OD, ($p<0.01$ for all comparisons with END). Dermatitis was, however, a more frequent finding in VIS than END, (17/22, [77%] vs. 5/21 [25%]; [$p<0.01$]). Eosinophilil counts were higher in END (geometric mean [GM] of 2352 for END vs. 1071 for VIS; [$p< 0.05$]), as was serum polyclonal IgE (GM of 22245 for END vs. 795 for VIS; [$p< 0.05$]). All individuals in both groups had elevated parasite-specific IgG and IgE levels and had a seropositive response to a panel of recombinant antigens for diagnosis of OV. In vitro culture of cryopreserved peripheral blood mononuclear cells from 3 END and 8 VIS revealed an increased capacity of cells from the END to produce the Th-2 like cytokines, IL-4 and IL-5, compared to the VIS, ($p<0.05$), whilst no difference in GM-CSF, IL-2 or Interferon γ were observed. We attribute the differences between VIS and END in clinical and laboratory findings, as well as in immunological reactivity to the chronicity and intensity of infection in the END.

110 CULTURAL, MOLECULAR AND ANTIGENIC CHARACTERIZATION OF THE ATYPICAL CANINE EHRLICHIOSIS (TROPICAL CANINE PANCYTOPENIA) AGENT. Kakoma I*, Hansen RD, Anderson BE, Hanley TE, Sims KG, Liu L, Bellamy C, Long MT, and Baek BK. University of Illinois, Urbana, IL; Center for Disease Control, Atlanta, GA; Bramer Animal Hospital, Evanston, IL; and Chonbuk National University, Chonju, Korea.

One hundred atypical canine ehrlichiosis cases reacted serologically in the indirect immunofluorescent antibody (IFA) test for *Ehrlichia canis* or *E. sennetsu* antigen. All of the cases were IFA and western immunoblot positive with titers of 1:10 to 1:640 to *E. risticii*. Three fatalities have been recorded so far. From the blood of 3 clinically well defined such cases, *Ehrlichia*- like agents were isolated by standard *in vitro* cultivation techniques. The isolates from all the three cases were culturally and morphologically identical and indistinguishable from the prototype *E. risticii*. The pattern of and products from the polymerase chain reaction (PCR) were identical to those of *E. risticii*. The entire 5' 16S ribosomal RNA sequences were distinct from *E. canis* and *E. ewingii*, but identical to those of *E. risticii*. The percent relatedness of the partial 16S rRNA gene of the ACE agent to *E. risticii*, *E. sennetsu*, *E. platys*, *E. phagocytophila*, *E. canis*, *E. chaffeensis and E. ewingii* was 100.0, 98.9, 83.7, 83.7, 83.0, 82.2, 81.8 and 81.5, respectively. These data fulfill criteria that are consistent with the identity of these isolates as *E. risticii*. The caninotropic characteristic of naturally acquired infections due to *E. risticii* are herein described for the first time and the epizootiological implications are discussed in relation to the host range of *E. risticii*, which includes dogs.

111 WHITE-TAILED DEER AS POTENTIAL RESERVOIRS OF *EHRLICHIA CHAFFEENSIS*, CAUSATIVE AGENT OF HUMAN EHRLICHIOSIS. Dawson JE*, Stallknecht D, Davidson R, Lockhart M, Nettles V, Biggie K, Olson JG, and Childs JE. Viral and Rickettsial Zoonoses Branch, CDC, Atlanta, GA; and Southeastern Cooperative Wildlife Disease Study, UGA, Athens, GA.

More than 298 cases of human ehrlichiosis have been diagnosed in 24 states in the United States since 1986, when the disease was first recognized. Fever, malaise, headache, myalgia, rigor, arthralgia, and nausea are common presenting symptoms. Seven fatalities have been associated with this disease. A majority of ehrlichiosis patients report an onset of illness, preceded by a tick bite, in the 6-month period between April and September. The vector(s) and reservoir host(s) of human ehrlichiosis remain unknown. However, a recent serosurvey of 1269 deer, in 17 states, found that 53% of these animals had antibodies reactive to *Ehrlichia chaffeensis*, the causative agent of human ehrlichiosis, suggesting that this species of deer may be a reservoir of *E. chaffeensis*. For the purpose of evaluating the susceptibility of these mammals to infection with *E. chaffeensis*, 2 white-tailed deer were inoculated with 2.9×10^6 infected cells. Baseline values for HCT, RBC, HGB, platelets, WBC, and serum chemistry were established. Leukocytes, separated from 10 ml of heparinized whole blood, collected on days 10, 13, 17, 20, 24, 27 and 31 postinoculation (p.i.), were co-cultured with DH82 cells. Serologic evidence of infection was found by day 10 p.i. *E. chaffeensis* was reisolated from the deer on days 13, 17, 20, and 24 p.i. These data document that white-tailed deer are susceptible to infection with *E. chaffeensis* and that wild deer are often exposed to *E. chaffeensis* or a closely related species. White-tailed deer may serve as a natural reservoir for *E. chaffeensis*, but further studies are required to confirm natural *E. chaffeensis* infections in wild deer by isolation and/or PCR procedures.

112 EPIDEMIOLOGY OF *ROCHALIMAEA* INFECTIONS IN CATS. Childs JE*, Olson JG, Fakile Y, Rooney JA, McGinnis R, Cooper JL, and Regnery R. Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA; Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA ; and College of Veterinary Medicine, University of Florida, Gainesville, FL.

Cats from four states and two foreign countries were tested for antibodies to *Rochalimaea henselae* and *R. quintana* by an indirect immunofluorescent antibody assay (IFA). These rickettsiae cause various clinical syndromes in immunocompromised humans and *R. henselae* is implicated as a causative agent of human cat scratch disease. Of 814 cats tested from the United States, 192 (23.6%) had antibody titers >1:64 to one or both *Rochalimaea* species. The highest antibody prevalence was from Maine (80.8%; N=52) and the lowest from Baltimore, MD (14.7%; N=592). Positive cats also were found from Egypt (5/42) and Portugal (1/14). Risk factors for *Rochalimaea* infection were investigated in a subsample of 592 cats from Baltimore. Evidence for *Rochalimaea* infection increased significantly with cat age and size, but was not associated with gender. *Rochalimaea* antibody was

significantly associated with *Toxoplasma gondii* antibody (24.4% of *T. gondii*-positive compared with 12.0% of *T. gondii*-negative cats). Antibody prevalence was higher among feral animals (44.4%) and feline immunodeficiency virus (FIV)-positive cats (33.3% compared with 13.3% of FIV-negative cats), although the small sample sizes precluded any definitive conclusions. The role of cats as reservoirs or mechanical vectors of human *Rochalimaea* -associated diseases remains speculative, but these findings indicate widespread infection of cats and suggest possible modes of transmission for *Rochalimaea* among cats.

113 COMMUNITY-BASED PROFILES OF RICKETTSIAL INFECTIONS IN THE NILE RIVER DELTA OF EGYPT. Corwin AL*, Olson JG, Habib MA, Dasch G, Kelly D, Richards A, Darwish MA, Botros BB, Watts DM, and Arthur RR. U.S. NAMRU-3, Cairo, Egypt; CDC, Atlanta, Georgia; CFAR, MOH, Cairo, Egypt; NMRI, Bethesda, MD; Ain Shams University, Cairo, Egypt; and NAMRID, Peru.

The purpose of this study was to determine the incidence of acute rickettsial infections in people living in a rural area of the Nile River Delta. Subjects enrolled in a community-based longitudinal studyin the Bilbeis area (60 km NE of Cairo) were monitored for febrile illness (>39°C) from 9/91-12/91. Among a cohort of 2000 villagers, sera were collected from 360 ill subjects (178 M, 182 F). 96% of subjects were <13 years of age. Single acute (A) or convalescent (C) phase serum specimens were available from 218 persons and A and C sera were available from 142 patients. Anti-*Rickettsia typhi* and *R. conorii* IgG titers were determined by ELISA and anti-*C. burnetii* phase I IgG by IFA. The overall prevalence of *R. typhi* and *R. conorii* antibodies (titer>1:100) and *C. burnetii* antibodies (titer ≥1:64) was 42%, 13% and 9%,respectively. No significant differences were observed in age or sex-related seroprevalence. 11 of 142 (7.7%) subjects had ≥4-fold IgG change to *R. typhi*, whereas, no change in *R. conorii* antibody was observed.4 of 125 (3%) patients had ≥ 4-fold change in anti-*C. burnetii* IgGtiter. The incidence of infections with *R. typhi* occurred more frequently in males than females, whereas *C. burnetii* infections were the same in both sexes. Incidence rates of *R. typhi* and *C. burnetii* were estimated to be 16.5 and 6 per 1000 per year, respectively.

114 UPDATE ON THE EPIDEMIOLOGY OF CHOLERA IN NORTH JAKARTA, INDONESIA: BASIS TO CONDUCT A LARGE-SCALE CHOLERA VACCINE TRIAL. Richie E*, Simanjuntak CH, Punjabi NH, Sukri N, Hisham MA, Pulungsih SP, Rifai AR, Supriharyanto E, Harahap DE, Rampengan TH, Sumual-Memah, and O'Hanley P. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; National Institutes of Health Research and Development, Jakarta, Indonesia; Infectious Diseases Hospital, North Jakarta, Indonesia; and Stanford University, Stanford, CA.

In this report, we describe the epidemiology of endemic cholera in North Jakarta based on retrospective chart reviews for 4 years (1988-1992) and since early 1992 by prospective surveillance for cholera at IDH. This is a densely populated metropolitan area that is frequently flooded by brackish waters during the rainy season. Many areas of North Jakarta have less than optimal sanitation and water systems. Annual estimates for clinically severe cholera incidence have been difficult to calculate precisely until recently because only one of the 8 hospitals in North Jakarta had routinely performed stool cultures for the isolation of *Vibrio cholerae* prior to this survey. The bacteriology, crude cholera incidence rates (according to age, gender, month of the year, residence location), clinical spectrum, and outcome for cholera disease have been compiled. Cholera occurs year round with increased number of cases per month during the peak rainy season (Dec-Jan) and peak dry season (Jun-Aug). Overall, ~12% of all acute diarrheal episodes among all age groups presenting at IDH in 1992 had *V. cholerae* strains cultured from stool, and ~95% of all clinical severe dehydrating cholera episodes had *V. cholerae* 01-Ogawa strains cultured from stool. These strains are uniformly sensitive to routine antibiotics, despite heavy antibiotic usage by the local inhabitants. The incidence of clinically severe cholera is variable, dependent on the age and the place of residence of the individual. Children <4 years of age have the highest incidence rates for severe cholera (e.g. >8 cases/ 1,000 children in certain locales). Also, we have determined that certain locales in North Jakarta have

annual severe cholera incidence of 1 case/ 1,000 individuals of all ages. The clinical presentation of patients with cholera versus *V. cholerae* culture-negative patients with diarrhea is distinctly different. There have been no deaths attributable to cholera at IDH during the last 5 years. A large cholera surveillance program recently established throughout North Jakarta will be used to assess the efficacy of an experimental recombinant oral cholera vaccine (CVD103-HgR) in preventing cholera over the next 3 years.

115 SAFETY, IMMUNOGENICITY AND TRANSMISSIBILITY OF SINGLE DOSE LIVE ORAL CHOLERA VACCINE CVD 103-HGR IN 2 TO 4 YEAR OLD INDONESIAN CHILDREN. Punjabi NH*, Simanjuntak CH, Suharyono, Hisham MA, Noriega F, Dykstra P, Pazzaglia G, Budiarso AD, Harun SR, Wasserman S, O'Hanley P, and Levine M. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; National Institute of Health Research and Development, Jakarta, Indonesia; Department of Pediatrics, University of Indonesia, Jakarta, Indonesia; Infectious Disease Hospital, Jakarta, Indonesia; Center for Vaccine Development, University of Maryland, Baltimore, MD; and Departments of Medicine and Microbiology, Stanford University, Stanford, CA.

A randomized, double-blinded study to evaluate the safety, immunogenicity, and transmissibility of a live oral cholera vaccine (CVD103-HgR) in 2 to 4 year old Indonesian children volunteers and their household contacts was conducted in North Jakarta during 1991 and 1992. A total of 301 healthy children was enrolled: 81 pairs were from the same households; 139 children were from separate households. Also, 177 household contacts among these children were followed. Healthy 2 to 4 year old children were administered orally either $5X10^9$ CVD103-HgR or $5X10^8$ *Escherichia coli* K-12. Each child was then examined 9 days thereafter to identify side effects. Serum samples were collected prior and at 9 and 28 days after administration among vaccinees, as well as, some household contact volunteers at the same time intervals. Serum was assessed for vibriocidal antibody titer. Seroconversion was defined by ≥4 fold rise in vibriocidal titer between day 0 versus day 9 and day 28. In addition, rectal swabs were obtained from the vaccinees and some of their household members for 5 days after vaccine administration to detect CVD103-HgR. Results were as follows: Low grade fever occurred more frequently in CVD103-HgR vaccinees compared to placebo recipients (18.1% vs 8.7%, $p = 0.045$). There were no significant differences in the occurrence of diarrhea, vomiting and abdominal cramps between the two vaccine groups. The seroconversion rate was 75% among CVD103-HgR recipients with an average of a 10-fold increase in the geometric mean titer. Seroconversion occurred among other volunteers in this study: 1 child who received *E. coli* and 4 household contacts. These individuals were associated with contact with a recipient of CVD103-HgR. CVD103-HgR excretion was confirmed by culturing rectal swabs in only 4 of 162 CVD103-HgR recipients and in only one person out of 177 unvaccinated family contacts of a CVD103-HgR recipient. In conclusion, a single oral dose of $5X10^9$ CVD103-HgR is safe and well-tolerated, is immunogenic in 2-4 years old Indonesian children, and has a low transmissibility based on culture and serological results among household contacts.

116 STOOL TUMOR NECROSIS FACTOR α TNF IN HUMAN SHIGELLOSIS. Murphy JR*, Mourad AS, Stevens S, and MacDonald TT. U.S. Naval Medical Research Unit #3, Cairo, Egypt and Center for Infectious Diseases, University of Texas, Houston; Faculty of Medicine, Alexandria University, Alexandria, Egypt; Celltech Ltd; and Paediatric Gastroenterology, Saint Bartholomews Hospital, London, UK.

To test the hypothesis that TNF would be increased in stool from patients with shigellosis, an invasive, inflammatory diarrhea, clinical and laboratory records were searched for cases of diarrhea from which *Shigella* (and no other bacterial pathogen) was isolated from stool and for examined healthy individuals who provided stool samples which were negative for both *Shigella* and other bacterial pathogens. Stool samples from 14 individuals with culture-proven *Shigella* infection were recovered from an archive (-70°C) and matched with stored stools from 24 individuals who were

symptom and bacterial pathogen free. TNF was found to be increased in *Shigella* infected (mean 278,0 pg/ml) as compared to the not-infected, not-ill individuals (35.4 pg/ml, $p < 0.05$). Associations were not found between TNF level in stool and the number of leukocytes in the sample or the presence of gross or occult blood. The discordance between results of TNF, leukocyte, and blood determinations shows that TNF is measuring an independent component of pathogenesis and may be useful in diagnosis and monitoring treatment.

117 GROUP III DENSOVIRUSES (DNV'S) ARE WIDESPREAD IN INSECT DISEASE VECTORS AND POTENTIALLY USEFUL AS GENE EXPRESSION VECTORS. O'Neill SL*, Kittayapong P, Braig HR, Gonzalez JP, and Tesh RB. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand; and Institut Franais de Recherche Scientifique pour le Developpement en Cooperation, Paris, France.

A PCR assay was developed to detect DNV genomes in insect cells and whole insect tissue. Application of this assay has revealed latent DNV infections in several mosquito cell lines and laboratory colonies. Phylogenetic analysis based on sequence data indicates that these isolates do not group with either group I or II DNV's but are more similar to other described mosquito DNV's. As such these viruses represent a new group of DNV's (Group III) which appear to be quite common in insects. Preliminary experiments indicate that some of these viruses are capable of infecting a number of different mosquito species both orally and parenterally and are transovarially transmitted. They appear to form largely avirulent infections. These viruses could prove useful as expression vectors in order to introduce genes conferring refractoriness to disease transmission into wild mosquito populations.

118 TRANSFECTION OF SALIVARY GLANDS FROM THE MOSQUITO, *AEDES AEGYPTI*. Morris AC* and James AA. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA.

The control of vector species has long been a mainstay in the control of transmission of parasitic diseases. The development of molecular biological techniques has led to the proposal that genetically-altered vectors may play a role in future control strategies. An important technical accomplishment for testing this proposal is the ability to stably introduce genes into target vector species. Efforts to develop transformation systems are underway in several laboratories. In the absence of such a system, transient assys are being developed so that DNA constructs may be evaluated prior to their application in germline transformation strategies. Transient assays have been developed using the salivary glands of the mosquito *Aedes aegypti*. The glands have been successfully transfected using a liposome-mediated technique following treatment of the glands with elastase to remove the surrounding basement membrane. Expression of a luciferase reporter gene has been detected using a DNA construct carrying this gene under the control of the *Drosophila* heat shock 70 promoter (hsp70). We report the use of this system to analyze a salivary gland specific putative promoter sequence.

119 FEMALE-SPECIFIC ARYLESTERASE ISOLATED FROM *AEDES AEGYPTI* SALIVARY GLANDS. Argentine JA* and James AA. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA.

Esterase activity was detected in *Aedes aegypti* female salivary homogenates using the substrate α-napthyl acetate. Activity in the female salivary glands was 0.05 nM/min/salivary gland or 49.8 μM/min/mg protein. Esterase activity in the male salivary gland was barely detectable and had a specific activity 10-fold less that of female salivary glands. α-napthyl butyrate was also hydrolyzed by

female salivary glands, albeit at a rate 41% relative to that of α-napthyl acetate. The esterase showed no activity towards the fatty acid α-napthyl caprate. Preliminary histological evidence indicates that most of the arylesterase activity is located in the distal-lateral region of the salivary gland, although staining also occurs in the proximal and medial lobes. The esterase inhibitors PMSF and DFP had little effect on activity, while paraoxon and PHMB completely inhibited activity. This indicates that the esterase activity from female *Ae. aegypti* salivary glands is an arylesterase. Efforts are currently underway to isolate the arylesterase by affinity chromatography using PHMB-coupled agarose beads. The female specificity of the salivary gland arylesterase indicates that it may have a role in bloodfeeding.

120 VASODILATORY TACHYKININS FROM SALIVARY GLANDS OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*. Champagne DE* and Ribeiro JM. Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ.

The saliva of the mosquito *Aedes aegypti* was previously reported to contain a 1400 Dalton peptide with pharmacological properties typical of tachykinins, an important class of vertebrate neuropeptides. In the present study this vasodilator has been purified to homogenity and found to consist two peptides: Sialokinin I, with the sequence Asn-Thr-Gly-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH2; and Sialokinin II, identical except for an Asp in position 1. These peptides are present in amounts of 0.62 and 0.16 picomoles respectively (711 and 178 ng) per salivary gland pair. When assayed for contractile activity on the guinea pig ileum, both peptides are as active as the mammalian tachykinin substance P, with $K_{0.5}$ values of 5.07, 6.58, and 4.94 nM for Sialokinin I, Sialokinin II, and substance P respectively. These are the first vertebrate-type tachykinins to be reported from an insect. Implications of the presence of this class of neuropeptides for the vectorial capacity of *Aedes aegypti* will be discussed.

121 THE SALIVARY APYRASE OF THE MOSQUITO *AEDES AEGYPTI* IS A MEMBER OF THE 5'-NUCLEOTIDASE FAMILY. Smartt CT, Champagne DE*, Ribeiro JM, and James AA. Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ; Department of Molecular Biology and Biochemistry, University of California, Irvine, CA.

The saliva of blood-feeding insects contains a variety of pharmacologically active substances which counteract the normal hemostatic response of their vertebrate hosts. The yellow fever mosquito, *Aedes aegypti*, secretes an apyrase (EC 3.6.1.5) that inhibits ADP-dependent platelet aggregation. Apyrase was purified as an active enzyme from 1,000 pairs of adult female salivary glands, using a combination of affinity (Cibacron Blue) and weak cation exchange HPLC. After desalting by RP C18 HPLC, the enzyme was digested with trypsin and two peptides, each 24 amino acids long, were microsequenced. The peptides matched the conceptual translation product of a cDNA clone isolated from an adult female salivary gland library. Sequence comparisons indicate similarities with a ubiquitous family of 5'-nucleotidases. The mosquito protein differs from other members of the family by lacking a carboxy-terminal hydrophobic region which is switched for a GPI anchor in membrane-bound nucleotidases. Comparison with vertebrate 5'-nucleotidases and bacterial UDP-glucose hydrolases indicates that a sequence, GKYVGR, associated with conserved domain 6, is a likely candidate for nucleotide binding. This is the first apyrase to be identified by primary sequence data and opens up the possibility of evaluating the evolution of this complex family of enzymes.

122 ENZYMES AND SUBSTRATES INVOLVED IN MELANOTIC ENCAPSULATION REACTIONS BY MOSQUITOES. Li J*, Zhao XL, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Recent studies in our laboratory using mosquito-filarial worm models indicate that phenol oxidase (PO), dopa decarboxylase (DDC) and an unclassified enzyme, that functions in the conversion of dopachrome to 5,6-dihydroxyindole, play significant roles in the formation of melanotic capsules that sequester these parasites. Significant increases in tyrosine, dopa and dopamine concentrations were detected in mosquito hemolymph following immune activation as compared to uninfected controls. Because PO, upon activation, functions in the hydroxylation of tyrosine to dopa and the oxidation of dopa and other *o*-diphenols (e.g., dopamine and *N*-acetyldopamine) to their respective *o*-quinones, the increased dopa concentration suggested that PO was activated by the parasites. High dopachrome conversion enzyme (DCE) activity also was detected in hemolymph samples. In vitro studies demonstrated that the combined effects of PO and DCE accelerated greatly melanization/pigmentation pathways when L-dopa was used as substrate. Our data suggest that melanotic encapsulation of filarial worms is initiated by PO activation by parasites, PO catalyzes the hydroxylation of L-tyrosine to dopa, dopa is either oxidized to dopaquinone by PO or decarboxylated to produce dopamine by DDC, and then dopamine is oxidized to form dopaminequinone. Both dopaquinone and dopaminequinone may react with nucleophilic residues on proteins to form melanotic materials. Because DCE greatly accelerates melanin formation and because of the high activity of this enzyme in mosquito hemolymph, eumelanin pathways also likely are involved in melanotic capsule formation.

123 SEQUENCE ANALYSIS OF A HEMOLYMPH POLYPEPTIDE PREFERENTIALLY EXPRESSED IN IMMUNE REACTIVE MOSQUITOES. Beerntsen BT* and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Because previous studies have shown the preferential expression of an 84 kd polypeptide in mosquitoes undergoing wound healing and melanotic encapsulation of filarial worms, the present studies were undertaken to identify this polypeptide and determine its role in these reactions. Amino acid microsequence data were obtained and used to design degenerate oligonucleotide primers. These primers then were used, in conjunction with PCR, to generate a clone from genomic mosquito DNA. Screening of mosquito cDNA libraries with this clone resulted in the selection of an approximately 500 bp clone that recognizes the same restriction fragments on a mosquito genomic blot as the original PCR-generated clone. Furthermore, sequencing of this clone indicates that it contains the same sequence as the PCR-generated clone. This 500 bp clone also recognizes an increase in expression of an approximately 1.8 kb message in mosquitoes undergoing melanotic encapsulation reactions, indicating a biologically relevant clone was selected that reflects the preferential expression of the 84 kd polypeptide. Sequence analysis of this clone and other relevant clones is being conducted to determine their identity and potential role in the immune responses of mosquitoes against filarial worms.

124 REPRODUCTIVE COSTS ASSOCIATED WITH RESISTANCE IN A MOSQUITO-FILARIAL WORM SYSTEM. Ferdig MT, Beerntsen BT, Spray FJ, Li J, and Christensen BM*. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

The mosquito *Armigeres subalbatus* can encapsulate and kill >80% of *Brugia malayi* microfilariae (mf) within 36 hr following ingestion. The cascade of biochemical events constituting this melanotic encapsulation response is also important in other mosquito biological events, including egg-chorion tanning. Certain biochemical entities, including a tyrosine precursor, are thought to be shared among these biological activities. Because of this purported tyrosine link, and because the bloodmeal both initiates egg development and is the source of mf, we evaluated the possibility that reproductive cost is incurred by the resistant host when undergoing a response to mf acquired in an infected bloodmeal. Mean time to oviposition was significantly longer for mosquitoes responding to parasites than for controls (77.68 vs 66.5 hrs). Tyrosine levels in ovaries from infected mosquitoes were less than half those of controls at 24 and 48 hrs, and were still significantly reduced at 72 hrs following bloodfeeding.

Ovary development, assessed via measurements and total protein content, also was delayed significantly in the experimental group, with ovary width and protein content never attaining levels found in control mosquitoes. Sections from 24 hr post bloodmeal ovaries demonstrated that the normal processes of egg development, including vitelline accumulation, was drastically altered as well. Biological implications of these results are considered.

125 ROLE OF DOPA DECARBOXYLASE IN MOSQUITO DEFENSE REACTIONS. Ferdig MT*, Li J, and Christensen BM. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Resistant mosquitos are able to sequester and destroy microfilariae (mf) via the production and deposition of melanotic substances on the surface of the parasite. The biochemical events involved in the production of melanotic materials are not fully known, but generally are considered to be derived from the phenol oxidase-catalyzed conversions of catechols derived from tyrosine. We have studied the potential involvement of dopa decarboxylase (DDC) in this reaction by assessing DDC activity in hemolymph of immune reactive mosquitoes using HPLC with electrochemical detection. Significantly increased enzyme activity was observed during the time periods when mf were being sequestered in melanotic capsules. As a consequence of these results we initiated studies to identify a mosquito DDC cDNA from an Aedes aegypti library by use of a Drosophila DDC probe. Sequence information demonstrates this clone contains regions homologous to published DDC sequences. Preliminary Northern analysis indicates increased transcription in mosquitoes responding to parasites. We are using this probe to evaluate the expression of DDC as relates to melanotic encapsulation reactions. Work is in progress to obtain the complete gene sequence for DDC in mosquitoes.

126 CHARACTERIZATION OF AN *AEDES AEGYPTI* YAC LIBRARY. Cook GA*, Christensen BM, and Severson DW. Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

Observations of parasite and mosquito vector interactions clearly reflect a complex genetic basis for vector competency. We are utilizing contemporary molecular techniques to identify and partition vector competency into discrete genetic components. The construction of a saturated RFLP genetic linkage map for the yellow fever mosquito *Aedes aegypti* has provided landmarks useful in identifying regions of the mosquito genome that contain the genes which contribute to the susceptible phenotype. We have constructed a genomic DNA library for *A. aegypti* in a Yeast Artificial Chromosome (YAC) cloning vector. Characterization of the YAC clones carrying genes involved in determining this vector competency have begun. Genomic DNA, isolated from pupae, was partially restriction enzyme digested to produce 200-500 kb fragments. Following separation in agarose gels, these fragments were ligated to the arms of a YAC vector. Yeast spheroplasts were transformed and those clones carrying YAC/*Aedes* constructs were identified by complementation selection. YAC clones flanking genomic regions carrying genes suspected of determining parasite susceptibility were identified using the RFLP markers. These YAC clones are the starting points for development of contiguous YAC clone physical maps across these regions.

127 EFFECT OF *ONCHOCERCA* INFECTION ON BLACKFLY REPRODUCTIVE PHYSIOLOGY. Hurd H* and Renshaw M. Centre for Applied Entomology and Parasitology, Department of Biological Sciences, Keele University, Keele, Staffordshire, UK.

Blackflies can act as vectors for filarial nematodes of the genus *Onchocerca*, some of which are the causative agents of onchocerciasis or "river blindness". A reduction in reproductive output has been observed in African and British Simuliidae infected with *Onchocerca*. In the UK host/parasite

association, *Simulium ornatum/ O. lienalis*, we have demonstrated that the process of vitellogenesis is affected by infection. Larvae or pupae were collected from local streams and reared to adults in the laboratory. 3-4 day-old females were blood-fed artificially through chick skin membranes. Flies were immediately infected with 20 microfilariae suspended in 199 medium, by intrathoracic injection, sham injected or left as controls. Ovarian protein sequestration was found to increase rapidly within 24h post blood-feeding. Ovaries from infected flies had sequestered significantly less protein than uninfected flies at 24, 34 and 50h post blood-feeding. Sham injections had no effect on protein uptake. Ovarian protein profiles, obtained by SDS PAGE, showed that simulium vitellin consisted of two subunits of 200kDa and 68kDa. Both of these subunits were depleted in ovaries from infected flies. Fat body synthesis and secretion of vitellogenin during a 1h *in vitro* incubation was significantly reduced by infection at 8 and 24h post blood-feeding. Immunoassays are being developed to further probe the mechanisms underlying fecundity depletion in this association.

128 ANALYSIS OF BINDING OF MONOCLONAL ANTIBODY TO A MALARIAL PEPTIDE BY SURFACE-PLASMON RESONANCE BIOSENSOR AND INTEGRATED RATE EQUATIONS. Wohlhueter RM*, Parek K, Udhayakumar V, Fang S, and Lal AA. Scientific Resources Program, National Center of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA; and Division of Parasitic Diseases, National Center of Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Using biosensor technology and integrated rate equations, we have developed procedures to determine kinetic parameters and binding affinities of antigen-antibody interactions. The peptide used in these studies represented the major B-cell epitope of the circumsporozite protein of *Plasmodium falciparum*, a promising vaccine candidate antigen. Measurements of association and dissociation rate constants of this peptide with the monoclonal antibody 2A10 were determined by fitting integrated rate equations to binding data. We examined two questions: first, whether accurate estimates of initial velocity and final equilibrium levels of binding of antibody to peptides can be obtained; and second, do kinetic rates and equilibrium constants obtained with systematic variation of the experimental parameters conform to a simple, bimolecular binding model? We found that initial velocity of association with antibody concentration reveals that the reaction rate varies linearly with antibody concentration, but it is not strictly first order with respect to peptide concentration. Using a series of four sensor cells with different peptide loads, the measured initial rates of association (at a constant antibody concentration) suggest that the reaction is nearly independent of peptide loading. Equilibrium analysis from the several experiments yields a range of values of association constants of 0.27 to 8.8 x 10^{-8} M. The method supports a critical examination of the assumptions on which the binding models are based, and suggests a systematic approach to refining such models. The procedure constitutes a simple approach to assessing antibody affinities in vaccine development, epitope mapping, and other immunochemical experiments.

129 EFFECTOR MECHANISMS OF HUMAN T CELL CLONES SPECIFIC FOR THE *PLASMODIUM FALCIPARUM* CS TH/TC EPITOPE. Moreno A*, Xu SG, Levi A, and Nardin E. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY.

Murine CD4+ T cells can be divided in two subsets based on functional activity and lymphokine production. In the rodent malaria model, CD4+ T cells are involved in protective immune response to the preerythrocytic stage of the malaria parasite, that is mediated by lymphokines, such as IFN-γ and TNF. These lymphokines are able to inhibit the development of exoerythrocytic forms (EEF) within the liver cells *in vitro* and *in vivo*. In addition, direct killing of infected hepatocytes by cytotoxic CD4+ T cells has also been suggested. Recently, we described the characterization and fine specificity of a series of human CD4+ T cell clones specific for a novel epitope in the*Plasmodium falciparum* CS protein. The epitope contained in the amino acid sequence 326-345 of the NF54 isolate, is unique in the sense that it is recognized by both cytotoxic and non-cytotoxic class II restricted CD4+T

cell clones, derived from sporozoite-immunized volunteers. In the present report we characterized the lymphokine profile of different peptide-specific T cell clones, by RT-PCR and ELISA. The T cell clones were found to be Th0, in the fact that they produced lymphokines characteristic of both subtypes of Th cells. Supernatants from peptide specific T cells were able to inhibit the intracellular development of EEF in different human cell lines. Inhibition of development was independent of the lytic mechanism and could be blocked by neutralizing antibodies against different lymphokines. The mechanism and *in vivo* relevance of this process will be discussed.

130 MONOCLONAL ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITE SURFACE PROTEIN 2 IDENTIFY THREE DISTINCT B CELL EPITOPES. Charoenvit Y*, Rogers WO, Fallarme V, Paul C, Yuan L, Kaur M, Aguiar CJ, de la Vega P, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

In previous studies we demonstrated that mice immunized with recombinant *Plasmodium falciparum* sporozoite surface protein 2 (PfSSP2) developed antibodies that reacted with *P. falciparum* sporozoites in an immunofluorescence antibody test (IFAT). These antisera identified a 90 kDa protein from *P. falciparum* sporozoite antigen extract in Western blot analysis, and inhibited 40-50% of *P. falciparum* liver stage parasite development in human hepatocyte cultures. In this study, we produced a series of monoclonal antibodies (MAbs) by immunizing mice with recombinant PfSSP2. Three MAbs (PfSSP2.1, PfSSP2.2 and PfSSP2.3) were selected for further characterization on the basis of class and subclass differences. PfSSP2.1 is an IgG1, PfSSP2.2 and PfSSP2.3 are IgG2a and IgM respectively. These MAbs are species specific; they react with *P. falciparum* sporozoites in IFAT, but not with *P. vivax*, *P. yoelii*, or *P. berghei* sporozoites. We synthesized on polypropylene pins overlapping 8 amino acid peptides representing the entire sequence of PfSSP2 and demonstrated that the 3 MAbs recognized distinct 7-11 amino acid epitopes. Work is underway to determine the effects of the Mabs on sporozoite invasion and development of hepatocytes. In addition the peptides recognized by the Mabs are being formulated with T helper epitopes and adjuvants to determine if they will induce in mice polyclonal antibodies that recognize sporozoites and block sporozoite invasion of hepatocytes.

131 INDUCTION OF MURINE CYTOTOXIC T LYMPHOCYTES AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITE SURFACE PROTEIN 2. Wizel B*, Houghten RA, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Johns Hopkins University, Baltimore, MD; and Torrey Pines Institute for Molecular Studies, San Diego, CA.

Mice immunized with P815 mastocytoma cells expressing *Plasmodium yoelii* sporozoite surface protein 2 (PySSP2) are protected against malaria by an immune response that requires CD8+ T cells, and adoptive transfer of CD8+ T cell clones against PySSP2 completely protects against sporozoite induced *P. yoelii* infection. To define CTL epitopes in the *Plasmodium falciparum* sporozoite surface protein 2 (PfSSP2), C57BL/6 mice were immunized with sporozoites or with recombinant PfSSP2 (amino acids 145 to 574) (rPfSSP2) purified by electroelution from preparative SDS-PAGE. Recombinant protein was delivered to mice with Lipofectin (Lpf), a cationic lipid. Class I MHC-restricted cytotoxic activity was detected in mice immunized with irradiated sporozoites and in mice receiving rPfSSP2/Lpf, but not in those who were immunized with rPfSSP2 alone or emulsified in complete Freund's adjuvant. In addition, antibodies against PfSSP2 were detected in sera from immunized animals. Only MHC-matched cells treated with rPfSSP2/Lpf were susceptible to lysis by effectors stimulated *in vitro* with similarly treated syngeneic cells. Using synthetic peptides derived from the PfSSP2 amino acid sequence, two different CD8+ CTL lines were generated from mice immunized with irradiated sporozoites by weekly cycles of stimulation with two different peptides. These results establish the presence of CTL specific to PfSSP2 in mice and provide evidence that recombinant proteins when delivered with cationic lipids induce both humoral and CD8+ T-cell responses.

132 ROLE OF γδ T CELLS IN IMMUNITY AGAINST THE LIVER STAGES OF MALARIA. Tsuji M*, Mombaerts P, Lefrancois L, Nussenzweig RS, Tonegawa S, and Zavala F. Department of Medical and Molecular Parasitology, New York University, New York, NY; Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; and Department of Medicine, University of Connecticut Health Center, Farmington, CT.

The functional role of γδ T cells in infectious diseasesremains largely unknown. To define the possible role of these T cells in immunity against the rodent malaria parasite *Plasmodium yoelii*, we investigated the immune response of αβ T-cell deficient mice, and of γδ T-cell deficient mice. The immunization of αβT-cell deficient mice with irradiated sporozoites induced a protective immune response that significantly inhibited the development of the parasite's liver stages. This protective response was abolished after transient *in vivo* depletion of γδ T cells. Two γδT cell clones were derived from malaria-immunized αβ T-cell deficient mice, and adoptive transfer of one of these γδ T cell clones to normal mice inhibited the development of liver stages upon sporozoite challenge. This protective γδ clone recognizes recombinant heat shock protein 65 of *Mycobacterial bovis* in a major histocompatibility complex unrestricted manner.

133 HUMAN B AND T CELL RESPONSES TO SYNTHETIC PEPTIDES REPRESENTING CONSERVED BLOCK 17 IN THE MEROZOITE SURFACE PROTEIN-1 (MSP-1) OF *PLASMODIUM FALCIPARUM*. Lal AA*, Kern M, Shi Y, Anyona D, Nahlen B, Weiss W, Oloo AJ, Bloland P, Ruebush TK, Udhayakumar V, Campbell CC, AND McCormick J. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease; Kenya Institute of Medical Research, Kenya; and NAMRI-detachment, Kenya.

In the MSP-1 antigen, block 17 has been implicated in providing protective immunity. We have characterized T-and B-cell epitopes within this region using overlapping 20 mer synthetic peptides. Serum samples from adults and children living in western Kenya were analyzed for the presence of antibodies against these peptides. Sera that had an OD more than three SD above the mean for control sera were scored as positive responders. Peptide 102 (CEDSGSSRKKITCECTKPDS) and 97 (CNSGCFRHLDEREECKCLLN) reacted with >50% of the sera. Other peptides reacted with less than 11% of the sera. Twenty-one adults were tested against these peptides for T- cell reactivity in a conventional T-cell proliferation assay. Although 19 of these adults showed significant T-cell reactivity against PPD, only a few individuals displayed reactivity against the MSP-1 block 17 peptides. Taken together, these results identify at least two dominant B-cell epitopes within block 17. Failure to detect any dominant T-cell epitope within this region suggests a need for including T-helper epitopes in the block 17 vaccine constructs. We are following these children in a longitudinal study to determine if antibodies to these B cell-epitopes correlate with protection.

134 IDENTIFICATION OF T AND B CELL EPITOPES ON *PLASMODIUM FALCIPARUM* MSP-1. Parra ME*, Roberts T, Quakyi IA, Berzofsky JA, and Taylor DW. Department of Biology, Georgetown University, Washington, DC; and Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, NCI, NIH, Bethesda, MD.

Identification of T and B cell epitopes from relevant *Plasmodium falciparum* antigens is an important step toward the development of a vaccine for malaria. Among the major problems in the development of a vaccine are that *P. falciparum* antigens frequently undergo antigenic variation and T cell responses are MHC-restricted. The malarial antigen, major merozite surface protein-1 (MSP-1) is a major vaccine candidate. We therefore sought to identify conserved T cell epitopes with minimal MHC-restriction. Five putative T cell epitopes in conserved regions of MSP-1 were identified using

AMPHI. The peptides(~20-22 a.a. long) corresponding to these regions were synthesized.Various B10 congenic strains of different MHC types were immunized with the peptides and anti-malarial responses were tested. Results show that two peptides from the C-terminal region of MSP-1 are of interest. One peptide stimulates T cell proliferation in 6/6 B10 strains and is IA restricted. It is capable of priming for help for Ab production. A second peptide stimulates proliferation of T cells in 4/4 IE^+ B10 strains. Thus, these peptides appear to be universal T cell sites. In addition, one peptide from the N-termial region was identified that has both a T and a B cell epitope. These 3 regions of MSP-1 have potential for inclusion in a vaccine for malaria.

135 LOCATION OF A CONSERVED SUB-DOMINANT T-CELL EPITOPE ON *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 (MSP-1), THAT INDUCES PARASITICIDAL T-CELLS. Quakyi IA*, Currier J, Fell A, Taylor DW, Roberts T, Houghten RA, England RD, Berzofsky JA, Miller LH, and Good MF. Georgetown University, Washington, DC; Queensland Institute of Medical Research, Queensland, Australia; Torrey Pines Institute for Molecular Studies, San Diego, CA.; Metabolism Branch, NCI, and Laboratory of Malaria Research, NIAID, NIH, Bethesda, MD.

The need for a malaria vaccine is now urgent due to widespread single and multidrug resistance of *Plasmodium falciparum*. T cells are crucial forimmunity for malaria, and short peptides (T cell epitopes) from malaria proteins often stimulate human CD4+ T cells and it is thought that thesemay be relevant for vaccine development. To verify whether synthetic peptides representing highly conserved regions of parasite antigens may contribute to a malaria vaccine, we synthesized 24 such peptides which represent 11 of the cloned and sequenced malaria asexual-stage antigens, and which were predicted by 2 algorithms, as reported earlier, to represent T cell epitopes and 6 peptides not predicted to be T cell epitopes. Seven of these peptides were used to generate T cell clones from individuals with an extensive history of malaria exposure. The T cell clones responded vigorously to the peptides but only a single clone which is specific for a peptide within MSP-1, as reported previously, and not previously defined to be a T cell epitope, reacted with malaria parasites. This clone was able to significantly inhibit parasite growth *in vitro*. Because this epitopeis subdominant, peptide immunization may elicit a potentially protective response not generated by exposure to parasite. Our data suggest that few conserved sequences within malaria parasites can be processed from the intact parasite. However, such peptides may be useful candidates for a vaccine aimed at inducing cellular immunity to malaria.

136 INDUCTION OF ANTIBODY RESPONSE BY IN VIVO CROSS-PRIMING USING HETEROLOGOUS *PLASMODIUM FALCIPARUM* MSP1 ALLELES. Hui GS*, Nikaido C, Hashimoto A, and Chang SP. Department of Tropical Medicine, University of Hawaii, Honolulu, HI.

The *Plasmodium falciparum* MSP1 protein exists in several allelic forms with conserved and allelic sequences intervening along the molecule. Hyperimmunization with MSP1 produced antibodies that extensively cross reacted with other MSP1 alleles and inhibited the *in vitro* growth of parasites carrying heterologous MSP1s,suggesting that conserved B epitopes are immunodominant and biologically active. In this study, we investigate the relative importance of allelic vs conserved sequences in inducing T cell help for the production of anti-MSP1 antibodies. Using FUP- and FVO-MSP1 as representatives of two alleles, outbred Swiss mice were primed with a sub-optimal dose of one MSP1 allele and challenged with the heterologous MSP1 antigen. Priming with heterologous MSP1 was as effective as homologous MSP1 in inducing a secondary antibody response to MSP1. Cross-priming with heterologous alleles induced primarily antibodies to conserved epitopes, as indicated by similar ELISA binding with different MSP1 alleles and similar efficiencies of homologous and heterologous MSP1s as inhibitors in competitive binding ELISAs. Cross-priming was also effective in inducing antibodies specific for conformational epitopes at the C-terminal 42 kDa fragment of MSP1. These data suggest that conserved sequence(s) or motifs of MSP1s are effective in

inducing T helper responses for the production of broadly cross reactive anti-MSP1 antibodies and may provide a rational basis for the identification and selection of T-epitopes in MSP1-based vaccines.

137 CRYSTALLOGRAPHIC STUDY OF TRANSMISSION BLOCKING ANTI-MALARIA FAB 4B7 WITH CYCLIC AND LINEAR PEPTIDES FROM THE PFS25 PROTEIN OF *PLASMODIUM FALCIPARUM*. Stura EA*, Kang AS, Stefanko RS, Calvo J, Gaardner KL, Kaslow DC, and Satterthwait AC. Department of Molecular Biology, The Scripps Research Intitute, La Jolla, CA; and Laboratory of Malaria Research, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

X-ray quality crystals of a transmission-blocking monoclonal antibody 4B7 (MAb 4B7) against a sexual stage protein Pfs25 of *Plasmodium falciparum* have been obtained. Both the intact immunoglobulin and an elastase produced Fab fragment have been crystallized both free and complexed with cyclic and linear peptides. While MAb 4B7 binds a linear peptide, cyclic peptides modelled on a predicted β-hairpin loop of the third EGF-like domain of Pfs25 bind better and are readily co-crystallized with the Fab. Several cyclic peptides and their linear counterparts have been synthesized. The affinity of MAb 4B7 for the different peptides varies widely. X-ray data have been collected from various peptide-complexed and free Fab crystals. The packing arrangement of the Fab in three independent crystal forms has been determined by molecular replacement and refinement of the structures is in progress. The genes for the variable domain of the Fab have been cloned, sequenced and the primary amino acid sequence deduced. The three-dimensional structure will aid in an understanding of the mode by which this antibody recognizes and prevents transmission of the parasite. The system presents unique opportunities to understand neutralization from the comparison between the bound forms of differently constrained cyclic peptides and the linear counterparts and from the comparison of the free and antigen-bound MAb. The study is being extended to include the structure determination of Pfs25 and its complex with MAb 4B7.

138 MODULATION OF THE TRANSMISSION OF *PLASMODIUM VIVAX* TO MOSQUITOES BY ANTI-MOSQUITO ANTIBODIES. Ramasamy R*, Ramasamy MS and Srikrishnaraj KA. Division of Life Sciences, Institute of Fundamental Studies, Kandy, Sri Lanka.

Antisera were raised in rabbits against head/thorax, midgut and rest of abdomen tissues of *Anopheles tessellatus*, an established vector of human malaria. The antisera were mixed with erythrocytes infected with *Plasmodium vivax* and fed *in vitro* to female *An. tessellatus* mosquitoes. Significant transmission blocking compared to controls fed on normal rabbit sera was observed in different experiments. IgG purified by ammonium sulphate precipitation followed by DEAE cellulose chromatography from rabbit anti-midgut sera was effective in mediating the reduction of transmission. A significant reduction in the transmission was seen with 125 μg/ml anti-midgut IgG in the ingested blood meal. The numbers of infected mosquitoes were reduced when fed on anti-midgut antibodies but the numbers of oocysts per infected mosquito remained unchanged. The transmission blocking effect was observed in the absence of complement showing that cytolytic effects are not involved in the process. Ingestion of antibodies in a second blood meal also did not enhance the antibody - mediated blocking of the transmission of parasites ingested in the first blood meal.

139 THE LIFE HISTORY AND ULTRASTRUCTURAL FEATURES OF A SPECIES OF *HEPATOZOON* (APICOMPLEXA: ADELEINA) OF THE NORTHERN WATER SNAKE. Smith TG* and Desser SS. Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

The development of a species of *Hepatozoon* of the Northern water snake (*Nerodia sipedon sipedon*) was studied by transmission electron microscopy. Gametogenesis, fertilization and sporogonic development were observed in experimentally infected, laboratory-raised mosquitoes (*Culex pipiens*,

C. territans). The oocysts grew rapidly and, following nuclear division, underwent extensive invagination leading to the formation of sporocysts. Mature oocysts, containing from 500 to 800 sporocysts, were first seen in the hemocoel at about 28 days post-feeding. Each thick-walled sporocyst contained eight sporozoites and a residual body. A crystalloid body in each sporozoite consisted of a dense array of regularly-arranged vesicles. Following ingestion of an infected mosquito by a water snake, sporozoites penetrated the visceral organs, forming meronts which contained a variable number of merozoites. Free merozoites were observed in the bloodstream of a snake 32 days post-inoculation, and appeared at peak levels approximately every 30 days thereafter. The absence of intraerythrocytic gamonts in inoculated snakes indicated that the merozoites do not fully mature in the snake host and that an intermediate vertebrate host, on which the snake feeds, is probably required to complete the life cycle. The possibility of merogonic development of this parasite is being studied in a common prey species, the Northern leopard frog (*Rana pipiens*).

140 ULTRASTRUCTURE OF *CARYOSPORA BIGENETICA* GROWN IN VIPER SPLEEN CELLS. Van Houten R* and Sundermann CA. Department of Zoology & Wildlife Science, Auburn University, AL.

Caryospora bigenetica is a coccidian that utilizes viperine snakes as a primary host and certain mammals as a secondary host. The fine structure of early developmental stages was studied in cultures of viper spleen cells grown at 29C. Cells were inoculated with sporozoites purified from an *in vitro* excystation of oocysts from rattlesnake feces. At various days post-inoculation (DPI), infected monolayers were prepared for transmission electron microscopy using standard procedures. Rhoptries, micronemes, subpellicular microtubles, lipid bodies and amylopectin granules were present in early and late-forming zoites; anterior and posterior refractile bodies were also observed and persisted through 27 DPI. Rhoptries were located anterior to the nucleus and were more numerous than in stages grown at higher temperature (37°C); micronemes were both anterior and posterior to the nucleus. In zoites, amylopectin granules were concentrated around the posterior refractile bodies, and in meronts they were located centrally. From 2-27 DPI, host cells displayed unusual cytoplasmic vacuole development, large accumulations of microtubules, and large membranous whorls. This morphology appeared to result from heavy infection and deterioration of host cells. Overall, the cell structure of the parasite was different from that observed in developmental stages occurring at 37°C; one of the most obvious differences was the persistance of refractile bodies late into development.

141 SCREENING GAMONT-SPECIFIC MONOCLONAL ANTIBODIES USING A CELL CULTURE-ADAPTED STRAIN OF *EIMERIA TENELLA*. Wilson E*, Zhang J, Yang S, and Healey MC. Department of Animal Dairy and Veterinary Science, Utah State University, Logan, UT.

The purpose of this research was to interrupt the life cycle of *Eimeria tenella*, the parasite that causes cecal coccidiosis in chickens, by blocking the fertilization process with a panel of 13 monoclonal antibodies (Mabs) specific for the gamont (sexual) stage of the parasite. The screening of Mabs necessitated the establishment of parasite development and reproduction in a reliable and quantitative cell culture system. Monolayers of primary chicken kidney cells (PCKC) were grown in 24-well tissue culture plates in 89% RPMI, 10% fetal bovine serum, and 1% penicillin/ streptomycin (pH 7.2) at 40.5°C in an atmosphere of 5% CO_2. The monolayers were then incubated with 100,000 sporozoites of *E. tenella* for 24 hours. Medium was then aspirated from each well and fresh media added every other day. On day 7 postinfection the resultant oocysts were harvested and subsequently passaged through chickens. Oocysts were then collected from chicken manure and passaged back into the PCKC system. This process of alternating from cell culture to chickens and back to cell culture was repeated several times. We have now developed a strain of *E. tenella* that is consistently producing over 250% more oocysts in cell culture than the original strain, and over 6 times as many oocysts as

reported by other investigators. This cell culture system is presently being used to screen our panel of Mabs for their ability to inhibit *E. tenella* fertilization *in vitro*.

142 ROLE OF NITRIC OXIDE (NO) IN SUPPRESSION OF LYMPHOCYTE PROLIFERATION DURING *PLASMODIUM CHABAUDI* AS INFECTION IN C57BL/6 MICE. Ahvazi B* and Stevenson MM. Institute of Parasitology and Centre for the Study of Host Resistance, Montreal General Hospital, Montreal, Quebec; and Research Institute, McGill University, Montreal, Quebec.

Following intraperitoneal infection with 10^6 *Plasmodium chabaudi* AS parasitized RBC, C57BL/6 mice develop a moderate level of peak parasitemia and resolve the infection by 28 days. In vitro proliferation of spleen cells from infected mice in response to the mitogens ConA, PHA and LPS was found to be suppressed, with the most severe suppression occurring between days 5 and 21 post infection. Recent evidence suggests that NO, a product of IFN-γ activated macrophages, mediates suppression of lymphocyte proliferation. Therefore, we investigated the role of NO and its relationship to the suppression of spleen cell proliferation in *P. chabaudi* AS infected mice at 7 days post infection. Addition of 0.5 mM N^G-monomethyl-L-arginine (NMMA) or 0.1 mM aminoguanidine (AG), specific inhibitors of NO synthase, partially abrogated the suppression in response to ConA and completely abrogated the suppression in response to PHA. There was also a significant increase in spleen cell proliferation of day 7 infected mice in response to parasite antigen. Coculture of spleen cells from normal mice with peritoneal macrophages from either normal or day 7 infected mice demonstrated that the addition of 5-10 x 10^4 peritoneal macrophages from infected mice significantly suppressed Con A- or PHA-stimulated proliferation of normal splenocytes. Furthermore, suppression correlated with increased macrophage production of NO and could be reversed by the addition of NMMA or AG. These results suggest that increased NO production by macrophages within the first week after infection with *P. chabaudi* AS contributes to immunosuppression associated with blood-stage malaria.

143 DETECTION OF BIOGENIC AMINES IN *BIOMPHALARIA GLABRATA* INFECTED WITH *SCHISTOSOMA MANSONI* USING HPLC-ED. Manger PM*, Li J, Christensen BM, and Yoshino TP. University of Wisconsin-Madison, Madison, Wisconsin.

Previous studies have shown that the reproductive behavior of *Biomphalaria glabrata* snails is adversely affected by infection with *Schistosoma mansoni*. This phenomenon, known as parasitic castration, manifests itself as a downregulation of egg-laying in *B. glabrata* between 14 and 21 days postexposure (PE) to the parasite. Studies using other molluscs have implicated biogenic amines in reproductive behaviors. Sexually mature M-line *B. glabrata* snails were exposed to 15 miracidia of *S. mansoni*. The bioamine levels of *B. glabrata* were monitored over the course of 28 days using high performance liquid chromatography with electrochemical detection (HPLC-ED). Three tissue types were used in this investigation: cerebral ganglia, other ganglia, and cell-free hemolymph (plasma). Samples were collected from exposed and unexposed snails on 7, 14, 21, and 28 days PE. Detection of epinephrine, L-DOPA, norepinephrine, dopamine, and serotonin were made using an oxidative potential (750 mV) of the working electrode. Trends in biogenic amine localization within *B. glabrata* tissues were reflected over the course of infection. Most notably, serotonin, which was present in plasma of uninfected snails, was undetectable in exposed snails by day 7 PE and remained as such through day 28 PE. In addition, L-DOPA and dopamine were sharply downregulated in each tissue type of exposed snails at day 28 PE compared to the control group. Thus, it is hypothesized that larval schistosomes may modulate biogenic amines in the snail, leading to alterations in host feeding behavior, nutritional status, and ultimately disruption of reproductive activities.

144 PERSISTENCE OF IRRADIATED *PLASMODIUM BERGHEI* PARASITES IN THE HOST LIVER AND THEIR POSSIBLE ROLE IN THE INDUCTION OF PROTECTIVE IMMUNITY. Scheller LF* and

Azad AF. Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD.

Immunization with irradiated sporozites (irr-spzs) induces protective immunity against a challenge infection. Here we report the sequence of events that lead to the acquisition of protective immunity in irr-spz immunized animals. Intrahepatic portal inoculation was used to direct spzs into a defined area of the liver. Spzs were irradiated with varying doses of γ-rays and inoculated into animals. Spzs irradiated with 10,000 rads were found to retain their ability to invade hepatocytes although complete schizogony was not observed. In contrast, irr-spzs (20,000 rads) were unable to invade hepatocytes. The quantitative presence of irradiated parasites in the host liver as a function of time, was monitered. Interestingly, spzs irradiated with 10,000 rads were not cleared immediately as reported by others. Although the number of irradiated parasites decreased by 2 fold within the first month post-inoculation, persistent parasites were detected as late as 6 months after primary inoculation. Upon challenge, focal infiltrates were observed in their liver, surrounding parasites resulting from the challenge. In addition to studying the antigenic profile of persisting parasites, immunohistochemical analysis was also performed to identify the nature of the cellular infiltrates. Plausible mechanisms leading to protective immunity in irr-spz immunized animals will be discussed.

145 MECHANISMS OF PARASITE-INDUCED IMMUNOSUPPRESSION IN AN INVERTEBRATE HOST. DeGaffe GH* and Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM.

Excretory-secretory products (ESP) of larval stages of the trematode *Echinostoma paraensei* have adverse effects on the spreading, phagocytic activity and encapsulation of trematode larvae by hemocytes of the host snail, *Biomphalaria glabrata*. To assess the *in vivo* relevance of these effects, the infectivity of different batches of *E. paraensei* parasites for snails was correlated with the ability of ESP derived from these same parasites to prevent hemocyte spreading. A positive correlation between *in vivo* infectivity and efficacy of ESP in the *in vitro* assay was found, suggesting trematode-mediated inhibition of hemocyte function contributes to infectivity. Further studies have compared the vulnerability to ESP of hemocytes from adult and juvenile snails. To simulate the larger volume of hemolymph normally found in adults, greater hemolymph pool volumes from adults than juveniles were utilized in a modified hemocyte spreading assay. Under these conditions, parasite ESP was not as effective against hemocytes from adults as it was against hemocytes from juveniles. However, when the assays were done after equilibrating the number of hemocytes and volume of hemolymph from juvenile and adult snails, no significant difference was seen in hemocyte response to parasite ESP. Our results suggest that the refractory status of adult snails to infection stems from quantitative rather than qualitative differences between the internal defense systems of juvenile and adult snails.

146 PLASMA INTERACTIONS OF SUSCEPTIBLE AND RESISTANT *BIOMPHALARIA GLABRATA* WITH SCHISTOSOME EXCRETORY-SECRETORY PRODUCTS. Davids BJ* and Yoshino TP. University of Wisconsin-Madison, Madison, WI.

Plasma polypeptides may be important in regulating internal defense reactions in the snail *Biomphalaria glabrata*. Previous studies have shown that *Schistosoma mansoni* excretory-secretory products (ES) bind to cellular components of the snail internal defense system and that ES are able to modulate various hemocyte functions. It is not known whether similar binding interactions between plasma and ES also occurs in *B. glabrata*, or whether plasma polypeptides of susceptible (S) and resistant (R) snail strains differentially bind to ES of early larval schistosomes. Plasma was separated by SDS-PAGE under non-reducing conditions and electrotransferred to nitrocellulose. Strips of separated plasma polypeptides were preincubated with glycoproteins or a cocktail of monosaccharides (sugars), and subsequently exposed to biotinylated ES (bES), HRP-labeled streptavidin and

chromogenic substrate. Additionally, bES polypeptides were preexposed to glycoproteins or sugars and applied to strips of separated snail plasma, followed by exposure to HRP-streptavidin and substrate. Only in the R strain did preincubation of blotted snail plasma or parasite ES with mucin affect the plasma-ES binding profile. Preincubation of bES with the glycoproteins fetuin and asialofetuin, partially inhibited ES binding to S and R plasma, whereas no inhibition of ES binding was seen when the same glycoproteins were used to pretreat separated and blotted plasma components. Therefore, these results suggest that plasma associated carbohydrates may serve as receptors for the binding of *S. mansoni* ES.

147 ROLE OF γδ T CELLS IN INTESTINAL INFLAMMATION INDUCED BY *NIPPOSTRONGYLUS BRASILIENSIS*. Barratt RA* and Scott AL. Department of Immunology and Infectious Diseases, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

T cells bearing the γδ TCR may be involved in inflammation induced by diseases such as leprosy, multiple sclerosis, and coeliac disease in humans, and *M. tuberculosis* and *L. monocytogenes* in mice. A rodent hookworm infection *Nippostrongylus brasiliensis* causes inflammation at three epithelial surfaces that are rich in γδ cells. These cells may play an important role in the worm-induced inflammatory response in the murine intestine. Female Balb/c mice were challenged subcutaneously with 600 *N. brasiliensis* infective-stage larvae. Groups of four animals each were dissected at 6,9,11,12 and 14 days post-infection (PI). Their intestines were processed for either plastic or cryostat sectioning and at least 5 complete villi were counted per section. The number of epithelia-associates lymphocytes (EAL) per 100 epithelial cells were enumerated by hematoxylin and eosin staining. The number of CD3+ or γδ+ lamina propria lymphocytes (LPL) per mm^2, and CD3+ or γδ+ EAL per unit perimeter (mm) were counted on immunostained frozen sections. H & E staining showed a decrease in EAL from 7/100 epithelial cells in normal guts to 4/100 epithelial cells in guts sampled at day 6 to 12 PI. Immunostaining also showed that the numbers of CD3_ LPL and CD3_ EAL decreased during days 6 to 12 PI, with a trend towards normal levels by day 14 PI. γδ+ EALs did not decrease significantly from Day 6 to 12 PI, which suggests that γδ+ EAL may constitute a larger percentage of CD3+ EAL present during *N. brasiliensis*-induced inflammation. This modulation in the number and ratio of T cell subsets indicates a possible dynamic rose of γδ T cells in gastrointestinal immunity against *N. brasiliensis*.

148 MODULATION OF COMPLEMENT RESISTANCE AND VIRULENCE OF *NAEGLERIA FOWLERI* AMOEBAE BY ALTERATIONS IN GROWTH MEDIUM. Toney DM* and Marciano-Cabral FM. Virginia Commonwealth University/Medical College of Virginia, Richmond, VA.

Highly pathogenic, mouse-passaged *Naegleria fowleri* amoebae are complement-resistant. Prolonged growth of *N. fowleri* in axenic culture results in a decrease in virulence and concomitant loss in resistance to complement lysis. Passage of *N. fowleri* through mice restores virulence to the amoebae and resistance to complement lysis. The present study evaluates the susceptibility of highly pathogenic *N. fowleri* to complement lysis and the loss of virulence of the amoebae after growth in different axenic media. Pathogenic *N. fowleri* maintained in enriched growth medium are highly virulent for mice and resistant to complement lysis. In contrast, growth of these amoebae in minimal medium results in increase susceptibility to complement and a decreased virulence for mice. Complement-sensitive *N. fowleri* grown in minimal medium can be rendered complement-resistant by shifting he amoebae to growth in enriched medium for 2 hours prior to addition of serum complement. Inhibition of protein synthesis with cycloheximide blocks the ability of the amoebae to become complement-resistant following a shift to growth in enriched medium. Metabolic labeling was used to identify proteins synthesized by *N. fowleri* following a shift in growth from minimal to enriched medium. Polyclonal antiserum to these proteins was used in an *in vitro* lytic assay to determine whether specific antibodies are capable of increasing susceptibility of complement-resistant

N. fowleri to complement lysis. Preincubation of amoebae with antiserum to a 42-44 kDa polypeptide (GP42-44) followed by addition of complement resulted in a modest increase in the susceptibility of *N. fowleri* to complement lysis. The role of GP42-44 and other proteins in modulating virulence and resistance to complement in being investigated.

149 THE ROLE OF CD4+ T CELLS IN THE EXPANSION OF THE SPLENIC γδ T CELL SUBSET DURING MALARIA. van der Heyde HC*, Manning DD, and Weidanz WP. University of Wisconsin-Madison, Department of Medical Microbiology and Immunology, Madison, WI; and University of Alabama at Birmingham, Department of Medicine, Birmingham, AL.

The previously observed expansion of the splenic γδ T cell subset was examined during the course of murine malaria to determine whether CD4+ T cells are required. Flow cytometric analysis during the course of *Plasmodium chabaudi adami* malaria in both C57BL/6 and BALB/c mice revealed that the maximal number of CD4+ T-cell blasts occurred during the period of ascending parasitemia, whereas the maximal numbers of γδ T cells and blast cells occurred during the period of descending parasitemia. Depletion of CD4+ T cells at the time of initiating the infection prevented both the γδ T-cell subset expansion and the resolution of malaria in immune T cell-reconstituted SCID mice. Transfer of enriched populations of CD4+ cells (>75%) containing <0.9% γδ T cells from immune BALB/c donor to SCID mice led to a population of γδ T cells that constituted 37% of the splenic T cells in the recipients and allowed them to resolve their infections. Transfer of the CD4- fraction did not suppress parasitemia. These results suggest that CD4+ T cells are activated early during the infection and are required for the subsequent expansion of the γδ T-cell population. Furthermore, the maximal γδ T-cell blast response during the period of descending parasitemia and the detection of high levels of these cells only in models that resolved their infections suggest that these cells may function in the resolution of blood-stage malaria.

150 INHIBITION OF *PLASMODIUM FALCIPARUM* GROWTH *IN VITRO* BY HUMAN γδ T CELLS. Elloso MM*, van der Heyde HC, vande Waa JA, and Weidanz WP. University of Wisconsin-Madison, Department of Medical Microbiology and Immunology, Madison, WI; University of Alabama at Birmingham, Department of Medicine, Birmingham, AL.

During malaria, the γδ T cell population expands in the peripheral blood of humans and in the spleens of both humans and mice. However, the function of γδT cells in malaria remains to be determined. It has been suggested by others that these cells may contribute to the immunopathology of malaria. Alternatively, γδT cells may be involved in the resolution of blood stage malaria. To determine whether γδT cells isolated from healthy human subjects can inhibit blood stage *Plasmodium falciparum* growth *in vitro*, we have developed a novel flow cytometric method using hydroethidine, a vital fluorescent dye, which allows erythrocytes containing viable parasites to be distinguished from those containing dead or no parasites. Data obtained by this method, which was confirmed by morphological examination, show that the γδT cell subset can inhibit parasite growth by as much as 45%, whereas control cells of the αβ T cell subset show no significant inhibition. Morever, inhibition of parasite growth by γδT cells was dose-dependent. Additionally, our data demonstrate that intraerythrocytic parasites (rings, trophozoites and schizonts) are not susceptible to inhibition by γδT cells, suggesting that the likely targets are the extracellular merozoites. The fact that γδT cells can recognize targets unrestricted by MHC glycoproteins may allow for the interaction between these cells and merozoites, leading to parasite death *in vivo*.

151 GENETIC VARIATION AND RESISTANCE TO PARASITES: AN EMPIRICAL TEST OF THE CORRELATION. Meagher S*. Mammal Division, Museum of Zoology, University of Michigan, Ann Arbor, MI.

Many authors claim that populations without significant genetic variation are more vulnerable to parasites than are genetically variable populations. Few empirical studies of animals support or refute this supposition, however. I tested whether genetically homogeneous populations display higher levels of parasitism by comparing the prevalence of a liver-inhabiting nematode, *Capillaria hepatica*, among mainland and island populations of the deer mouse, *Peromyscus maniculatus*. Among these host populations, the prevalence of *C. hepatica* is negatively correlated with average allozyme heterozygosity (Spearman r=-0.72, p=0.039), providing the first support for the predicted correlation from natural populations of obligately sexual hosts. Another factor expected to play a role in parasite prevalence is host population density: high densities may produce high transmission rates and high prevalence of parasitic infection. In these same deer mouse populations, prevalence of *C. hepatica* is positively correlated with host population density (r=0.76, p=0.030). Controlled laboratory infection experiments are required to determine whether the observed pattern of prevalences is due to genetic variability, density or other unmeasured ecological parameters of these populations.

152 ZINC DEFICIENCY AFFECTS *HELIGMOSOMOIDES POLYGYRUS* DURING BOTH PRIMARY AND CHALLENGE INFECTIONS IN MICE. Shi HN*, Scott ME, Stevenson M, and Koski K. Institute of Parasitology, McGill University, Quebec, Canada; Center for Host Resistance, Montreal General Hospital, Montreal, Quebec, Canada; and School of Human Nutrition and Dietetics, McGill University, Quebec, Canada.

BALB/c mice were divided into three dietary groups: zinc sufficient (ZN+, 60 ppm), zinc deficient (ZN-, 1 ppm) or pair-fed (PF). Four weeks after the experiment began, 15 of the 20 mice of each dietary group were infected with 100 *Heligmosomoides polygyrus* larvae. Nine days p.i., 3 infected mice per group were killed to confirm the infectivity of the larvae. The remaining infected mice were treated with pyrantel on days 9 and 14, and reinfected on day 21. The 5 previously uninfected animals per group were given a primary infection as a control for the challenge exposure. Total daily egg production was measured weekly and mice were killed after 4 weeks. Dietary zinc deficiency significantly reduced food intake and growth of mice. During the primary infection, larvae matured to adults more rapidly in the ZN- and PF groups, compared with the ZN+ group. This effect was more pronounced in ZN- mice than PF mice. As infection progressed, consistently more worms were found in ZN- mice than in ZN+ mice. Although the size of female worms was reduced in ZN- mice, egg hatchability was not affected. Results following the challenge infection revealed that no protective immunity was elicited in ZN- mice, whereas partial protection was observed in PF mice, and very good protection occurred in ZN+ mice. This study demonstrates that dietary zinc deficiency has a significant impact on *H. polygyrus* and that calorie restriction has a lesser effect.

153 WILD RATS: A VECTOR OF HANTAVIRUS DISEASE (HVD) IN N. IRELAND?. McKenna P*, Clement J, McCaughey C, and Coyle P. The Belgian Zoonosis Workgroup, Queen Astrid Military Hospital, Brussels, Belgium; and The Department of Microbiology and Immunology, Regional Virus Laboratory, The Royal Victoria Hospital, Belfast, N. Ireland.

Hantavirus Disease (HVD) is an acute febrile illness caused by several virus strains belonging to the recently defined Hantavirus (HV) genus of the family Bunyaviridae. Since to our knowledge, no clinically document cases of HVD have been reported in N. Ireland, a sero-epidemiological study was performed to assess the degree of HV immunity in a group of 627 Northern Irish patients presenting with symptoms suggestive of HVD, plus 100 normal controls. IFA screening for IgG Hantavirus specific antibodies was carried out with a panel of up to 9 different HV antigens. IgM screening was

performed using a commercially available μ-capture ELISA based upon two recombinant HV nucleocapsid antigens: "Asian" serotype *Hantaan* 76-118 (HTN) and "European" serotype *Puumala* (PUU). An overall IFA seropositivility of 2.2% (16/727/ was recorded, with an almost exclusive reaction against a rat-derived R22VP30 strain of another "Asian" serotype called *Seoul*. Sole reliance upon non-rat-derived classic screening antigens: HTN and PUU would have resulted in the detection of only 2/16 (12.5%) of cases in the IgG IFA, and 9/15 (60%) of the cases in IgM ELISA. The majority of the HV seropositive cases had reported sighting wild rats around their dwellings. Screening for leptospirosis (LPS) (a spirochetal illness also transmitted to man via infected rodents) however, appeared negative. Our findings indicate that fro the first time in Europe, non-laboratory outbreaks of HVD may be caused by wild rats acting as a reservoir for a *Seoul*-like Hantavirus. Since both LPS and HVD can be transmitted by the rat and since both clinical pictures are very similar (acute kidney and liver involvement), it seems mandatory to screen such cases not only for LPS, but also for HVD, with a supplementary rat-derived HV antigen such as R22VP30.

154 PREVALENCE OF ANTIBODY TO JUNIN VIRUS IN SMALL MAMMALS OF THE ARGENTINE PAMPA. Mills JN*, Ellis BE, Childs JE, McKee KT, Maiztegui JI, Peters CJ, Ksiazek TG, and Jahrling PB. Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Department of Immunology and Infectious Diseases, Johns Hopkins University, Baltimore, MD; Centers for Disease Control, Atlanta, GA; Preventive Medicine Service, Fort Bragg, NC; and Instituto Nacional de Enfermes Virales Humanes, Pergamino, Argentina.

We completed indirect immunofluorescent antibody screening for antibody to Junin virus on 1112 sera from small mammals captured on two mark-recapture grids in the epidemic area of Argentine hemorrhagic fever. Antibody was found in three species of Cricetid rodents, *Calomys musculinus, C. laucha* and *Bolomys obscurus*; and a small carnivore that preys on rodents, *Galictis cuja*. Most seropositive animals (27/30 individuals) were *C. musculinus* and, over 30 months, the prevalence in this, the primary reservoir species, was 8%. Approximately half of infected individuals simultaneously carried serum antibody and antigen in blood and saliva, some for up to 61 days. Except for *C. laucha*, which was captured in crop habitats, seropositive animals were strongly associated with the relatively rare roadside and fence-line habitats. Seropositive *C. musculinus* were predominantly males in the oldest age classes and heaviest body mass classes, and seropositive males were twice as likely to have body scars as seronegative males. These observations provide additional evidence implicating horizontal transmission as the primary mode of infection and aggressive encounters among large, adult males in relatively densely populated roadside and fence-line habitats as the principal mechanism of transmission of Junin virus within reservoir populations.

155 LASSA FEVER EPIDEMIC IN PLATEAU STATE, NIGERIA - 1993. Rollin P*, Wilson L, Childs J, Peters C, Tomori O, Nasidi A, and Ksiazek T. Centers for Disease Control, Atlanta, GA

In December 1992, a woman living in Plateau State, Nigeria died of a febrile illness identified as Lassa fever. Serosurveys of hospital staff and village occupants were conducted to determine the seroprevalence of Lassa antibody and determine primary routes of transmission. Suspected patients and their contacts were interviewed and sera collected. Rodents were trapped in households to estimate the population density and antibody status of the rodent carriers. Indirect fluorescent antibody tests and IgG and IgM ELISA tests were used to determine Lassa infection. Six of 10 sera from suspected patients contained IgM antibodies, indicating recent infection with Lassa virus and a single Lassa virus isolate was obtained from the serum of one of the IgM positive patients. Lassa antibody prevalence was 21.1% (n-317) among hospital staff and 6.1% (n-196) among village residents. Lassa virus antibody prevalence was 17% (n-53) among contacts of confirmed Lassa patient. Antibody was not detected nor virus isolated from rodents collected in the surveyed villages (79 total, 52 *Mastomys* spp.). Thus, evidence indicated that the epidemic was propagated by person-to-person and nosocomial transmission, rather than rodent-to-human transmission.

156 YELLOW FEVER IN NIGERIA, 1986-1993: CONSIDERATIONS ON EPIDEMIC PREPAREDNESS AND CONTROL. Tomori O*, Nasidi A, and Spiegel R. Department of Virology, University College Hospital, Ibadan, Nigeria; Federal Ministry of Health, Epidemiology Division, Lagos, Nigeria; and CCCD-USAID, Lagos, Nigeria.

A devastating outbreak of yellow fever (YF) occurred in Nigeria between 1986 and 1993. Field investigations, including community and hospital based surveys, as well as laboratory and entomological studies were carried out in response to the reports of the epidemic. The epidemic started in the south eastern zone of the country and was reported in 28 of the 30 states in the country, with many states experiencing annual outbreaks during the period 1986-1993. The officially notified number of cases and deaths were 20,000 and 4,000 respectively. However, epidemiological investigations indicated that morbidity and mortality figures were between X10 and X100 higher than the official figures. In many of the states, majority of the cases and deaths occurred among children below the age of 15 years. A significant entomological finding was the discovery for the first time in continental Africa of breeding populations of *Aedes albopictus*, (the Asian tiger) mosquitoes.

157 EPIDEMIOLOGIC ASPECTS OF A YELLOW FEVER OUTBREAK IN NORTHWEST KENYA, 1992-93. Marfin AA*, Tukei PM, Agata NN, Sanders EG, den Boer JW, Reiter IP, McLean RG, Cropp CB, Moore PS, and Gubler DJ. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado; Virus Research Center, Kenya Medical Research Institute, Nairobi, Kenya; and Kenya Ministry of Health, Nairobi, Kenya; World Health Organization, Nairobi, Kenya.

Outbreaks of yellow fever (YF), a mosquito-borne viral hemorrhagic disease with case-fatality of 30-50%, have never been reported from Kenya. In September 1992, the Kenyan Ministry of Health identified a cluster of hemorrhagic fever cases in the Kerio River Valley and, with CDC, serologically confirmed two cases of YF. Cases of hemorrhagic fever were identified by medical record review and hospital-based disease surveillance using clinical case definition. 53 persons with hemorrhagic fever were identified from 2 districts of Rift Valley Province (AR: 8.4 per 100,000). Serologic evidence of YF infection was found in 21 of 53 persons; 3 cases were confirmed by YF virus isolation. Earliest onset of illness was September 10, 1992, and the latest was February 18, 1993. Of 53 persons, 28 (53%) were 10 to 29 years old and 36 (68%) were males. All confirmed hemorrhagic YF cases lived in remote areas within dense forest and brushlands. *Aedes africanus* was the predominant mosquito species captured near homes of these cases; no *Aedes aegypti* were found. A review of hospital records from 1990-91 suggested that YF may have occurred but was not recognized. This is the first documented YF outbreak in Kenya and resulted from transmission by sylvatic mosquitoes. Mass vaccination was initiated to control this outbreak because efforts to reduce mosquito larval habitats and peridomestic pesticide application would have little effect. Although there is no evidence of urban transmission, surveillance should be strengthened to detect the early emergence of an urban epidemic.

158 YELLOW FEVER IN THE KERIO VALLEY, RIFT VALLEY PROVINCE, KENYA, 1992-93: ENTOMOLOGICAL INVESTIGATIONS. Reiter P*, Cordellier R, Ouma J, Tukei PM, Okelo GB, Agata N, Cherogony SC, Marfin AA, Cropp CB, Savage HM, McLean RG, and Gubler DJ. CDC Dengue Laboratories, Division of Vector-borne Infectious Diseases, San Juan, Puerto Rico; Institut Français de Recherche Scientifique pour le Développement en Coopération; Ministry of Health, Division of Vector-borne Diseases, Nairobi, Kenya; Kenya Medical Research Institute, Nairobi, Kenya; Headquarters, Ministry of Health, Nairobi, Kenya; Provincial Medical Headquarters, Kabarnet, Kenya; CDC, and Division of Vector-borne Infectious Diseases, Fort Collins, CO.

An outbreak of Yellow Fever (YF) in south Kerio Valley, Kenya, the first recorded in that country, was reported in September 1992, peaked in January 1993 and ended in February. The ecology of the valley

is complex, from semi-arid thorn scrub and acacia savannah below 1200m to pine forest at 2400 m. Monkeys of several species occur throughout. Most confirmed cases lived in wooded areas between 1500 m and 1920 m. We made human bait collections (March 1993) in (i) non-thorny bush/woodland, associated with the majority of confirmed cases; (ii) gallery woodland on the semi-arid floor of the valley, associated with early cases; (iii) banana orchard/thornbush thicket. In the non-thorny bush/woodland *Aedes africanus* was often abundant, and by far the most commonly collected species, although some sites yielded significant numbers of *Ae. ingrami* and *Ae. keniensis*. In the discontinuous gallery woodland *Ae. luteocephalus, Ae. metallicus* and *Ae. vittatus*, all implicated as YF vectors in semi-arid or arid regions of the continent, were present. *Ae. bromeliae*, an important peri-domestic YF vector, was collected in the thornbush thicket but not in banana orchards. To date, we have isolated yellow fever virus from *Ae. africanus* (1 pool) and *Ae. keniensis* (2 pools). Water storage is not practiced in the area; domestic vectors were rare and there was no indication of domestic transmission. We conclude that this was a sylvatic outbreak in which human cases were directly linked to an epizootic and were independent of each other. The epidemiologic data fully corroborate this interpretation. There is an urgent need to identify potential sylvatic corridors that could conduct the enzootic from the Kerio Valley to adjacent areas where water storage practices support domestic vectors and the human population has not been vaccinated.

159 NATURAL VERTEBRATE HOSTS IN THE TICK-BORNE ENCEPHALITIS VIRUS TRANSMISSION CYCLE: AMPLIFICATION OF INFECTION PREVALENCE BY NONVIREMIC TRANSMISSION. Labuda M*, Kozuch O, Eleckova E, Zuffova E, and Nuttall PA. Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia; and NERC Institute of Virology and Environmental Microbiology, Oxford, U.K.

Tick-borne encephalitis (TBE) is the most important human arboviral disease in Europe with TBE virus activity permanently high in some countries (e.g. Austria), and apparently on the decline in others (e.g. Slovakia). From blood and target organs of more than 6,000 small terrestrial mammals live-trapped in selected territories of Central Europe during 1964 to 1991, 48 TBE virus isolates were recovered; 20 isolates were obtained from *Apodemus flavicollis* and 22 from *Clethrionomys glareolus* species. About 15% of these abundant rodent species had neutralizing antibodies to TBE virus. The field collected data raise the guestion: how do these rodent species support the spread of infection into newly feeding ticks? Laboratory experiments were designed to mimick natural conditions of virus transmission by allowing infected and uninfected *Ixodes ricinus* ticks to feed together on uninfected hosts. The greatest numbers of infected ticks were obtained from *Apodemus* field mice, even though they had undetectable or very low levels of viremia. In contrast, bank voles (*C. glareolus*) and pine voles (*Pitymys subterraneus*) developed substantial levels of viremia but gave rise to 4- to 5-times fewer infected ticks compared with field mice. The results suggest that *Apodemus* mice are the most important amplifying hosts of TBE virus and "nonviremic" transmission is an important mechanism for the survival of TBE virus in nature.

160 HOST-PARASITE BIODIVERSITY: THE INTERFACE OF FIELD PARASITOLOGY, SYSTEMATICS, AND MAMMALOGY. Gardner SL*. Department of Nematology, University of California, Davis, CA.

From 1984-1993, approximately 10,000 mammals and their parasites have been collected from throughout Bolivia. Techniques of field parasitology-mammalogy developed during the period of this study have been applied to other disciplines such as botany, herpetology, and ornithology. During our field studies and subsequent laboratory analyses, the highest priority continues to be the acquisition and tracking of accurate data. These data can be used for studies from the level of molecular phylogenetics through ecosystem ecology. Accurate logging of habitat types through the use of field notes, photographs, and now video tape, and digital photography is extremely important for documentation and archival storage of data. In the field, specimens are collected, habitat noted,

associated symbionts collected, and the data are transferred to a growing computerized database. Accurate identifications of the hosts of the symbionts that are collected is requisite before any "real" taxonomic or systematic-ecological interpretation can be made of the symbionts themselves. All hosts or symbiotypes must be sent to recognized museums to maintain the integrity of the data. We have found that a well organized field-party with carefully assigned tasks is necessary to keep the data-stream unambiguous. Data on parasites collected during the past 9 years have been used to test theories of historical ecology, continental drift, biogeography, coevolution, and molecular systematics.

161 NEMATODE DIVERSITY OF NATIVE GRAPE IN CALIFORNIA. Al-Banna L*.

From 1990 through 1992 soil samples taken from the rhizosphere of native species of grapes from four areas of northern California and two areas from Southern California. The objective was to quantify the diversity and relative abundances of species of nematodes associated with these grape roots. Results showed that the native grapes of California harbored many more species than cultivated forms of *Vitis vinifera*. Grouping of the nematode aggregation among all localities was done using both clustering and ordination methods. By performing 3-dimensional ordination analysis, a slightly different view of the community structure of the nematode fauna of the species if the genus *Vitis* was obtained relative to the 2-dimensional view with the UPGMA cluster analysis. Analysis of the data at the levels of species, families, or number of individuals (quantitative analysis) provided different levels of insight in the aggregations of nematodes among the different collections localities. However, analysis based on the presence/absence of species of nematodes provided the most realistic interpretation of the pattern of diversity and composition of the nematode communities among localities. Results of this survey indicate that both species of California Native Grape could act as reservoirs for plant parasitic nematodes that have the potential to infest grapes and other crops of economic importance in California.

162 MICRODISTRIBUTION OF SELECTED GILL PARASITES OF THE SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*. Riekerk G* and Runey M. Marine Resources Research Institute, South Carolina Wildlife and Marine Resources Division, Charleston, SC; and College of Charleston, Charleston, SC.

The microdistribution of three monogenetic trematodes and two copepods was examined from the gill cavity of the spotted seatrout, *Cynoscion nebulosus*. The monogenetic trematode *Diplectanum bilobatum* was the most abundant as well as the most widely distributed within the gill filaments. The preferred microhabitat for this species was the proximal ends of the primary filaments, significantly more were found on the medial surface of the arch. Abundance for this species was significantly higher on the first and fourth arches. *Cynoscionicola heteracantha* was the second most abundant parasite. It was primarily found on the second and third arches, attached to the medial and distal areas of the primary filaments. *Neoheterobothrium cynoscioni* was found on smooth oral surfaces such as the roof of the mouth and the gill arches. Highest abundance within the gill arches occurred on the lateral surface of the first arch. The two copepods, in the genus *Lernanthropus*, were distinguished by size and microhabitat preferences. The larger, sexually mature form, was found most often on the second and third arches, always attached to the inside edge of the primary filament. The smaller form was found more evenly distributed among the arches and was primarily attached to the outside edges of the filaments. Spacial overlap seems to be minimized in the sections of highest abundance. Where overlap occurs, feeding type (blood vs. mucus) differed.

163 TWO-HOST LIFE CYCLE IN THE MYXOSPOREA AND CONSEQUENT TAXONOMIC IMPLICATIONS IN THE MYXOZOA. Kent ML*, Margolis L, and Whitaker DJ. Department of Fisheries & Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada.

ABSTRACTS

The phylum Myxozoa is comprised of two classes, Myxosporea Butschli, 1881 primarily of fishes and the Actinosporea Levine et al., 1980 primarily of aquatic oligochaetes. Until recently the life cycles of these parasites were poorly understood. We conducted transmission studies on *Myxobolus arcticus*, a myxosporean that infects the brain of Pacific salmon *(Oncorhynchus spp.)*. Transmission was achieved when hatchery-reared (in well water) sockeye salmon fry were exposed to the actinosporean spores *(Triactinomyxon sp.)* collected from *Stylodrilus heringianus*, from a lake where M. arcticus infections are common in this fish. We also induced triactinomyxon infections in parasite-free *S. heringianus* by exposure to *M. arcticus* spores. Thus, the life cycle of *M. arcticus* involves transformation into a actinosporean stage in *S. heringianus*. Alternate development of myxosporeans in aquatic oligochaetes has been established or implicated for 10 other species of myxosporeans belonging to 5 genera in 4 families, but this is the first report of alternate development in a lumbriculid worm. There is now sufficient evidence that confirms a two host life cycle within the Myxosporea, which involves transformation into actinosporeans in oligochaetes. These results indicate that the class Actinosporea should be suppressed in favor of the older taxon Myxosporea. To date, all myxosporean that have been shown to have actinosporean developmental stages belong to genera that predate the corresponding actinosporean genera and, therefore, would take precedence in nomenclature.

164 A NEW SPECIES OF GYMNOPHALLOIDES (TREMATODA: GYMNOPHALLIDAE) FROM HUMANS IN KOREA. Lee SH*, Chai JY, Hong ST, and Choi MH. Department of Parasitology, Seoul National University College of Medicine, Seoul, Korea.

A new species of *Gymnophalloides* is reported as a new intestinal trematode of humans in Korea. The first case of human infection was found from a seashore village of southwestern part of Korea in 1988, whose clinical impression was acute pancreatitis. Further human infections were found from the same and nearby villages. An epidemiological survey showed that fecal egg positive rate among the inhabitants was 49.0%, and worm burden by individual 1-26,373 (median 850, average 3,119). Oysters, *Crassostrea gigas*, which were collected from the locality were found to carry the metacercariae. The number of the larval trematodes per the oyster varied 2-4,792 (average 610). Metacercariae of were 0.31-0.39 (average 0.346) x 0.20-0.26 (average 0.228) mm, pyriform, and not encysted. Adult worms from humans or experimental mice were 0.40-0.49 (average 0.433) x 0.27-0.31 (average 0.294) mm. They were equipped with a well-developed muscular oral sucker, slit-shaped ventral pit, small ventral sucker, inconspicuous genital pore, two compact masses of vitelline glands, and V-shaped excretory bladder. The eggs were 0.022-0.026 (average 0.023) x 0.012-0.016 (average 0.014) mm, and elliptical in shape. The egg shell was thin and transparent. Since *G. tokiensis* Fujita, 1925 is the only species in the genus *Gymnophalloides*, which was described only by its metacercarial morphology, we hereby report the worms we saw as a new species based on the morphology of both metacercariae and adults.

165 A NEW SPECIES OF CESTODE IN *UROTRYGON CHILENSIS* FROM THE GULF OF NICOYA, COSTA RICA. Berman RL*, Brooks DR. University of Toronto, Department of Zoology, Toronto, Ontario.

Cestodes collected in spiral valves of specimens of the stingray *Urotrygon chilensis*, from the Pacific coast of Costa Rica, represent an undescribed species of Tetraphyllidea. Current taxonomy subdivides tetraphyllideans into the monophyletic Onchobothriidae, diagnosed by hooks on the scolices of adult worms, and the paraphyletic Phyllobothriidae, characterized by the plesiomorphic absence of hooks. Phyllobothriids comprise species with apical suckers and no bothridial loculi and species with bothridial loculi and no apical suckers. The first group, diagnosed by symplesiomorphies, is paraphyletic; the second group may or may not be monophyletic, depending on the homology of the bothridial loculi. There has been no evidence supporting any hypothesis of relationship between the two "groups" of phyllobothriids. By possessing the apomorphic bothridial loculi as well as the

plesiomorphic apical sucker on each bothridium, the new species may indicate such a relationship. The new species also possesses globular structures irregularly arranged on the surface of the bothridia. We found similar structures on the bothridial faces of *Trilocularia acanthiaevulgaris*, possibly indicating close phylogenetic relationships with the new species. This possibility is enhanced by the new observation that the bothridia of *T. acanthiaevulgaris* comprise two loculi and an apical sucker, rather than three loculi.

166 SPECIFICITY IN THE GREGARINE ASSEMBLAGE PARASITIZING *TENEBRIO MOLITOR*. Clopton RE*. School of Biological Sciences, University of Nebraska, Lincoln, NE.

Host-specific exsporulation responses, long-term viability, and pre-infective development time in the oocysts of *Gregarina niphandrodes*, *Gregarina cuneata*, and *Gregarina polymorpha* were examined using an experimental exsporulation protocol. No difference was observed in exsporulation response that would suggest exsporulation specificity among homologous and heterologous host-parasite combinations. No pre-infective period was observed for any of the 3 parasite species examined. Exsporulation assays demonstrate that the oocysts of all 3 parasite species remain viable for at least 728 days. Host- stadium specific infection and establishment responses have been previously demonstrated in these species. Disparate developmental niches (along temperature and humidity axes) that are associated with host use have also been defined. In combination with previous studies, these data suggest that although host-specificity may be maintained across several ontogenetic stages in a parasitic life cycle, sufficient flexibility may be maintained to facilitate host transfer and subsequent host capture events.

167 OBSERVATIONS ON THE LIFE CYCLE STAGES OF A NUMBER OF *DIPLOSTOMUM* SPECIES MAINTAINED IN THE LABORATORY. Irwin SW*, McKeown CA, and Field JS. Department of Biological and Biomedical Sciences, University of Ulster, Northern Ireland.

Metacercariae of *Diplostomum* species occur in the eyes of fresh water fish. As they are larval forms, devoid of useful diagnostic features such as reproductive organs, difficulty arises when attempting to distinguish between species. In this study naturally occurring metacercariae from the eyes of wild and farmed fish were provisionally identified using a key devised by A.A. Shigin in 1986.The life cycle of each 'species' was established under identical conditions in the laboratory. Newly hatched domestic chickens were used as definitive hosts and laboratory reared snails *Lymnaea stagnalis* and Rainbow trout *Oncorhynchus mykiss* acted as intermediate hosts. Utilization of identical species minimised host induced morphological variations at each stage of development. Light and electron microscopy was employed to study the larval and adult forms. The presence of distinct species was confirmed and they differed from one another in terms of the anatomy of the adults, their location in the hosts and their developmental rates.

168 THE EFFECTS OF CONCURRENT *NIPPOSTRONGYLUS BRASILIENSIS* AND *ECHINOSTOMA CAPRONI* INFECTIONS IN GOLDEN HAMSTERS. Huffman JE*, Holben DM, and Fried B. Department of Biological Sciences, East Stroudsburg, University, East Stroudsburg, PA; and Department of Biology, Lafayette College, Easton, PA.

Hetrologous parasitic infections are common in nature. Concurrent infections with *Nippostrongylus brasiliensis* and *Echinostoma caproni* effects the susceptibility of the host to the parasite. Hamsters infected with a primary dose of *N. brasiliensis* demonstrate a significant resistance to *E. caproni* . The dry weight of those trematodes which do establish is also decreased. The recovery of the nematode is unaffected by the presence of *E. caproni* . A Significant increase in the number of lymphatic nodules, eosinophils and neutrophils occurred in infected animals. Adrenal weights were unaffected with a slight increase in splenic weight noted in infected animals. Histologically an increase in intestinal

mast cells, basophils and goblet cell proliferation was accompanied by an increase in muccous in the intestine. The immune response of the hamster to the nematode infection appears to play a role in the expulsion of *E. caproni* .

169 PARASITES OF THE ORECTILOBIFORM SHARK FAMILIES OTHER THAN RHINCODONTIDAE: WHAT HAPPENED TO *PEDIBOTHRIUM*? Caira JN*. Department of Ecology and Environmental Biology, University of Connecticut, Storrs, CT.

According to Dingerkus (1986), the nurse shark family Rhicodontidae is one of only 5 families of sharks in the order Orectilobiformes. The family Rhincodontidae is unusual in that it currently contains only 5 genera of sharks each of which is monotypic. To date 3 of these genera have been examined for onchobothriid cestodes; all 3 have been found to host species of tapeworms belonging to the genus *Pedibothrium*. In the present study representatives of the remaining 4 families in the order Orectilobiformes were surveyed for cestodes to determine the distribution of *Pedibothrium* within this host order. The spiral intestines of 4 to 30 individuals of each of the following orectilobiform sharks were examined: the epaulette shark and the brownbanded bambooshark (both members of the family Hemiscyllidae), the collared carpet shark (family Parascyllidae), both of the known species of blind sharks (family Brachiluridae) and two species of wobbegongs (family Orectilobidae). None of these species of hosts was found to harbor *Pedibothrium*. Species of the onchobothriid genus *Acanthobothrium* were found in all of the sharks species examined except for sharks of the family Hemiscyllidae. The epaulette shark was found to host a new species of proteocephalidean cestode. The onchobothriid genera *Yorkeria* and *Spiniloculus* were found in the brownbanded bambooshark. A species that appears to represent a new genus of phyllobothriid was found in one of the species of blind sharks. It would appear that *Pedibothrium* is restricted to a single family of orectilobiform sharks.

170 THE TAXONOMY AND SYSTEMATICS OF NON-HOOKED TETRAPHYLLIDEANS: A SPECULATIVE JOURNEY INTO THE REALM OF THE UNKNOWN. Ruhnke TR* and Jacob BA. Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs CT.

In 1986, Schmidt compiled a taxonomic account of species in the Tetraphyllidea. He recognized four families (Onchobothriidae, Phyllobothriidae, Triloculariidae and Cathetocephalidae) containing 47 genera and 296 species in the order. Since 1986, the family Cathetocephalidae has been accorded ordinal status. Evidence exists for the monophyly of only one of the remaining families, the Onchobothriidae, or hooked tetraphyllideans, consisting of 14 genera and 120 species. The monophyly of the Trilocullariidae and Phyllobothriidae should be considered suspect because they appear to be "not-A" groups sensu Eldredge and Cracraft (1980), relative to the onchobothriids. Our current understanding of the taxonomy and systematics of trilocullariids and phyllobothriids is poor. The Trilocullariidae contains 3 monotypic genera. The Phyllobothriidae contains 171 species in 27 genera, 13 of which are monotypic. Eight of the 27 phyllobothriid genera are systematically enigmatic. The genera *Anthobothrium* and *Phyllobothrium* should be considered taxonomic wastebaskets. Hayden and Campbell (1981) suggested that the species in 3 of the remaining genera (*Rhinebothrium, Caulobothrium* and *Rhabdotobothrium*) are actually congeners. Some evidence suggests that *Psuedanthobothrium, Phormobothrium, Clydonobothrium, Echeneibothrium* and even the trilocullariid *Zyxibothrium* comprise another group. At the present time, the other 4 genera and the newly erected *Clistobothrium* and *Paraorygmatobothrium* should be regarded as valid. No explicit phylogenetic scheme exists for the relationships among the phyllobothriid genera.

171 LIFE CYCLE OF *GNATHOSTOMA BINUCLEATUM* ALMEYDA-ARTIGAS, 1991 (NEMATODA), ONLY KNOWN CAUSAL AGENT OF HUMAN GNATHOSTOMIASIS IN MEXICO. Almeyda-Artigas RJ*, Alcolea-Herrera E, Mosqueda-Cabrera MA, and Saldana-Martinez G.

Laboratorio de Sanidad Acuicola, Department El Hombre y su Ambiente, Universidad Autonoma Metropolitana-Xochimilco, Mexico.

Recently, it was demonstrated that the third-stage larva (L_3) described in 1989 from cichlid fishes of Temascal dam, Oaxaca belongs to *Gnathostoma binucleatum*. It differs from its closed related form, *G. spinigerum*, essentially in: a) L_1 length: 194-256 μ (231) *vs.* 255-286 (269); b) L_2 cephalic bulb hooklets: I- 37-41 (38.3), 40-47 (43.2); II- 39-44 (41.7), 37-49 (44.8); III- 41-47 (44.1), 42-52 (46.7); IV- 44-53 (47.9), 48-58 (52.3); c) L_2 IV-I: 9.7, 9.1; d) L_3 cephalic bulb hooklets: I- 35-44 (38.7), 39-49 (44.3); II- 38-47 (42.4), 42-54 (47.3); III- 40-49 (44.7), 45-56 (49.6); IV- 43-52 (48.2), 45-58 (52.0); e) L_3 IV-I: 9.5, 7.7; f) adult corporal cuticular spine dentition, density, and arrangement. It is the fifth species known to occur (together with *G. turgidum*, *G. americanum*, *G. procyonis*, and *G. miyazakii*) in the New World. Four cyclopoid copepod species serve as experimental first intermediate hosts (*Eucyclops agilis*, *E. macrurus*, *Mesocyclops leuckarti*, and *M. edax*); members of the five classes of vertebrates as second intermediate; ocelots and feral cats as natural definitive hosts. It is the fourth confirmed species as the causal agent of human gnathostomiasis (together with *G. spinigerum*, *G. doloresi*, and *G. nipponicum*), common in lower Papaloapan river basin inhabitants, discovered in Mexico 28 years ago. Raw fish flesh consumption, mostly of *Oreochromis* spp. and *Gobiomorus dormitor* as "cebiche", produces man infection due to the fixed habit in the area.

172 HIRUDINEAN PHYLOGENY: IMPLICATIONS FOR THE EVOLUTION OF ECTOPARASITISM AND VECTOROLOGY. Siddall ME*, Burreson EM, and Desser SS. Department of Zoology, University of Toronto, Toronto, Ontario, Canada; and Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA.

A phylogenetic analysis of the leeches, a largely ectoparasitic clade of oligochaetes, was undertaken including representative species of the hirudiniform, erpobdelliform, piscicolid and glossophoniid leech types. In addition to other intermediate taxa, the type-species of type-genera of each hirudinoidean subfamily listed in recent leech classifications were examined. Character analysis revealed that transformations of internal reproductive anatomy and jaw morphology characterizes the major phenotypic evolutionary events in the arhynchobdellid lineage, whereas changes in coelomic architecture have occurred throughout the macroevolutionary history of the rhynchobdellids. The evolution of sanguivorous habits remains complex, having been adopted and abandoned a number of times during the evolution of leeches. The hypothesis that the evolution of the rhynchobdellid intestinal blood sinus was significant in facilitating transmission of blood parasites is presented. Moreover, this phylogeny represents a necessary first-step in the investigation of co-speciation between leeches and the haemogregarines and trypanosomes that the rhynchobdellids vector.

173 DIFFERENTIATION OF STRONGYLE EGGS FROM CATTLE FECES USING GENUS SPECIFIC DNA PROBES. Christensen CM*, Gasbarre LC, Zarlenga DS. Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Biosystematics Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

The purpose of this work was to identify and characterize DNA sequences for members of four common cattle nematode genera. Such sequences might be used with DNA hybridization techniques for the semi-quantitative identification of morphologically similar parasite eggs excreted in feces. Genomic DNA libraries were developed from *Ostertagia ostertagi*, *Haemonchus placei*, *Cooperia oncophora*, and *Oesophagastomum radiatum*. Sublibraries were screened with homologus DNA as well as heterologous DNA to identify specific sequences. One clone from each parasite was chosen and the specificity verified by Southern blot and dot blot analysis. Clones were further tested for specificity within their own genus. Strongyle egg DNA was blotted on nylon membranes and screened with radiolabelled cloned DNA probes. The probes were found to differentially hybridize to

eg DNA from the homologous genus, and thus, may be used diagnosticaly to identify strongyle eggs isolated from cattle feces.

174 LYME BORRELIOSIS SURVEILLANCE IN THE UNITED STATES. Dennis DT*, Ettestad PJ, Campbell GL, and Craven RB. Centers for Disease Control, NCID, Division of Vector-Borne Infectious Diseases, Fort Collins, CO.

Surveillance for Lyme disease (LD) was initiated by the Centers for Disease Control and Prevention (CDC) in 1982, and in 1990, the Council of State and Territorial Epidemiologists (CSTE) approved are solution making LD nationally reportable. Forty-nine states and the District of Columbia now require reporting of LD. The CSTE/CDC case definition for reporting of LD, implemented nationally in 1991, requires the presence of an erythema migrans rash or at least one objective sign of musculoskeletal, neurologic, or cardiovascular disease and laboratory confirmation of infection.During 1991, 47 states reported 9,465 cases of LD to CDC; during 1992, 45 states reported a provisional total of 9,677 cases,representing a 19-fold increase over the 497 cases reported by 11 states in 1982. Enzootic cycles of *Borrelia burgdorferi*,the causative agent of LD, have been identified in 20 states; these states accounted for 94% of cases reported during 1991-1992.The overall incidence rate of reported LD during 1992 was 3.9 per 100,000 population. During 1992, Connecticut (53.6 cases per 100,000), Wisconsin (10.7), and California (0.8) reported highest rates in the northeast, north central, and Pacific coastal regions, respectively. Rates in some counties in California, Connecticut, Massachusetts, New York, and Wisconsin exceeded 200 cases per 100,000; the incidence was highest in Nantucket County, Massachusetts (449.1). The number of reported cases in Connecticut and Rhode Island increased 48% and 93%, respectively, over 1991. Among 7507 cases analyzed for which patient age was given, the largest numbers were reported for persons aged 0-9 years [1,087(14.5%)], 30-39 years [1,272 (16.9%)], and 40-49 years [1,271(16.9%)]. Of 7,642 cases, 3,770 (49.3%) occurred among males. More than 97% of cases were white.

175 FIELD AND LABORATORY STUDIES OF *BORRELIA BURGDORFERI* IN TICKS USING AN OSPA ANTIGEN CAPTURE ELISA. Burkot TR*, Wirtz RA, Patrican LA, and Piesman J. Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado; Department of Entomology, Walter Reed Army Institute of Research, Washington DC; and Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY.

To facilitate laboratory and field studies on Lyme disease, an outer membrane surface protein (OspA) antigen capture ELISA to detect *Borrelia burgdorferi* in ticks was developed. The ELISA was compared to an IFA for determining infection rates in wild caught *Ixodes scapularis* from Westchester, NY. The ELISA was also used in laboratory based studies to investigate the kinetics of OspA in *I. dammini*. OspA levels were followed from exposure of uninfected larvae to the JD1 strain of *B. burgdorferi* in infected mice through the ticks' development to the adult stage. In the field evaluation, both the IFA and the ELISA found 54% of adult male *I. dammini* to be infected with *B. burgdorferi* with a 90% concordance between the two tests. ELISA results on the transmission dynamics of OspA showed a rapid increase in OspA following repletion, reaching a maximum of 20,000 spirochete antigen equivalents 4-6 weeks later before dropping to less than 5000 after molting. During nymphal blood feeding, OspA antigen rose rapidly from 24 hrs post attachment and reached a maximum amount at 72 hrs post attachment. In the 12 hours immediately prior to detachment, OspA levels dropped significantly. Completion of nymphal feeding led to a rise in OspA antigen to >30,000 spirochete equivalents. OspA levels dropped significantly after the molt to the adult stage.

176 ENHANCED TRANSMISSION OF LYME DISEASE SPIROCHETES BY PARTIALLY REPLETE VECTOR TICKS. Shih CM* and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

To determine how rapidly Lyme disease spirochetes (*Borrelia burgdorferi*) can be transmitted by partially fed vector ticks (*Ixodes dammini*), attached nymphs were removed from their hosts at various intervals post-attachment and subsequently permitted to refeed to repletion on noninfected mice. Ticks deposit Lyme disease spirochetes in the skin of mice mainly after 48 hours of attachment. Those that have been removed from a host within this interval can reattach and commence feeding. Spirochete-infected nymphs that had previously been attached to a host for 24 hours become infectious to other hosts within another day. Noninfected nymphs acquire infection from spirochete-infected hosts within 24 hours of attachment and become infectious to other hosts 3-5 days later. Virtually all ticks transmitted infection when reattaching to a host after first feeding for 2 days. We conclude that partially replete nymphal ticks transmit spirochetal infection more rapidly than do ticks that had never been in contact with a host and that infected ticks become infectious within the same instar.

177 ECOLOGICAL STUDIES OF *IXODES SCAPULARIS* IN GEORGIA: IMPLICATIONS FOR LYME DISEASE EPIDEMIOLOGY IN THE SOUTHEAST. Durden LA* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

The life cycle of *Ixodes scapularis*, a tick vector of the Lyme disease spirochete, was studied in coastal Georgia. Warm summers and cool winters influence the life cycle of *I. scapularis* in the Northeast where overlapping 2-year tick cohorts result in early summer nymphal peaks, followed by late summer larval peaks. Adult *I. scapularis* in this region are most common during the cooler months of autumn, winter, and spring. This life cycle, coupled with feeding by most immature ticks on spirochete-competent white-footed mice, results in an efficient transmission cycle for *Borrelia burgdorferi* in the Northeast. The life cycle of *I. scapularis* in coastal Georgia differs greatly from this scenario. Here, probably as a consequence of the warmer climate, most *I. scapularis* populations complete their life cycle in 1 year with larval peaks either preceeding or approximately coincident with nymphal peaks. Furthermore, population peaks of immatures are not sharply defined with collections of both larvae and nymphs extending from February through November. However, adult *I. scapularis* are again most abundant during the cooler months. Cotton mice and cotton rats appear to be the principal spirochete-competent reservoir hosts for *B. burgdorferi* in Georgia. Although both rodent species are parasitized by immature *I. scapularis*, tick infestations are much lower than on white-footed mice in the Northeast. However, reptiles (notably glass lizards and some species of skinks) are, in general, heavily parasitized by immature *I. scapularis* in Georgia. These reptiles are presumed to be spirochete-incompetent hosts. The epidemiology of Lyme disease in the Southeast will be discussed in relation to these findings.

178 NIDICOLOUS TRANSMISSION OF THE LYME DISEASE SPIROCHETE. Pollack RJ*, Katavolos P, and Spielman A. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The force of transmission of the agent of Lyme disease *Borrelia burgdorferi*, is driven mainly by the intensity of contact between its tick vector, *Ixodes dammini*, and its white-footed mouse reservoir host, *Peromyscus leucopus*. To measure the intensity of vector-host contact, we designed and field-tested mouse nesting boxes that collect host-associated ticks. Ticks that detach from the mice resting within the box become trapped on an adhesive-coated collecting plate below the nesting chamber. We installed 36 nesting boxes in sites of intense tick infestation in coastal Massachusetts and Rhode Island, and monitored the collecting plates monthly from April - November 1992. Of the 442 larval deer ticks collected on the plates, 56.8% (X=6.97, sd=10.61) were engorged; the remainder (X=6.16, sd=9.59) appeared non-fed. Similarly, of the 45 nymphs observed, 66% (X=0.83, sd=1.77) were engorged; the remainder (X=0.42, sd=0.97) appeared non-fed. Ticks deposited within the nest may thereby encounter another suitable host without the necessity of exiting from the nest. Nymphs,

derived from nest-deposited engorged larvae, may attach to other mice visiting the nest during the following spring or summer. Additionally, non-fed nymphs that had detached from mice may parasitize other mice visiting that nest. Either interaction is likely to incease the capacity of the vector to transmit zoonotic agents by intensifying host-vector contact. Thus, mouse nests serve as potential venues for transmission of certain tick-borne pathogens.

179 SPECIES CONCEPTS AND *IXODES* TICKS. Telford SR*. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

A recently published analysis of the status of the northern deer tick, *Ixodes dammini*, illustrates the problems of defining a species in parasite systems. Morphological, karyotypic, and molecular evidence, synthesised with hybridisation data, indicated that the main vector of Lyme disease in the United States is a northern population of *I. scapularis*, the blacklegged ticks (Oliver et al. 1993). Such a comprehensive report was considered to be definitive, and synonymy of the 2 species was recommended. Scrutiny of the presented evidence indicates that such an action is unwarranted. Hybridisation evidence, in particular, was considered to be conclusive. Absence of fertile hybrids is evidence for isolation. But, the converse, often used as evidence for conspecificity, is at most inconclusive (Mayr 1963). At any rate, unpublished evidence documents asymmetrical fertility (Gowan and Oliver 1978) between the 2 ticks. Morphological analyses obscured relevant taxonomic characters, and ignored the possible presence of mixed populations in geographically intermediate sites. Molecular evidence was erroneously interpreted with an insect "clock". Elegant experiments that were not emphasised indicated asymmetry in mate choice, suggesting that a specific mate recognition system (SMRS; Paterson 1980) and thus some isolation has been achieved. These considerations, coupled with behavioral distinctions that reduce the vectorial capacity of *I. scapularis*, suggest that these *Ixodes* species are not conspecific. The biological species concept shall be discussed in the light of recent concepts in evolutionary theory, and related to issues in parasitology and public health entomology.

180 PHYLOGENY OF TWO EMERGING HUMAN PATHOGENIC ARENAVIRUSES FROM SOUTH AMERICA. Gonzalez JP*, Thayu M, and Rico-Hesse R. Institut Franais de Recherche Scientifique pour le Développement en Coopération, Paris, France; and Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT.

There are now five arenaviruses recognized as pathogenic for humans in the Americas: Junín, Machupo, Flexal, Guanarito and SPH114202. In the 1960's, two arenaviruses had been shown to cause disease in humans in the Americas, Junín and Machupo. Flexal virus, isolated in 1975, appeared to be mildly pathogenic for humans, after a laboratory infection. In 1989, Venezuelan hemorrhagic fever, caused by Guanarito virus, was first detected. 105 presumed cases and 26 deaths were reported by 1992 and several strains isolated from humans and rodents. In 1991, two cases, including one fatality, of Brazilian hemorrhagic fever have been documented and one virus strain identified, SPH114202. We studied the molecular epidemiology and origin of these two last emerging arenaviruses by comparing them genetically to others from the Americas. We obtained nucleotide sequences (by primer extension of the viral S-RNA segment) of 8 of the 12 arenaviruses circulating in the Americas, to determine their phylogenies. Guanarito virus and the SPH114202 strains were genetically distinct (approx. 30% of divergence) from other human arenaviruses. This support the hypothesis that theses viruses have been evolving independently in their endemic focus, for some time. Specific diagnostic tools (PCR/oligonucleotide probes) have been developed for clinical and epidemiological studies. These reagents allow for a rapid identification of these viruses, without the need for biological amplicfication.

181 STUDY OF MOLECULAR EPIDEMIOLOGY OF HANTAVIRUS INFECTION IN SMALL MAMMALS BY POLYMERASE CHAIN REACTION. Avsic-Zupanc T*, Poljak M, Lavrencak J, Krystufek B, and Trilar T. Institute of Microbiology, Medical Faculty, Ljubljiana, Slovenia; Natural History Museum of Slovenia, Ljubljana, Slovenia.

Results of previous epidemiological, clinical and serological studies of Hemorrhagic Fever with Renal Syndrome (HFRS) in Slovenia indicated simultaneous circulation of different Hantaviruses. to elucidate the variability of hantaviruses spreading by their natural hosts, small mammals were collected in HFRD foci in Slovenia. Recent advances in the polymerase chain reaction (PRC) technology that allow detection of viral RNA in patient specimens and that proved DNA fragments for restriction endonuclease analysis was applied to study naturally infected animals. RNA was extracted from 92 lung and kidney tissues of seropositive and seronegative small mammals trapped in three locations in Slovenia. For screening, a genus-reactive oligonucleotide primer pair, flanking a 365 bp region of the G2 glycoprotein gene was used. Hantavirus-specific RNA was detected in 10 (83,3%) of 12 seropositive small mammals and in 4 seronegative animal tissues. The PCR products were digested with 5 restriction endonucleases and cleavage patterns were using 3 sets of primers designed from unique sequences of M genomic segment such that each primer set was specific for only one serological type of Hantaviruses. Both typing strategies used in our study revealed coexistence of three hantavirus serotypes in Slovenia: Hantaan, Puumala-like and Dobrava. Hantaan virus was detected in yellow-necked mouse *Glis glis*. Bank voles *Clethronomysglareolus* are the only reservoir of Puumala-like (Bashkiria) hantavirus and Dobrava virus is spread in nature by *A. flavicollis*. The results obtained in our study suggested that PCR is a specific and sensitive method for detecting and characterizing of hantaviruses represents a suitable tool for epidemiological studies of hantavirus infection.

182 EMERGENCE OF A NEW EPIDEMIC/EPIZOOTIC VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN SOUTH AMERICA. Rico-Hesse R*, de Siger J, and Salas R. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Instituto de Investigaciones Veterinarias, Centro Nacional de Invest. Agropecuarias, Maracay, Venezuela; and Instituto Nacional de Higiene "Rafael Rangel", Ministerio de Salud, Caracas, Venezuela.

The last documented epizootic of Venezuelan equine encephalitis (VEE) occurred in Venezuela, in 1973. Since then, only sporadic reports of disease in equines have been made, but no virus for definitive identification has been obtained. We have documented the emergence of a new epidemic/epizootic VEE virus (variety IC) in the State of Trujillo, Venezuela, in Dec. 1992-Jan. 1993. Both equines (26 cases, including 10 deaths) and humans (5 cases) presented with disease, and subclinical infections were prevalent in both groups. Phylogenetic analysis of 4 virus isolates from this outbreak have indicated that this epidemic virus probably originated from an enzootic VEE virus (variety ID) focus in northern South America, confirming previous evolutionary studies. These results point to the need for continued vaccination of equines in these areas, and surveillance and genetic monitoring of enzootic VEE virus foci throughout the Americas.

183 EXPRESSION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS PROTEINS BY RECOMBINANT BACULOVIRUSES. Hodgson LA*, Ludwig GV, Lind CM, and Smith JF. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD.

To study the feasibility of using purified structural proteins of Venezuelan equine encephalitis (VEE) virus as subunit vaccines suitable for human and veterinary use, we developed recombinant baculoviruses capable of high-level expression of these proteins. With cDNA clones as templates, PCR amplification products were produced corresponding to the entire 26S region as well as to the individual sequences encoding C, E3-E2, 6K-E1, and E3-E2-6K-E1. Initiation and termination sequences were included in the PCR primers where appropriate. The amplified DNA was cloned into

the Bam HI and Hind III sites of the pBlueBac III transfer vector (Invitrogen) and transfected with linearized Autographa californica baculovirus DNA into Spodoptera frugiperda (Sf9) cells. Recombinant baculoviruses were isolated and the expressed VEE viral proteins were analyzed antigenically in immunoprecipitation, western blot, and immunofluorescence assays. Based on these assays, the proteolytic processing of precursor polypeptides in SF9 cells appeared normal, and monoclonal antibodies defining multiple epitopes on the capsid, E1 and E2 proteins reacted to high titer with these products. The immunogenicity and protective capacity of these expression products will be discussed.

184 BIOLOGICAL STUDIES ON WEE COMPLEX VIRUSES. Sabattini MS*, Bianchi TI, Aviles G, Daffner J, and Monath TP. Virology Institute "Dr. J.M. Vanella" and SENASA, Argentina; INEVH, Pergamino, Argentina; and OraVax, Inc., Cambridge, MA.

The presence of 3 antigenically distinct viruses or subtypes belonging to the Western equine encephatitis (WEE) complex in Argentina and the periodic occurrence of epizootics raise questions about the biologic relevance of antigenic variation. We experimentally infected mice, guinea pigs, rabbits, chickens and horses and compared mortality, average survival times, virus replication in brain, viremia and antibody (Ab) responses. Results defined three patterns of virulence: (1) viruses that were both neurovirulent and neuroinvasive (WEE McMillan, WEE Cba 87 and WEE CIV180, representing epizootic strains from temperate areas of North and South America); (2) viruses that were neurovirulent but not neuroinvasive [WEE Y62-33 strain from Russia, Highlands J (HJ) virus from North America, a virus that causes rare and sporadic human and equine cases]; (3) viruses that were not neurovirulent [Fort Morgan (FM) virus from North America, Aura virus, and WEE AG80-646, a virus from South America with no known disease association]. Cross-protection studies in adult mice provided unexpected patterns of cross-protection and enhancement of virulence. Immunization with epizootic strains protected animals against the homologous or heterologous epizootic strains. Auras immunized mice were protected against homologous virus but showed enhanced virulence of WEE Cba 87 and CIV 180 epizootic strains. WEE AG80-646 protected animals challenged with WEE Cba 87 but enhanced WEE CIV 180 infection. Taken together, the results raise a challenging set of questions regarding the natural history and interactions of WEE complex viruses with overlapping geographic distributions in the Americas.

185 THE MODE OF NEUROINVASION BY JAPANESE ENCEPHALITIS VIRUS FOLLOWING INTRAPERITONEAL INOCULATION IN MICE. Dubois DR*, Hase T, Summers PL, and Eckels KH. Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

The mode of neuroinvasion of Japanese encephalitis (JE) virus inoculated intraperitoneally (IP) into mice was investigated. Following IP inoculation, mice developed a bimodal viremia. A transient, low-level primary viremia, which originated directly from the inoculum, was detected within 1 h postinoculation (p.i.) and was cleared by 4 h p.i. A more sustained, higher-level secondary viremia, which was believed to originate form virus replicating in peripheral tissues, was detected at 12 h p.i. and was cleared by day 4 p.i. Enhanced neuroinvasion which occurred following a sham intracerebral (sIC) inoculation was effective during the primary viremia but not during the secondary viremia. This indicated that the virus circulating during the primary viremia was responsible for the neuroinvasion. The failure of the nervous system (CNS) was probably due to a host response arising from peripheral viral replication since the same virus was shown to cause fatal encephalitis was inoculated IC into naive adult mice. The present study, therefore, indicates that the "window of opportunity" for invasion of the CNS by JE virus is very brief and that subsequent infection and disease is caused by the virus in the inoculum. In addition, virus infection of the CNS is limited not only the blood brain barrier but also by suppression of viral replication as a result of a host response induced soon after infection.

186 NONVASCULAR DELIVERY OF ST. LOUIS ENCEPHALITIS AND VENEZUELAN EQUINE ENCEPHALITIS VIRUS BY INFECTED MOSQUITOS DURING FEEDING ON A VERTEBRATE HOST. Turell MJ* and Tammarielo RF. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

We determined whether mosquitoes infected with St. Louis encephalitis (SLE) or Venezuaelan equine encephalitis (VEE) virus inoculate infectious particles extravascularly or directly into the vascular system. Mosquitos infected with SLE or VEE virus were allowed to feed on the distal third of the tails of suckling mice. Mice whose tails were amputated at the midpoint within 10 minutes of mosquito feeding had significantly higher survival rates than their siblings whose tails remained intact. Even when tails were amputated 1 to 6 h after SLE virus-infected mosquitoes fed, the median time to death was significantly longer in mice with amputated tails (171 h) than in those mice with intact tails (139 h). The results of this study and a previous one with Rift Valley fever virus indicate that mosquitos inoculate virus extravascularly, rather than directly into the vascular system, when feeding on a vertebrate host. Extravascular, rather than intravascular, delivery of a pathogen by a transmitting mosquito may affect disease pathogenesis, drug therapy, and vaccine efficacy.

187 AN IMMUNOCYTOCHEMICAL STUDY OF THE DISTRIBUTION OF DENGUE VIRUSES IN *AEDES AEGYPTI*. Linthicum KJ*, Platt K, Myint KS, and Lerdthusnee K. Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok Thailand; Departent of Microbiology, Iowa State University, Ames,Iowa; and Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand.

The mechanisms responsible for intra- and interspecific variation in the ability of *Aedes (Stegomyia)* species mosquitoes to become infected with and transmit dengue (DEN) viruses is not well understood. In an attempt to better elucidate these mechanisms an immunocytochemical technique was developed to identify and localize DEN antigen *in situ* in serial paraffin sections of infected *Ae. aegypti*. Mosquitoes were infected by intrathoracic inoculation of virus or by feeding on hanging drops composed of human blood cells, virus suspensions, and sucrose. The DEN 1 and DEN 3 viruses used to infect mosquitoes were originally isolated from human serum. Antigen was visualized with an avidin-biotin-complex technique using an anti-DEN monoclonal antibody (4G2), capable of reacting with all DEN viruses, as the primary antibody, and a biotinylated secondary antibody. Infected mosquitoes were placed in an incubator at 30°C and sampled every other day for 21 days. In parentally infected specimens DEN 3 antigen was first seen at day 7 postinfection (PI) in only a limited portion of fat body tissue localized near the site of inoculation and in the cells of the intussuscepted foregut. Salivary gland infection first appeared at day 10 PI, followed by very extensive infection of all nervous tissue by day 12 PI. Infection of the fat body tissues appeared to subside by day 12 PI. Further studies with different strains and species of mosquitoes are needed to determine routes of virus dissemination to salivary glands.

188 CYTOCHROME OXIDASE ACTIVITY AND CYANIDE-INSENSITIVITY IN *BLASTOCYSTIS HOMINIS* MITOCHONDRIA. Hollebeke NL* and Mayberry LF. Department of Biological Sciences, University of Texas at El Paso.

Blastocystis hominis is an anaerobic protozoan parasite that contains large numbers of mitochondria and cardiolipin, a mitochondrion-associated lipid. It has been suggested that these mitochondria do not contain cytochrome oxidase similar to those found in mammalian mitochondria. Positive staining of mitochondria with Janus Green B, a vital stain, is dependent on the presence of a cytochrome oxidase system. Since we and others have shown *B. hominis* stains positive with Janus

Green B, a paradox in terms of the activity and function of the mitochondria is implied. Cyanide sensitivity analysis completed by this laboratory suggests that *B. hominis* respiratory chain is cyanide-insensitive. Cyanide-insensitivity coupled with the presence of cardiolipin suggests that the mitochondria of *B. hominis* may be a modified cytochrome system perhaps including flavoproteins or quinones. Research is being conducted on superoxide dismutase activity and oxygen consumption of *B. hominis* to determine more precisely the function(s) of the mitochondria in this organism.

189 OVEMB-1: A GENE EXPRESSED BY EMBRYOES OF IMPORTANCE FOR EMBRYOGENESIS IN *ONCHOCERCA VOLVULUS*. Triteeraprapab S*, Richie TR, Neubert T, and Scott AL. Department of Immunology and Infectious Diseases, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD; NAMRU-2, Jarkata, Indonesia; and Howard Hughes Research Institution, University of Washington School of Medicine, Seattle, WA.

Onchocerca volvulus is a filarial nematode causing onchocerciasis which is endemic in Latin America and Africa. Despite recent advances in vector control and chemotherapy, the disease is still the major public health problem causing blindness in much of Africa. One aspect of filarial biology that has received little attention is the reproductive process. A better understanding of embryogenesis will uncover unique aspects of filarial biology that can be used as targets for intervention and control the disease.The gene OVEmb-1 was identified by using polyclonal antibodies against embryonic stages of *O. volvulus* to screen an *O. volvulus* female cDNA expression library. *In situ* hybridization showed that OVEmb-1 mRNA was expressed at high levels in late blastocyst/early morula stage embryos. The level of OVEmv-1 expression diminished in later embryonic stages and microfilariae. The body wall of adults also showed trace amounts of expression. The OVEmb-1 cDNA was subcloned into pMAL-p2 vector and the fusion protein was used to produce the OVEmb-1-specific antibodies. The antibodies recognized a complex of high m.w. proteins (180-220 Kd, 110Kd, 75 and 60Kd.) in extracts of *O. volvulus* adult females. A similar complex of high m.w. proteins were recognized in extracts of *Brugia malayi* and *Brugia pahangi* females. Further studies of the OVEmb-1 gene and gene products should significantly increase the knowledge concerning embryonic development in nematodes. Inhibition of embryogenesis may provide an alternative strategy for blocking the diseases, pathology and transmission of onchocerciasis.

190 PURIFICATION AND CLONING STRATEGIES FOR CYTOCHROME c PEROXIDASE IN *SCHISTOSOMA MANSONI*. Campos EG*, Smith JM, and Prichard RK. Institute of Parasitology, MacDonald College, McGill University, Quebec, Canada.

In mammalian cells, hydrogen peroxide (H_2O_2) is usually detoxified by enzymes such as glutathione peroxidase (GPO) and catalase. The human parasite *Schistosoma mansoni* does not have catalase. Removal of H_2O_2 is dependent on the presence of GPO and cytochrome c peroxidase (CcP). CcP catalyses the reduction of H_2O_2 using cytochrome c as the electron donor. CcP is absent in mammalian tissues and its presence in the parasite may be important as a potential target for chemo or immunotherapy. The objectives of this study are the purification and cloning of CcP in *S. mansoni*. CcP from *S. mansoni* binds to DEAE anion exchanger under low ionic strength. Mitochondrial proteins extracted using the detergent digitonin were separated using FPLC. A protein band with a molecular weight of 33.5 KDa was identified in the fractions containing CcP activity. Investigation of peroxidases sequences sharing homology with yeast CcP has revealed two conserved regions surrounding the distal and proximal histidines which interact with heme in the native proteins. Two DNA primers were synthetized based on these regions. Using the polymerase chain reaction (PCR) these primers were used to amplify the region between them by using cDNA from *S. mansoni* as a template. A product of around 350 nucleotides long was obtained and cloned in a PCR^{tm}-vector. The partial DNA sequence of this clone codes for amino acids sequence which share homology with the conserved region of the distal histidine in peroxidases.

191 MOLECULAR CHARACTERIZATION AND COMPLETE SEQUENCE ANALYSIS OF THE EXTRACHROMOSOMAL DNA ELEMENT IN *NAEGLERIA GRUBERI*. Mullican JC* and Tracy SM. Pathology and Microbiology Department, University of Nebraska Medical Center, Omaha, NE.

Members of the free-living amoebae genus *Naegleria* maintain ribosomal RNA (rRNA) genes exclusively on circular extrachromosomal DNA molecules. These extrachromosomal ribosomal DNA (rDNA) elements range in size from ca. 12-18 kbp depending on the species and are present on the order of 1,000 copies per cell. Our interest is in determining the function(s) the non-rRNA sequence (NRS) plays in the biology of these rDNA elements. To facilitate our studies, we cloned the entire rDNA elements from the pathogenic *N. fowleri*, LEE and the non-pathogenic *N. gruberi*, EG_B termed pFOWL and pGRUB, respectively. We sequenced the complete pGRUB element to provide a fundamental tool to study the molecular biology of this element. Complete sequence analysis of pGRUB revealed a length of 13,978 kbp and an overall G+C content of 40%. The rRNA cistron is approximately 6 kbp in length and has a typical eukaryotic organization (5'-SSU-5.8S-LSU-3'). S-values for the mature rRNA molecules were determined to be 18S, 5.8S-like and 25S by sucrose gradient sedimentation. The 5' ends of the mature rRNAs were determined by alignment to known rRNA sequences and by directly sequencing the RNA using the dideoxy chain termination method. A sequence approximately 450 bp upstream of the 18S gene has 78% identity to an RNA polymerase I promoter consensus sequence. The NRS in pGRUB has revealed a large number of different size repeat sequences. We are sequencing the NRS of pFOWL to determine if it has similar repeat sequences. To date, sequence comparison of the NRSs from pFOWL and pGRUB show little identity to each other.

192 INCREASED SURFACE EXPRESSION OF MHC CLASS I ON *TRYPANOSOMA CRUZI*-INFECTED MURINE CELLS. Stryker GA* and Nickell SP. Department of Immunology & Infectious Diseases, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD.

$CD8^+$ T cells have been implicated in the protective immune response to Trypanosoma cruzi. In order to study this response, we have established short-term $CD8^+$ T cell lines that lyse parasite-infected target cells. $CD8^+$ T cells recognize foreign peptides in association with MHC class I molecules. In order to establish conditioned for the optimal generation of these effectors, we have examined the effect of IFN-γ on the expression of MHC molecules in *T. cruzi*-infected 3T3 fibroblasts and J774 macrophages using FACS analysis. Surprisingly, in the absence of added IFN-γ, we observed a 2-3 fold increase in class I expression by *T. cruzi*-infected cells compared to uninfected cells as measured by log mean fluorescence. This 2-3 fold increase in expression was present in cell cohorts representing several different size classes and was similar in magnitude to that seen in uninfected cultures treated with IFN-γ. Additional treatment of infected cells with IFN-γ did not dramatically increase the already enhanced level of class I expression. Class II was not expressed by either of these cell lines, as has been previously reported. we are currently examining *T. cruzi*-upregulated class I and II expression in other cell types, particularly peritoneal macrophages, Upregulated expression of class I (or class II) by *T. cruzi* infection could be an important factor in both immunity and pathogenesis.

193 CYCLIN DEPENDENT KINASES IN *PLASMODIUM FALCIPARUM*. Wang H*, and Mikkelsen RB. Departments of Radiation Oncology and Microbiology/Immunology, Medical College of Virginia, Richmond, VA.

Cyclin-dependent kinases are key components in the regulation of the eukaryotic cell cycle. The role of $p34^{cdc2}$ (cdk1) in both the G1-S and G2-M cell cycle transitions has been established for all eukaryotes. In contrast, cdk2 has only been found in multicellular organisms and is essential for the G1-S transition in these higher eukaryotes. The activities of cdk1 and cdk2 during the cell cycle are

regulated by association with specific cyclins and phosphorylation on tyrosines and threonines. Using antibodies specific for human cdk1 and cdk2, we have demonstrated the presence of both kinases in erythrocytic stages of *Plasmodium falciparum* by Western blot analysis and by metabolic labeling followed by affinity purification. Relative levels of cdk1 and cdk2 do not fluctuate with parasite maturation when monitored with cultures synchronized by sorbitol lysis. In contrast, tyrosine phosphorylation of both kinases as followed with a phosphotyrosine specific monoclonal antibody increases dramatically from ring to trophozoite stage and subsequently decreases in schizont stage. The significance of cdk2 in a unicellular organism such as *P. falciparum* is unclear but may reflect the adaptation of the parasite to the human host.

194 MYXOZOAN PHYLOGENY DETERMINED BY DNA SEQUENCE ANALYSIS OF THE 18S RIBOSOMAL RNA GENE. Smothers JF*, Smith LH, and Spall RD. Department of Biological Sciences, Idaho State University, Pocatello, ID.

Myxozoans are oligocellular parasitic organisms of marine and freshwater fish and some amphibians. Little is known about the phylogeny of these organisms or the degree to which different taxa within their Class are related to one another. Phylogenetic studies that employ comparative DNA nucleotide sequence analysis are robust on both theoretical and empirical grounds and have become a widely accepted method for the determination of phylogenetic relatedness among different taxa. Partial sequences of the 18s ribosomal RNA gene have been used extensively in such comparisons. We used the polymerase chain reaction (PCR) and site-directed primers to amplify and sequence the 18s rRNA gene from each of five species of myxozoans. PCR-amplified gene products were subcloned into the pCR™ plasmid (Invitrogen, Inc.) and nested deletions then generated by Exonuclease III digestion (Pharmacia, Inc.) for DNA sequencing. Sequence alignment comparisons and base sequence homologies of the 18s rRNA gene were made using MacVector© and MacClade©DNA analysis software. In addition to myxozoan sequence data, 18s rRNA gene sequences of other organisms retrieved from the Genbank® database were incorporated into these alignments. Finally, alignments were analyzed using PAUP© (Phylogenetic Analysis Using Parsimony) for generation of the most parsimonious trees. An intra/interclass cladistic phylogeny is proposed for these organisms based on these results.

195 EPITOPE SPECIFICITY OF A MOUSE MONOCLONAL ANTIBODY AGAINST A 50 KDA MAURER'S CLEFT-ASSOCIATED ANTIGEN OF *PLASMODIUM FALCIPARUM* MALARIA. Cohen SJ*, Lindler LE, Stoute JA, and Klotz FW. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; and Department of Microbiology & Immunology, The George Washington University, Washington, DC.

Numerous malaria antigens contain amino acid sequences homologous to host proteins. Such homologous amino acid sequences may induce the production of autoantibodies commonly detected during acute malaria. We characterized the epitope specificity of a monoclonal antibody, Mab FC2, reactive with a 50 kDa *Plasmodium falciparum* blood stage antigen, a 12 kDa primate leukocyte antigen and 20 and 30 kDa primate platelet antigens. Using a phage epitope library, we identified 52 clones reactive with Mab FC2. Inserted into the coat protein of these phage clones is a single peptide sequence, QEETHK. We also isolated two clones from a *P. falciparum* cDNA library which react with Mab FC2. We predict that these cDNA clones encode a 258 amino acid protein. Contained within the predicted malaria protein are amino acid repeats, HEEIHK, HEEVHK, HEENHK, PEEVHK, PEEFHK, HEKVHK, PGEIHK and HRKLHK. The use of the phage epitope library combined with cDNA cloning allowed us to identify nine potential epitopes that may react with Mab FC2. Overall, our results demonstrate the ability to screen cDNA libraries and epitope libraries to detect immunologically cross-reactive epitopes in order to design safer malaria vaccines.

196 IMMUNITY TO *HYMENOLEPIS DIMINUTA* AS A LABORATORY EXERCISE FOR UNDERGRADUATE PARASITOLOGY STUDENTS. Woodmansee DB*. Department of Biology, Wilmington College, Wilmington, OH.

An experiment designed to demonstrate immune-mediated clearance of *Hymenolepis diminuta* from mice was adapted for use as a laboratory exercise in a Junior/Senior level parasitology course. the exercise was designed to fit into regular 3 hour lab sessions which met once per week. Students were provided with a protocol and were required to conduct the experiment, analyze the results and produce a manuscript conforming to the "Guidelines and Policy of Authors" of the Journal of Parasitology. The experiment successfully demonstrated clearance of worm infections as well as enhanced clearance of challenge infections. The exercise gave students an opportunity to develop several important research skills including handling of experimental animals, data analysis and scientific writing. All students successfully completed the exercise and student evaluations of the exercise were uniformly favorable.

197 *ANCYLOSTOMA CEYLANICUM* IN THE HAMSTER: A LABORATORY EXERCISE TO DEMONSTRATE THE RELATIONSHIP BETWEEN INFECTION AND DISEASE. Nolan TJ* and Schad GA. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

The difference between infection and disease is a distinction that students frequently fail to make. We make use of *Ancylostoma ceylanicum* infections in hamsters to demonstrate the effect of parasite numbers on the development of clinical signs in the host. *A. ceylanicum*, a hookworm of man, dogs and cats, will develop to patentcy in hamsters and the infected hamster is a good model for demonstrating the classical features of hookworm disease. We use packed cell volume (PCV) and hemoglobin concentration as markers of disease, while eggs per gram of feces (EPG) and numbers of adult parasites are followed as parasitological indicators of the presence and magnitude of infection. When given orally, 60 to 70 % of the third-stage larvae will develop to adults (as assessed at 3 weeks post-infection). When there are fewer than 15 adults there is usually no indication of anemia and 60 to 100 adults are sufficient to produce fatal disease. The prepatent period is about 2 weeks and a "self-cure" takes place between 4 and 6 weeks post-infection. This "self-cure" can be followed by both EPG and PCV and leads to a chronic infection in which hamsters (at 9 weeks post-infection) harbor only a few worms, pass low numbers of eggs in the feces, and have normal blood values.

198 STUDENT MEMBERSHIP IN ASP: STABLE OR WORRISOME?. Siddall ME*. Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

The American Society of Parasitologists has, in recent years, undertaken initiatives to highlight the importance and the representation of students in the society. Trends both in the number of students in ASP and in the proportion of total membership represented by students were compared with similar data obtained from other biological societies. The latter were compiled both for societies related to the parasitological field and for some of broader biological scope. The ASP student membership has remained reasonably stable over the past ten years and falls roughly in the mid-range of those societies investigated. The fact that ASP student membership is not increasing gives cause for concern for the health of the society as it enters the next century. A short-term goal of a student proportion of 15% by 1996 is suggested with a longer-term objective of 20% by 2000. Methods for achieving these levels are also presented.

199 THE RELATIONSHIP BETWEEN HUMORAL RECOGNITION OF SCHISTOSOMAL ANTIGENS, CLASS II HLA TYPE AND *SCHISTOSOMA JAPONICUM* INFECTION IN A CHINESE VILLAGE POPULATION. Wasley AM*, Yuan HC, Zhang SJ, and Harn DA. Department of Tropical

Public Health, Harvard School of Public Health, Boston, MA; Department of Epidemiology, Shanghai Medical University, Shanghai, PRC; and Jiangxi Provincial Institute of Parasitic Diseases, Nanchang, PRC.

In Schistosomiasis mansoni, certain isotypes, particularly IgM and IgG4, are associated with non-protective "blocking" responses. The targets of these "blocking" antibodies are thought to be carbohydrate epitopes. To clarify the importance of different isotypes in mounting an effective immune response against *S. japonicum* infection, the relationship between pre-treatment intensity of infection and the isotupe specific response against native and deglycosylated *S. japonicum* SEA and SWAP were determined in a group of 200 infected and uninfected persons from a rural Chinese village population. Using multiple linear regression to adjust for the effect of age and sex, levels of IgM against S.j. SEA and levels of IgG4 against S.j. SWAP were found to be significant positive predictors of pretreatment levels of infection. In addition, levels of IgG2 against both SWAP and SEA were negatively correlated with infection, suggesting a role for IgG2 antibodies in the expression of protective immunity against *S. japonicum* infections. To further characterize the immunological makeup of this population, we have used PCR-SSO analysis to determine the HLA profile at the class II loci for these individuals. Class II molecules may influence the way in which an individual responds to schistosomal infection by determining which T cells are activated and thus affecting which cytokines are produced and which B cells are stimulated. We are evaluating the importance of class II HLA as a determinant of susceptibility to *S. japonicum* infection and pathology by looking for linkage between particular HLA haplotypes and susceptibility/resistance to either infection or hepatic pathology.

200 LYMPHOID-STIMULATORY IDIOTYPE EXPRESSION ON ANTIBODIES TO *SCHISTOSOMA HAEMATOBIUM* EGG AND WORM ANTIGENS. Abdel-Salam E, Fouad SA, Abdel-Meguid IE, Mansour MM, and Kamal KA*. Faculties of Medicine and Science, Cairo University, Cairo, Egypt; and U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Antibodies (Abs) against *Schistosoma haematobium* soluble egg (SEA) and adult worms NP-40 extract (NP-40 WA) antigens were immunopurified from sera of patients infected with *S. haematobium*. The Abs were free of antigenic contaminations as determined by silver stained SDS-PAGE separated materials. The specificity of the Abs resided primarily in the IgG1 and IgG4 subclasses. Both Abs uniformly recognized surface antigens on 3 hr-old *S. haematobium* schistosomula and induced *in vitro* complement-mediated damage to > 90% of this developmental stage. These multi-clonal idiotypic (id) preparations were tested for their ability to stimulate proliferation of peripheral blood T lymphocytes from both *S. haematobium* infected and from normal individuals. Proliferative responses of T cells from 17 patients to NP-40 WA-and SEA-related ids ranged from 2060±379 to 32,407±1721 and from 1582±191 to 57,307±10,216 net cpm, respectively. These ids failed to stimulate normal T cells. Moreover, normal human IgG did not stimulate cell proliferation. The present study demonstrate the presence of anti-idiotypic T cells in the circulation of *S. haematobium* infected patients which may play important role in the immunoregulatory system of human *S. haematobium* hosts.

201 EOSINOPHILIA, ANTI-EGG RESPONSES, AND SUSCEPTIBILITY IN *SCHISTOSOMA JAPONICUM* INFECTION OF MICE EXPRESSING AN IL-5 TRANSGENE. Kuriyama T*, Amano T, Tominaga A, Takatsu K, Colley DG, and Minami M. Yokohama City University School of Medicine, Yokohama, Japan; Kumamoto Univ. Med. Sch., Kumamoto, Japan; Faculty of Medicine, University of Tokyo, Tokyo, Japan; and Parasitic Diseases Branch, DPD/NCID/Centers for Disease Control, Atlanta, GA.

IL-5 transgene expression leads to high blood and tissue eosinophilia. Normal and IL-5 transgenic C3H male mice were infected with 20 or 60 cercariae of *Schistosoma japonicum* and monitored for 13

weeks for peripheral blood leukocytes, size of hepatic egg-focused granulomas during infection, lymphocyte responsiveness, serologic reactivity, and levels of innate susceptibility. The background of 20%-30% eosinophilia of IL-5 transgenic mice rose early in infection, plummeted to 5% at 5 and 6 weeks, then rose and remained elevated until 13 weeks. After 6 weeks of infection, eosinophilia in C3H mice remained elevated at 5%-8%. Spleen cell proliferative responses and IgG antibody levels to *S. japonicum* soluble egg antigen were equal in both groups. Hepatic egg granulomas were more eosinophil-rich in the IL-5 transgenic mice. After 6 week peaks in size, granuloma formation modulated in both groups by 9 and 13 weeks of infection, but at all points lesions in IL-5 transgenics were larger than in the controls. IL-5 transgenic mice were significantly more susceptible to infection with 60 *S. japonicum* cercariae, than were normals. Constitutive eosinophilia alters this host-parasite relationship in unexpected ways.

202 PHENOTYPIC SPLEEN CELL DIFFERENCES IN DISTINCT CHRONIC CLINICOPATHOLOGIC SYNDROMES IN MICE WITH SCHISTOSOMIASIS MANSONI. Freeman, Jr. GL*, Nix NA, Colley TA, and Colley DG. Parasitic Diseases Branch, DPD/NCID/Centers for Disease Control, Atlanta, GA; Division of Oncology, Department of Medicine, Vanderbilt University, Nashville, TN; and Oberlin College, Oberlin, OH.

Male CBA/J mice exposed to 45 *Schistosoma mansoni* cercariae develop either chronic hypersplenomegaly syndrome (HSS) or moderate splenomegaly syndrome (MSS). HSS mice have much more severe clinicopathology and differ immunologically in regard to TNF-α and cross-reactive idiotypes on their anti-SEA antibodies. Using flow cytometry we now report that the proportions of splenocyte populations in MSS and HSS mice differ.

Clinical Form	Percentage of Phenotypes						
				CD4+ Cells			
	CD4+	CD8+	CD4:CD8	CD45RBlo	CD44hi	Ia+	CD25+
Acute	17.2	9.5	1.9	59.8	27.9	38.5	5.3
MSS	17.5	12	1.6	59.9	20.9	21.4	2.9
HSS	10.8	10.5	1.1	67.5	30.5	29.3	6.7

Based on CD44hi, Ia+, and CD25+ cell activation markers, CD4+ cells of ACUTE (8 weeks) and HSS mice appear to be immunologically hyperactive, while cells of MSS mice appear down-regulated from ACUTE infections. These indicators imply that from 8 to 20 weeks of infection, mice either down-regulate their apparent immune responses or continue high levels of reactivity and become HSS.

203 SUPPRESSION OF THE IMMUNE RESPONSE TO DIPHTHERIA TOXOID IN MURINE SCHISTOSOMIASIS. Craig JP* and Haseeb MA. Department of Microbiology & Immunology, State University of New York Health Science Center, Brooklyn, New York, NY.

Mice infected with *Schistosoma mansoni* and uninfected controls were immunized with three doses at 2-week intervals of unadjuvanted diphtheria toxoid (DTd) at varying intervals following or preceding infection with 100 cercariae. Serum diphtheria antitoxin levels were determined by measuring their capacity to neutralize the lethal effect of diphtheria toxin in VERO cells. Antitoxin titers were expressed as the mean of two sera collected 7 and 21 days after the 3rd DTd injection. 71% of immunized, uninfected mice developed serum diphtheria antitoxin levels which would be considered protective against diphtheria in humans, i.e., the equivalent of Schick negativity. The mean titer was 0.046 antitoxin units/ml. In mice infected with schistosomes 112 to 14 days before, on the same day as, and 28 days after the first of the 3 DTd injections, mean antitoxin levels were 0.003 to 0.016 antitoxin units per/ml ($p = 0.002$ to 0.115). Antitoxin levels were significantly ($p = <0.05$) lower than controls in animals infected 112, 84, 56 and 14 days prior to, simultaneously with and 28 days

after the first dose of DTd. Only in mice infected 28 days prior to the first dose of DTd was the p value >0.05. Overall, only 28% of mice infected with schistosomes achieved diphtheria antitoxin levels equivalent to Schick negativity in humans. If similar immunosuppression occurs in human schistosomiasis, these findings may have implications for childhood immunization programs in areas where schistosomiasis is endemic.

204 *SCHISTOSOMA MANSONI*-INDUCED THROMBOCYTOPAENIA IN MICE : AN IMMUNOLOGICAL BASIS?. Wambayi EJ*, Ngaiza J, and Doenhoff MJ. Kenya Medical Research Institute, Nairobi, Kenya.; The New York Hospital, Cornell Medical Center, Division of Hematology-Oncology, New York, NY; and University of Wales, School of Biological Sciences, Bangor, Gwynedd, UK.

Normal mice with bisexual infections of *Schistosoma mansoni* became thrombocytopaenic. The onset of thrombocytopaenia coincided with initiation of egg production by the parasite, and appeared to be immune-dependent. Thus, intravenous injections of freshly isolated *S. mansoni* eggs induced a thrombocytopaenia in naive normal mice, but not T cell-deprived mice. 51Cr-labelled platelets survived for a shorter time than in normal mice, suggesting an increased rate of destruction of circulating platelets as a result of infection. Thrombocytopenia was induced by transfer of serum from chronically infected mice (CIS) into normal uninfected mice. Antibodies in such sera reacted with washed normal mouse and human platelets in an ELISA assay and the reactions of these sera were significantly more intense than those from uninfected control mice and single sex infected mice. In immunoblot analysis, IgG from mouse CIS recognized antigens on both mouse and human platelets, the reactive antigens having similar molecular weights in the two species of platelets. The *in vitro* aggregability of mouse platelets in response to thrombin was modifiable by IgG isolated from CIS. It is concluded that antiplatelet antibodies are produced during the course of a schistosome infection in mice and that such antibodies are induced mainly by the egg stage. These antibodies may cause destruction of some platelets and modulate the function of the remainder.

205 EFFECT OF CASTRATION AND TESTOSTERONE TREATMENT ON SCHISTOSOMIASIS MANSONI IN MALE MICE. Nakazawa M*, Eloi-Santos S, Olsen NJ, Kovacs WJ, and Colley DG. Parasitic Diseases Branch, DPD/NCID/Centers for Disease Control, Atlanta, GA; Faculty of Medicine, University of Federal Minas Gerais, Belo Horizonte, Brazil; and Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN.

Parallel *Schistosoma mansoni* infections of male and female mice results in fewer adult worms in male than female mice. To study this phenomenon, male CBA/J mice were castrated or sham castrated. Some castrated mice received a regimen of testosterone, and 1 week later all 3 groups, and a group of females, were subcutaneously exposed to 45 cercariae. Eight weeks later organomegaly and worm burdens were determined on some of each group, and survival rates were then monitored in the rest. By 16 weeks >85% of the female or castrated mice (circulating testosterone = <0.2 ng/ml) were dead, while <30% of the sham-castrated (mean 3.2 ng/ml) or castrated & testosterone treated (mean > 188 ng/ml) mice succumbed to infection. The mean number of worms recovered from castrated mice was comparable to that from female mice, and greater than that from sham-castrated or castrated & testosterone treated males. Organomegaly correlated with worm burdens. When male CBA/J mice were castrated or sham-castrated 5 weeks after existing *S. mansoni* infection, no significant alteration in host survival occurs. The host sex bias we observe in parallel-infected male and female mice appears to be related to the presence of male gonadal tissue or testosterone during the schistosome maturational process.

206 CHARACTERIZATION OF CLONED TH0 AND TH2 LYMPHOCYTES OBTAINED FROM LIVER GRANULOMAS OF *SCHISTOSOMA MANSONI* INFECTED MICE. Boros DL*, Zhu Y, and

Lukacs NW. Imunology/Microbiology Department, Wayne State University School of Medicine, Detroit, MI.

In murine schistosomiasis mansoni $CD4^+$ T helper lymphocytes induce the granulomatous responses around disseminated eggs. In the present study T cells were cloned from isolated, collagenase-dispersed, vigorous (V) or modulated (M) liver granulomas (Gr) of acutely or chronically infected mice. Soluble egg antigens (SEA)- stimulated T blast cells were cloned by limiting dilutions in the presence of feeder cells, IL-2 and SEA. All the T cell clones were CD4+ and responded to SEA (proliferation index E/C: 3-22). The SEA-specific clones were recloned. Three clones obtained from VGr produced IL-2, IL-4 and IFN-γ in response to SEA (TH0 type cells). Two of 3 clones from MGr produced IL-2, IL-4 (TH0) and 1 clone produced only IL-4 (TH2 type cell). A concentration of $1x10^6$ feeder cells (control) or VGr, MGr-derived clones were injected i.v. together with 2,000, 60μ size SEA-coated beads or live schistosome eggs into normal recipients. Four days later lungs were processed and granuloma areas were measured in stained histologic sections. Feeder cell-induced mean granuloma area ($MGAx10^{-3}$ $\mu m^2 \pm$ SEM) around beads was 5.37 ± 0.2. Clones of VGr or MGr evoked a range 6.6 - 8.4 of Gr sizes (significance $p < 0.05$) compared with control. MGA values around eggs were: feeder cells 3.9 ± 0.2, VGr, MGr derived T clones: 6.6 - 9.4 ($p < 0.05$). No differences were seen in bead, egg Gr sizes induced by VGr or MGr T clones. VGr compared with MGr T clones responded to different SEA fractions. It is concluded that both precursor TH0 and TH2 type helper cells are present in liver Gr throughout the infection, indicating the complexity of T cell-mediated granulomatous inflammation.

207 IDENTIFICATION AND CHARACTERIZATION OF A SECOND FORM OF TROPOMYOSIN FROM *SCHISTOSOMA MANSONI*. Osman A*, Karim A, Abdel Fattah M, Thakur A, and LoVerde P. Department of Microbiology, State University of New York, Buffalo, NY; and Department of Biochemistry, Ain Shams University, Cairo, Egypt.

A 43 kDa acidic (pI = 4.6) polypeptide from a NP-40 3 hr schistosomula extract was shown by western blot of a two-dimensional gel to be highly immunogenic when probed with sera either from animals immunized with irradiated parasites or from humans with chronic infection. Rabbit anti-43 antibodies identified a cDNA clone (1369 bp) from a cercarial λ gt11 cDNA library. The amino acid sequence of the 43 kDa antigen as predicted from the cDNA sequence identified a 284 amino acid peptide that showed significant homology to different members of the tropomyosin family. The highest score (66%) was shared with *Schistosoma mansoni* tropomyosin, SMTM-I. Polyclonal sera made against recombinant tropomyosin, SMTM-II, shared epitopes with SMTM-I as demonstrated by western blot, ELISA and immunoprecipitation of *in vitro* translation products. However, only 2 of 6 monoclonal antibodies specific to SMTM-I reacted with SMTM-II. Popiteal lymph node cells from animals vaccinated with irradiated parasites showed a strong blastogenic response to recombinant SMTM-II. Epitopes for SMTM-II were detected on both live and acetone fixed 3 hr schistosomula by indirect immunofluorescence. Current studies focus on evaluating SMTM-II as a vaccine candidate.

208 ANTI-CARBOHYDRATE ANTIBODIES MAY BE PROTECTIVE AGAINST *SCHISTOSOMIASIS MANSONI* IN HUMANS. Zimon AE*, Secor WE, Reis MG, Ramos EA, Carmo TM, Reis EA, Mattos EP, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, Bahia, Brazil.

There is an increased focus on the role of antibody isotypes in resistance and susceptibility to schistosomiasis. Studies have suggested that human carbohydrate-specific antibodies perform a flocking function. However, in the murine model, certain monoclonal antibodies specific for carbohydrate epitopes are partially protective. Also, resistance resulting from vaccination with irradiated cercariae correlates with levels of anti-carbohydrate antibodies. This study examined the association between carbohydrate-specific antibody isotype responses and differences in fecal egg

output or in the form of clinical disease in a population of *Schistosoma mansoni*-infected patients in northeastern Brazil. One-hundred and twelve patients were examined for their isotypic antibody responses to adult, carbohydrate epitopes. High egg excreting individuals (putatively susceptible) were compared with age-matched low egg excreting individuals (putatively resistant). Also, intestinal patients were compared with age-matched hepatosplenic patients. No differences were seen in anti-carbohydrate egg-excreting individuals tended to have a stronger proportion of their antibody responses directed against carbohydrate epitopes than high egg-excreting individuals. This is consistent with the results in the murine model and suggests that the antibodies which are directed against carbohydrates may be performing a protective function.

209 MHC CLASS II HAPLOTYPE EFECTS ON CLINICAL FORM AND IMMUNOREACTIVITY TO *SCHISTOSOMA MANSONI* ANTIGENS IN AN ENDEMIC POPULATION IN NORTHEASTERN BRAZIL. del Corral E*, Secor WE, Reis MG, Ramos EA, Carmo TM, Reis EA, Mattos EP, Zimon AE, AND Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro de Pesquisas Goncalo Moniz, Bahia, Brazil.

Although most individuals with schistosome infections do not experience severe disease, a small proportion of patients progress to hepatosplenism and death. Pathology resulting from schistosome infection is due to granuloma formation, an event largely mediated by CD4+ T cells. Because major histocompatibility complex (MHC) haplotypes influence the repertoire of T cell responses and the CD4+T cell subset reacts with antigens presented by cells bearing class II molecules, the MHC class II phenotype of a given individual is likely to influence the severity of disease. Although there have been a limited number of studies on the role of MHC phenotype in schistosomiasis, the significance of class II in *Schistosoma mansoni* has not been examined. The MHC class II alleles of 400 patients living in an endemic area in northeastern Brazil were characterized at four loci (DRB1, DPB1 and DQA1) using the polymerase chain reaction followed by allele-specific oligonucleotide hybridization. The population in this area is ethnically heterogeneous and the MHC composition has not been described. This study examined the correlation of MHC haplotypes with: (i) clinical status (intestinal vs hepatosplenic), (ii) fecal egg output, (iii) peripheral mononuclear cell proliferative responses, and (iv) specific anti-parasite antibody isotypic responses to various schistosome antigens. Preliminary analysis of the data indicates a positive correlation between hepatosplenism and DQA allele 0102 and a negative correlation between hepatosplenism and DQA allele 0501. Other analyses with immunoreactivities are currently in progress.

210 IL-10 INHIBITS SCHISTOSOMAL EGG ANTIGEN-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTIONS AS WELL AS EGG GRANULOMA FORMATION IN VIVO. Flores-Villanueva PO*, Reiser H, and Stadecker MJ. Tufts University School of Medicine, Boston, MA; Harvard Medical School/Dana-Farber Cancer Institute, Boston, MA.

We have previously shown that spleen cell supernatants from schistosome-infected mice contain IL-10 in amounts that inhibit the capacity of splenic antigen-presenting cells (APC) to stimulate cloned schistosomal egg antigen (SEA)-specific CD4+ Th-1-type lymphocytes. We now show that local injection with murine recombinant IL-10 inhibits the capacity of such clones to mediate local DTH reactions *in vivo*. Furthermore, IL-10 administered systemically inhibits antigen-specific DTH reactions, as well as synchronous lung granuloma formation around embolized eggs in SEA-sensitized mice. Inhibition of *in vivo* parameters is accompanied by a marked decrease in SEA-specific lympho-proliferation and IL-2 production *in vitro*. Our data raise the possibility that IL-10 plays a significant role in the immunomodulation of granuloma formation in schistosomiasis. Consistent with this hypothesis, we have observed in preliminary experiments that *in vivo* injections with IL-10 can block the induction of costimulatory antigen B7 expression on peritoneal exudate cells.

211 ANALYSIS OF FRACTIONATED SCHISTOSOMAL EGG ANTIGENS USING T CELL HYBRIDOMAS. Hernandez HJ*, Brodeur PH, and Stadecker MJ. Tufts University School of Medicine, Boston, MA.

We have generated a panel of T cell hybridomas from C57BL/6 mice which respond to soluble egg antigens (SEA) of *Schistosoma mansoni*. Southern blot analysis of rearranged T cell receptor β loci indicates that all hybrids are derived from independent clones. Reactivity to fast performance liquid chromatography-fractionated SEA identifies at least four antigen-containing fractions. Three of these fractions also stimulate previously reported T cell clones and the hybridoma-stimulating fractions induce the peak responses of polyclonal SEA-sensitized lymph node T cells. These results suggest that SEA reactive T cell hybridomas reflect the *in vivo* anti-SEA repertoire and provide a strategy to identify the immunodominant determinants and potential targets for immunotherapy in the granulomatous inflammatory response of schistosomiasis.

212 CHARACTERISATION OF *SCHISTOSOMA MANSONI* ANTIGEN SM480. Cooper RO*, Miller CM, Fallon PG, Probert AJ, Doenhoff MJ. University of Wales Bangor, School of Biological Sciences, Brambell Bld, Deniol Road, Bangor, Gwynedd, UK.

Sm480, a polymeric, glycosylated antigen originally identified in the soluble fraction of *Schistosoma mansoni* egg homogenate, has been implicated in the induction of protective immunity against *Schistosoma mansoni* infection in mice. Its immunoprophylactic potential has been demonstrated both by passive transfer of specific rabbit antisera, and by active immunization with immunoelectrophoresis precipitin arcs containing the antigen. Consistent with its immunoprophylactic potential, Sm480 has also been detected on the surface of *S. mansoni* lung schistosomula by indirect immunofluorescence, and it is present in immunoprecipitable form in crude adult worm extracts.In immunoblotting rabbit and mouse antisera specific for Sm480 yield complex patterns of reactivity. Furthermore, it has been found that the egg form of the antigen, but not the adult worm form, has peptidolytic activity on chromogenic substrate solutions, and the worm form of Sm480 induces a marginally higher degree of protective immunity than the egg form.An attempt has been made to resolve the apparent inherent complexity of Sm480, (i) by elucidating the structural characteristics of the immunologically cross-reactive forms of the antigen that are present in different stages of the life-cycle, and (ii) by identifying the most immunogenic subunits of the antigen in each stage. The direction future work on this *S. mansoni* candidate vaccine antigen is likely to take will be outlined.

213 INVESTIGATIONS INTO THE REASONS FOR OBSERVED INFLAMMATION CAUSED BY EXTRACTS OF *SCHISTOSOMA MANSONI* CERCARIAE. McNeice C*, Hellewell PG, Williams TJ, Doenhoff MJ, and Teixeira MM. School Of Biological Sciences, University of Wales, Bangor, Deniol Road, Gwynedd, UK.; and Department of Applied Pharmacology, National Heart and Lung Institute, Dovehouse Street, London, UK.

Extracts of *Schistosoma mansoni* cercariae have been shown to induce inflammation when injected intradermally (i.d.) into mouse skin. Experiments were carried out to investigate possible reasons for this observed inflammatory response. Using intravenously injected radio-labelled markers, oedema, neutrophil and eosinophil accumulation were monitored in a guinea pig skin model of inflammation. *S. mansoni* larval extracts were injected i.d. 5 minutes after intravenous injection of the radio-labelled markers. High levels of cell accumulation and oedema were recorded at the sites of i.d. injection. Selected inhibitors of inflammation were added to the larval extracts to elucidate the mechanisms involved. By this means platelet activating factor and a 5-lipoxygenase product were found to be at least partially responsible for eosinophil accumulation. An inhibitor of bradykinin receptors inhibited the oedematous response. Pre-incubation of the larval extracts with serine

protease inhibitors also significantly inhibits the inflammatory responses being measured. An intradermal injection of cercarial homogenate just prior to infection with *S. mansoni* cercariae, at the skin site over which the infection takes place, resulted in a significant increase in the number of adult worms that were subsequently perfused from the mice. A possible relationship between proteolytic activity, intradermal inflammation and successful migration of schistosome larvae through host skin will be discussed.

214 AN ALBUMIN-LIKE MOLECULE ASSOCIATED WITH *SCHISTOSOMA MANSONI*. Riley SL*, Fallon PG, Doenhoff MJ. School of Biological Sciences, University of Wales, Bangor, Gwynedd, UK.

A carbohydrate-rich antigen, K3, can be identified with rabbit antisera in immunoelectrophoreses of *Schistosoma mansoni* soluble egg antigens (SEA). Most antisera raised against K3 have been found also to react against a second distinct anionic antigen. Antisera raised against the anionic antigen did not, however, react against K3. Extracts of different stages in the life cycle of *S. mansoni* have been found to contain the anionic antigen, and attempts were made to further characterise its possible structure and function. The anionic K3-contaminant antigen has a molecular weight of approximately 60kD in Western immunoblots of SDS-PAGE gels. While it was found to have immunological cross-reactivity with different mammalian serum albumins, it also cross-reacted with *S. mansoni* egg antigens of relative molecular size, 30-43kD, and with key-hole limpet hemocyanin (KLH). Potential roles for this albumin-like molecule in the schistosome-host relationship will be discussed. These include evasion of immunity through antigenic mimicry, and inhibition of platelet activation.

215 GRANULOMA FIBROBLASTS ARE THE MAJOR SITE OF VIRAL REPLICATION IN *SCHISTOSOMA MANSONI* INFECTED MICE CHALLENGED WITH RECOMBINANT VACCINIA VIRUS. Actor JK*, Eltoum IA, Pimenta P, Buller RM, Berzofsky JA, and Sher A. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, MD; Metabolism Branch, NCI, National Institutes of Health, Bethesda, MD.

BALB/c mice infected 7 weeks previously with *Schistosoma mansoni* and challenged with a recombinant vaccinia virus expressing the HIV envelope protein gp160 show a marked delay in viral resolution as compared to mice infected with vaccinia alone. The delay in viral clearance is greatest in the liver, the major site of granuloma formation during schistosome infection, with virus persisting up to 3 weeks apparent in that tissue. In order to investigate the role of egg granuloma formation in viral persistence, experiments were initiated to localize the virus in tissue and to explore the effect of local granuloma formation on viral expansion. Immunohistochemical staining of doubly infected liver sections with a rabbit anti- vaccinia virus revealed high levels of viral epitopes in 8.9% of granulomas examined. Staining was distributed throughout the lesion with the exception of the egg itself. Further examination using electron microscopy identified fibroblasts as the major site for viral replication with no virus present in either macrophages, lymphocytes, polymorphonuclear cells or surrounding hepatocytes. EM analysis also failed to reveal any association of the virus with adult schistosomes perfused from the portal region. Induction of pulmonary granulomas by intravenous injection of 5000 *S. mansoni* eggs into mice presensitized by natural infection led to a slight increase in local (lung), as well as peripheral (liver), persistence of the recombinant virus. Immunohistochemical staining demonstrated viral epitopes in approximately 10% of the lung induced granulomas. As seen in the liver, these viral epitopes were localized to fibroblasts. These experiments suggest that egg granulomas, together with the cytokine imbalance present during schistosome infection, can promote the expansion of vaccinia virus and possibly other viral agents by providing a source of fibroblast target cells for viral replication.

216 IMMUNOLOGIC CHARACTERIZATION OF INDIVIDUALS WITH LOW AND HIGH LEVELS OF ANTIFILARIAL IgG4. Marley SE*, Eberhard ML, and Lammie PJ. Department of Zoology, University of Georgia, Athens, GA; and Division of Parasitic Diseases, Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA.

Antifilarial IgG4 levels have been shown to correlate with active infection of humans with *Wuchereria bancrofti*, suggesting that the presence of adult worms leads to a shift inthe isotype of antifilarial antibody. However, previous studies in Haiti have suggested that up to 10% of microfilaremic individuals may have low to nonexistent antifilarial IgG4. To explore this issue, antifilarial IgG4 levels in 85 sera collected from microfilaremic (MF+) individuals were measured by ELISA. The geometric mean (GM) antifilarial IgG4 response was 51.3 μg/ml (range 0.371 to 605 μg/ml) compared to 6.6 μg/ml and 4.3 μg/ml for antigen negative symptomatic (n=55) and microfilaria negative individuals (n=22), respectively. Thirteen of the 85 individuals had antifilarial IgG4 levels lower than 10 μg/ml; four of these had responses lower than 1 μg/ml. In order to gain insight into how individuals with low responses differed from those with high responses, total IgG4 and IgE, and filaria-specific IgE, IgG1 and IgG2 were analyzed. Individuals with lower antifilarial IgG4 had lower total IgG4 levels than those with higher parasite-specific responses (GM 307.3 μg/ml vs 1201.3 μg/ml, respectively; normal range 30-1330 μg/ml). Of the low responders, one of 13 had total IgG4 level of less than 10 μg/ml. Neither parasite specific nor total IgG4 levels were significantly correlated with microfilaremia level. Individuals with low antifilarial IgG4 also had lower parasite-specific and total IgE levels than high responders; antifilarial IgG2 levels were comparable for the two groups. These results are consistent with a number of hypotheses: lower antifilarial IgG4 levels may reflect decreased TH2 activation, a lower frequency of IgG4- and IgE-producing B cells, or regulatory differences including differential lymphokine responsiveness or production.

217 ANALYSIS OF SPECIFIC IgG SUBCLASSES IN A POPULATION NATURALLY EXPOSED TO *LOA LOA*. Akue JP*, Devaney E, Egwang TG, Vincent J, and Hommel M. Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK; Centre International de Recherches Medicales de Franceville, Gabon; Department of Veterinary Parasitology, Unversity of Glasgow, Glasgow, Scotland; and Malaria Branch, NCID, Centers for Disease Control, Atlanta, GA.

To have an insight on protective immunity in a population naturally exposed to *Loa loa* infection, we used 56 adult volunteers from Ambinda Village, south east Gabon. This study population was divided into four groups, according to their parasitological and clinical status: high and low microfilaremic, amicrofilaremic and endemic controls. By comparing these groups using an ELISA technique with IgG subclass reagents and heterologous antigens (*Brugia pahangi*), a significantly higher level of IgG1 was observed in the amicrofilaremic group, whereas the level of IgG4 was high in all other groups, with the exception of the endemic controls. The levels of IgG2 and IgG3 were similar in all groups. A negative correlation was observed between the levels of microfilaremia and the level of IgG1 for L3 antigens (Spearman's $r = -0.562$; $P < 0.05$), adult ($r = -0.469$, $P < 0.05$), and microfilaria antigens ($r = -0.498$, $P < 0.05$). No relationship was found between the levels of *Loa loa* microfilariae and IgG4. Qualitative analysis by Western blotting of antigen recognition showed a differential recognition of 28-31 kDa antigens by IgG1 in amicrofilaremic individuals; whereas IgG4 from microfilaremic and from the majority of individuals exposed to *Loa loa* infection, recognized specifically low molecular weight antigens (18-30kDa) of homologous adult *Loa loa*.

218 DIROFILARIASIS IN THE ARABIAN GULF: AUTOCHTHONOUS HUMAN INFECTION IN KUWAIT? Hira PR*, Madda JP, Al-Shamali MA, and Eberhard ML. Faculty of Medicine, Kuwait University, Kuwait; and Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

Filarial worms in the genus *Dirofilaria* cause sporadic zoonotic infections in humans. Thus far, there are no reports of human dirofilariasis from the Arabian Gulf. We present the first report of human dirofilariasis from this region. A 50-year old Kuwaiti man complained of pain and swelling in the right lower quadrant (RLQ) of the abdomen. On palpation, an elongated, mildly tender and fixed mass was felt deep in the abdominal wall; the mass was excised. A worm was identified in H & E stained tissue sections of the abscess. History of residence in an "endemic" geographic area, location of the nodule in subcutaneous tissue, the diameter of the worm, the multilayered cuticle, prominent longitudinal cuticular ridges, an internal cuticular ridge, and abundant somatic muscles suggested that the worm is *D. (Nochtiella) repens*, a natural parasite of dogs and cats in Asia, Africa, and Europe. The worm could be distinguished from others in the *Nochtiella* subgenus that infect subcutaneous tissue and also *Onchocerca* from man and camels in this geographic area. Without microfilaraemia, there is no need to administer antifilarials; the excisional biopsy is both diagnostic and therapeutic. The identification of *D. repens* in Kuwait raises several questions. Is there indigenous transmission of the parasite in Arabian Gulf countries? Appropriate mosquito vectors are present in Kuwait and the definitive host, dogs, are plentiful. Since outside activity increases the likelihood of being bitten by infected mosquitoes, it is conceivable that military personnel still active in the country after Operations Desert Shield and Desert Storm could be exposed to zoonotic filarial infections.

219 GERBIL-*ACANTHOCHEILONEMA VITEAE* MODEL: SUPPRESSION OF MICROFILARIA LEVELS ASSOCIATED WITH PREGNANCY. Dickerson JW*, Walker EM, and Eberhard ML. Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

Epidemiological analysis of data from various filarial endemic areas of the world indicates a lower prevalence and density of infection in women of reproductive age. This cannot be explained solely by differences in exposure to infective vectors and may be due in part to suppression of infection by females during childbearing years. We attempted to test this premise using the gerbil-*Acanthocheilonema viteae* model. Seven-week-old female jirds were inoculated with 30 *A. viteae* L3's. At 10 wks post- inoculation, microfilaria (mf) levels were monitored weekly by sub-orbital eyebleeds. When mf levels had stabilized, jirds were mated, arrival of litters recorded, and weekly monitoring of mf levels continued. Results from 8 pairings indicate that mf levels remained at or were below pre-mating levels (range from 13 to 25.5 mf/female) over the periods of time when females were bearing litters. When males were removed after the pair produced 2 to 3 litters, the range of mf counts in females increased to 59 to 308 mf/female. In addition, mf levels of paired female jirds who did not produce litters did not exhibit the same periods of suppression of mf counts demonstrated by females who bore litters. These results, which may be due to physiologic changes during pregnancy, may help explain the lower incidence and density of microfilaremia in women of childbearing age.

220 EFFECTS OF REPEATED CHALLENGE INFECTIONS OF RHESUS MONKEYS WITH INFECTIVE LARVAE OF *BRUGIA MALAYI* ON LYMPHATIC PATHOLOGY, AND IMMUNE RESPONSES. Lasater BL, Dennis VA*, Lowrie, Jr. RC, and Frantz RC. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

Five rhesus monkeys which had been infected 96 weeks previously with a single large infective larvae inoculum of *Brugia malayi* (200 L3; single infection) and subsequently had become amicrofilaremic were challenged by repeated trickle inoculations over an 89 week period (25 L3; 22 trickle challenge infections). The effects of such infections on lymphatic pathology, microfilaremia and the immune response in animals were examined as a function of time. After reinfection, 2 animals (F660, F712) developed lymphedema of the leg and remained amicrofilaremic; 3 animals remained asymptomatic with low numbers of microfilariae (21-82 mf/ml). Two animals which served as controls for the trickle inoculations only had high numbers of microfilariae (2300-4200

mf/ml). Eosinophilia of the 5 animals with single infection was marked initially, but declined with onset of patency. Eosinophilia of these animals after the first trickle challenge infection was lower than those of the single infection (945-2070 vs 845-7700 eosinophil/mm3). PBMC blastogenic response to adult worm antigens (Ad Ag) occurred rapidly in all 5 animals after the single infection; it was marked but decreased with time. After reinfecting these animals, Ad Ag response was detected after the 11th trickle challenge infection in only 3 animals. At this time, responses were transient and lower than those of the single infection (6.68-22 vs 19.83-165 SI). Eosinophilia and Ad Ag response in the 2 trickle control animals were similar to those of animals with the trickle challenge infections. Antibody (Ab) response to Ad Ag developed rapidly in animals after the single infection. However, with chronicity of infection, Ab responses declined but developed again after the 1st trickle challenge infection, albeit lower than those of the single infection (25-75 vs 45-109 ELISA units). Of significance, was the higher Ab response after the first 12 trickle infections in animals that were challenged, especially those with lymphedema, than in the 2 trickle control animals (25-75 vs 18-31 ELISA units). The absence of or low microfilaremia in rhesus monkeys receiving the challenge trickle infections suggests resistance to reinfection. The lower response in animals with repeated trickle infections suggests a different immune regulation compared with animals receiving the single large L3 infection.

221 *BRUGIA MALAYI* EXCRETORY/SECRETORY ANTIGENS: SUPPRESSION OF CON A-INDUCED BLASTOGENIC RESPONSES OF T-CELLS FROM UNINFECTED RHESUS MONKEYS. Bakeer MK*, Dennis VA, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

T-cell hyporesponsiveness to the filarial parasite *Brugia malayi* is often seen in infected human patients, especially those who are asymptomatic and microfilaremic. Parasite antigen(s) that may be involved in downregulating the T-cell response in these individuals are not fully characterized. During the parasite's developmental cycle, the host is exposed to several antigenic components chief amongst which are the antigens exported by live worms, i.e. excretory/secretory (E/S) molecules. The role played by E/S antigens in T-cell hyporesponsiveness in filariasis is not known. Likewise, little is known about the host's immune responses to these antigens in general. Earlier studies in our laboratory showed that PBMC from *B. malayi*-infected rhesus monkeys respond to adult worm somatic antigens but not E/S antigens in blastogenesis assays. The present study, the first of several to characterize E/S antigens, was conducted to determine if these molecules can suppress the *in vitro* Con A-induced blastogenic response of PBMC from normal rhesus monkeys. E/S antigens co-cultured with Con A could suppress the Con A-induced blastogenic response of normal monkeys' PBMC. The suppression was found to be dose-dependent, i.e. suppression was greater with 10 μg/ml than with 1 μg/ml of E/S antigens (39-44% vs 9-11%). Cells (viability >94%) treated with E/S for five days were washed and subsequently co-cultured with freshly obtained syngeneic cells in the presence of Con A. Co-cultures of fresh syngeneic cells and E/S-treated cells produced a response to Con A that was 37-42% lower than cultures with cells lacking E/S treatment. Only 5-15% suppression was obtained when adult worm somatic antigens were used rather than E/S antigens. This study suggests that E/S antigens contain a suppressive component(s). Current studies are being conducted to characterize this component biochemically and also to determine the mechanism(s) of E/S suppression on the Con A-induced blastogenic response.

222 DIAGNOSIS OF ONCHOCERCIASIS: DEVELOPMENT AND FIELD EVALUATION OF A SIMPLE DOT BLOT ASSAY ADAPTABLE FOR FIELD USE. Lavebratt C*, Dalhammar G, and Akuffo HO. Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden; and Department of Infectious Diseases, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden.

Development of better diagnostic methods for onchocerciasis with all its debilitating symptoms is important since existing detection techniques are inconvenient. Results indicate that a dot blot assay analysing the total IgG response to an easily available and simply prepared fraction of whole female worm (BSF), might be applicable in the field as a first screening method. The specificity of the assay is further improved using a semi purified fraction of BSF, PakF, dominated by a 23 kD protein. Sera from 194 individuals from the onchocerciasis endemic country Ghana including confirmed patients, parasitologically negative controls in a highly endemic rural area (endemic controls) and apparently healthy controls in an urban area (urban controls) were screened. The sensitivity of the assay was 100%. The specificity was 93% using sera from the urban controls. However, the specificity was 68% when sera from the endemic controls were screened. The assay was further adapted for field use and evaluated by screening 516 individuals living in highly and lowly onchocerciasis endemic areas in Ghana using fingerprick blood on filter-paper. Individuals with other parasite infections, skin or eye lesions from an urban area were included. Initial results indicate that the dot blot assay can compete with and in some instance is better than existing diagnostic methods considering sensitivity, technical practicality, patient comfortability, and spread of other infections.

223 EVALUATION OF A PILOT VILLAGE BASED EPIDEMIOLOGICAL SURVEILLANCE SYSTEM FOR DRACUNCULIASIS ELIMINATION IN BURKINA FASO. Hutin YJ*, Ouedraogo JB, Fabre-Teste B, Soula G, Hien R, Guigemde TR. Service d'Epidemiologie, de Statistique et d'Information Sanitaire du Secretariat General de l'O.C.C.G.E., Bobo-Dioulasso, Burkina Faso; Centre Muraz (O.C.C.G.E.), Bobo-Dioulasso, Burkina Faso; and Direction de la Medecine Preventive, Ministere de la Sante, Ouagadougou, Burkina Faso.

A pilot dracunculiasis surveillance system was organised in 1989 in two provinces of Burkina Faso (Bam and Oubritenga). Primary health workers (PHW) were asked to enquire monthly about all guinea worm emergence cases in their village, and to notify them to the reference dispensary. Data were analysed at the provincial level. A sample of villages was visited in early 1993 using door to door visits (villages reported as endemic in 1992 by PHW) and local informants meetings (villages in which no case was notified in 1992 by PHW). Results were compared with data from the surveillance system. The surveillance system was simple and well accepted. 30 villages that had not notified cases were visited: 28 were truly non endemic, but 2 were in fact endemic (1 case per village). In villages that notified cases in 1992, sensitivity for case detection by PHW was 78% (95% C.I. 69-87) in Bam and 67% (95% C.I. 56-78) in Oubritenga. Incidence in 1992 in these villages, as estimated by the validation survey, was 1.9 % (95% C.I. 0.9-3) in Bam and 2.2 % (95% C.I. 0.7-3.7) in Oubritenga as compared with 1.7% and 0.8% respectively (surveillance system data). An village-based surveillance system based upon active case search by PHW is a valuable tool to monitor dracunculiasis eradication efforts.

224 THE PREVALENCE OF ONCHOCERCIASIS IN THE NORTHWESTERN PROVINCE OF CAMEROON. Siegel JA*, Nguefeu CN, and McKerrow J. Department of Anatomic Pathology, University of California, San Francisco, CA; and C.U.S.S. Department of Immunology and Biotechnology, University of Yaounde, Republic of Cameroon.

We performed an epidemiological survey of villagers living on an island (Mbissa) surrounded by a new reservoir which developed after the construction of a dam across the Noun river in 1974. The local health care workers have claimed that there is an increased incidence of onchocerciasis since the dam was built due to the production of breeding sites for *Simulium damnosum*. This was this first screening study in the entire province. There were 331 participants out of an estimated population of 3000. A medical history, physical exam, visual acuity test, four skin snips, 5 cc of plasma, blood smears, and urine were taken from each participant. Based on skin snip, there was an infection prevalence of 41%. There was a significantly greater prevalence of parasitic infection in areas closer to the dam. 87% of the skin snip positive individuals were over 20 years of age. The prevalence of blindness was approximately 2%. This data will be further analyzed to correlate physical signs and

symptoms of onchocerciasis with microfilarial load and antibodies against specific recombinant antigen. From this study we expect to gain new insights into the impact of onchocerciasis in this region and provide data for the local health care workers to aid in treatment of infected individuals and to control infection.

225 PROGRESS IN THE GHANA GUINEA WORM ERADICATION PROGRAM. Bugri SZ*. Medical Director, Northern Region, Ministry of Health, Ghana.

As part of the global effort to eradicate dracunculiasis (Guinea worm disease), Ghana began its eradication program in 1988. The program developed a plan of action and set a target date for eradication of the disease in Ghana by the end of 1993. A national village-by-village case search in 1989 enumerated 179,556 cases of the disease, in 6,873 villages. Previously, routine surveillance had recorded less than 5,000 cases annually. The program trained at least one village-based health worker in each of the endemic villages to report cases monthly, conduct health education of villagers, distribute cloth filters, and help provide topical treatment of lesions. Other important intervention strategies include priority provision of new sources of safe drinking water to affected villages, and vector control using temephos (Abate). Surgical extraction of mature pre-emergent worms has recently been introduced. The percentage of endemic villages being reached by each of these measures is being monitored by district, region and nationally. Only 33,464 cases were reported in 1992, in 3,185 villages. Health workers in over 90% of endemic villages regularly submit reports of cases within thirty days of the end of the reporting month. Ghana is confident it will eradicate dracunculiasis by the global target date of 1995.

226 RAPID ASSESSMENT OF ONCHOCERCIASIS IN CAMEROON: A STUDY IN SAVANNA AND IN FOREST-SAVANNA MOSAIC. Boussinesq M, Prod'hon J, and Chippaux JP*. Antenne ORSTOM, Centre Pasteur, Yaounde, Cameroon.

The prevalence of nodules and the prevalence of leopard skin are recommended to consider large scale ivermectin treatment in communities with *Onchocera volvulus*, We compared the relationships between clinical and parasitological indices in tewo endemic areas in Cameroon. The study was conducted in 23 villages of savanna area and in 27 vilages of forest-savanna mosaic area. Three parasitological indicators were calculated: the community microfilarial load (CMFL) and the prevalence of microfilatia both in the population above 5 years old (PMFP) and males aged 20 years old and over (PMFM). Four clinical indicators have been measured: the prevalence of nodules and the prevalence of leopard skin, in both the population above 5 years old (PNP and PLSP) and males aged 20 years and over (PNM and PLSM). In savanna, the PNP and the PNM were closely related to the CMFL ($P<10^{-2}$) and to the PMFP and the PMFP and the PMFM ($P<0.05$). The PLSM was only related to the CMFL ($P<0.05$). In the forest-savanna mosaic area, the PNP and PMFM ($P<10^{-3}$). The PLSP and the PLSM were related to the CMFL and the PMFP ($P<10^{-3}$). WHO proposes that large cale ivermectin treatment should be considered urgent in communities is which the CMFL is higher than 10 microfilariae per snip. This threshold corresponds to PNP and PNM values of 30 and 50% respectively in the savanna area and 48 and 65% in the forest-savanna mosaic area.

227 SUPPRESSION OF CELLULAR IMMUNE RESPONSIVENESS IN RHESUS MONKEYS EXPERIMENTALLY INFECTED WITH *LOA LOA*. Osae-Addo GA*, Dennis VA, and Lasater BL. Department of Parasitology, Tulane Regional Primate Research Center, and Covington, LA; and Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

In rhesus monkeys with experimental loiasis infections, PBMC blastogenic response to parasite antigens declines after patency, and persists with chronicity of infection. This parasite-specific

hyporesponsiveness, which is common in human filarial infections, has been attributed to either antigen-specific suppressor T cells or to tolerance. Studies in our laboratory indicate, that with progression of infection, CD4/CD8 cell ratios are decreased mainly because of a greater percentage of CD8+ cells. The percentage of CD8+ cells expressing IL-2 receptors are also increased in antigen-driven PBMC cultures, especially those stimulated with microfilarial (mf) antigens. The present study examined the role of CD8 cells and *Loa loa* mf antigens in down regulating PBMC blastogenic response in infected rhesus monkeys. Depletion of CD8+ cells prior to stimulation of cells with mf antigen could not reverse the antigen-specific hyporesponsiveness. Neither did addition of exogenous IL-2 to CD8+ depleted cell cultures restore the ability of cells to respond to antigen. The role of mf antigens in down regulating the immune response was assessed by *in vitro* co-culture experiments. PBMC from infected animals were either untreated or treated with mf antigens for 5 days, washed and co-cultured with fresh autologous cells plus Con A. Untreated cells from 9 of 12 infected animals suppressed the Con A response of fresh autologous cells by 12-38%. This suppression was exacerbated in mf-treated co-cultures of 6 of 12 animals (2-24% suppression). This suggests that cells from infected animals are either in a state of tolerance or have been primed *in vivo* towards suppressor activity. Similar levels of suppression were also achieved with *B. malayi* adult worm antigens. Significant suppression was not obtained with untreated or antigen-treated naive monkey cells. Likewise, co-cultures of live mf with naive cells failed to suppress the Con A response which indicates that *in vitro*, CD8+ cells or mf antigens may not be directly responsible for the down-regulation of the immune response. We conclude that T-cell hyporesponsiveness in *L. loa* infected animals probably occurs *in vivo* via several mechanisms including tolerance.

228 ROLE OF SUBSETS OF T LYMPHOCYTES IN MURINE RESISTANCE TO THE HUMAN FILARIAL PARASITE, *BRUGIA MALAYI*. Rajan TV*, Greiner DL, Killeen NL, Littman DV, Shuiltz LD, and Yates J. University of Connecticut Health Center, CT; and Jackson Laboratory, Bar Harbor, ME; University of Massachusetts Medical Center, Worcester, MA; University of California San Francisco, San Francisco, CA; and Oakland University, Rochester, MI.

The ability of C.B.17 scid/scid mice (SCID mice) to support the growth and development of the human filarial parasite *Brugia malayi*, and the failure of normal immunocompetent mice to support such growth strongly imply that the antigen specific (adaptive) immune system is critical in mediating the resistance of immunocompetent mice to filarial parasites. In order to determine the component of the antigen specific immune system responsible for this resistance, we have used mice that have been engineered to lack defined components of the antigen specific immune system. Earlier studies revealed that mice lacking CD8+ T lymphocytes are non-permissive to filarial parasites, implying that this subset of T lymphocytes is not required. We have investigated the role of CD4+ T lymphocytes using two models. In one model, mice were injected with a monoclonal antibody directed against CD4+ T lymphocytes. In the second model, we used mice in which the gene encoding the CD4 antigen had been disrupted by homologous recombination. We find that in both cases, the CD4+ T cell depelted mice are completely resistant to the growth of *B. malayi*, suggesting that CD4+ T lymphocytes are not an obligate requirement for resistance. Taken together, these data imply that whereas T lymphocytes are required for filarial resistance, this resistance can be mediated either by CD4+ cells or CD8+ cells.

229 HISTOPATHOLOGICAL CHANGES IN THE SKIN OF SUDANESE ONCHOCERCAL PATIENTS UNDERGOING TREATMENT WITH IVERMECTIN. Barouka O, Mackenzie CD*, Mahmoud B, Magdi M, Williams J, and Campbell K. NIH Sudan Medical Parasitology Project, Khartoum, Sudan; Department of Pathology, Michigan State University, East Lansing, MI; and Department of Microbiology, Michigan State University, East Lansing, MI.

Patients were treated with regular treatments of ivermectin for onchocerciasis infection acquired through their residence in the endemic areas of the Ethiopian border region of Sudan. A series of

patients with chronic reactive onchocercal dermatitis ("sowda") were biopsied 3-4 times over a period of two weeks as part of a study designed to investigate the effects of this drug on this severe form of dermatitis. Histological samples were examined for inflammatory cell profile using the following antibodies: UCH-1, Leu-7, lysozyme, Factor VIII, PCNA, Q Endo, AE1 and B cell antigens. The finding indicate the development of an active cellular immune response during this treatment cycle. The consequences of this on clinical management will be discussed.

230 WESTERN BLOT ANALYSIS OF *ONCHOCERCA VOLVULUS* USING SERUM FROM MICE IMMUNE TO INFECTIVE STAGE LARVAE. Brigandi RA*, Lange AM, Yutanawiboonchai W, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

Previous studies have demonstrated that BALB/cBYJ mice can be immunized against infection with larval *Onchocerca volvulus* . In this model, elevated parasite-specific and non-specific IgE responses are associated with immunity to *Onchocerca volvulus* infection. Depletion of IgE by anti-IL-4 treatment eliminates immunity to *O. volvulus* infective third stage larvae (L3) in immunized mice. The goal of the present study is to identify antigens recognized by parasite-specific IgE. Sera obtained from mice which are protectively immunized against L3 are used for this analysis. Initially anti-mouse polyvalent immunoglobulin (IgG, IgA, IgM) is used to screen immune sera. Proteins with an apparent molecular weight of 200, 100, 85, 70, 55, and 39 kDa are identified in the L3 stage as uniquely recognized by protectively immunized mouse sera. Additional analysis reveals that IgE from immune mouse sera, but not from normal mouse sera, clearly recognizes a 39 kDa band in the L3 stage. Immune sera do not recognize a 39 kDa band in adult/microfilarial stage protein preparations. Recognition by IgE in immune mice and specificity to the infective stage and not the pathological stage indicates that the 39 kDa protein is a good candidate for further study. Future investigation will then determine if this or any of the other proteins are protective or are usable for detection of prepatent infection.

231 ABSENCE OF PROTECTIVE RESISTANCE TO HOMOLOGOUS CHALLENGE INFECTION IN JIRDS WITH CHRONIC, AMICROFILAREMIC INFECTIONS OF *BRUGIA PAHANGI* . Lin DS, Coleman SU*, Petit TA, Jones KS, Weil GJ, and Klei TR. Department of Veterinary Microbiology & Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA; and Washington University School of Medicine, St. Louis, MO.

Studies of experimentally infected cats suggest that some individuals that become amicrofilarenic are resistant to reinfection with filariae, and express a form of concomitant immunity. This hypothesis was tested in the jird-*Brugia* model by determining the 25 day old larval burden following challenge infection in 6 groups of chronically infected jirds. These groups were; amicrofilaremic antigenemia negative (N 11); amicrofilaremic, antigenemic (N 13); high microfilaremic (>400/20 µl) (N 10); low microfilaremic (<4/20 µl); and uninfected controls (N 10). Nonchallenged age matched controls included jirds with both high (N7) and low (N6) microfilaremias. Challenge and existing adult worm populations were readily differentiated by size. Amicrofilaremic jirds had significantly fewer adult worms than microfilaremic jirds. No differences in recovery of 25 day old parasites were seen between any challenged group. Based on mean numbers of lymph thrombi and spermatic cord lymphatic dilation, lesion severity was greater in microfilaremic jirds than in amicrofilaremic jirds and was not apparently altered by the challenge infection. Existing infections appeared to inhibit the challenge infection induced eosinophilia observer in controls. These data indicate that the development of low level chronic occult infections in jirds is not accompanied by acquired resistance to reinfection and supports the hypothesis that long term multiple inoculations of L3 may be necessary to induce the phenomena of concomitant immunity in filariae.

232 IDENTIFICATION OF IMMUNOGENIC EXCRETORY-SECRETORY PROTEINS OF *ONCHOCERCA VOLVULUS* LARVAE. Irvine M*, Brotman B, Prince AM, and Lustigman S. The Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY; and Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia.

The success of live and irradiation attenuated L3 vaccines in a variety of animal models of filariasis and other helminths suggests that products actively synthesized and released (excreory-secretory (ES) products) by developing larvae may be prime protective immunogens. Our study is directed towards the identification of E-S antigens of *Onchocerca volvulus* that are immunogenic and correlated to immune status. Culture supernatants of day 1 and day 2 L3 were analyzed by Western blot and immunoprecipitation using a variety of sera. The same five antigenic proteins were immunoprecipitated from ^{35}S-methionine labelled supernatants by antibodies from two chimpanzees immunized with X-irradiated L3 larvae of *O. volvulus*. One of the chimpanzees (chimp 300) became patent after challenge; the other chimpanzee (chimp 305) never became patent and is therefore regarded as being putatively protected. In addition, a unique stage-specific 15 kDa product was recognized by antibodies from chimp 305. E-S products of L3 day 1 were purified, concentrated 25 times and analyzed by Western blot analysis. Using rabbit antisera raised against larval stages of *O. volvulus*, specific antigens of 120, 32, 25 and 15 kDa were recognized. Sera from both patent and non-patent humans residing in onchocerciasis endemic areas identified two immunoreactive E-S products of 55 and 50 kDa. Substrate gel electrophoresis has indicated that some of the L3 E-S products have specific serine and metalloprotease activities. This analysis makes it possible to clone and characterize unique antigenic E-S products and evaluate their potential role in protection.

233 IDENTIFICATION AND CHARACTERIZATION OF TWO RECOMBINANT FILARIAL ANTIGENS RECOGNIZED BY IgE OF *WUCHERERIA BANCROFTI*-INFECTED INDIVIDUALS. Mollis SN*, Raghavan N, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD.

The molecular nature of the parasite antigens (Ag) which induce the characteristic IgE response of human filarial infections has not been well defined. Thus, to identify and characterize those filarial Ag capable of inducing an IgE response, a *Brugia malayi* adult cDNA library was screened using a serum pool from filaria-infected individuals. Further screening of the expressed fusion proteins, using a protein-G adsorbed pool of sera from patients with the tropical pulmonary eosinophilia (TPE) syndrome and elevated serum IgE levels, identified two recombinants with M_rs of 157 kD (Wb1.2) and 144 kD (Wb2.1) that were strongly recognized by IgE from the patients with TPE. PCR amplification of the phage DNA showed the Wb1.2 insert to be 620 bp; sequence analysis revealed a single open reading frame (ORF) of 153 amino acids (aa) and no sequence similarity to known sequences. Wb2.1 has a 340 bp insert, a single ORF of 88 aa and 43% aa sequence identity with several of the members of a high mobility group (HMG-1/-2) protein family. Because IgE and IgG_4 antibodies show parallel Ag recognition, the two expressed fusion proteins were screened for IgE and IgG_4 recognition by serum from individuals infected with *W. bancrofti* and controls. Wb1.2 was recognized by IgE from 9/10 TPE patients and 7/7 microfilaremic (MF) individuals and by IgG_4 from 1/9 TPE and 5/7 MF patients; and Wb2.1 was seen by IgE from 5/7 TPE and 6/7 MF and by IgG_4 from 4/9 TPE and 5/7 MF patients. Further characterization of these filarial Ag may help define the signals necessary for induction of IgE and IgG_4 antibody production and give insights into the regulation of immediate hypersensitivity responses.

234 MOLECULAR CLONING AND SEROLOGICAL CHARACTERIZATION OF A *BRUGIA MALAYI* PEPSIN INHIBITOR HOMOLOG. Xu M *, Dissanayake S, Petralanda I, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and CAICET, Puerto Ayacucho, Venezuela.

By screening a cDNA library of adult male *Brugia malayi* with sera from microfilaremic donors with bancroftian filariasis, we identified a microfilaremia-associated recombinant antigen with diagnostic potential (Bm33). The cDNA clone contains an open reading frame coding for 221 amino acid residues continuous with β-galactosidase of λgt11. Nucleotide and protein sequence analysis indicates that Bm33 is a member of the nematode pepsin inhibitor family. At the protein level, Bm33 is 60 % homologous to the *Onchocerca*-specific antigen called Ov33 or Oc 3.6. Identical amino acids are present in 134 positions of the *Brugia, Onchocerca* and *A. viteae* molecules, which share an additional 28 identical residues with the *Ascaris* homolog. The *Brugia* molecule contains only the four central amino acids of the LVRDLT motif suggested by others to be the "active site" of the pepsin inhibitor. Although the proteins are very similar, the antigenic specificity of Bm33 differs markedly from that reported for Ov33/Oc3.6. Western blot analysis indicates that sera from ~ 60 % of microfilaremic and ~ 37 % of amicrofilaremic persons with bancroftian filariasis contain specific antibodies to recombinant Bm33. In contrast, positivity rates of sera from African or Venezuelan donors with skin snip proven onchocerciasis are 17 and 10 %, respectively. Reactivity of the latter sera with Bm33 is also much weaker than that of sera from donors with bancroftian filariasis. None of 17 sera from patients with mansonellosis, dracunculosis or loaiasis reacted with Bm33.

235 MOLECULAR CLONING AND PARTIAL CHARACTERIZATION OF A *LOA LOA* ALLERGEN. Akue JP*, Egwang TG, and Ajuh PM. Centre Internationale de Recherches Medicales de Franceville (CIRMF), Franceville, Gabon.

A ladder antigen of *Loa loa* was identified on Western blots of a detergent-solubilised extract probed with a pool of resistant sera. The smallest subunit has a molecular weight of about 15.0 kilodaltons (KD) on SDS/PAGE, and larger subunits represent increments of 15 KD : 30, 45, 60, 75, 90 etc. The antigen is not recognised by the sera of individuals singly infected with *Mansonella perstans* nor *Onchocerca volvulus*, two filarial parasites that a sympatric with *L. loa* in Gabon. The antigen is recognised by sera of *L. loa*-infected mandrills before patency, suggesting its usefulness as a specific marker for prepatent or occult infections. However, only 44 out of 80 loaiasis sera react with this antigen. The immune response to this antigen may, therefore, by subject to HLA restriction. An 1800-bp genomic *L. loa* λ gt11 clone encoding this antigen was isolated out of a genomic expression library using a pool of resistant sera. A lysate of *Escherichia coli* containing this clone produced multiple bands when probed with resistant sera on Western blots. A mouse polyclonal serum to this lysate reacted with the ladder antigen. Restriction mapping and Southern blot analysis indicates that the genomic clone contains tandemly repetitive DNA of 430 bp. Nucleotide sequencing has revealed that the 1800-pb genomic DNA contains no introns and encodes 4 repeats of 140 amino acids each. Each repeat shows 40-60% homology to ABA-1 allergen of *Ascaris suum* and the filarial homologue described in *Dirofilaria immitis*. The availabiltiy of recombinant allergen of *L. loa* may facilitate immunological studies of the pathogenesis of Calabar oedema and pruritis.

236 CHARACTERIZATION OF A 39 KILODALTON *DIROFILARIA IMMITIS* LARVAL SPECIFIC PROTEIN WITH IMMUNOPROPHYLACTIC POTENTIAL. Tripp CA*, Frank RS, Frank GR, Mika-Grieve M, and Grieve RB. Paravax, Inc., Fort Collins, CO.

Filarial nematodes, including the infectious agent of canine heartworm disease, *Dirofilaria immitis*, cause considerable morbidity in their respective vertebrate hosts. Unlike natural infections, a significant protective immune response can be detected in dogs receiving chemically abbreviated *D. immitis* larval infections. Sera from immune dogs were used to identify putative protective, larval specific proteins which were not recognized by sera from infected cohorts. The genes corresponding to these antigens were cloned from larval cDNA libraries and are being studied as potential vaccine candidates. This report focuses on the characterization of a clone encoding a 39 kD developmentally regulated protein that was isolated from the L3 cDNA library by immunoscreening. Both the native larval 39 kD protein and the recombinant protein are uniquely recognized by immune sera. Clone

specific antibodies were used to verify this gene encodes a native larval protein of 39 kD. The coding region has been subcloned into expression vectors to optimize production for immunization studies.

237 RELATIONSHIP BETWEEN *IN VITRO* KILLING OF MICROFILARIAE, PATENCY, AND HUMORAL RESPONSE TO *ONCHOCERCA VOLVULUS* ANTIGENS. Johnson EH*, Kass PH, Irvine M, Prince AM, and Lustigman S. Vilab II, The Liberian Institute for Biomedical Research, Robertsfield, Liberia; School of Veterinary Medicine, University of California, Davis, CA; and The Lindsley F. Kimball Research Institute, The New York Blood Center, New York, NY.

The *in vitro* cellular killing of microfilariae (mf) of *Onchocerca volvulus* mediated by neutrophils and freshly obtained autologous serum was compared in three groups of individuals: patently infected (N=41), non-patent endemic controls (N=35) and non-endemic controls (N=8). Infected individuals and endemic controls killed nodular mf at significantly higher levels (91.5 and 91.8% respectively) than non-endemic controls (32.2%, $p<0.001$). These findings were consistent at all serum dilutions tested. The level of killing in infected individuals varied from 59 to 100% when the mean mf count/skin snip was less than 35. Killing was 95 to 100% in individuals with higher mf levels. Among infected individuals and endemic controls, mean killing of nodular mf was significantly higher than killing of skin mf. The killing of skin mf in both groups is significantly higher than in the non-endemic controls. The antibody response to five *O. volvulus* antigens: OVAg, GST-OV7, GST-OV103, GST-OV3.6 and MBP-OV16 revealed serological differences between the infected individuals and endemic controls. Infected individuals had a higher frequency of response to OVAg (95.2 vs 57.1%, $p<0.01$) and OV7 (66.7 vs 37.1%, $p<0.1$) than endemic controls, and were also more likely to respond positively to all antigens except OV103 (38 vs 3%) (risk ratio = 13.33; 95% confidence interval 1.86, 95.60). Preliminary observations using Western blot analysis suggest that sera from certain endemic controls respond quantitatively at higher levels to 40, 45 and 47 kDa proteins than infected individuals. These studies suggest that a high proportion of endemic controls have been, or are infected, but are still immunologically unique in their ability to limit development of mf *in vivo*, as no patency was observed in these individuals.

238 FIELD EVALUATION OF A RECOMBINANT ANTIGEN-BASED ANTIBODY ASSAY FOR DIAGNOSIS OF BANCROFTIAN FILARIASIS IN EGYPT. Ramzy RM*, Helmy H, Chandrashekar R, Faris R, Gad AM, and Weil GJ. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt; and Washington University School of Medicine, St. Louis, MO.

Prior studies have shown that most sera from Indian patients with bancroftian and brugian filariasis have IgG4 antibodies to a *Brugia malayi* recombinant antigen BmM14. The present study evaluated this test with a large collection of well-characterized sera from an Egyptian village that is highly endemic for *W. bancrofti*. Positive tests were observed in 124 of 136 microfilaria (MF) carriers (91.1%), 56 of 63 antigen positive endemic normals (88.9%), 6 of 9 clinical cases (67%), and 175 of 320 antigen negative endemic normals (54.7%). Sera from Egyptians with no history of residence in a filariasis endemic area were uniformly negative in the test. These included 42 sera from healthy Egyptians and 47 sera from people with a variety of nonfilarial helminth infections. Advantages of the test are its simplicity, sensitivity in MF carriers, and specificity for filariasis. Weaknesses include relative insensivity for clinical cases and the high prevalence of antibodies in endemic normals without MF, clinical disease or antigenemia. This test may be useful as a means of primary surveillance for filariasis endemicity in communities not known to be endemic for filariasis and as a means of rapidly assessing areas where the disease may have been recently introduced.

239 CANINE EHRLICHIOSIS IN EGYPT: SEROEPIDEMIOLOGICAL SURVEY. Botros BA*, Elmolla MS, Salib AW, Calamaio CA, Dasch GA, and Arthur RR. NAMRU-3, Cairo, Egypt; Police Academy, Cairo, Egypt; and Naval Medical Research Institute, Bethesda, MD.

Ehrlichiosis or tropical canine pancytopenia is a tick-borne hemorrhagic disease of dogs caused by *Ehrlichia canis* (EC). Ehrlichiosis has been reported in the Middle East and E. Africa but not in Egypt. This study assessed the prevalence of EC infection in dog populations in Egypt. A total of 374 dogs, 252 from five military kennels (MD) and 122 household pets (HP), were tested for EC antibody (Ab). Sera were tested at a 1:20 dilution by IFA using EC cell culture antigen slides (ProtaTek). The overall prevalence of EC Ab was 33%. Ab prevalence among MD (29%) was lower than among HP (41%; P<0.05). The EC seroprevalence among dogs infested with ticks (*R. sanguineus*) was higher (44%) than that among uninfested dogs (31%; P=0.08). The seroprevalence among MD varied from 21% to 46% at the five kennels; lower prevalences were observed in kennels with higher sanitary and hygienic conditions. Age and sex-related EC Ab prevalence between MD and HP was not significantly different, although adult male HP had the highest seroprevalence (45%). Three dogs with epistaxis, a clinical sign suggestive of EC infection, had EC Ab titers >1:320. These data demonstrate the first laboratory evidence of EC infection among dogs in Egypt and suggests that infection is more prevalent in dogs with higher risk of tick exposure.

240 SEROLOGIC DIAGNOSIS OF LOUSEBORNE TYPHUS IN ETHIOPIA. Messele T, Tzianabos T*, and Olson J. National Institute of Health, Addis Ababa, Ethiopia; and Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

Enzyme immunoassays (EIAs) employing *Rickettsia prowazekii* lipopolysaccharide (LPS) antigen were compared with the indirect immunofluorescence assay (IFA) for the serologic confirmation of louseborne typhus. Between August 1990 and February 1991, patients suspected of having typhus were referred to the National Research Institute of Health for serologic testing. Of 173 patients with a history of fever for 10 days or less and from whom acute- and convalescent-phase sera were collected, 43 (25%) had illnesses confirmed as typhus by IFA. The diagnostic sensitivities and specificities of a direct IgG EIA, a capture IgM EIA and the Weil Felix microagglutination test (WF) were calculated, using the IFA result as the true diagnosis. Sensitivity was highest for the IgG EIA (96%), followed by the IgM EIA (87%) and the WF (66%). Specificity was highest for the WF (86%), followed by the IgM EIA (85%) and the IgG EIA (83%). The diagnostic utility of the IgM EIA on single acute-phase serum specimens was 72% sensitive and 84% specific. EIAs were both sensitive and specific for the diagnosis of typhus and had several advantages over the other assays evaluated.

241 PROTECTION OF GUINEA PIGS FROM EXPERIMENTAL ROCKY MOUNTAIN SPOTTED FEVER WITH BACULOVIRUS EXPRESSED *RICKETTSIA RICKETTSII* ROMPA PROTEIN. Sumner JW*, Sims KG, Jones DC, Olson JG, and Anderson BE. Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

Previously available Rocky Mountain spotted fever vaccines have been shown to be only partially effective in human vaccine trials. Currently, a commercial vaccine is not available. Two *Rickettsia rickettsii* surface antigens, the rOmpA and the rOmpB proteins, have been shown to be protective in animal studies. The 2,249 amino acid rOmpA protein has been cloned and expressed in *Escherichia coli*, but was cytotoxic and appeared to be severely degraded by proteases. Using the pAcDSM baculovirus transfer vector, we constructed baculovirus recombinants that express the *R. rickettsii* rOmpA protein. Monoclonal antibodies specific for rOmpA reacted with recombinant infected Sf9 cells in indirect immunofluorescence assays (IFA). Sodium dodecyl sulfate- polyacrylamide gel electrophoresis and immunoblotting of recombinant infected Sf9 cell lysates with a specific monoclonal antibody, or with serum from a rabbit immunized with *R. rickettsii*, showed the recombinant expressed rOmpA protein migrated slightly below the native *R. rickettsii* expressed protein. Guinea pigs immunized with sonic lysates of recombinant infected Sf9 cells developed antibodies reactive with *R. rickettsii* by IFA, and were protected against challenge with *R. rickettsii*,

indicating the baculovirus expressed rOmpA protein could be useful for the production of subunit vaccines, and for studies of the immune response to *R. rickettsii* infection.

242 WESTERN BLOTTING ANALYSIS OF SERA FROM MILITARY PERSONNEL EXHIBITING SEROLOGICAL REACTIVITY TO SPOTTED FEVER GROUP RICKETTSIAE. Dasch GA, Kelly DJ*, Richards AL, Sanchez JL, and Rives CC. Naval Medical Research Institute, Bethesda, MD; Walter Reed Army Institute of Research, Washington, DC; and Centers for Disease Control and Prevention, Atlanta, GA.

Military personnel may have significant risk of tick-transmitted diseases due to extended exposure during field training exercises. In the spring of 1989 a military unit from Maryland trained in Arkansas and Virginia. Two individuals experienced acute febrile episodes requiring hospitalization and seroconverted by indirect fluorescent antibody (IFA) to *Rickettsia rickettsii*. An additional 16 of 109 individuals in the unit were anti-immunoglobulin IFA positive post-exposure at >1:32 dilution. When the sera were screened by IgG IFA (1:64 cutoff) and IgG ELISA (1:100 dilution), 5 and 28 of the 109 were reactive against *R. rickettsii*, respectively. We analyzed the reactivity of 12 of these positive sera against *R. typhi* and 9 different species of spotted fever rickettsiae (SFG) found in North America by Western blotting to confirm that these individuals had been exposed to rickettsiae and to define which agent may have been responsible for the seroconversion observed. Nine of the 12 sera had IgG antibodies to SFG lipopolysaccharide (LPS) but not typhus LPS. The other 3 sera had IgM antibodies to *R. bellii* LPS only. IgG and/or IgM anti-major surface protein antigens (SPA) were detected in all of the sera. Most of the sera showed similar IgM reactivity toward *R. rickettsii*, *R. montana*, *R. parkeri*, *R. rhipicephali*, *R. akari*, *R. typhi*, 364-D, 85-1034, and Hlp#2 SPAs. Five sera exhibited significant IgG specificity toward SPA of 85-1034 which had been isolated from *Amblyomma americanum*, a tick commonly found in Arkansas. These results confirm that the soldiers had experienced mild spotted fever disease but some may have been exposed to 85-1034 rather than virulent *R. rickettsii*.

243 ANTIBIOTIC SUSCEPTIBILITY OF *RICKETTSIA TSUTSUGAMUSHI* FROM PATIENTS WITH SEVERE SCRUB TYPHUS IN NORTHERN THAILAND. Strickman D, Bodhidatta D, Kelly D*, Dasch G, Chouriyagune C, Watt G. Walter Reed Army Institute of Research, Washington, DC; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Naval Medical Research Institute, Bethesda, MD; and Prachanuchoa Hospital, Chiangrai, Thailand.

We have tested antibiotic susceptibility of eight isolates of *Rickettsia tsutsugamushi* (RT) made from human patients in northern Thailand, some of whom suffered severe disease or death. Isolates were grown in mouse fibroblast cells (L929), partially purified, and titered according to amount of cell damage. Susceptibilities to doxycycline, chloramphenicol, and ciprofloxacin were assayed in cells infected with RT immediately prior to addition of the antibiotics. The extent of rickettsial growth during 5 days was assayed by L929 cell uptake of neutral red vital stain, by observation of cytopathic effects (CPE), and by microscopic examination of rickettsial growth in Giemsa-stained preparations. Neutral red uptake proved to be the least sensitive means of assaying growth, probably because cytotoxicity was at least partially independent from rickettsial growth. The minimum inhibitory concentration (MIC) of antibiotic necessary to maintain CPE below 40% of the cells varied between 0.25 and 1.0 μg/ml for chloramphenicol, 4 and 16 μg/ml for doxycycline, and 4 and 8 μg/ml for ciprofloxacin. By NCCLS criteria, all of the strains were susceptible to chloramphenicol, one was resistant and four were of only intermediate susceptibility to doxycycline, and all were resistant to ciprofloxacin. The isolate most resistant to doxycycline showed marked growth of rickettsial particles 30 hours after infection even in the presence of 16 μg/ml of doxycycline. Tests to determine virulence of RT or associate virulence with particular strains might improve clinical therapy in the future by providing an early indication of the likely course of disease.

244 VALUE OF TRANSAMINASE AND PLATELET EVALUATIONS IN ACUTE TYPHOID FEVER PATIENTS. El-Masry NA*, Bassily S, Farid Z, Sultan Y, Abu-Elyazeed R, and Hibbs RG. U.S. Naval Medical Research Unit No. Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

Thirty-six blood culture-positive acute typhoid fever patients admitted to Abbassia Fever Hospital, Cairo, Egypt, were retrospectively evaluated during 1991-1992 to determine the effect of typhoid fever and treatment on transaminase (AST) and platelet counts. Hepatosplenomegaly (81%) and abdominal tenderness (67%) were the most prominent presenting signs. Thirty-three (92%) patients had AST values > 45 μ/l (1.5 times normal value of 30 μ/l) and 28 (78%) patients had platelet counts <200,000/mm^3 (lowest 20% of the normal range of 150 -400,000/mm^3). Fourteen days post-treatment there was a 30-80% decrease in AST values in 27 of 35 (77%) patients. The mean values significantly dropped from 149 to 60 (P<.01). Also 29 of 36 (81%) patients had 30-90% increase in platelet counts. The mean counts significantly increased from 160 to 315 (P <.01). When pre-treatment values of AST and platelet were compared to 22 patients with presumptive diagnosis of acute typhoid, but blood culture-negative, AST values were significantly greater (149 vs 56 μ/l, P <0.02) and platelet values were significantly lower (160 vs 244/cumm, P <0.02).Thus, clinical picture together with changes in AST and platelet values may be useful in the of diagnosis and follow-up of typhoid patients.

245 LYMPHOCYTE PROFILES AMONG INDONESIANS WITH TYPHOID FEVER. Pudjoprawoto N*, McGladdery S, Punjabi NH, Pulungsih S, and O'Hanley P. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Infectious Diseases Hospital, North Jakarta, Indonesia; and Stanford University, Stanford, CA.

Enumeration of lymphocyte subset profiles in clinically defined populations provides a basis to understand host cellular immune responses to specific disease states. We report here on the lymphocyte profiles of 51 culture-confirmed typhoid fever and 66 acutely febrile non-typhoid fever Indonesian patients between the ages of 14-60 years. Most patients received a full course of chloramphenicol. Blood was obtained from the patients during the acute illness (A) and during the convalescent period (C) up to 90 days after initial symptoms. Lymphocytes were prepared for flow cytometry utilizing a battery of fluorescein-labeled monoclonal antibodies. Results from these clinically ill patients were compared with normal Indonesian (H) reference standards for age and sex. In brief, flow cytometric data indicated that there was an overall decrease of lymphocyte subsets in all age groups and in both sexes among acutely ill patients irrespective of the duration of symptoms prior to hospitalization. This decrease was most marked in younger age groups and in males. The only exceptions to this trend were that there was an increase in total T and natural killer (NK) cells levels acutely among females of all ages. The trend during the convalescent period was for all lymphocyte subsets to return to normal ranges within 7 days of hospitalization. This study provides additional information about the relative immunodeficiency occurring during typhoid fever.

	Mean Total number cells/μl of blood											
	Total T		T4		T8		T4:T8		Total B		Total NK	
	M	F	M	F	M	F	M	F	M	F	M	F
H-Adult N=152	1168	1012	621	542	863	626	0.79	0.92	241	236	545	300
A-Typhoid N=51	832	1202	345	462	493	637	0.70	0.73	170	184	265	460
C-Typhoid N=34	1848	2077	709	711	859	900	0.82	0.79	248	227	337	316

246 EFFECT OF DEXAMETHASONE ON ACUTE INFLAMMATORY CYTOKINE LEVELS FROM CULTURED MONOCYTES OF TYPHOID FEVER PATIENTS. McGladdery S, Larasati R, Silitonga N, Punjabi NH, Lesmana M, Pulungsih SP, and O'Hanley P. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Infectious Diseases Hospital, North Jakarta, Indonesia; and Stanford University, Stanford, CA.

Mortality is reduced by dexamethasone treatment in severe typhoid fever; however, the mechanism of action of corticosteroids is unknown. One hypothesis to account for efficacy is that corticosteroids inhibit *in vivo* acute inflammatory cytokine responses. In this report, cytokines were evaluated in the supernatants from monocyte cultures of 6 patients with severe, culture-documented typhoid fever before and 48 hours after starting high dose dexamethasone treatment (HD). Cytokine levels were compared with 19 typhoid patients who did not receive dexamethasone. There were no significant differences between the 2 patient groups in initial cytokine levels from monocyte cultures acutely before patients received HD. Levels of tumor necrosis factor (TNF), interleukin-1 (IL-1) and IL-6 in monocyte cultures were invariably suppressed to non-detectable levels within 48 hours of receiving HD. By contrast cytokine levels from the same time course fell to 10-50% of the initial value but never reached non-detectable levels among typhoid patients not receiving dexamethasone. Gamma-interferon (γ-IFN) levels were not effected by HD and remained elevated in all patients regardless of dexamethasone treatment or not. In a further study, dexamethasone was shown to uniformly suppress production of TNF, IL-6 and IL-1 *in vitro* from monocyte cultures of 14 patients with confirmed typhoid fever, in a concentration-dependent fashion, compared to media alone ($p<0.05$). In addition, TNF levels in monocyte cultures from 2 healhty volunteers were compared after priming with increasing concentrations of lipopolysaccharide (LPS), with and without pretreatment with low dose dexamethasone (LD). No suppression of TNF production occurred when LD and LPS were given simultaneously; however, overnight incubation with LD prior to exposure to LPS led to a reduction of approximately 50% in the TNF response to increasing concentrations of LPS. In conclusion, dexamethasone suppresses the production of some acute inflammatory cytokines both *in vivo* and *in vitro* and may explain the mechanism by which corticosteroids reduce mortality in severe typhoid fever.

247 PRELIMINARY EVALUATION OF *VIBRIO CHOLERAE* NON-01 AGGLUTINATING STRAINS ASSOCIATED WITH ACUTE DIARRHEAL DISEASE IN NORTH JAKARTA. O'Hanley P*, Manurung N, Richie E, Peetosutan K, Punjabi N, Pulungsih SP, Masbar T, Widjaja D, Wangsasaputra F, Hanurawati W, McGladdery S, and Simanjuntak CH. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia.; Stanford University, Stanford, CA; Infectious Disease Hospital, North Jakarta, Indonesia; and National Institutes of Health Research and Development, Jakarta, Indonesia.

North Jakarta is a densely populated metropolitan area where cholera is endemic with an annual incidence of clinically severe cholera $\geq$ 1 case/1,000 total population. At one hospital during 1992, *Vibrio cholerae* 01, Ogawa strains accounted for 509 (95%) of all 535 severe cholera cases; 12 (2%) were due to 01, Inaba; and 14 (4%) were due to *V. cholerae* non-01 strains. An unusually high number of *V. cholerae* non-01 strains were isolated recently from the diarrheal stool of cholera patients in North Jakarta. They accounted for 15% and 20% of all culture-documented cholera cases during March and April 1993, respectively. There were no temporal, geographic, or household associations to suggest a focal outbreak for these non-01 cases. None of these 14 strains belonged to the 0139 serotype that has been associated with the recent epidemic outbreak in Bangladesh. The biochemical, hemagglutination patterns, antibiotic sensitivity, toxin production, and genetic diversity profiles have been compiled for these strains. Half of the cases were among children (mostly < 2 years) and occurred equally in each gender. Stool consistency and frequency were reported to be uniformly watery and averaged 9.9 stools/24 hrs prior to the first healthcare visit. From 50%-70% of the patients complained of vomiting, fever, and abdominal pain. In contrast to the moderate to severe dehydrating cholera disease caused by 01 strains here, non-01 strains that were isolated from diarrheal stool were associated with less dehydrating disease (DD): 1 pt had severe DD; 4 pts had moderate DD; 3 pts had mild DD; and 7 pts had no DD. Continued intensive surveillance for and characterization of non-O1 strains will elucidate the local epidemiology of this (these) enteric pathogen(s) and strategies for prevention by immunoprophylaxis and education.

248 DOSE-RESPONSE STUDY OF PASTEUR MERIEUX ' INACTIVATED HEPATITIS A VACCINE. Vidor E*, Garin D, Fanget B, Brasseur P, Delolme H, Caron F, Humbert G, Mojon M, Wallon M, Gravey D, Flehmig B, and Peyron F. P.M. sv., Marnes La Coquette, France; HIA Desgenettes, Medical Biology, Lyon, France; P.M sv., Lyon, France; CHR Charles Nicolle, Rouen, France; HIA Desgenettes, Lyon, France; CHU Croix Rousse, Lyon, France; and Hygiene Institute, Tubingen, Germany.

A formalin-inactivated aluminium hydroxide adsorbed hepatitis A vaccine has been evaluated in a dose-response study over 195 healthy male adults (age ranging : 18-31 years) in France (Lyon, Rouen). Doses ranging from 20 to 160 RIA antigen units have been IM administered with two injections over a 6 months period. 32 subjects (16.4 %) were found to be positive (> 20 mIU/mL) against HAV antigen (Total Ig RIA HAVAB assay, ABBOTT Laboratories) at time of the first vaccine injection and were excluded from the analysis of the immunogenicity criteria. Fourteen days after the first vaccine injection, 80 % (95 % CI : 62-90) of subjects having received the 160 RIA antigen units dose seroconverted with a GMT of 43 mIU/mL (95 % CI : 33-56). Seroconversion was 100 % (95 % CI : 91-100) at one month with a GMT of 95 mIU/mL (95 % CI : 80-112). Statistical analysis revealed a significant dose-effect on GMT by multivariate analysis and variance analysis with repeated measures. Biological safety was evaluated and ALAT and ASAT levels were similar before and fourteen days after the first injection in the four groups. No serious adverse events were reported and reactogenicity after the first injection was similar in the four groups: 6.1 % of subjects reported immediate reactions after vaccination (feeling sick, spontaneous pain, headache), 8.8 % reported local reactions at the site of injection (spontaneous pain, hematoma, local adenopathy) and 13 % reported general reactions ("Flu-like" syndrome, digestive troubles, fatigue, headache). Immunogenicity and safety results after booster injection of this ongoing study will be presented, however according to these data the 160 RIA antigen units dose will be used to confer a rapid protection against HAV during the next steps of the clinical development of this vaccine.

249 COMPARATIVE CONTROLLED STUDY OF THE IMMUNOGENICITY AND SAFETY OF TWO DOSING SCHEDULES OF HB VAX II HEPATITIS B VACCINE IN NEONATES. Bassily S*, Kotkat A, Gray GC, Hassan N, Imam Z, and Hibbs RG. U.S. Naval Medical Research Unit No. Three, Cairo, Egypt; High Institute of Public Health, Alexandria, Egypt; and Clinical Epidemiology Naval Health Research Center, San Diego, CA.

Healthy newborns of hepatitis B (HB) seronegative (HBsAg and HBeAg) mothers were randomly enrolled in one of three vaccination schedules.Two and one-half mcg Recombinant HB vaccine (HB vax II) was given at the ages of 2, 4 and 9 months (group A) or 0, 2 and 6 months (group B).These two groups and the third control group (group C) were given the other conventional vaccines (BCG, DPT, Polio and Measles) as recommended by the Egyptian Ministry of Health. There were 250 infants in each group. Blood samples, taken one month after the third HB vax II dose, were tested for HBsAg, anti-HBc and quantitatively for anti-HBs usingAbbott's commercial enzyme immunoassay kits. The vaccine was well tolerated and side-effects were limited to local soreness, redness or temporary swelling. Thus far, 522 of 750 infants have been followed and sampled. Good (51-300 mIU anti-HBs/mL) or excellent (>300 mIU anti-HBs/mL) immunogenic response occurred in 95% of infants in group A and in 92% in group B. Geometric mean antibody titres (GMT) of anti-HBs were 2042 mIU/mL in group A and 513 mIU/mL in group B. In conclusion, the HB vax II is safe and immunogenic when given in 3 doses of 2.5 mcg in either regimen.

250 ACUTE HEV INFECTIONS IN DJIBOUTI AND SENEGAL. Rodier GR*, Polycarpe D, Michel P, El-Zimaity DT, Arthur RR, Carl M, and Hyams KC. U.S. NAMRU-3, Cairo, Egypt; Service Medical Interentreprises, Djibouti; Institut Pasteur, Dakar, Senegal; and Naval Medical Research Institute, Bethesda, MD.

The role of hepatitis E virus (HEV) in acute viral hepatitis (ALT and AST >3X normal value) occurring in Senegal (2/92-2/93) and Djibouti (11/92-4/93) was investigated. Sera were collected during the acute phase of illness from 42 patients in Djibouti, East Africa and from 34 in Dakar, Senegal in W. Africa. Convalescent- phase sera were collected from approx. 50% of patients. The mean ages (range) of patients in Djibouti and Senegal was 27.6 (1-70) and 29.3 yrs. (1-66), respectively. Sera were tested by EIA for anti-HAV IgM, anti-HBc IgM, HBsAg, total anti-HDV if HBV markers were positive and by a Western blot assay for anti-HEV IgM. Sera negative for acute HAV and HBV markers were tested for anti-HCV IgG by EIA and anti-HEV IgM using a Western blot assay. Sera were positive for anti-HEV IgM in 65.3% of acute hepatitis cases in Djibouti versus only 7.5% in Senegal. HAV infection occurred in 33.3% and 17.5% of cases in Djibouti and Senegal, respectively. Hepatitis B was diagnosed in only 2.4% in Djibouti vs. 30% in Senegal; whereas, HBV-carriers were identified in 14.3% of patients in Djibouti and 7.5% in Senegal. Five (9.3%) sera from Djibouti reacted positively for both anti-HAV and anti-HEV. Sera were negative for all viral markers in 14.3% of the cases in Djibouti and 45% in Senegal.Fifteen epidemiological variables, risk factors of parenteral inoculation, and 16 clinical variables were analyzed.

251 EVALUATION OF POXVIRUS-HANTAAN VACINES IN HAMSTERS. McClain DJ*, Summers PL, Henchel E, Dalrymple JM, and Schmaljohn CS. U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

A hamster model for protection against challenge with Hantaan virus was used to evaluate candidate Hantaan vaccines. Hamsters were immunized intramuscularly with poxviruses which expressed the envelope glycoprotein and nucleocapsid protein encoded by cDNA from the M and S genomic segments, respectively, of the 76-118 strain of Hantaan virus. The animals were subsequently challenged with wild-type Hantaan virus. Protection was judged by the absence of Hantaan antigen in the lung and kidney as detected by direct immunofluorescence microscopy. A vaccinia-vectored M+S construct induced protective immunity after either 1 or 2 injections. Evidence of protection was also seen after 2 injections of an M+S construct vectored by either a NYVAC (attenuated vaccinia virus) or ALVAC (canarypox virus) recombinant. Repeat experiments comparing the M-only vs. the M+S vaccinia construct indicated protection after 1 or 2 immunizing doses of either vaccine. However, examination of the animals' sera showed higher geometric mean titers by ELISA in those receiving the M-only construct. Plaque-reduction neutralization titers will be discussed and related to the degree of protection observed.

252 AN EVALUATION OF A MEASURE OF RODENT DENSITY AS AN INDICATOR OF RISK FOR ARGENTINE HEMORRHAGIC FEVER. Calderon GE*, Sabattini MS, Mills JN, Feuillade MR, and Maiztegui JI. Instituto Nacional de Enfermedades Virales Humanas, Pergamino, Argentina; and Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

Junin virus (JV), etiologic agent of Argentine Hemorrhagic Fever (AHF) is transmitted to human by infected rodents, primarily *Calomys musculinus*. Progressive extension of the AHF endemic area has created a dynamic pattern of three epidemiologically-defined geographic zones. Historic areas are older parts of the endemic area, with currently low incidence of disease; epidemic areas have high and variable incidence; non-endemic areas are peripheral zones where no cases have been reported. This pattern results in distinct geographic variations in the risk for AHF. In search for a rapid, inexpensive predictor of risk, we compared trap success (TS), a measure of the relative density of rodents, among 3 historic, 6 epidemic and 3 non-endemic localities. Between June 1991 and April 193, we captured 3,130 rodents (including 473 *C. musculinus* and analyzed total TS (all species combined) and specific TS for *C. musculinus*, by season. During periods of maximum rodent density (autumn and early winter), total TS was higher in epidemic and historic than in non-endemic localities.

However, total TS was not uniformly higher than in historic areas for all years/seasons, and specific TS values for *C. musculinus* in epidemic localities were equal to, or lower than, values in historic localities. This observation may reflect the deleterious effect of JV on populations of *C. musculinus* in epidemic areas. TS may not be a useful marker of geographic variation in risk, but may help explain temporal variation. The integration of data from ongoing virological, ecological, and genetic studies of *C. musculinus* populations in different epidemiological areas should contribute to an understanding of the natural history of JV.

253 ELEVATED LEVELS OF TOTAL AND SPECIFIC IgE IN SCANDINAVIAN TYPE OF HEMORRHAGIC FEVER WITH RENAL SYNDROME. Alexeyev OA*, Ahlm C, Billheden J, Settergren B, Wadell G, and Juto P. Department of Virology, Department of Infectious Diseases, University Hospital of Northern Sweden, Umea, Sweden.

One previous study showed increased levels of total IgE in patients with hemorrhagic fever with renal syndrome (HFRS). The aim of the present study was to investigate whether specific IgE is produced during the course of the disease. For this purpose an ε-capture ELISA was developed. In total 72 patients with HFRS caused by Puumala virus were studied. Three different control groups were included: 20 blood donors, 20 patients with other viral diseases (influenza A and B, acute Epstein-Barr virus and acute cytomegalovirus infections) and 5 subjects with high levels of total IgE (median 1070 kU/l, range 773-5740). Total IgE was significantly higher during the acute phase of HFRS (median 34.5 kU/l, range 0-907) as compared to blood donors (median 10 kU/l, range 0-63) and other viral disease (median 1 kU/l, range 0-216, $p<0.05$). All patients developed specific IgE in the acute phase of the disease (median 55 AU/l, range 24-123) as compared to the different control groups in which no specific IgE was detectable. Both total and specific IgE decreased in convalescence as compared to acute phase of HFRS ($p<0.002$ and $p<0.001$, respectively). In conclusion, we have shown that both total and specific IgE are increased in HFRS as compared to other viral diseases. The possible pathogenic role of the specific IgE response in HFRS remains a prospect for further studies.

254 HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS): PRESENT SITUATION IN RUSSIA. Tkachenko EA* and Drozdov SG. Institute of Poliomyelitis and Viral Encephalitides, Russian, Academy of Medical Sciences, Moscow, Russia.

Total number of cases registered in Russia during 1978-1992 was 68,796 (66,089 cases in European and 2,707 in Asian part of the country). The most cases are registered in 20-45 years age group, female/male ratio among all the patients is 1:6. Children up to 14 years of age compose about 4% of the total number of diseased persons. Clinical picture of HFRS is variable, nevertheless in Far Eastern foci, where viruses of Hantaan serotype are predominant, severe forms of disease are also predominant. In European foci, where clinical cases in human beings are caused mainly by viruses of Puumala serotype, most cases are moderate or mild. The proportion of fatal cases as an indicator of severity composes 1-2% in European foci and 10-15% in Far-Eastern ones. Untio 1985 the HFRS cases were registered in 38 of 89 administrative territoria of Russia. In 1985-1992 the cases of HFRS were registered in 23 more regions. About 300,000 small mammals belonging to 63 species and collected in all landscape zones of Russia were studied for the presence of hantaviruses. The HV antigen was found in the mammals of 45 species and in 13 species of birds.

255 MOLECULAR CHARACTERIZATION OF DEN-1 VACCINE CANDIDATE 45 AZ5. Puri B*, Nelson WM, Howland DF, Henchal EA, and Hayes CG. Viral and Rickettsial Disease Program, Infectious Disease Division, Naval Medical Research Institute, Bethesda, MD; Department of Molecular Virology, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD; Geo-Centers, Inc., Fort Washington, MD; and Virology Division, U. S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

The molecular basis of dengue virus attenuation is unknown. While significant differences have been observed in the growth characteristics of vaccine candidates in humans, monkeys, mice and model cell culture systems, little attempt has been made to link nucleotide base sequence changes to observed biological phenotypes. In an effort to identify the changes accumulating in the nucleotide base sequences of dengue viruses after attenuation, DEN-1 vaccine candidate 45 AZ5 (PDK 27) have been cloned and sequenced by a fluorescent chain terminating method on a Genesis 2000 automated sequencer. Two changes have been observed in E region of vaccine candidate as compared to parent strain. Comparison of nucleotide base sequences of vaccine candidate with the parent virulent strain will permit conserved and unique changes important for attenuation. In addition, evaluating sequences from the noncoding 5' and 3' portion of the genome will allow comparison of potentially important structural properties of the nucleic acids of virulent and avirulent dengue viruses.

256 GENETIC STUDY OF DENGUE-3 VIRUSES. Chungue E*, Deubel V, Cassar O, Laille M, and Martin PM. Institut Territorial de Recherches Medicales Louis Malarde, BP 30, Papeete, French Polynesia; Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex, France; and Institut Pasteur de Noumea, Noumea, New Caledonia.

Only limited sequence data and genetic informations on dengue-3 viruses are available. In this study, the nucleotide sequences of a short fragment of the E-protein gene encoding amino acids 25 to 89 of 27 dengue-3 viruses were determined by direct sequencing of polymerase chain reaction (PCR) amplified products, and compared regarding their time and geographic distribution. This method can characterize reliably unadapted dengue-3 isolates and provided sufficient information for estimating genetic relationships among them. The published nucleotide sequence of the reference dengue-3 strain (H-87) was included in this study. Four distinct genotypic groups were discerned at 6 % divergence between nucleotide sequences. The first group contains isolates from South Pacific (1988-1992), Singapore (1973) and Indonesia (1973-1991). The second group comprises viruses from Asia (1956-1989) including the reference strain H-87. The third was composed of one isolate from Thailand (1971), and the fourth includes the early strains from French Polynesia (1964-1969) and from Puerto Rico (1963). Furthermore, difference between early and recent strains from South Pacific was as high as 12.3 %. This observation suggests that the recent epidemics in South Pacific were likely the consequence of the spread of a new variant that merged from New Caledonia.

257 DEVELOPMENT OF ELISAS TO DETECT FLAVIVIRUSES: GENUS SEROGROUP AND COMPLEX SPECIFIC ANTIGENS. Lewis TE*, Rossi CA, Montoya RR, Korch G, Hile J, and Mangiafico JA. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD.

We developed an ELISA to identify members of the *Flavivirus* genus and then, if necessary, to identify complex- specific antigens in samples. This method can initially distinguish flaviviruses from other arboviruses and, through further evaluation, identify the sero-complex to which the flavivirus belongs. The viral antigen is first captured from samples by using genus-specific antibodies. The bound antigen is then detected by using a broadly cross-reactive antibody followed by a horseradish peroxidase-labeled secondary antibody. If positive, the sample can then be evaluated simultaneously against a number of flavivirus complex-specific capture antibodies. Complete evaluation requires 2 ml of sample. Sensitivity and specificity data for the various members of the *Flavivirus* genus are presented.

258 ENZYME IMMUNOSORBENT ASSAY FOR DENGUE 2 VIRUS USING HUMAN IMMUNOGLOBULINS. Teruel-Lopez E, Costa-Leon L, Valero-Fuenmayor N, Fuenmayor D,

Gutierrez L, and Martinez M. Instituto de Investigaciones Clinicas and Laboratorio Regional de Referencia Virologica, Universidad del Zulia, Maracaibo, Venezuela.

Dengue virus is endemic in Zulia state (Venezuela) and the population has very high titer of antibodies against dengue virus as measured by IHA test (>1:2560) making the sera an important source of immunoglobulins. Indirect ELISA method was developed in our laboratory and IgG was isolated by chromatographic separation in microcolumns of Sephacryl and identified by radial immunodifussion with anti-human IgG; several concentrations of the isolated IgG were used to sensitized microplates together with monoclonals antibodies against dengue 2. IgG concentration of 5 μg/ml was selected for the standarization of the test and the results showed excellent correlation between both assays; when IgG was used as sample for determining antibody titer there was a significant difference ($p<0.01$) between negative sera and its respective IgG but not between positive sera and its IgG while the differences with both IgG were extremely significant ($p<0.0001$) as expected. This finding are very useful for field studies for their low cost and because they can be used simultaneously as primary antibody,as standards controls (negative or positive), and for immunoglobulins titration in convalescent sera.

259 DEVELOPMENT OF A GENOMIC TYPING ASSAY UNIVERSAL FOR MOSQUITO-BORNE FLAVIVIRUSES: APPLICATION TO THE GROUPING OF AFRICAN WEST NILE VIRUS ISOLATES. Berthet FX*, Zeller HG*, Pierre V, Digoutte JP, and Deubel V. Arbovirus Research Unit, Institut Pasteur, Dakar, Senegal; and Department of Virology, Institut Pasteur, Paris, France.

In order to assess the genetic variability of mosquito-borne flaviviruses, we developed a genomic typing assay based on reverse transcription/polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism (RFLP) analysis. A pair of degenerate oligonucleotide primers was designed by compiling available flavivirus nucleotide sequences. These primers flank a region encompassing the end of NS5 and part of the 3' non coding sequences, allowing the PCR amplification of about 5% of the viral genomic RNA. The versatility of these generic primers was confirmed by the successful amplification of more than fifteen mosquito-borne flaviviruses, including those of medical interest (Dengue serotypes 1 to 4, Yellow Fever, Japanese Encephalitis, West Nile.). This approach was used to identify West Nile (WN) virus genomic variants among a collection of 42 strains isolated from 10 african countries. Digestion of WN amplicons with 4 frequent cutter restriction enzymes (*AluI*, *DdeI*, *Hae*III, *Hpa*II) provided more than 20 RFLP alleles combined as 15 different genotypes. One genotype was found to be predominant and widely spread in Africa whereas others seem to be specific topotypes. Furthermore, identical RFLP patterns shared between French (Rhône delta) and Algerian (Djanet) WN strains support the implication of bird migrational movements in the continent-to-continent dissemination of the WN virus.

260 EXPERIMENTAL TRANSMISSION OF POWASSAN VIRUS BY *IXODES DAMMINI* TICKS. Costero A*and Grayson MA. Department of Entomology, McGill University, Montreal, Canada; and New York State Department of Health, Wadsworth Center for Labs. and Research, Arbovirus Laboratory, Albany, N.Y.

Powassan (POW) virus, the cause of human encephalitis in the northeastern U.S. and Canada, is transmitted by tick bite. Since the geographic and host distribution patterns of *Ixodes dammini* and POW virus overlap, it was decided to explore the potential of this tick to transmit POW virus. Infection experiments were conducted with viremic hamsters and immature *I. dammini* from a POW-free colony. Oral infection rates in larvae and nymphs tested 13-23 days after detachment were 10% and 40%, respectively. Transtadial transmission from the larval to nymphal stage was demonstrated by isolation of POW virus from 4 of 42 (9.5%) flat nymphs orally infected in the larval stage. Transtadially infected nymphs were able to transmit the virus to 3 of 4 clean hamsters which developed HI antibodies to POW virus 2 weeks after exposure. From these results we conclude that:

larvae and nymphs of *I. dammini* can become infected with POW virus when feeding on experimentally infected hosts; there is transtadial transmission of POW virus from larvae to nymphs; transtadially infected nymphs can transmit virus orally to clean hosts. Experiments are underway to determine: if transtadial transmission of POW virus occurs from infected nymphs to adults; if transtadially infected adults can transmit the virus by bite; if clean adults can become infected when feeding on POW-infected hosts; and if there is transovarial transmission of POW virus.

261 EVALUATION OF IMMUNOASSAYS TO DETECT ANTIBODIES TO VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS AND CHIKUNGUNYA VIRUS IN SERUM SAMPLES. Rossi CA*, Mangiafico JA, McClain D, Danner DK, Lewis TE, and Korch G. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; and Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

We evaluated sera from individuals vaccinated against either Venezuelan equine encephalomyelitis virus (VEE, N=41) or Chikungunya virus (CHIK, N=117) for IgM and IgG antibody titers by ELISAs to evaluate sensitivity and specificity of ELISA compared with the plaque-reduction neutralization test (PRNT). Positive titers by PRNT indicated true seropositivity. Sera were tested beginning on 10 day post-vaccination. Forty-four CHIK sera were positive by PRNT (80% reduction), 40 of these sera were positive by ELISA, and four were false negatives. Sixty-nine of these sera were negative by both ELISA and PRNT, while four sera yielded false positives. The ELISA sensitivity was 91% and specificity was 95%. Of the 41 sera tested to date, 18 of 41 of the VEE samples were PRNT positive (80% reduction), 13 of these were positive by PRNT and ELISA, and 22 were negative by both methods. One false positive and five false negatives by ELISA were observed. The ELISA sensitivity was 88% and specificity was 93%. These sera will be screened further for cross-reactivity by PRNT and ELISA.

262 EVALUATION OF IMMUNOCAPTURE PROCEDURES FOR PREPARING VENEZUELAN EQUINE ENCEPHALITIS (VEE) VIRUS SAMPLES FOR IDENTIFICATION BY RT-PCR. Knauert FK*, Parrish BA, Ibrahim MS, Kondig JP, and Korch GW. Applied Research Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

Methods for the isolation of nucleic acid from samples for polymerase chain reaction (PCR) procedures are typically labor intense and often use hazardous materials, which limit their routine use in a diagnostic laboratory. We evaluated several immunocapture procedures to improve sample preparation of VEE virus for reverse transcriptase (RT)-PCR. In one method, samples were added to tubes coated with virus-specific antibodies that selectively adsorbed the virus from the sample milieu. Tubes were washed to remove nonspecific contaminating material, leaving the captured viral particles attached to the tube in a relatively pure form. The second immunocapture procedure used antibody-coated magnetic beads to specifically concentrate viral particles before RT-PCR. The RNA from the captured particles was subsequently released by a proteinase K digestion. Proteinase K was inactivated by heating to 95°C for 5 min, and the samples were subjected to standard RT-PCR procedures. These immunocapture procedures are comparable to a guanidine isothiocyanate extraction procedure for preparing VEE virus for RT-PCR. They have the advantage of being simple, less labor intensive; and they eliminate the use of hazardous chemicals, and can be adapted to microtiter plate formats.

263 DEVELOPMENT OF AN IN SITU ELISA FOR THE DETECTION AND IDENTIFICATION OF SANDFLY VIRUSES. Summers PL*, Silverstein JA, and McClain DJ. Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

Sandfly fever (SF) is a acute viral infection transmitted to humans by the sandfly of the genus Phlebotomus. Fever usually lasts 2-3 days with detectable viremia 1 to 3 days before and during the onset of fever. However, detecting virus directly from sera by plaque assay is insensitive and inconsistent. Other techniques such as immunofluorescence (IFA), antigen-capture ELISA and intrathoracic inoculation of sandflies are used more often. IFA appears to be the most sensitive test but interpretation is subjective. We combined the objectivity and sensitivity of an ELISA with virus amplification in cell culture to create a single assay system to titrate and immunologically identify sandfly viruses with SF-infected cells as antigen. Vero cells grown in 24- or 96-well plates were infected with the Sicilian or Naples strains of sandfly virus. After 4-5 days of incubation, culture medium was removed, and the monolayers were fixed. The plates were then incubated with blocker buffer, washed, and incubated with SF mouse hyperimmune ascitic fluid. The plates were again washed and incubated with peroxidase-labeled anti-mouse IgG. Results were read visually or spectrophotometrically after adding ABTS substrate. The entire in situ ELISA required 5 hours. Tissue culture infectious dose 50% (TCID50) results from this assay were obtained earlier and compared well with titers from plaque assays. The *in situ* ELISA is a simple, rapid, and sensitive method to measure and distinguish viruses and has been used successfully to titrate and identify vaccinia, Venezuelan equine encephalitis, and Hantaan viruses.

264 PHLEBOTOMINE SANDFLIES AND ISOLATIONS OF ARBOVIRUSES FROM A SAHELIAN REGION IN SENEGAL. Fontenille D*, Traore-Lamizana M, Zeller HG, Trouillet J, Leclerc A, Mondo M, Ba Y, and Digoutte JP. ORSTOM, BP 1386, Dakar, Senegal; Institut Pasteur, BP 220, Dakar, Senegal; and Departement de Biologie Animale, Universite CAD, Dakar, Senegal.

Longitudinal surveys on the ecology of sandflies and arbovirus transmission by insects were conducted around temporary ground pools in a sahelian region in Senegal from November 1991 to December 1992. Approximately 34,000 sandflies were collected by CO_2 light-traps with a peak of abundance in March and April, one month after the complete drying of the temporary ground pools. Eleven sandfly species were identified from 4,191 specimens caught by sticky traps, including *Phlebotomus duboscqi*, a leishmaniasis vector, *Sergentomyia adleri*, *S. clydei*, *S. magna* and *S. schwetzi*, which can feed on mammals. An average of 136 sandflies per m^2 were caught by sticky traps. One strain of Chandipura virus, four strains of Saboya virus, and one strain of a not yet identified virus were isolated. These are the first isolations of arboviruses from phlebotomine sandfly pools in West Africa. Chandipura virus, a Rhabdovirus from the VSV group of the genus vesiculovirus, was first isolated from patients in India, then from hedgehog in Nigeria. The Saboya Flavivirus was already isolated from small rodents (*Tatera kempi*, *Mastomys* sp, *Arvicantis niloticus* and *Mus musculus*) in Senegal. Its transmission cycle probably occured between rodentophilic sandflies and rodents. All these viruses were pathogenic for new born mice. No isolation of Rift Valley fever phlebovirus was obtained, despite its recent circulation in the survey area, either from mosquitoes or sandflies.

265 VIRAL SURVEY OF TICKS IN SAUDI ARABIA. Tantawy TA, Al-Khalifa MS, Elyan DE, Diab FM, Al-Asgah NA, Hussein HH, Botros BA, and Arthur RR. Virology Division, Naval Medical Research Unit No. 3, Cairo, Egypt; and Department of Zoology, College of Science, King Saud University, Saudi Arabia.

Tick samples collected from indigenous livestock in Saudi Arabia at locations that excluded any possible intermingling with imported animals were investigated to determine the possible viruses that they may carry. From Sept. 1991 to Dec. 1992 a total of 1295 ticks were collected from camels, cattle, sheep, goats, dogs, cats, rodents, chicken and pigeons in diverse areas of the country. Ticks were separated by species, sex and engorgement status into 172 pools (maximum of 30 ticks per pool). They represented 7 species of *Hyalomma* (134 pools), 2 species *ofRhipicephalus* (24 pools), *Argas persicus* (11 pools), *Haemaphysalissulcata* (2 pools) and *Boophilus kohlsi* (1 pool). With few exceptions ticks

were received live at NAMRU-3, were frozen (-70°C) and stored until being processed for culture. Frozen tick pools were triturated in MEM containing antibiotics and inoculated into suckling mice (IC), and into cultures of Vero and BHK-21 cells. Viruses were detected in 7 pools of female *Hyalomma* ticks (*H. dromedarii*, 3 pools from camels in Qasim Province; *H. impeltatum*, 3 pools from camels and cattle in Qasim and Gazan Provinces; *H. anatolicum*, 1 pool from goats in Eastern Province). Preliminary data obtained by immunofluorescent staining of infected cells suggests that 2 of 7 viruses are alphaviruses. Specific identification of all viruses is currently in progress.

266 SUSCEPTIBILITY OF THREE BREEDS OF INDIGENOUS SHEEP IN NIGERIA TO EXPERIMENTAL INFECTION WITH THE ZINGA STRAIN OF RIFT VALLEY FEVER VIRUS. Olaleye OD* and Tomori O. Department of Virology, College of Medicine, University College Hospital, Ibadan, Nigeria.

Rift valley fever (RVF) is an arthropod-borne viral disease primarily affecting ruminants causing abortion in adult animals and death in young offsprings. Since the first isolation of the virus during an epizootic in the Rift valley of Kenya in 1930, several outbreaks of RVF involving human and animals have occurred in different parts of Africa. In Nigeria, the role of domestic ruminants in the epidemiology of RVF is not fully understood. In this study, we sort to determine whether or not other breeds of sheep in Nigeria are also susceptible to RVF virus as previously shown for West African dwarf. Experimental infection of three indigenous breeds of sheep in Nigeria, namely, West African dwarf, ouda and yanka with Zinga strain of RVF virus resulted in marked haemorrhage which was very severe in Yankasa. There was a significant fall in mean PCV, hemoglobin concentration and total erythrocyte counts. Carcass of all the infected animals showed significant gross and microscopic lesions typical of RVF infection. This study shows that contrary to the reports that indigenous breeds of sheep in Nigeria may be relatively resistant to RVF virus infection, the three breeds of sheep used in this study are susceptible to Zinga strain of RVF virus. The severity of clinical outcome of infection with the virus varied slightly with breeds of sheep.

267 IMMUNO-ELECTRON MICROSCOPY OF INFLUENZA HAEMAGGLUTININ (HA1 & HA2) AND M2 PROTEIN MOLECULES AND THEIR INTRACELLULAR TRANSPORT. Ciampor F*, Vareckova E, Mucha V, Betakova T, Cmarko D, Hanincova J, and Zavodska E. Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia.

Haemagglutinin (HA) was detected on the surface of influenza virus infected cell with monoclonal antibodies against both HA glycopolypeptides, HA1 and HA2, however, the reactivity of HA2-specific monoclonal antibodies was remarkably lower as compared with HA1-specific monoclonal antibodies. We found that anti-HA2 monoclonal antibody reacted with intact virus and purified undissociated HA. This reactivity of anti-HA2 monoclonal antibodies usually increased after pH 5 treatment. Quantitative analysis revealed that HA2 epitope was reachable for antibody only in minor subpopulation of HA representing approximately 7% of all molecules. M2 protein is transported with HA molecules from endoplasmic reticulum through Golgi complex, trans-Golgi network to the plasma membrane. Anti M2-protein antibodies against cytoplasmic domain of molecules labeled with colloidal gold particles visualized the M2 protein in the transporting vesicles, coated vesicles, in plasma membrane and in the membrane of influenza virus particles. The immuno-electron microscopic labeling of HA with 10 nm gold particles and M2 protein with 5 nm gold particles visualize the topography of molecular interactions between structural components of influenza virus.

268 A DIFFERENTIAL SEROLOGICAL SCREEN IDENTIFIES NOVEL AND PUTATIVE PROTECTIVE MALARIAL EPITOPES. Lobo CA* and Sharma S. Molecular Biology Unit, Tata Institute of Fundamental Research, Bombay, India.

Many genes coding for antigens of *Plasmodium falciparum* have been cloned by screening libraries with clinical sera from natural infection. An unusual feature of almost all malarial antigens studied so far is the extensive array of tandemly repeating amino acid sequences. These repeats are immunodominant and most of the human antibody response is directed towards them. It has been suggested this hyperstimulation of irrelevant B cells leads to a less effective immune response against critical epitopes and in this sense, the repeats are acting as a smoke-screen. Thus, Ags with arrays of identical repeating immunodominant epitopes will be heavily represented in any screen using natural antibodies. A differential serological screen of an expression library of *P. falciparum* was thus initiated to focus on rare and putative protective antigenic determinants of the parasite. The sera used were either from (i) patients, actively presenting malarial symptoms at the time of sera collection (patient sera) or (ii) healthy, permanent, adult residents of malaria endemic areas who had not had malaria for the previous 3-12 years (immune sera). The initial screening was done with a single clinically immune serum sample. 48 positive clones from this screen were selected for the differential serological analysis. These clones were immuno-screened with 73 individual patient sera. Of these, only 8 were found to react exclusively with the healthy endemic serum sample. These 8 were then checked for reactivity against 84 other clinically immune sera. All clones reacted with more than 50% of the sera, although some were more widely recognized. Therefore, these clones express domains that are recognized exclusively by the immune sera and hence may encode protective determinants. Two of these have been sequenced. Genbank analysis reveals that they do indeed encode novel *P. falciparum* sequences. Thus, the differential screen was successful in its attempt to cut through the smoke-screen of immunodominant epitopes and identify rare and putative "host-protective" antigens.

269 MEFLOQUINE PROPHYLAXIS FAILURES IN DUTCH UNITED NATIONS TRANSITIONAL AUTHORITY IN CAMBODIA (UNTAC) TROOPS. Hopperus Buma AP*, Ohrt C, van Thiel PPAM, Tendeloo CH, and Kyle DK. Royal Netherlands Navy, Medical Service, The Hague, Netherlands; US Army Medical Component, Armed Forces Research Institute of Medical Science, Bangkok, Thailand; and Unit of Infectious Disease and Tropical Medicine, Academic Medical Center, Amsterdam, Netherlands.

The Dutch contingent of 750 troops took mefloquine prophylaxis while stationed in Western Cambodia during their first 6 month rotation. Approximately 135 soldiers were in an intensely malarious area. They experienced 14 cases of *Plasmodium falciparum* and no cases of *Plasmodium vivax* while in Cambodia. After returning to Holland, 10 cases of *P. falciparum* and 11 cases of *P. vivax* occurred (16-72 and 30-130 days after stopping mefloquine respectively). Seven *P. falciparum* isolates from local Cambodians showed a range of sensitivity to mefloquine, but all 4 specimens from Dutch soldiers were moderately to highly mefloquine resistant. Four of 700 screening thick smears on return to Holland were found to be positive for *P. falciparum*; two of which had gametocytes present. Three of 4 of these soldiers were foundto be smear positive 34-43 days after the screening smear; two were symptomatic. Mefloquine prophylaxis appeared to select for resistant*P. falciparum* strains and late prophylaxis failures were common. Departure thick smears identified asymptomatic parasitemia and had a low sensitivity for identifying those who would develop symptomatic malaria. These findings are concerning regarding the spread of highly drug resistant malaria in contingents returning to malarious countries.

270 MALARIA IN DUTCH MARINES DURING AND AFTER DEPLOYMENT IN CAMBODIA. van Thiel PPAM*, Hopperus Buma AP, Tendeloo CH, van Gool T, Kager PA. Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, Amsterdam, The Netherlands; Royal Netherlands Navy, Medical Service, The Hague, The Netherlands; and Department of Medical Microbiology, Academic Medical Centre, Amsterdam, The Netherlands.

Plasmodium falciparum infection was diagnosed in 24 out of 759 Dutch Marines deployed on the Cambodian-Thai border from June until December 1992. 15 infections were diagnosed during and 9, 16 to 72 days after weekly mefloquine prophylaxis. Halofantrine (H) 500 mg TDS on days 1,(2),8 was successful in 12/16, H1,2,3 in 6/7 and quinine 10 mg/kg TDS plus doxycycline 200 mg OD for 7 days (Q7,D7) in 1/1. The 5 failures (totally 11 recrudescences) were finally cured with Q7,D7 (2), mefloquine 25 mg/kg (M25) plus D7(1) or artesunate 600 mg OD over 5 days(2). 1 *P. vivax* infections was diagnosed during and 10 infections were seen 30-130 days after mefloquine prophylaxis. All were cured with chloroquine. The incidence of malaria varied in the 5 different locations from 0.8 - 22.3%. All *P. falciparum* failures and *P. vivax* infections came from the same location. Conclusion: in general mefloquine prophylaxis gave good protection in all but one location. H1,2,3, and Q7,D7 appeared to be good first-line treatment; however, for final cure artemisinin derivatives were essential.

271 DOXYCYLINE AND CHLOROQUINE COMBINATION FOR MALARIA CHEMOPROPHYLAXIS IN AUSTRALIAN SOLDIERS DEPLOYED TO CAMBODIA. Roessler PM, Travers T, Barnett AK, Edstein MD, Shanks GD*, and Rieckmann KH. Army Malaria Research Unit, Ingleburn, Australia.

Long-term protection from multi-drug resistant malaria is a real problem in South East Asia. Nearly 600 Australian soldiers are serving in the United Nations Transitional Authority in Cambodia with varying exposures to malaria over a 1 year period. The assigned regimen was daily doxycycline (50 or 100 mg) and weekly chloroquine (300 mg). Questionaries and blood were collected from the soldiers to estimate the tolerance, compliance, and blood drug concentrations. Blood smears were examined from any soldier suspected of having malaria. Exposure to malaria was minimal in the capital Phnom Penh but increased markedly at border control posts. Questionaries indicated that compliance with the daily doxycycline regimen varied between 50-80% decreasing with time in Cambodia. The medications were generally well-tolerated but doxycycline was discontinued in 2 persons who developed skin rashes and 5 who developed oesophagitis. 3 of the individuals with oesophagitis were able to restart doxycycline within 4-6 weeks. As of May 1993, 5 cases of malaria (2 PF, 3 PV) had been diagnosed in Cambodia, all of them associated with doubtful compliance with the prophylactic regimen. One vivax relapse occurred in Australia. Doxycycline and chloroquine a useful combination that can be considered in long-term travellers to malarious areas of South East Asia.

272 CHLOROQUINE CHEMOPROPHYLAXIS IN INDONESIAN SOLDIERS DEPLOYED TO CENTRAL CAMBODIA AS PART OF UNTAC PEACE-KEEPING OPERATIONS. Widodo S*, Mustadjab A, Purnomo, Richards AR, Shanks GD, and Corwin AL. Central Army and Gatot Soebroto Hospitals, Jakarta, Indonesia; U.S. NAMRU No. 2 Jakarta, Indonesia; and Army Malaria Research Unit, Australia.

Multi-drug resistant malaria in South East Asia has made the choice of chemoprophylactic regimens difficult. The possibility of the introduction of highly-drug resistant strains of *Plasmodium falciparum* from Cambodia into Indonesia through Indonesian military forces serving in Cambodia has raised serious public health questions. Epidemiologic surveillance was initiated in two Indonesian battalions deployed to central Cambodia in 1992 as part of the United Nations peace-keeping forces (UNTAC). Blood smears for parasitemia were done before, 3 and 6 weeks after deployment, and when malaria symptoms occurred. Chloroquine 300 mg base per week was the assigned chemoprophylaxis due to the expected low attack rates in central Cambodia and chloroquine's high level of safety and ease of compliance. Serologic tests for rickettsia were done at the same time as blood smears. From the nearly 2000 men screened, 17 vivax and 2 falciparum parasitemias were found prior to deployment to Cambodia. During their 6-9 month stay in central Cambodia, 10 vivax cases were reported. Blood smears 3 and 6 weeks following return to Indonesia detected no malaria cases. Rickettsial serologies are in progress. Chloroquine chemoprophylaxis may

be adequate for persons deployed to very low malaria endemic areas of central Cambodia although this does not apply to the border regions of Cambodia.

273 ANTIMALARIAL EFFECTS OF NEEM: GAMETOCIDAL ACTIVITY. Udeinya IJ*, Quakyi I, Brown N, and Ajayi FO. Howard University College of Medicine, Washington, DC; Department of Biology, Georgetown University, Washington, DC; and Walter Reed Army Institute of Research, Washington, DC.

The neem tree (Azadirachta Indica AJ) also called dogonyaro in Nigeria is a source of various medicinal preparations in Nigeria and many other African and Asian countries. Medicines from neem are used in a variety of diseases including malaria, diabetes and skin infections. Various reports have shown that extracts from neem leaves and seeds contain principles with activity against asexual malarial parasite *in vitro* and *in vivo*. In the present report, we show that neem leaves extract also inhibit the sexual stages, gametocytes of the malarial parasite *Plasmodium falciparum in vitro*. Neem leaves were suxhlet extracted using acetone-water (50/50) mixture as solvent. Following solvent evaporation the isolated extract was fractionated by reverse phase HPLC, and the antigametocidal activity was tested using mature, and mixed-gametocyte populations of the NF54 (3D-7) isolate of *P. falciparum*. Gametocyte and asexual parasitemia was reduced to less than 50% of control by unfractionated extract 0.5 μg/ml during 72 hours incubation. Fractionated extracts 10-2ng/ml caused complete disappearance of gametocytes and asexual forms during the 72 hour incubation period. When only mature gametocytes (stages 4 and 5) were incubated with the isolated fractions, 10-2 ng/ml, the gametocytes completely disintegrated within 72 hours. Two isolated active fractions named respectively IRDNA and IRDNB had similar effects on both gametocytes and asexual forms of the tested isolate. These results contribute further evidence for the potential of the neem as a source of active ingredient for development of agents for malarial therapy and control.

274 PRE-TRAVEL HEALTH ADVICE TO INTERNATIONAL TRAVELERS BY PRIMARY CARE PHYSICIANS. Lobel HO*, Kozarsky PE, Barber AM, Blass M, Waterman SH, and Campbell CC. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; Emory University School of Medicine, Atlanta, GA; and San Diego County Health Department, San Diego, CA.

To assess the quality of health advice to travelers to the developing world, a questionnaire using 3 travel scenarios was mailed in 1992 to a random sample of 1,062 registered primary care physicians in the Atlanta and San Diego areas. Those who gave health advice were asked their recommendations for immunizations and malaria prevention. The responses were assessed by comparison with the 1991 CDC recommendations. Of 545 respondents, 195 (36%) gave pre-travel health advice. Of these, 23 (12%) provided correct malaria recommendations in all scenarios, 32 (16%) in 2 scenarios, 60 (31%) in 1 scenario, 33 (17%) did not give correct advice in any scenario, and 47 (24%) had no opinion. Problems included prophylaxis for areas without malaria and failure to recognize drug-resistant areas. The advice on immunizations by 1 (0.5%) physician was appropriate in all scenarios, by 5 (3%) in 2 scenarios, by 69 (35%) in 1 scenario, 78 (40%) did not give correct advice in any scenario, and 42 (22%) had no opinion. Frequent errors were advising unnecessary immunization and not considering special health risks. Pre-travel advice is very inaccurate. Educational efforts and improved access to accurate health information are needed.

275 COMPLIANCE WITH ANTIMALARIAL CHEMOPROPHYLAXIS - A MATCHED CASE - CONTROL STUDY. Gyrokos TW, Svenson JE, and MacLean JD. Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada; and McGill University Centre for Tropical Diseases, Montreal, Quebec, Canada.

The association of compliance with antimalarial chemotherapy and malaria was examined in a matched case-control study of 157 patients with malaria and recent travel to a malaria-endemic area and 157 control patients. In all, only 152 patients (48%) (72 cases and 80 controls) had been prescribed chemoprophylaxis prior to travel. Chemoprophylaxis use was correlated with region and purpose of travel. Cases were found to be significantly less compliant (53%) than controls (76%) [OR =.35 (CI:0.27, 0.73)]. Using multiple logistic regression, compliance with antimalarials was found to be associated with region and purpose of travel and with the number of drugs taken. These results indicate that compliance with antimalarial chemoprophylaxis is protective against development of malaria. There must be increased efforts of both health prectitioners and travel agents in the promotion of chemoprophylactic use during travel to risk areas.

276 QUANTITATIVE PCR TO PREDICT *PLASMODIUM FALCIPARUM* TREATMENT FAILURE. Kain KC*, Kyle DE, Brown AE, Mirabelli L, Webster HK, and Looareesuwan S. Tropical Disease Unit, Division of Infectious Disease, The Toronto Hospital, Canada; Department of Immunology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Multidrug resistance in falciparum malaria is increasing in all malaria endemic areas. Rapid methods to monitor patients' response to therapy and to predict treatment failure would facilitate management and control of drug resistant infections. The objective of the current study was to examine whether quantitative PCR might be useful in predicting treatment failure in patients with *Plasmodium falciparum* malaria in Thailand. Eleven patients with acute uncomplicated falciparum malaria were prospectively followed over a treatment course by conventional light microscopy and by PCR of *P. falciparum* DNA eluted from filter paper blood samples. A 206 bp sequence, specific for *P. falciparum*, was enzymatically amplified and quantitated by HPLC. All patients were admitted to hospital for 28 days to exclude re-infection. During the 28 day follow-up, 1 patient treated with quinine/tetracycline and 1 treated with mefloquine/tetracycline, recrudesced at day 16 and day 24, respectively. PCR clearance of *P. falciparum* DNA was delayed in both patients (216 and 432 hrs) compared with a mean of 120 hours (95% C.I. 102-138 hrs; P=.01, Mann-Whitney) in successfully treated patients. PCR remained positive until greater than or equal to day 9 in both recrudescent patients versus less than or equal to day 6 in 9 patients without treatment failure (P=.018, Fisher's exact two-tailed). Quantitative PCR levels did not drop to below 50% of pre-treatment levels until > day 3 in both treatment failures. These results suggest that quantitative PCR performed on filter paper blood samples may be useful as a method to monitor patients response to therapy and to predict subsequent treatment failure.

277 HALOFANTRINE PHARMACOKINETICS OF AN EXTENDED DOSE REGIMEN IN PATIENTS WITH ACUTE FALCIPARUM MALARIA AND HEALTHY VOLUNTEERS. Ohrt C *, Watt G, Teja-Isavadharm P, Loesuttiviboon L, Webster HK, Schuster B, and Fleckenstein L. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Surasinghanat Army Hospital, Aranyaprathet, Thailand; College of Pharmacy, University of Iowa, Iowa City, IA; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

The pharmacokinetics and tolerance of a seven day halofantrine regimen were evaluated in 10 Thai malaria patients and 10 non-infected volunteers. To maximize bioavailability, a 4.5 gram regimen was given orally over seven days after meals. Halofantrine peak plasma concentrations (Cmax 0-1d) and bioavailability (AUC 0-1d) on the first day of treatment, were significantly lower in malaria patients than in healthy volunteers (p=0.0005). Halofantrine elimination half-life (t1/2) was significantly shorter in malaria patients than healthy controls (9.5 versus 15.8 days). These data demonstrate a distinct effect of acute malaria on the absorption and elimination of the drug. In addition, marked inter-subject and intra-subject variability in daily trough halofantrine levels was

observed, indicating variable drug absorption. This dosing regimen was effective and well tolerated, with mild, transient diarrhea during the first few days of treatment in both groups. To consistently produce effective drug levels, the currently recommended dosing regimens may be suboptimal. Slow halofantrine elimination raises concern for induction of parasite resistance when the drug is used in endemic areas.

278 ENHANCEMENT OF VIRUS REPLICATION BY ANTIMALARIAL TREATMENT IN CD-1 MICE. Sklarsh JW*, Sidhu GS, and Maheshwari RK. Uniformed Services University of the Health Sciences, Bethesda, MD.

The spread of AIDS has been rapid in tropical Africa, where there is a high incidence of malaria. The frequent treatment has been chloroquine (CHL). The Herpes Zoster virus is normally uncommon in younger children. However, its incidence was markedly increased in immunosuppressed children who were being treated with CHL following *Plasmodium falciparum* and *P. vivax* malaria infections. Recently it has been shown that TAT protein purified from Human Immunodeficiency Virus Type-1, the causative agent of AIDS, transactivates the viual promoter and this transactivation is dramatically increased by CHL. So far, most of these studies indirectly suggest that CHL may be involved in the enhancement of virus replication. Results presented here show convincingly that certain antimalarials (chloroquine, Primaquine, quinine hemisulfate, pyrimethamine and sulfadoxine) do enhance virus replication in CD-1 mice, infected with the Semliki Forest virus and encephalomyocarditis virus. Preliminary studies show Simplex virus type-1. Possible mode of action by chloroquine may be by its ability to interfere with antigen presentation, macrophages to T-cell subsets. These results suggest that the widespread use of these antimalarials in malaria endemic areas may predispose the treated population to the risk of significant viral infections, including HIV.

279 AN INVESTIGATION INTO THE ANTIMALARIAL ACTIVITY OF CYMBOPOGUN CITRATUS ON *PLASMODIUM BERGHEI BERGHEI IN VITRO.* Obih P*, Makinde J, and Ojo J. College of Pharmacy, Xavier University of Louisiana, New Orleans, LA; and University of Ibadan, Nigeria.

For many years now, many people have used one medicinal herb after another for the treatment of malaria. Lemon grass happens to be one of such herbs. Often times the users have reported being cured of malaria, yet the medicinal herbs have remained only at the background and failed to be investigated, either because the symptomatic relief that users confessed of were regarded as non-existing or psychological. This work was carried out to test the efficacy of lemon grass as an antimalarial agent. In the four day schizontocidal test, both aqueous and chloroform extracts of lemon grass were tested against drug-sensitive *Plasmodium berghei berghei* in mice, chloroquine was used as the reference. A repository test was also run by administering the extracts to mice for three days running before challenging them with *P. berghei berghei.* The mice were observed for parasitemia 72 hours later. In both tests, lemon grass extracts showed promising results by suppressing parasitemia. The concentrated extracted given orally produced as high as 67% chemosuppression.

280 RAPID DEVELOPMENT OF PYRIMETHAMINE RESISTANCE (PYR^R) *IN VITRO.* Krogstad FM* and Krogstad DJ. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

These studies were performed to select for transfection of the 3D7 pyrimethamine-succeptible (Pyr^S) strain of *Plasmodium falciparum* (IC_4 = 2-4 ng/ml) *in vitro* after electroporation or exposure to the biolistic particle delivery system using a dihydrofolate reductase-thymidylate synthase (DHFR-TS) construct containing the Pyrimethamine-resistance (Pyr^R) determinant from the Pyr^R Indochina I

strain of *P. falciparum*. After elecroporation or exposure to the particle delivery system, Pyr^S *P. falciparum* exposed to selection with pyrimethamine concentrations 50 to 100-fold greater than their IC_{50} (200 ng/ml) reached parasitemias > 5% within 2 weeks, and then had an IC_{50} of 500 ng/ml. This observation does not represent transfection because it occurred similarly in controls that were not exposed to the Pyr^R DHFR-TS construct, and because the IC_{50}s observed with these isolates are more than 10-20 fold less than that of the Pyr^R Indochina I parent strain ($IC_4 \geq 4{,}000$ ng/ml). These results demonstrate that a 100-fold increment in pyrimethamine resistance can be produced within 2 weeks *in vitro* (beginning with an initial inoculum of 5×10^7 parasites). They suggest that similar phenomena may occur when pyrimethamine is used widely, and that countries which have changed from chloroquine to pyrimethamine for the treatment of *P. falciparum* infection may inadvertently select for the rapid evolution of pyrimethamine resistance.

281 PHARMACOKINETICS OF ARTEMISININ AFTER ORAL ADMINISTRATION IN HEALTHY VOLUNTEERS. Loutan L*, Paris M, Plessas C, Benakis A, and and Andrial M. Parasitic Disease Division, Geneva University Hospital, Geneva, Switzerland; Department of Pharmacology, University Medical Centre, Geneva, Switzerland; and Medical Department Mepha Ltd., Basel, Switzerland.

Artemisinin is the natural compound extracted from the *Artemisia annua* plant, which has been shown in numerous Chinese studies to be an effective anti-malaria agent. Although some of its derivatives have been found to be more active, the difficulty in obtaining them has resulted in the widespread use of orally administered artemisinin for the treatment of malaria. The present study was conducted therefore to establish pharmacokinetic data after oral adminstration of 2 X 250 mg tablets of a new pharmaceutical form of artemisinin (lactab MEPHA) in 6 healthy volunteers. In addition to artemisinin, a metabolite, was also quantified in plasma, using HPLC with electrochemical detector operating in reductive mode. After oral adminstration of artemisnin, the Cmax value was 0.405 µg/ml measured at 90 min. The absorption rate constant k_a : 0.87±0.168 h^{-1}; $t_{1/2}$ of absorption: 0.89±0.20 h; the elimination rate constant: k_e : 0.562±0.022 h^{-1} and the $t_{1/2}$ of elimination: 1.23±0.05 h.

282 RELATIONSHIP OF THE 3-DIMENSIONAL STRUCTURE OF HALOFANTRINE TO ANTIMALARIAL ACTIVITY. Karle JM*. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC.

The 3-dimensional structure of halofantrine was determined by x-ray crystallography in order to correlate its 3-dimensional steric and electronic structure with antimalarial acitvity. The ultimate goal is to design more effective antimalarial agents. The absolute configuration of (-)-halofantrine hydrochloride was determined to be the S configuration. (-)-Halofantrine hydrochloride crystallized as a tertiary amine salt with the hydrogen atom from the HCl salt residing on the nitrogen atom of halofantrine. The N...O distance of 4.170 Angstroms is comparable to the N...O distance of 4.389 Angstroms in the crystal structure of racemic halofantrine hydrochloride, but substantially longer than the 2.791 Angstrom N...O distance in the crystal structure of racemic mefloquine hydrochloride, a somewhat less potent amino alcohol antimalarial agent *in vitro* on a molar basis. Mefloquine has one less methylene group between the N and O atoms. Although the steric bulk of halofantrine is greater than mefloquine, the electronic charge of the van der Waals potential surface of the two molecules is quite similar. The crystal structure of (-)-halofantrine demonstrates the flexibility of the acyclic portion of the molecule in two ways. First, one of the n-butyl groups assumes two different positions in a ratio of 64:36. Second, the N-H bond points in a different direction in relation to the C-O bond than occurs in the crystal structure of the racemate as demonstrated by the O-C...N-H torsion angle of 33.0° for the (-)-isomer and 172.6° for the racemate. The flexibility of the amine group may explain why the (+)- and (-)-isomers of halofantrine are equipotent against *Plasmodium falciparum in vitro* whereas the more rigid mefloquine molecule has displayed stereospecific activity.

283 EFFECT OF POLY ICLC AGAINST A MALARIAL INFECTION IN RHESUS MONKEYS. Maheshwari RK*, Levy HB, Dutta GP, Puri SK, and Kamboj VP. Uniformed Servies University of the Health Sciences, Bethesda, MD; NIAID, National Institutes of Health, Bethesda, MD; and Central Drug Research Institute, Lucknow, India.

We have tested the prophylactic effect of poly ICLC, a double-stranded RNA, which is a potent interferon inducer, against a sporozoite-induced *Plasmodium cynomolgi* B malaria infection in rhesus monkeys. The course of the infection of rhesus monkeys with *P. cynomolgi* B closely resembles that of relapsing *P. vivax* malaria in humans, Data showed that a single dose of poly ICLC (1 mg/kg) given 24 hr before infection completely protected monkeys against sporozoite-induced *P. cynomolgi* B infection. Current studies are in progress to evaluate the effect of poly ICLC against trophozoite-induced infection and on the anti-relapse efficacy.

284 MOLECULAR ANALYSIS OF *PFMDR1* IN *PLASMODIUM FALCIPARUM* ISOLATES FROM SUB-SAHARAN AFRICA. Basco L, Le Bras J, Rhoades Z, and Wilson CM*. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Laboratoire de Parasitologie, Hopital Bichat-Claude Bernard, Paris, France.

The purpose of this study was to extend previous molecular studies of *pfmdr1* to fresh clinical isolates of *Plasmodium falciparum* from sub-Saharan Africa. DNA was isolated from newly obtained clinical isolates within 48 hrs of clinical presentation. The isolates were from patients either returning or immigrating from widely dispersed geographic locations in sub-Saharan Africa. Analysis of 1) drug susceptibility, 2) reversal modulator effect on drug susceptibility, 3) the sequence of the five previously identified regions of polymorphic alleles and the poly-asparaginated region of *pfmdr1*, 4) DNA fingerprinting using a PCR-based technique, 5) and gene copy number of *pfmdr1* was completed for all of the isolates. A full-length sequence analysis of *pfmdr1* in five of these sample revealed no new sequence polymorphisms. Using criteria previously established for the prediction of CLQ resistance, only 33 of 42 (79%) isolates analyzed were predicted correctly. The predictions were essentially based on a single allele (amino acid 86) because none of the isolates had the second resistance genotype variation. Other observations were: 1) gene copy number of *pfmdr1* did not correlate with mefloquine/halofantrine resistance as it did in isolates from Thailand; 2) the poly-asparaginated linker region was much more polymorphic than previously observed; 3) a PCR fingerprint analysis worked well to distinguish these isolates.

285 THE *IN VITRO* ACTIVITY OF CIPROFLOXACIN AND CHLORAMPHENICOL AGAINST *PLASMODIUM FALCIPARUM*. Yeo AE* and Rieckmann KH. Army Malaria Research Unit, Ingleburn, Australia.

Tetracyclines, such as doxycycline, are being extensively used for the treatment and prophylaxis of malaria because there are few or no alternatives, particularly in areas where parasites have become resistant to Fansidar and mefloquine. In view of recent concerns about the possible emergence of tetracycline- resistant strains of *Plasmodium falciparum*, we decided to take another look at the *in vitro* activity of 2 non-tetracycline antibiotics, ciprofloxacin and chloramphenicol, and compare their activities with doxycycline. The minimum inhibitory concentrations (MIC) of these drugs were determined against a chloroquine sensitive (FC) and a chloroquine resistant (K1) isolate of *P. falciparum* using a modification of the Rieckmann microtest. After 48 hours of incubation, the MICs of doxycycline and ciprofloxacin were greater than 10 μg/ml and the MIC of chloramphenicol was greater than 100 μg/ml for both isolates. However, at 96 hours, mean values of doxycycline, ciprofloxacin and chloramphenicol were 1.0 μg, 2.8 μg and 10.7 μg/ml for the FC isolate and 1.3 μg, 4.4 μg and 12.5 μg/ml, respectively for the K1 isolate. Even lower concentrations of these drugs were able

to inhibit parasite growth when incubation periods were extended beyond 96 hours. These results indicate that, like doxycycline, the multiplication of parasites can be inhibited by clinically achievable concentrations of ciprofloxacin and chloramphenicol provided that exposure to these drugs is prolonged over several asexual life cycles. During further evaluation of these slow-acting drugs, they should be given to malaria patients for at least 7 days to determine their value as curative agents. As with acute infections treated with tetracyclines, they will probably also have to be taken in conjunction with a rapidly- acting but non-curative drug regimen, such as a 3 day course of chloroquine, quinine or one of the arteminisin derivatives.

286 MEPACRINE AND PYRONARIDINE UPTAKE BY *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES. Elueze EI*, Wu LJ, Croft SL, and Warhurst DC. Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, UK; and Institute of Parasitic Diseases, Shanghai, China.

The uptake of the acridine antimalarials, mepacrine and pyronaridine, by chloroquine sensitive (T.996 clone) and chloroquine resistant (K1 strain) *Plasmodium falciparum* infected erythrocytes was evaluated using the techniques of flow cytometry, fluorescence spectrometry and radiometry. In both fluorometric techniques, mepacrine uptake was seen to be rapid (steady state reached in 10 mins) and glucose and temperature dependent. The Kd for mepacrine uptake by infected erythrocytes determined from studies using flow cytometry and fluorescence spectrometry were 4 and 11 μM respectively. Preincubation with N-ethylmaleimide(NEM), dicylohexyl-carbodiimide(DCCD), oligomycin and carbonylcyanide-chloro-phenylhydrazone (CCCP) resulted in a decrease in total mepacrine uptake. Ouabain and vanadate had no effect on mepacrine uptake. The increase in apparent drug concentration in the cytoplasmic compartment detected by flow cytometry in the presence of NEM, DCCD and oligomycin suggest that the drug is being exported from the lysosome into the cytoplasm. These results will be compared with the kinetics of the uptake of tritiated pyronaridine. Electron microscopy revealed changes in *P. falciparum* trophozoites after 30mins exposure to 0.2 μM of both drugs. Changes included enlargement of the food vacuole, formation of intravacuolar membrane whorls and a decrease in pigment granules.

287 MOLECULAR EPIDEMIOLOGY OF ANTIFOLATE RESISTANT *PLASMODIUM FALCIPARUM* IN MALI. Plowe CV*, Boare M, Wellems TE, Peterson DS, and Doumbo 0. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD; and Malaria Research & Training Center, National School of Medicine & Pharmacy, Bamako, Mali.

As chloroquine resistance spreads from East to West Africa, antifolate drugs are becoming first-line choices for malaria treatment and prophylaxis. Clinical and *in vitro* resistance to antifolates has been reported in Africa, and there is a need for ongoing surveillance to map resistance rates and to measure the effects of increased usage on resistance. An assay suitable for large-scale epidemiological surveys of drug resistance will be an important tool in efforts to design optimal drug distribution and treatment strategies. Specific point mutations in the *P. falciparum* dihydrofolate reductase (DHFR) gene have been associated with *in vitro* resistance to the DHFR inhibitors pyrimethamine and proguanil. PCR assays have been developed that exploit these sequence differences to detect parasites resistant to pyrimethamine and proguanil. The assays are suitable for use in developing countries, requiring only a small amount of parasitized blood and utilizing a simplified method for cell lysis and DNA extraction. Epidemiologic mapping of antifolate resistance is being initiated in Mali. Preliminary findings indicate that proguanil resistance is present in an isolated area where proguanil is being used in a prenatal malaria prophylaxis program.

288 DEVELOPMENT OF AN *IN VITRO* PHARMACODYNAMIC MODEL TO ASSESS IBI ARTEETHER ANTIMALARIAL ACTIVITY. Li X*, Brewer TG, Miller RE, Figueroa L, Gerena L,

Oduola AJ, Nuzum EO, and Milhous WM. Division of Experimental Therapeutics, Walter Reed Army Institute of Research Washington DC; USA Medical Reserach Unit, Brazil, Rio de Janiero, Brazil; and Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.

An *in vitro* bioassay system has been developed to assess antimalarial pharmacodynamics of *b*-arteether (AE) *in vivo*. The model chosen for metabolism of arteether *in vitro* was the isolated perfused rat liver (IPRL), a surgically isolated liver maintained using an oxygenated, erythrocyte-containing perfusate which is recirculated by means of a pump. This model provides the advantages of intact hepatic metabolism and access to repeated perfusate sampling for measurement of drug level, and it eliminates confounding effects of *in vitro* models. The standard IPRL model was modified by addition of RPMI 1640 and human albumin. Since arteether has been shown to be rapidly cleared and extensively metabolized I*in vivo*l and *in vitro*, prolongation of antimalarial activity would indicate active metabolites that are more persistent than parent drug. Parasites of the D6 *P. falciparum* was used in this study. Bioassay of arteether perfusated samples (25 µl) was quantitated by matching *in vitro* values of the perfusate with a standard curve generated from different concentrations of parent compound and dihydroquinghaosu (DQHS) using the semi-automated microdilution method. Preliminary HPLC findings show that the peak level of arteether appears at 1 minute after drug administration with log linear decrease to undetectable levels (10 µl/ml) within 35 minutes. This HPLC pattern matched with that of bioassay for the first 30 minutes after drug administration. However, perfusate *in vitro* antimalarial activity of arteether persists beyond 30 minutes. The data indicated that this sensitive bioassay system can be used to reveal the relationship between drug pharmacokinetics and antimalarial pharmacodynamics.

289 ANTIMALARIAL AND CYTOTOXIC ACTIVITY OF NATURAL BISBENZYLISOQUINOLINE ALKALOIDS. Angerhofer CK*, Guinaudeau H, Lin LZ, Likhitwitayawuid K, Wongpanich V, Pezzuto JM, Ruangrungsi N, and Cordell GA. Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, College of Pharmacy, Chicago, IL; and Department of Pharmacognosy, Faculty of Pharmacy, University of Angers, France.

Bisbenzylisoquinolines comprise a class of natural alkaloids that are known to occur in several plant families. Members of this class have recently been reported to us, and by other groups, to inhibit the growth of cultured malarial parasites with IC_{50} values in the nanomolar range. Furthermore, a synergistic antimalarial effect has been found to occur between chloroquine and certain members of this class (e.g., tetrandrine, 7-*O*-demethyltetrandrine, limacine), while other bisbenzylisoquinolines appera to be addative or antagonistic with chloroquine (e.g., phaeanthrine). As part of an ongoing collaborative effort to discover new anitmalarial agents from natural sources, we have tested a large series of bisbenzylisoquinolines for their antimalarial and cytotoxic activities against chloroquine-sensitive and chloroquine-resistant clones of *P. falciparum in vitro*. We have also examined the most potent and selective compounds in this series for their possible interactions with known antimalarial drugs such as chloroquine, mefloquine, and artemisinin. We have attempted to determine the correlations that may exist between the structures, their stereochemistry and the substitution patterns of the compounds we have studied and their *in vitro* antimalarial and cytotoxic activities. Emerging relationships will be analyzed and discussed.

290 PRELIMINARY STUDY OF INTRA-RECTAL QUININE ADMINISTERED TO *PLASMODIUM FALCIPARUM* MALARIA CHILDREN IN NIAMEY, NIGER. Barennes H*, Kahistani F, Clavier F, Meynard D, Ndinfountawoo S, and Verdier F. French Cooperation, Ministry of Public Health, Niamey, Niger; Hôpital National de Niamey, Niger; INSERM, Unité 13, IMEA, Paris, France; and Faculté des Sciences de la Santé, Niamey, Niger.

In order to decrease the complications of intra-muscular quinine (Q), the intra-rectal (i.r.) route, already empirically used with success, was evaluated in Niamey. Fifty three *Plasmodium falciparum* malaria children aged from 2 to 10 yrs, hospitalized, were included in the study. They were given 20mg/kg diluted injectable Q twice a day for 3 days (i.r.group). Five other malaria children, were treated with 12.5mg/kg Q through a slow perfusion over 4hrs twice a day for 3 days (i.v.group). Blood samples were withdrawn from 23 children (18 i.r. and 5 i.v.) for a kinetic study of Q, the plasma levels of which were assayed according to a HPLC method. Kinetic analysis was performed using APIS® pharmacokinetic software. Both modes of administration were well tolerated. At 48hrs, 13 children (25%) (i.r.), presented positive blood smears; temperature was>37°C in 22 children (44%). All children i.v. were apyretic and aparasitemic. At 72hrs, all children (i.r.) but one were aparasitemic ; temperature was>37°C in 2 children (4%). At 96hrs, all children were apyretic. Results of the kinetic study with Q plasma levels obtained from the i.r. and i.v. routes were respectively: Tmax: 2.7±1.7 and 1.8±1.5 hrs; Cmax: 4.9±2.1 and 16.4±6.5 nmol/l; areas under the curve (AUC), extrapolated to the 12.5mg/kg dosage were: from 0 to 8hrs: 19±7 and 85±31 nmol/l h-1; from 0 to 48hrs: 144±57 and 501±58 nmol/l h-1 defining a bioavailability of 23% (0 to 8hrs) and 29% (0 to 48 hrs) of intra-rectal Q. In conclusion, tolerance and efficacy through the i.r. route were good; bioavailability will be likely improved by the use of a Q formulation specifically adapted to this route. Such a study is in progress at that time in our group.

291 USE OF A SERUM-FREE MEDIUM IN THE DETERMINATION OF *PLASMODIUM FALCIPARUM* DRUG SENSITIVITY. Ofulla AV, Orago AS, Githure JI, Burans JP, Aleman GM, Johnson AJ, and Martin SK*. Kenya Medical Research Institute, Nairobi, Kenya; Kenyatta University, Nairobi, Kenya; Naval Medical Research Institute, Bethesda, MD; and United States Army Medical Research Unit, Nairobi, Kenya.

We recently described a serum-free medium that can eliminate the need for serum in the continuous *in vitro* cultivation of *Plasmodium falciparum*. One of the potential uses of such a medium is in the standardization of parasite antimalarial drug sensitivity assays. Using the *in vitro* 48 hour [^{3}H] hypoxanthine uptake method, we compared IC_{50} values obtained simultaneously in serum-free and serum supplemented media against eight antimalarial drugs. Both laboratory adapted strains (n=7) and fresh isolates from Kenya (n=4) and Somalia (n=4) were used for our comparisons. There was good correlation between IC_{50} values by regression analysis: bovine albumin versus control medium ($r=0.87$, $n=11$, $p<0.001$); bovine albumin-glucose-lipid versus control medium ($r=0.996$, $n=26$, $p<0.001$). These results are further evidence that our serum-free medium is an effective substitute for the routinely used serum supplemented medium.

292 SITE PREPARATION FOR MALARIA DRUG AND VACCINE TRIALS IN BRAZIL. Pang LW*, Milstrey EG, Martins OR, Arias JR, Milhous WK. USA Research Unit-Brazil, American Consulate Rio de Janeiro, Brazil; Municipal Secretary of Health, Peixoto, Mato Grosso, Brazil; and PAHO, WHO, Brasilia, Brazil.

As a collaborative effort, we are evaluating feasibility for conduct of clinical intervention studies against falciparum malaria in the Amazon region of Brazil. Clinical trials will include chemotherapeutic treatment of uncomplicated malaria, chemoprophylaxis, and vaccine studies. Peixoto is a municipality of Mato Grosso state located 700 km north of the state capital of Cuiaba. In the past several years it has increased in size to its present population of 70,000, largely due to the influx of gold miners from neighboring states. The predominant vector in this area is *Anopheles darlingi* which enters huts and feeds during the first half of the evening. The vector breeds in partially shaded riverine pools and can fly 6-7 km between feedings. For the month of January 1993 there were 4800 slide confirmed cases of malaria (60% falciparum & 40% vivax). There are about 10 deaths per month from malaria. Falciparum malaria is treated with quinine + tetracycline for 7 days. 5-10% of cases fail this regimen and are given mefloquine (1000 mg) or oral artesunate + tetracycline

(4 + 5 days, respectively). There is an urgent need to characterize strains, perform clinical trials and implement a rational and prudent approach for the introduction of new therapies.

293 INTRINSIC ACTIVITY OF SULFONAMIDES/SULFONES AGAINST FALCIPARUM MALARIA. Lucia L*, Miller RE, Pang LW, Schuster BG, and Milhous WK. USA Research Unit-Brazil, American Consulate Rio de Janeiro, Brazil; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Multiple Drug Resistance (MDR) due to enhanced efflux or exocytosis continues to confound antimalarial drug development. The mechanism of resistance of antifolate antimalarials, though, is independent of drug transport and due to differential binding affinities to parasite dihydrofolate reductase (DHFR). New observations on lack of cross resistance of proguanil analogs, triazines & quinazolines has stimulated interest in DHFR inhibitors as second generation Fansidar-like drugs. Severe allergic reactions such as Stevens- Johnson syndrome and toxic epidermal necrolysis curtailed use of Fansidar, sulfadoxine (SDX) plus pyrimethamine, for malaria prophylaxis, as reported previously. National adverse drug reaction registers in Sweden and the UK, as reported previously, suggest less risk for severe allergic reactions with the sulfas with short elimination half-lives, sulfamethoxazole (SMX), low dose dapsone (DDS), or sulfadiazine (SDZ). We examined SMX, SDZ & DDS *in vitro* against falciparum malaria parasites from various geographic regions and found them to be more potent than SDX and exhibited less cross-resistance against SDX resistant strains. These drugs may represent safer and more potent companion drugs for new antifolate antimalarials drug combinations.

294 IN VITRO DRUG SENSITIVITY OF *PLASMODIUM FALCIPARUM* STRAINS FROM 3 ENDEMIC CONTINENTS TESTED AGAINST 4 CLASSIC ANTIMALARIALS AND 4 QINGHAOSU DERIVATIVES. Gay F*, Bustos DG, Venturin C, Ciceron L, Counali JB, Sampang J, Nadire M, Saniel MC, and Gentilini M. Department de Maladies Tropicales et Sante Publique, Groupe Hospitaliere Pitie-Salpetriere, Paris, France; Research Institute for Tropical Medicine, Alabang, Metro Manila, Philippines; and Service Departemental de Desinfection de la Guyane, Cayenne, Guyane.

The derivatives of Qinghaosu, artemisinin (AS), artemether (AT), arteether (AE) and artesunate (AN) could be integrated in the treatment of acute uncomplicated or severe complicated falciparum malaria, either alone or in combination with the standard antimalarials chloroquine (CQ), quinine (QN), mefloquine (MQ) and halofantrine (HL), depending onthe existing sensitivity patterns of the standard antimalarials perendemic region. Strains from Africa, Asia and South America composed of 44 isolates of imported malaria from West and Central Africa, 38 isolates from the Philippines and 37 isolates from French Guyana weretested using the radioisotope microtest method. The IC_{50} of 8 antimalarials were calculated for all isolates, and EC_{50}, EC_{90} and EC_{99} determined by log-probit transformation per region of origin. Multi-drug resistance does not exist in Africa; the African strains were comparatively more sensitive to the 8 drugs than from the other regions. Resistance to CQ was comparable between the French Guyana (EC99 = 998 nmol/l) and Philippine strains (1029 nmol/l). French Guyana had higher values for QN (3558 nmol/l) and AE (38.7 nmol/l). Philippine strains had unusually elevated values for MQ (334 nmol/l), AT (171 nmol/l) and AS (148 nmol/l). The EC_{99} for AN was comparable for all 3 regions ranging from 10.9 to 14.2 nmol/I. Comparison between regions and drugs showed disparities in terms of their chemoresistance profile and drug correlation. The *in vitro* results obtained question work currently done on drug associations, henceforth should serve as informative guidelines in the protocol of therapeutic trials for malaria.

295 CARDIAC EFFECTS OF STANDARD DOSE HALOFANTRINE THERAPY. Matson PA*, Luby SP, Redd SC, and Meriwether RA. Division of Field Epidemiology, Centers for Disease Control and

Prevention, A; Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; and Communicable Disease Section, North Carolina Department of Health, Raleigh, NC.

Halofantrine, a 9-phenanthrene-methanol compound effective against multi-drug resistant *Plasmodium falciparum*, was recently associated with prolonged QT intervals and possible cardiac arrhythmias when administered at a higher than standard dosage. To investigate the potential cardiac effects following standard dose (500 mg q. 6 hr. x three doses) halofantrine therapy, we studied 48 adult refugees from Southeast Asia being treated for P. falciparum. Each patient had a baseline electrocardiogram and a follow-up electrocardiogram 24 hours after therapy started, when serum halofantrine levels are highest. We noted no arrhythmias on follow-up studies. The rate- corrected Q-T interval (QTc) was longer on follow-up (mean 0.44 sec, S.D. 0.04) than at baseline (mean 0.40 sec, S.D. 0.03). Thirty-nine patients displayed an increase in QTc of at least 0.01 seconds after therapy ($p < 0.01$, paired t-test). However, no patients, before or after halofantrine, had a QTc greater than 0.55 seconds, and only two patients had the QTc lengthened more than 25% during the treatment period. These findings show that standard dose halofantrine can lengthen the QTc interval, but not to the degree that would likely be associated with cardiac arrhythmias.

296 TWO NOVEL APPROACHES FOR DETERMINING THERAPEUTIC DRUG LEVELS OF ARTEMISININES IN BIOLOGICAL FLUIDS. Mount DL*, Green MD, and Todd GD. Malaria Branch, Centers for Disease Control. Atlanta, GA.

Existing methods for determining artemisinines (QHS), naturally occurring, sesquiterpene lactone endoperoxides used in the treatment of malaria infections, have their particular drawbacks making them impractical for routine use in determining therapeutic drug levels. Thus, we have continued the search for a better method. One new approach utilizes supercritical fluid chromatography with supercritical carbon dioxide to elute QHS from a high performance liquid chromatographic column, followed by electron capture detection. With this approach, we have been able to achieve a limit of detection of 20 ng/ml extracted from 1 ml of whole blood. The second new approach involves the use of reversed-phase, high-performance liquid chromatography (HPLC) with chemiluminescent detection. After elution from the HPLC column, QHS is mixed with hemin and luminol in a post-column reactor, producing chemiluminescence that is monitored at a wavelength of 425 nm. We can currently detect about 25 ng of QHS injected onto this chromatographic system. Further optimization is ongoing and the development of sufficient sensitivity with either method will represent a major step toward routine analysis of QHS.

297 CLINICAL EFFICACY OF MICRONIZED HALOFANTRINE FOR ACUTE UNCOMPLICATED FALCIPARUM MALARIA IN NONIMMUNE PATIENTS. Bouchaud O*, Basco LK, Gillotin C, Gimenez F, Genissel B, Farinotti R, LeBras J, and Coulaud JP. Service de Maladies Infectieuses et Tropicales, Hopital Bichat-Claude Bernard, Paris, France; Service de Parasitologie, Hopital Bichat-Claude Bernard, Paris, France; and Service de Pharmacie, Hopital Pitie-Salpatriere, Paris, France.

Halofantrine is effective against multi-drug resistant *Plasmodium falciparum*. One of its major drawbacks is poor absorption, leading to treatment failures in some case. To pallidate this problem, a newly formulated, micronized halofantrine hydrochloride was administered (250 mg PO x 3 doses at 6 hr interval) to nonimmune patients returning from sub-Saharan Africa. Twenty-five patients, 9 French travelers and 16 African immigrants, with an acute uncomplicated falciparum malaria were included and followed up for 28 days. Plasma concentrations of halofantrine and its metabolite, N-desbutylhalofantrine, were measured 24, 48, and 72 hrs after the first dose. Pharmacokinetic studies were conducted in 8 patients. The mean fever clearance time was 49 hrs; the mean parasite clearance time was 59 hrs. Twenty-two patients were cured (type S response). In 3 patients, parasitemia was cleared before day 7 but recrudesced (type RI response). The RI type response was associated with poor drug absorption in 1/3 patients (halofantrine IC_{50} 1.27 nM). In 2/3 patients (IC_{50} 7.40 and 14.0 nM;

resistance threshold > 6 nM), plasma concentrations of halofantrine and N-desbutylhalofantrine were within the range of values observed in cured patients, suggesting halofantrine resistance. Drug absorption was highly variable and even poor in some patients. Our study suggests that micronized formulation of halofantrine is effective and well-tolerated but higher dose (500 mg x 3) may be required to attain an adequate plasma concentration.

298 COMPARISON OF PHARMACOKINETICS, BIOAVAILABILITY AND HYDROLYSIS OF DIHYDROARTEMISININ, ARTEETHER, ARTESUNATE AND ARTELINATE IN RATS. Li Q *, Peggins JO, Masonic K, and Brewer TG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

There is much information in the literature regarding the antimalarial activity of Qinghaosu (QHS) and its various analogs. However, there is scant data available on the pharmacokinetics of these compounds. The pharmacokinetics and bioavailability of dihydroartemisinin (DQHS), arteether (AE), sodium artesunate (AS) and artelinate (AL) were investigated in rats following single i.v., i.m. and intragastric (i.g.) administrations of 10 mg/kg are reported here. Plasma was separated from serial blood samples collected at various times after dosing. After extraction plasma samples were analyzed for parent drugs by HPLC using reductive electrochemical detection. Samples from rats dosed with AE, AS and AL were also analyzed for DQHS which is a known to be a major metabolite of these drugs. Plasma levels of all parent compounds declined in a biexponential manner which could be reasonably fit using a 2 compartment open model. The pharmacokinetic profiles were substantially different not only between drugs but also between routes of administration for the same drug. The highest plasma level after i.v. injection was found for AL (AUC = 10194 ng·h/ml), followed by DQHS (3184 ng·h/ml), AS (738 ng·h/ml), and AE (611 ng·h/ml). This resulted in the lowest apparent volume of distribution (0.16 l) for AL, increasing thereafter for DQHS (0.50 l), AE (0.75 l) and AS (0.87 l). The clearance for AL was slowest for all three routes of administration (17-21 ml/min/kg) compared with DQHS (55-64 ml/min/kg), AS (191-240 ml/min/kg) or AE (263-323 ml/min/kg). In addition, the longest terminal half-life after i.v. dosing was found for AL (1.4 hr), followed by DQHS (1.0 hr), AE (0.4 hr) and AS (0.4 hr). These results indicated that the rank order of elimination and biotransformation was AS ≥ AE > DQHS > AL. Bioavailability was highest for AS (105%), AL (95%) and DQHS (85%) after intramuscular injection, and lowest for AE (51%). After intragastric dosing, however, the highest bioavailability was observed for AE (49%), then AL (31%), AS (30%) and DQHS (19%). In vivo AE, AS and AL were converted to DQHS, the highest percentage conversion was found for AS (25 - 73%), followed by AE (9 - 16%) and AL (2 - 4%) after i.v., i.m. and i.g. administration. These results indicate that AS could be considered a prodrug for DQHS.

299 PHARMACOKINETICS AND METABOLISM OF A NEW 8-AMINOQUINOLINE PRIMAQUINE ANALOG, WR242511. Marino MT*, Peggins JO, Brown LD, Idowu OR, Urquhart M, and Brewer TG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

WR242511 is an 8-aminoquinoline analog of primaquine shown to be superior to primaquine in animal models for radical cure and causal prophylaxis of malaria and is therefore a potential replacement for primaquine. Pharmacokinetic studies were done in dogs with both intravenous and oral dosing. A reverse phase high performance liquid chromatography method was developed to study pharmacokinetics of this drug. The method has a limit of quantitation of 25 ng/ml from plasma. The drug has a bioavailability of 68%, a distribution half life of 9 minutes, a terminal half life of 32 hours and a volume of distribution at steady state of 15 L/Kg. Radiolabeled kinetics have shown a plasma half life in dogs of greater than 100 hours suggesting that the drug has one or more metabolite(s) with a long half life. One of the drugs side effects, methemoglobinemia, also lasts greater than 240 hours which supports the drug having metabolites with a long half life. Preliminary metabolism studies in rat microsomes show that the drug has a complex metabolic scheme with

multiple metabolites. Further studies are in progress to try to isolate metabolite(s) which are responsible for methemoglobin production and antiparasitic activity.

300 EARLY COMPARISON OF PERMETHRIN IMPREGNATED-BED NETS AND CURTAINS AND LAMDACYHALOTHRIN HOUSE SPRAYING FOR MALARIA CONTROL IN EASTERN NIGERIA. Sexton JD*, Breman JG, Ekanem OJ, Ezike VI, Roberts JM, Onyido AE, and Herrington JE. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Federal Ministry of Health, Nigeria; Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA; International Health Program Office, Centers for Disease Control and Prevention, Atlanta, GA; and Federal Ministry of Health, Nigeria.

A community-based malaria control trial comparing permethrin impregnated bed nets (PINs) or curtains (PICs) (0.5 gm/m^2 of material) and lamdacyhalothrin (ICON®) residual house spraying (HS) (25-30 mg per m^2) with a control (Co) began in August 1992 in Enugu State, Nigeria. Approximately 1,500 people living in 5 to 10 villages were assigned to each group. In August 1992 and February 1993, 1,100 bed nets and 2,600 curtains were impregnated with permethrin, and 300 houses were sprayed. Parasite prevalence, parasite acquisition in children <5 years of age, clinical episodes, human behavior, vector identification and behavior, and insecticide susceptibility were measured. Baseline malaria prevalence and fever episodes were similar in the 4 groups. After 6 months, prevalence was 14% (HS), 16% (PIC), 18% (PIN), and 23% (Co); only the spray group differed significantly from the control. Prevalence of fever episodes were 28% (PIN), 30% (PIC), & 21% (HS); these were all significantly lower than the 42% in the control group. Dry season vector assessment has yielded few *Anopheles*. Pyrethrum knock down and resting collections produced only 171 *Anopheles gambiae*, of which 72% were from control group houses.

301 THE PUBLIC HEALTH IMPACT OF THE RAPID RESETTLEMENT OF A GROUP OF SOUTHEAST ASIAN REFUGEES. Paxton LA*, Schultz LJ, Luby SP, Meriwether RZ, and Slutsker LM. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta, Georgia; and North Carolina Department of Environment, Health, and Natural Resources, Raleigh, NC.

In November 1992, the U.S. State Department airlifted to North Carolina 403 Montagnard refugees who had continued to fight the North Vietnamese for 17 years in a densely forested region along the Cambodia-Vietnamese border characterized by intense transmission of multi drug-resistant *Plasmodium falciparum*. These soldiers and their families underwent only cursory health examinations before resettlement. To evaluate reports of malaria illness, we collected malaria blood smears and determined recent fever history. Of 315 Montagnards screened (27% female, 20% children < 10 years of age), 181 (57%) had *Plasmodium* infection. Among children, infection prevalence was 87%. Only 17% of those with documented parasitemia reported fever. Because of the high infection prevalence and the presumption of sub-patent levels of parasitemia, the entire group was treated with halofantrine. This group accounted for almost one-third of all reported malaria cases and 58% of all smears reviewed at the CDC reference lab in 1992. The outbreak taxed the capacity of the public health system and highlighted deficiencies in U.S. preparedness for management of a complex tropical disease. Refugees from tropical areas require pre-immigration medical screening and treatment; furthermore, medical resources need to be allocated to localities where the refugees settle.

302 RAPID DIAGNOSIS OF MALARIA BY USE OF FLUORESCENT PROBES. Caramello P*, Negro C, Lucchini A, Dal Conte I, Pollono AM, Tanpradist S, and Gioannini P. Institute of Infectious Diseases, University of Turin, Italy; and Malaria Division, Ministry of Public Helath, Bangkok, Thailand.

This study was undertaken to compare Giemsa stain technique with the fluorescent stain DAPI/PI for:a) sensitivity of both thick and thin smear; b) speed of reading (thin smears only); c) identification of parasite species and stage on DAPI/PI stained smears. Two thin and two thick smears were obtained from 103 symptomatic patients in a malaria endemic area in Thailand. A thin and a thick smear were stained each with either of the two staining techniques. For DAPI/ PI, thick smears were dehaemoglobinized in distilled water for approximately 1'; then both thick and thin smears were fixed with methanol for 1'. 4,6 diamidine- 2-phenilindolo(DAPI) was applied for 1' and then one drop of propidium iodide (PI) was added, a coverslip was placed on top and the slide observed at 400x (thin smears) or 1000x (thick smears) magnification under a fluorescent microscope. RESULTS:species identification on DAPI/PI stained smears was correct in all cases. With DAPI/PI stained thick smears, 39 out of 40 Giemsa positive thick smears were identified, giving a sensitivity of 97.5%. Of 63 Giemsa negative thick smears, 62 were correctly recognized as such with DAPI/PI (specificity 98.4%). On thin smears, the time required to identify one parasite on Giemsa stained specimens or two on DAPI/PI ones, was remarkably shorter with the latter technique. In conclusion, DAPI/PI staining technique may prove useful especially in non-endemic countries where technicians are not experienced in reading Giemsa stained thick smears and the availability of a fluorescent microscope is not a constraint. In developing countries, it could be utilized in epidemiological surveys of populations with low density parasitaemias, where it enables a faster examination of slides.

303 USING PCR TO DETECT MALARIA DIRECTLY FROM BLOOD SAMPLES IN THE VENEZUELAN AMAZON. Laserson KF, Petralanda I, Hamlin DM, Almera R, Fuentes M, Carrasquel A, and Barker, Jr. R*. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Centro American Amazonico Para la Investigacion y Control de Enfermedes Tropicales "Simon Bolivar", Venezuela.

We have examined the reproducibility, sensitivity, and specificity of detecting *Plasmodium falciparum* using PCR and the *P. falciparum*-specific probe, pPF14, under field conditions in the Venezuelan Amazon. Up to 8 samples were field collected from each of 48 consenting Amerindians presenting with symptoms of malaria. Sample processing and analysis were performed at the Centro Amazonico para las Investigacion y Control de Enfermedades Tropicales "Simon Bolivar" (CAICET). A total of 229 samples from 48 patients were analyzed by PCR methods using four different *P. falciparum*-specific probes and 1 *P. vivax*-specific probe and by conventional microscopy. Samples in which results from PCR and microscopy differed were analyzed at high sensitivity by microscopy. Results suggested the microscopy-negative, PCR-positive were true positives and that microscopy-positive and PCR-negatve are true negatives. Sensitivity of the DNA probe/PCR method was 78% and specificity was 97%. The positive predictive value of PCR method was 88%, and the negative predictive value was 95%. Through the analysis of multiple blood samples from each individual, the DNA probe/PCR methodology was found to have an inherent reproducibility that was highly statistically significant.

304 MICROSCOPIC DIAGNOSIS OF MALARIA FOLLOWING A CYTOCONCENTRATION TECHNIQUE AFTER SAPONINE TREATMENT TO CAUSE HEMOLYSIS. Petithory JC*, Dufour M, Garnier R, Ardoin F, and Brumpt E. Controle de Qualite National en Parasitologie, Laboratoire Centre Hospitalier, Gonesse, France.

The diagnosis of malaria generally depends upon the microscopic demonstration of organisms in blood smears. However,this can be difficult and time consuming when the parasitemia is low.The use of thick blood films can improve efficiency in diagnosis but it takes time of smears to dry and often the organisms present may be distorted in appearance. We have tried a new technique which consists of hemolyzing the blood prior to cytoconcentration .75 µl of blood is diluted in isotonic solution, saponine is added and it is followed by a cytocentrifugation with a cytospin 3 apparatus Shandon for 10' at 2000 rpm (350xg).The sediment obtained,about a 6 mn diameter circle within a 32

mm^2 area is immediatley stained by the May-Grunwald-Giemsa technique.The results obtained to date, on 13 proven cases of malaria, are satisfactory. In one case of Tropical splenomegaly syndrome in which thin and thick blood films were negative for parasites we were able to demonstrate trophozoites of *P. falciparum* in the sediment. In another case of blackwater fever white cells containing pigment were numerous and had good cell morphology. A case of loiasis was also diagnosed with this technique. In conclusion, this newly described technique has given us better results than use of ordinary thick films and deserves continuing investigation.

305 ASSESSMENT OF A RAPID MANUAL TEST FOR THE DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA IN *AOTUS* MONKEYS. Millet PG, Grady KK*, Maret SM, Sullivan JS, and Collins WE. Malaria Branch, DPD, NCID, Centers for Disease Control, Atlanta GA; and Becton Dickinson Advanced Diagnostics, Baltimore MD.

We evaluated the sensitivity of a rapid manual test, ParaSight™F, in monitored experimental *Plasmodium falciparum* infections in monkeys. This test is performed on a strip, using whole blood, and offers a qualitative detection of HRPII (histidine rich protein II), a specific *P. falciparum* soluble antigen. Specificity of the test for *P. falciparum* was first evaluated using whole blood from monkeys and/or chimpanzees infected with human and nonhuman primate malaria parasites, and with *Babesia* sp. The test was negative with all heterologous parasites, including *P. vivax, P. ovale,* and *P. malariae.* In *Aotus* monkeys infected with blood stages of *P. falciparum,* the parasite was detected both by thick blood film and ParaSight™F at a mimimum concentration of 0.003% parasitemia (10 parasites per µl). Asexual parasites were not detected on thick film 7 days after treatment with chloroquine (15 mg/day for 2 days). However, circulating gametocytes were found up to 21 days, and the dipstick test remained positive for up to 28 days after treatment, suggesting, for these nonimmune animals, either a slow drug effect or a long-term clearance of circulating HRPII antigens. Such results were not obtained in previous studies involving patients. An IFA test conducted with an anti-HRPII monoclonal antibody demonstrated that mature gametocytes do not express this protein. In malaria-endemic zones, the usefulness of the ParaSight™F is yet to be determined. However, its specificity, sensitivity, and simplicity indicate potential usefulness for the field diagnosis of *P. falciparum* malaria.

306 EVALUATION OF CLINICAL DETERMINANTS IN PREDICTING MALARIA INFECTION. Svenson JE*, MacLean JD, and Gyorkos TW. Department of Epidemiology and Biostatistics, McGill University; McGill University Centre for Tropical DiseasesMontreal, Quebec, Canada; and Division of Clinical Epidemiology, Montreal General Hospital, Montreal, Quebec, Canada.

Data obtained during a matched case-control study that examined the association between malaria and compliance with antimalarial chemoprophylaxis were used to determine if signs and symptoms of the clinical presentation could predict malaria infection. The study population included 157 cases and 157 controls recently returned from a malaria-endemic area who had presented to the clinic complaining of fever and who sub- sequently had the same labortory work-up. There was no significant difference between cases and controls in the period of time since last travel in an endemic area, the season of travel or the mean number of days of reported fever before seeking care. GI symptoms of malaise and fatigue were reported less frequently in cases than controls. Cases were more likely than controls to have a temperature >38oC and palpable spleno- megaly [OR= 12.5;CI:3.33,33.00], but only 20% of cases had this sign. There was no difference in other physical signs or in laboratory values for bilirubin, creatinine, AST or glucose. Forward logistic regression identified symptom duration less than 7 days, tertian fever pattern, immune status, temperature >38°C and splenomegaly as having the highest predictive values (ranging from 40% to 82%) but corresponding low sensitivities. Because no sign or symptom (singly or in combination) could accurately predict the presence of malaria, testing for malaria should be done in all patients complaining of fever who have a history of travel to a malaria-endemic area.

307 CLINICAL RECOGNITION OF ANEMIA IN MALAWI. Kazembe PN*, Redd SC, Luby SP, Ziba C, Nwanyanwu OC, Franco C, Chitsulu L, Wirima JJ, and Olivar MA. Kamuzu Central Hospital, Lilongwe, Malawi; Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; Child Health Science Unit, Ministry of Health, Malawi; and University of Malawi, College of Medicine, Blantyre, Malawi.

Malaria-associated anemia is a common problem in young children living in sub-Saharan Africa, and guidelines for recognition and treatment are needed but not available. As a first step in developing such guidelines, we evaluated the ability of health workers to use clinical findings to identify children with anemia, defined as hemoglobin below 8 gm/dl. Trained health workers examined and obtained blood for hemoglobin concentration from children under age 5 years at either of two outpatient departments in rural Malawi. Among the first 476 children, conjunctival, tongue, nailbed, or palmar pallor were 35, 32, 59, and 62 percent sensitive in diagnosing anemia and 86, 91, 72, and 72 percent specific, respectively. Splenomegaly was 54% sensitive and 70% specific in diagnosing anemia. Either splenomegaly or palmar pallor was 79% sensitive and 48% specific in diagnosing anemia. For severe anemia, (defined as hb <5 gm/dl) palmar pallor was 81% sensitive and 64% specific. Even without laboratory support, which is often unavailable in rural Africa, clinical findings can identify the majority of children with anemia who need to be either treated or protected from malaria.

308 CLINICAL RECOGNITION OF MALARIA, MALAWI. Luby SP*, Redd SC, Ziba C, Nwanyanwu OC, Franco C, Kazembe P, Olivar MA, Cullinan T, Chitsulu L, and Wirima JJ. Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; Child Health Science Unit, Ministry of Health, Lilongwe, Malawi; Kamuzu Central Hospital, Lilongwe, Malawi; and University of Malawi, College of Medicine, Blantyre, Malawi.

Treating children who would benefit from antimalarial therapy while preventing over-treatment is difficult in Africa where microscopy is not generally available. To evaluate alternative clinical case definitions for malaria, we obtained a medical history, physical examination, and blood samples from children who attended either of two outpatient clinics in rural Malawi during the high transmission season. We defined malaria disease as parasitemia >10,000/mm3 or any level of parasitemia in a patient with hemoglobin <8.0 gm/dl. Among 399 children, 336 (84%) had a history of fever as part of their presenting illness and 138 children (35%) met the criteria for malaria disease. History of fever was 92% sensitive and 20% specific for malaria disease, and would result in 209 children (52% of all the children) treated unnecessarily. A clinical case definition using rectal temperature >37.5 C or pallor on physical exam was 76% sensitive, 52% specific and would result in 125 children (31%) treated unnecessarily. Physical exam findings can increase the specificity of the malaria clinical case definition and so reduce over-treatment with antimalarial chemotherapy, but would result in more persons with malaria who are not treated.

309 A COMMUNICATION STRATEGY FOR THE VILLAGE-LEVEL PROMOTION OF INSECTICIDE-IMPREGNATED MOSQUITO NETS IN BAGAMOYO DISTRICT, TANZANIA. Winch PJ*, Makemba AM, Kamazima SR, Premji Z, Minjas JN, and Shiff CJ. Department of International Health, Johns Hopkins University, School of Hygiene & Public Health, Baltimore.; Bagamoyo Bed Net Project, Dar es Salaam, Tanzania; and Department of Parasitology & Medical Entomology, Muhimbili Medical Centre, Dar es Salaam, Tanzania.

If insecticide-impregnated mosquito nets are to have a significant impact on malaria transmission in holoendemic areas of Africa, it will be necessary to achieve high rates of continuous usage and high rates of regular reimpregnation with insecticide among the local population. This paper will present the experience in communication with villagers about mosquito nets from a large intervention trial

in Bagamoyo District, Tanzania. It is not enough to just promote the general concept of net usage. Villagers are already familiar with nets, but lack information on proper usage. Messages need to be specific and focus on the importance of their use by children under five and women, groups who may be the last to gain access to the nets, and the importance of sleeping under a net even during seasons when mosquitoes are relatively few in number. Communication channels which were used included face-to-face promotion by members of village mosquito net committees, village meetings, education in schools, pamphlets, posters, calendars and plays staged by the Bagamoyo College of Fine Arts. The impact of this communication strategy will be discussed, including the strengths and weaknesses of specific channels.

310 MALARIA EPIDEMICS IN AMAZONAS, VENEZUELA: 1989 -1992. Almera R*, Fuentes M, Felicita S, Lopez A, Garcia M, Hung S, and Petralanda I. CAICET, Puerto Ayacucho, Amazonas, Venezuela.

Malaria in Amazonas affects Amerindian groups living in the southeastern border region with Brazil (SP) and in the northwestern part of the state (PY). Between 1990 and 1992, 1141 clinical cases of malaria were referred to the reference center of CAICET for diagnosis. This constitutes 12 % of all reported malaria cases from the entire Amazonas state during this 2 year period. Clinical illness interfered with normal daily activities in all Amerindians with malaria, which was confirmed by standard microscopic examination of duplicate slides. 59 % of all cases were due to *Plasmodium vivax* and 41 % to *P. falciparum*. A single case caused by *P. malariae* was observed. During each of the two years, peak incidence of malaria occurred between April to August and October to January. Each epidemic of *P. vivax* malaria lasted longer (3 - 5 months) than *P. falciparum* (2 months). There were no significant sex or age differences in the prevalence of cases due to either *Plasmodium* species. 39 % of all malaria cases occurred in the 10 to 30 year age group. Children below the age of 9 constituted 25 % of cases in SP and 14 % in PY. The average number of malaria attacks per individual per year in a sentinel population of SP (N = 2353) varied from 0.6 in 0 - 4 year old children to 1.3 in 20 - 40 year old adults. These results indicate that malaria epidemics are a major cause of morbidity among Amerindians in Amazonas.

311 EVALUATION OF MALARIA CONTROL IN AFRICA: CHANGING THE RULES. Ntahobari S*, Roungou JB, Nguyen-Dinh P, Bryce J, Naimoli JF, and Hersh B. Ministry of Public Health, Burundi; Ministry of Health, Central African Republic; Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA; and International Health Program Office, Centers for Disease Control and Prevention, Atlanta, GA.

Program managers from several African countries have identified program evaluation as a priority strategy for improving malaria control in sub-Saharan Africa. Such evaluation should be an activity built into program operations and provide immediate feedback on progress toward program objectives. For example, a 1990 survey in 87 health facilities in the Central African Republic (CAR) documented that 40% had no chloroquine in stock; only 35% of the patients were administered the correct dose of chloroquine; and satisfactory patient education was provided in fewer than 40% of cases. This information led CAR to take actions to improve the flow and availability of antimalarial drugs and the performance of health workers. Such evaluation data, tracking key program outcomes and implementation activities, can alert managers to operational areas that need improvement and can identify the corrective actions needed. This approach complements more traditional malaria program evaluations that emphasize the measurement of mortality and morbidity and rely on visits at multi-year intervals by outside experts. Guidelines for the evaluation of malaria control programs have been collaboratively developed by program managers and will be field tested.

312 AREA-SPECIFIC RISKS OF MALARIA IN THAILAND. Thimasarn K, Suvannadabba S, Jatapadma S, Sirichaisinthop J, and Wongsrichanalai C*. Ministry of Public Health, Bangkok, Thailand; and Department of Immunology and Parasitology, Armed Forces Institute for Medical Sciences, Bangkok, Thailand.

The extent of multi-drug resistant strains of *Plasmodium falciparum* and current area-specific risks of malaria in Thailand are usually not known to most physicians outside of the country. Computerized malaria surveillance database has been accumulated by the Thai Department of Communicable Diseases Control since 1986. The recorded number of cases detected by a district malaria office, proportion of cases having contacted malaria within the district, and the known total district population allow for estimation of a monthly cumulative incidence (CI), or "average risk," of malaria within that particular district. For example, in Tah Song Yang District of Tak, a northern province on the Thai-Myanmar border, all of the 55,088 inhabitants live in areas with malaria transmission. The CIs of malaria among these semi-immune individuals were estimated to be 0.49% for April and 1.40% for July 1992. The malaria situation in Tak represents one of the worst in northern Thailand and, therefore, these data suggest that under the national control scheme, the overall risk of malaria in Thailand is relatively low. Country-wide area-specific CI estimates based on the monthly 1992 statistics will be presented.

313 A LONGITUDINAL STUDY OF ANTIBODY LEVELS IN AN AREA OF LOW MALARIA ENDEMICITY USING THE INDIRECT FLUORESCENT ANTIBODY (IFA) TECHNIQUE. Pasay CJ, Bustos DG*, Belizario VY, Lansang MA, and Saul AJ. Research Institute for Tropical Medicine, Department of Health, Alabang, Metro Manila, Philippines; and Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Australia.

A longitudinal study was conducted in the low endemic municipality of Morong, Bataan, Philippines, to provide baseline information on the dynamics of antibody response to malaria using the Indirect Fluorescent Antibody Technique (IFAT). 125 malaria positive individuals were followed-up for 9 months at 3-month intervals. On follow-up, cases were examined for malaria-associated symptoms, and blood collected for thick and thin smear and serology. IFA was done to detect antibody levels,particularly for the isotypes IgM and IgG. Results showed that on the initial survey, 87% of the study population had no detectable IgM while 99.2% had IgG titers of 1:64 and above. On the 3rd & 6th month, IgG and IgM fluctuations were seen suggesting active transmission. Infection rate was < 11% for 3 follow-up periods, but geometric mean IgG titers ranged from 1:256-1:16384 in areas with no parasitemic cases, indicating potential for transmission. Geometric mean IgM (p=.03,.01 &.02 on the 3rd, 6th and 9th month, respectively) and IgG (p=.007 and.004 on the 6th & 9th month) titers of infected cases were usually higher than non-infected cases suggesting a probable association between malarial smear positivity and antibody levels. IgG levels increased with age.The presence of high IgG levels (>1:4096) was not protective against either plasmodial species, but was negatively associated with symptoms.

314 GEOGRAPHIC VARIATION IN THE INCIDENCE OF CEREBRAL MALARIA IN A ZAMBIAN COMMUNITY. Thuma PE*, Njungu M, Thuma EN, Biemba G, and Gordeuk VR. Department of Pediatrics, Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA; Macha Mission Hospital, Choma, Zambia; and Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.

To determine if geographic clustering in the incidence of cerebral malaria exists in Zambia, we estimated the occurrence of cerebral malaria in four adjacent regions served by Macha Mission Hospital during 1987-1989. Macha Hospital is a 200 bed rural hospital which serves as the primary referral center for four traditional chiefdoms within a 50 kilometer radius. The relative incidence of cerebral malaria in each region was estimated by analyzing all pediatric admissions for the period, and

calculating the proportion of children 14 years and under admitted with cerebral malaria as compared to the children of this same age group admitted with all forms of infection with *Plasmodium falciparum*. Results were determined as the percentage of all malaria cases that had cerebral malaria. The four regions showed a significant difference as follows: 7.2% of 237 admissions, 9.7% of 696 admissions, 12.3% of 308 admissions and 13.2% of 876 admissions. ($P < 0.05$) These data raise the possibility that there is clustering of cerebral malaria cases in this rural African community. Possible explanations for this pattern would include variations in virulence of *P. falciparum* strains, as well as genetic or nutritional differences in the population at risk.

315 AN IMPORTED FOCUS IN NORTH CAROLINA OF 402 MONTAGNARD REFUGEES INFECTED WITH FOUR *PLASMODIUM* SPECIES: DIAGNOSTIC EVALUATION. Sulzer AJ*, Long EG, Gracia LS, Millet PG, Grady KK, Schultz LJ, Paxton LA, Slutsker LM, Luby SP, Robertson GC, and Turner LS. Malaria Branch, DPD, National Center for Infectious Disease, Centers for Disease Control, Atlanta, GA; Division of Bacterial Diseases, NCID, Centers for Disease Control, Atlanta, GA; UCLA Medical Center, Los Angeles, CA; and Division of Health Services, Dept of Environment, Health, and Natural Resources, Raleigh, NC.

In November and December, 1992, 402 Montagnard refugees arrived in the United States after residing for many years in a holoendemic malaria area on the Vietnam-Cambodian border. Within 1 month of arrival, the group was screened for malaria parasitemia, revealing 47% of the refugees were infected with *Plasmodium vivax*, *P. falciparum*, or *P. malariae*. Detailed re-examination of the blood films revealed 58% were parasitemic. Seven cases of *P. ovale* were identified, confirming a 1970 report that *P. ovale* is indigenous to Southeast Asia. Screening identified 24 persons infected with more than one species, whereas detailed exams revealed 26 infections with two species, and 11 infections with three species. Among those infected with multiple species, *P. falciparum* was often less apparent than *P. vivax* or *P. malariae*. Children <10 years accounted for 82% of multiple infections, indicating age-specific differences in immune response. To ensure proper management, malaria smears should be examined carefully to rule out infection with multiple species, particularly when potentially life threatening, drug-resistant strains may be present.

316 CHARACTERIZATION OF THE CELLULAR RESPONSES IN PATIENTS WITH TEGUMENTARY LEISHMANIASIS USING T-CELL IMMUNOBLOTTING. Ortiz-Ordonez JC* and Saravia NG. Fundacion Centro International de Entrenamiento e Investigaciones Medicas, Cali, Colombia.

Little is known about the antigens involved in eliciting immunity against *Leishmania (V.) panamensis*, the most common cause of tegumentary leishmaniasis in Colombia and parts of Central America. A T-cell Western protocol was adapted to analyze the cell-mediated responses to *L. panamensis* antigens using peripheral blood lymphocytes from patients with parasitologically-confirmed tegumentary leishmaniasis. For these studies, *Leishmania* antigens were separated by one or two-dimensional SDS-PAGE and transferred to nitrocellulose and PVDF membranes. Although controlled experiments demonstrated partial inhibition of lymphocyte proliferation by the membranes, immunodominant antigens were identifiable. Experiments utilizing peripheral blood mononuclear cells from patients with the active disease (n=11) showed predominant proliferative responses to be elicited by proteins in the relative molecular weight ranges of 67-43 kd (82% of patients), 43-30 kd(90%), and below 14 kd (64%). Cytokine responses associated with these immunoreactive areas are also being assessed (IFN-γ, IL-2, IL-10 and TNF). This approach will also be applied to putative susceptible and resistant individuals from endemic foci.

317 ELEVATED SERUM ARGINASE LEVELS IN EXPERIMENTAL ANIMALS AND IN PATIENTS WITH VISCERAL LEISHMANIASIS. Evans TG, Fratkin M*, Hibbs JB, and Vasconcelos

WA. Infectious Diseases Section, Salem Veterans Administration Medical Center, Salem, VA; Division of Infectious Diseases, University of Utah, Salt Lake City, UT; and Nucleo de Medicina Tropical, Universidade Federal do Ceara, Fortaleza, Ceara, Brazil.

Synthesis of nitric oxide (N0) from arginine via cytokine-inducible NO synthase is critical to the outcome of visceral leishmaniasis in experimental animal models. Since the enzyme arginase competes with NO synthase for the substrate arginine, as previously described, we hypothesized that visceral leishmaniasis may be characterized by elevated levels of arginase as one of the mechanisms of reducing potential nitric oxide production. We measured serum arginase activity by detecting radiolabeled urea derived from L-guanido-C14-labeled arginine. Hamsters infected with [Leishmania chagasi] which were nearly endstage (N=15) had nearly fourfold the level of arginase compared to uninfected healthy adult animals (N=7) (224 ± 86 vs 61± 48, $p<0.001$). Patients from northeast Brazil with acute visceral leishmaniasis (N=7) had twice the serum arginase activity of normal individuals (N=13) (5.6 ± 2.3 vs 2.9 ± 1.3, $p=0.003$). We have also developed techniques for the measurement of arginase in experimental tissue as well as in human skin and will present data from individuals with different forms of leprosy. Experimental and human visceral leishmaniasis are associated with an increase in serum arginase levels which may reflect a down-regulation of NO synthase, or which may contribute to the depletion of arginine, the substrate for synthesis of nitric oxide.

318 T CELL RESPONSES AND CYTOKINE PRODUCTION IN *LEISHMANIA MAJOR*-INFECTED MICE TREATED WITH PENTOSTAM. Nabors GS* and Farrell JP. Department of Pathobiology, University of Pennsylvania, Philadelphia, PA.

Following successful drug therapy, previously non-responsive patients with visceral leishmaniasis become DTH+, and their peripheral blood cells produce IFN-γ and IL-2. In vivo studies in nude mice have demonstrated a requirement for CD4+ T cells, CD8+ T cells, IFN-γ or IL-2, for effective drug therapy. In this study, non-healing BALB/c mice infected with *Leishmania major*, were treated with Pentostam (250 mg/kg/day) to investigate whether the severity of infection contributes to the perpetuation of an established TH2 response. Lesion growth was suppressed in drug-treated mice which received high ($1x10^6$) infective doses of *L. major*, promastigotes. Limiting dilution analysis of lymph node, spleen and footpad tissue revealed that Pentostam treatment also reduced the parasite burdens 900-3,000 fold. However, following cessation of therapy, lesion growth resumed, indicating that the noted shift in cytokine profiles did not result in the development of resistance. In contrast, Pentostam treatment had a greater therapeutic effect in animals with chronic non-progressing lesions such as those observed in mice inoculated with low ($3x10^3$) numbers of parasites. Cells from treated animals produced increased levels of IFN-γ, but decreased levels of IL-4 and IL-5, suggesting a shift in the TH1/TH2 profile. Production of TGF-β, a cytokine associated with disease exacerbation, was also reduced following drug treatment, suggesting that the beneficial effects of Pentostam may not only be due to increased expansion of TH1-type responses. Current studies are investigating the effects of combing drug therapy with cytokines and /or anti-cytokine antibodies.

319 INFECTION OF HUMAN BONE MARROW DERIVED STROMA WITH VICEROTROPIC *LEISHMANIA TROPICA* PROMASTIGOTES. Rowton ED*, Leiby DA, Toro-Lopez L, and La Russa VF. Division of Communicable Diseases and Immuniology, Walter Reed Army Institute of Research, Washington, DC; and Division of Medicine, Walter Reed Army Institute of Research, Washington, DC.

Bone marrow is the target tissue for viscerotropic *Leishmania tropica* isolated from US soldiers returning from Operations Desert Shield and Storm (ODS). Human bone marrow-derived stromal cells from long-term marrow cultures (LTMC) established with marrow light density cells (<1.070) were investigated as potential cells for *in vitro* culturing of amastigotes. Replicate cultures of

adherent stromal cells from 3-4 week old LTMC were washed and inoculated with early stationary-phase promastigotes (3:1) in each of the media described below and incubated at room temperature for 3 hrs, then maintained in a CO2 incubator at 37 C for 3 days. Dulbeccos minimal essential medium (DMEM), Schneiders medium (SDM), and Long-term Culture medium (LTCM), supplemented with either 30% fetal bovine serum (FBS) or human serum (HS) were compared in this system for ability to support infection. Infection of stromal cells was measured by determining the % of stromal cells which contained 1 or more amastigotes 3 days post infection. The viscerotropic isolate (WR1063) and a cutaneous isolate (WR966) infected cultures maintained in SDM-30%FBS equally (27% and 29%). When these infected cultures were maintained in LTCM-30% FBS, both had equally-reduced numbers of infected cells (13% and 12%). Iscove's modified Dulbecco's medium (IMDM) supplemented with either 30% FBS or 30% HS showed 5% and 0% infected cells, and SDM-30% HS also showed low numbers of infected cells (3%). Stationary phase promastigotes were more efficient in infecting stromal cells than log phase parasites (29% and 3%). The growth of another ODS cutaneous isolate, WR1075 (*L. major*) was compared. The stationary-phase parasites from this strain were also more efficient in infecting stromal cells than log-phase parasites (68% and 36% respectively) and this species infected LTMC better than the viscerotropic isolate (68% vs 29%). These preliminary results suggest that adherent stromal cells from LTMC could be used as an *in vitro* model for the generation of *Leishmania* amastigotes. They also suggest that certain cells of the bone marrow microenvironment are target cells for *Leishmania*.

320 IN VITRO INFECTION CHARACTERISTICS OF TWO STRAINS OF *TRYPANOSOMA CRUZI*. Luo H* and Rowland E. Department of Biological Sciences, Ohio University, Athens, OH.

Differing characteristics of experimental murine Chagas' disease reported in the literature are often suggested to be due to the inherent qualities of the parasite strain used. In a previous report, work from our laboratory has shown that the Brazil strain of *T. cruzi* produces a more virulent *in vivo* infection than the Guayas (Ecuadorian) strain in C3H mice. This difference was indicated by a decreased parasitemia and degree of cardiomyopathy in the Guayas infected mice. In the present work *in vitro* infection characteristics of these two parasite strains were studied in cultures of macrophages, myocytes and fibroblasts by microscopic examination of stained slide cultures. The infection rate and the number of cell-associated parasites per infected cell were measured during the first 24 hours of interaction and during long-term infection up to 15 days (excluding macrophage cultures). In both short-term and long-term experiments Brazil strain infection was shown to have a greater infection rate with a higher number of parasites per cell than Guayas infection for all host cell types. There was no indication of different tissue tropisms in that both parasite strains showed an ability to infect myocytes and macrophages. Determination of trypomastigotes released by infected cells into culture medium indicated that the intracellular reproductive cycle is shorter for the Brazil strain (7-12 days) than that of the Guayas (12-15 days). The more aggressive *in vitro* infection displayed by Brazil parasites compared to Guayas organisms may be the cause for the more virulent characteristics seen in Brazil infected mice.

321 AMERICAN CUTANEOUS LEISHMANIASIS IN COLUMBIA: CELLULAR AND VASCULAR PROFILE OF CUTANEOUS LESIONS AND MONTENEGRO SKIN TESTS. Palma G*, Mackenzie C, Salinas G, Guarin N, and Saravia A. CIDEIM & Department of Microbiology, Universitas de Valle, Cali, Columbia; and Department of Pathology, Michigan State University, East Lansing, MI.

Cutaneous leishmaniasis lesions due to *Leishmania panamensis* and *L. brasiliensis*, together with biopsies of 48 hour standard Montenegro skin tests, were examined for the infiltrating cell profile and changes in vascular elements using immunocytochemical approaches. The following antibodies directed against cellular markers, vascular markers and normal skin elements were investigated: UCH1 (T and macrophage), B cell, LCA, Leu-7, lysosome, Factor VIII, PCNA, Q Endo and AEI. Cell activity was seen most prominent in different areas and this was related to the intensity of reaction.

These findings indicate the variation seen in the inflammation due to this infection. It is seen that the Montenegro test reflects an activity in some but not all aspects.

322 TRYPANOSOMES AND MICROFILARIAE IN FERAL OWL AND SQUIRREL MONKEYS MAINTAINED IN RESEARCH COLONIES. Steurer F, Sullivan JJ*, Benavides G, Tarleton RL, Eberhard ML, and Landry S. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Department of Zoology, University of Georgia, Athens, GA; and Malaria Vaccine Development Program, Office of Health, U.S.A.I.D., Washington, DC.

A group of 358 owl and squirrel monkeys imported from Colombia, Peru, and Bolivia for the USAID Malaria Vaccine Development Program was examined for trypanosomes and microfilariae. *Trypanosoma rangeli,* isolated by hemoculture from *Aotus nancymai, Saimiri b. boliviensis,* and *S. b. peruviensis,* accounted for 76.6% of all trypanosome infections. *T. cruzi* was isolated from 25 of 194 *S. b. boliviensis,* including two mixed infections with *T. rangeli.* Trypanosome identifications were confirmed by blinded tests with a panel of five rRNA probes on a subsample of cultures identified morphologically. Although no trypanosomes were isolated from *A. vociferans* or *A. lemurinus griseimembra,* positive serologic responses to *T. cruzi* were observed by indirect immunofluorescence in all species of monkeys examined and ranged from 42.1% among *S. b. peruviensis* to 92.3% among *A. vociferans.* Among *T. rangeli*-infected monkeys, 43.7% were seronegative for *T. cruzi.* No microfilariae were found in *S. b. boliviensis* or *A. l. griseimembra. Mansonella barbascalensis* and *Dipetalonema caudispina* were observed in *A. vociferans, M. panamensis* in *A. nancymai,* and *M. saimiri* and *D. caudispina* in *S. b. peruviensis.* Such naturally occurring infections in imported animal models are potential sources of accidental transmission to animal handlers and uninfected laboratory animals and can introduce confounding variables into otherwise well-planned studies.

323 DEGRADATION OF RABBIT IMMUNOGLOBULIN INGESTED BY *GLOSSINA MORSITANS MORSITANS* (WESTWOOD). Hampton RW*, Preston L, Narcisi EM, and Honigberg BM. Department of Pathology, University of South Alabama College of Medicine, Mobile, AL; and Department of Zoology, University of Massachusetts, Amherst, MA.

Anti-procyclic antibody ingested by *Glossina morsitans morsitans* (Westwood) is inactivated, failing to block transformation of *Trypanosoma brucei brucei* procyclic forms into epimastigote stages. The mechanism for the inactivation is not known. Conceivably, ingested antibody could be enzymatically degraded by endogenous proteases. To address this possibiity, 36 tsetse flies, starved for 7 days, were allowed to gorge on procyclic immunized rabits. The midgut contents from 2 flies were subsequently collected at 8 hr intevals over a 128 hr period. Midgut samples were analyzed for (1) total protein content and (2) presence of intact rabbit IgG. Results using a modified Lowry protein assay and Western blotting techniques indicate rabbit IgG could be detected in the midgut at 120 h post-ingestion. These results suggest *G. m. morsitans* employs a mechanisms other than enzymatic degradtion to inactivate antibody ingested in a bloodmeal.

324 THE IMMUNE RESPONSE OF DOGS IMMUNIZED WITH IRRADIATED L3 LARVAE OF *DIROFILARIA IMMITIS*: IDENTIFICATION OF POTENTIALLY PROTECTIVE ANTIGENS. Mejia JS* and Carlow CK. Molecular Parasitology, New England Biolabs, Beverly, MA.

The immune response of dogs that were vaccinated with irradiated L3 larvae of *Dirofilaria immitis* and challenged with normal infective larvae is characterized and compared to that of nonvaccinated dogs. The comparative analysis included a monitorization of the peripheral blood eosinophila, and the sequential IgM and IgG response to antigens from different stages of the parasite life-cycle. The humoral immune response of vaccinated and non-vaccinated animals was found to be very similar,

and while no antigen was found to be exclusively recognized by vaccinated dogs, they displayed an earlier response to an L3 antigen of 34kD, adult antigens of 20, 38, and 200kD and microfilarial antigens of 38, 40, 73 and 84kD. In addition to this temporal difference in the immune response, differential recognition of adult worm antigens of 14, 20, 34 and 55kD was suggested at the epitope level in competitive binding experiments. A more rapid onset of eosinophilia was also detected in vaccinated dogs. We propose that the rapid eosinophilia and antibody response to certain antigens/epitopes may be crucial in the induction of protective immunity. The extensive cross-reactive response to larval antigens observed late during natural infection may result in "blocking immunity" explaining the absence of natural immunity in dirofilariasis.

325 ANTIBODY RESPONSES IN MICE IMMUNE TO THE LARVAL STAGES OF *ONCHOCERCA VOLVULUS*. Yutanawiboonchai W*, Lange AM, Haberstroh FH, and Abraham D. Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA.

Little is known about the early phase of infection with *Onchocerca volvulus* due to the lack of suitable monitoring techniques. Recently, a mouse model using a diffusion chamber system was developed to assay parasite survival during the early phase of infection. Utilizing this model, it was shown that protective immunity could be induced in BALB/c mice using irradiated *O. volvulus* infective larvae (L3) as the antigen. To study humoral immune responses generated in the immunized mice, serum from immunized BALB/c was transferred to SCID mice which were then challenged with *O. volvulus* L3 in diffusion chambers. Significant reduction of worm survival was observed in SCID mice receiving immune serum, in comparison to SCID mice receiving normal mouse serum. Using an ELISA to measure total antibody levels, it was found that IgE was elevated in immunized mice, while other isotypes did not show any significant change from control value. Immunohistochemistry was performed on frozen sections of *O. volvulus* L3 to identify the specific antibody isotypes generated in immunized mice. It was found that there was a significant increase in specific IgE in immunized mice. In addition, IgG2b and IgG3 found in immune serum also recognized antigens of *O. volvulus* L3 in sections. Further studies will be done to confirm the role of antibodies in parasite killing both *in vivo* and *in vitro*. These findings will help in understanding protective immune responses against the early phase of *O. volvulus* infection and aid in identifying the protective antigens to be used in vaccine development.

326 EARLY DOWN REGULATION OF PARASITE ANTIGEN ASSOCIATED GRANULOMATOUS INFLAMMATORY RESPONSES IN BRUGIA-INFECTED JIRDS. Klei TR*, Horohov DW, Coleman SU, Nguyen C, Philpott MS, and Nasarre C. Veterinary Microbiology & Parasitology, School of Vet Medicine, Louisiana State University, Baton Rouge, LA.

Previous studies have shown that soluble adult worm antigen mediated granulomatous inflammation and splenocyte proliferative responses are down regulated in *Brugia*-infected jirds and that these phenomena are temporally associated with increasing microfilaremia. The kinetics of antigen mediated granulomatous inflammation, lymphocyte proliferation and antibody response to saline extracts of microfilariae, L3, L4 male and female worms were further assessed during early developmental phases of the parasite (7, 14, 28, 56 and 120 days post-infection (DPI)). Peak granulomatous responses to all antigens were reached by 7 DPI. Reduction in these responses began by 28 DPI. Similar reduced inflammatory responses were not seen in jirds inoculated with irradiated (25 krad) L3. Eosinophils were a prominent feature within these lesions at all times. Peripheral blood eosinophilia in the jird peaks near 28 DPI. These observations suggest that normally developing L3 induce an immune response, prior to adult worm maturation, which down regulates parasite mediated inflammation. Further, the eosinophilia seen indicated that prominent Th2 T-cell mediated events, not seen in irradiated L3 vaccinated jirds, temporally correspond to the induction of this down regulation.

327 IMMUNE REACTIVITY TO THE GP15/400 MOLECULE OF *BRUGIA MALAYI*. Allen JE*, Lawrence RA, and Maizels RM. Wellcome Research Centre for Parasitic Infections, Department of Biology, Imperial College, London, UK.

Gp15/400 of *Brugia* sp. is a surface-associated glycoprotein that is characterized on polyacrylamide gels by a regular series of proteins of increasing molecular weight with a base unit of 15 kDa. The antibody response to gp15/400 is restricted in mice by factors outside the H-2 complex such that BALB/c mice ($H\text{-}2^d$) make a vigorous antibody response while both CBA ($H\text{-}2^k$) and B10.D2 ($H\text{-}2^d$) mice are incapable of responding to the native molecule. We analyzed T-cell and B-cell responses to gp15/400 in mice that had been exposed to native antigen or mice that were immunized with an *E. coli* recombinant, p15. This evaluation demonstrated that although CBA mice were unable to elicit either T or B cell responses to the native molecule, they responded at least as well as BALB/c mice to the recombinant. Thus, the failure of CBA mice to make anti-gp15/400 antibody was not due to an absence of recognizable T cell determinants on the molecule. To map the T and B cell recognition sites, fusion proteins were constructed that represent overlapping fragments of the 15 kDA base unit. This analysis demonstrated that both antibody and T cell responses of BALB/c mice exposed to native glycoprotein are directed at the carboxyl region. In contrast, when either BALB/c or CBA mice are immunized with recombinant p15 the antibody response is directed primarily at the amino terminal region which is glycosylated in the native molecule. Our current hypothesis is that the inability of CBA mice to recognize the native molecule is the result of inadequate antigen processing due to the nature or location of posttranslational modifications on the native glycoprotein.

328 IMMUNITY TO ONCHOCERCIASIS: IDENTIFICATION OF A PUTATIVELY IMMUNE POPULATION IN A HYPERENDEMIC AREA OF ECUADOR. Elson LH*, Guderian RH, Calvopina M, Araujo E, Parredes W, Bradley JE, and Nutman TB. Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; Department of Clinical Investigation, Hospital Vozandes, Quito, Ecuador; and Department of Biology, Imperial College of Science and Technology, London, UK.

The existence of immunity to *Onchocerca volvulus* (Ov) infection is suggested by the presence of amicrofiladermic individuals in hyperendemic areas. A major barrier to the study of immunity has been the correct identification of immune (PI) individuals since very low level infections and occult infections are often not detected using traditional methods of diagnosis. To characterize more precisely a PI group in a hyperendemic focus in northwestern Ecuador, an extremely sensitive PCR technique based on the tandemly repeated O-150 sequence to identify Ov DNA in skin snips was used in combination with a panel (OV7, OV11, OV16, OV33) of recombinant antigen based ELISA assays (RA). The PI group (n=42), previously and repeatedly found to be infection free by clinical and parasitological examinations over the past 14 years, were compared to individuals with active infection (INF; n=45). While each of the INF were positive in the RA, 93% of the uninfected individuals were similarly positive. Of these, 3 individuals were found to contain skin DNA positive for the O-150 repeat and were therefore felt to be infected. The remaining PI individuals had significantly lower serum levels of *O. volvulus* specific IgG, IgG subclasses and IgE (p values all < 0.05) than the INF individuals (n=48). Analysis of proliferative responses of PI and INF PBMCs to parasite antigen (Ag), non parasite Ag and mitogens revealed no significant differences between the two groups although Ag driven cytokine analyses are underway. Nevertheless, the use of highly discriminating techniques for clinical categorization will allow for more precise definition of the PI state in Ov infection.

329 HLA-DQ ALLELES ASSOCIATED WITH SUSCEPTIBILITY AND RESISTANCE TO ONCHOCERCIASIS. Meyer CG*, Gallin M, Erttmann KD, Brattig N, Schnittger L, Begovich AB,

Erlich HA, and Horstmann RD. Bernhard Nocht Instute for Tropical Medicine, Hamburg, F.R. Germany; Department of Human Genetics, Roche Molecular Systems, Alameda, CA.

Human infections with the tissue nematode *Onchocerca volvulus* cause a spectrum of manifestations which include features of tolerance, pathology, and possibly protection. Evidence suggests that such a spectrum relates to variations in the immune response mediated by the polymorphic MHC class II products HLA-DR, -DQ, and -DP, which present peptides to select and stimulate T lymphocytes. Seventy-four subjects of a West-African area hyperendemic for human onchocerciasis were classified according to clinical and laboratory findings as presenting with generalized onchocerciasis, localized onchocerciasis, or in an onchocerciasis-free condition. MHC class II variants (DRB1, DQA1, DQB1, DPB1) were assessed by oligonucleotide typing and sequencing of the variable gene segments amplified *in vitro* from genomic DNA of peripheral blood lymphocytes. No significant difference in the distribution of HLA-D variants was found between patients with the two forms of the disease, generalized or localized. However, compared to individuals without onchocerciasis, either form of the disease was associated with the DR1-DQw5 haplotype (DRB1*01-DQA1*0101- DQB1*0501), whereby a strong association was found with DQA1*0101 but not with DQB1*0501. In addition, DQB1*0201 was independently associated with disease. Onchocerciasis was negatively associated with the DQ molecule encoded by DQA1*0501 and DQB1*0301 on DR11 and DR12 haplotypes. Thus, a group of protected individuals may be defined by exclusion. HLA-DQ alleles are associated with susceptibility and resistance to onchocerciasis.

330 MHC CLASS II ASSOCIATIONS WITH CLINICAL MANIFESTATIONS IN LYMPHATIC FILARIASIS OBSERVED IN SOUTH INDIANS. Zimmerman PA*, Phadke P, Kumaraswami V, Vijayan V, Naryanan PR, Ottesen EO, and Nutman TB. Laboratory of Parasitic Diseases, National Institutes of Heath, Bethesda, MD; and Tuberculosis Research Center, Madras, India.

As immune recognition of filarial parasite antigens is MHC class II restricted, the objective of the present study was to determine if class II genes are associated with disease status in bancroftian filariasis. Twenty-nine South Indians were studied; 8 were uninfected and 21 had various manifestations of infection (5 with asymptomatic microfilaremia, 8 with elephantiasis and 8 with tropical pulmonary eosinophilia [TPE]). For genotyping a new PCR-based technique, termed directed heteroduplex analysis (DHDA) was developed, using the DQA2 and DQB2 pseudogene second exon fragments as probes. Despite the small number of subjects studied a significant association between the presence of DQA1*0301 and elephantiasis was found ($p<0.05$; Fisher's exact test). There was also a tentative association between DQA1*0201 and TPE. These observations suggest that the previously reported genetic predisposition between developing pathology in lymphatic filariasis and HLA-B15 may be further strengthened by defining associations with MHC class II haplotypes or individual alleles; an extensive analysis involving more than 200 such patients is currently underway.

331 ANTIBODY RESPONSES TO EXTRACELLULAR MATRIX PROTEINS IN PATIENTS WITH ONCHOCERCIASIS AND LYMPHATIC FILARIASIS. Petralanda I*, Camico C, Carrasquel A, and Piessens WF. CAICET, Puerto Ayacucho, Amazonas, Venezuela; and Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

We have previously shown that filarial proteases cleave extracellular matrix (ECM) proteins such as collagen and elastin and that some cleavage products are immunogenic. We therefore examined the reactivity of sera from patients with onchocerciasis [Ov], bancroftian [Wb] and brugian [Bm] filariasis with endothelial/epithelial laminins, merosin, elastin, vitronectin, keratin and collagen types 1-7. Characteristic patterns of antibody [Ab] responses were observed. Ov sera reacted strongly [mean OD = 0.98] with some laminins and one peptide therefrom [laf1, mean OD = 0.75] but not with any other ECM proteins tested. Such Abs were mainly of the IgG2 subclass, increased with age and were more prevalent in areas with intense transmission and severe skin and eye pathology. In contrast, Wb and

Bm sera from patients with elephantiasis reacted strongly [mean OD > 0.4] with laminins, laminin-derived fragments, elastin, collagens type 2 and 4 and type 4 collagen fragments. Bm sera from microfilaremic donors contained high levels of antibodies to elastin and type 4 collagen. Wb sera from patients with hydrocoeles reacted with laminins but not with collagens, whereas sera from patients with chyluria failed to recognize any of the antigens tested. These findings reveal subtle differences in the patterns of host responses to ECM proteins between patients with onchocerciasis and lymphatic filariasis and between groups of patients with different clinical manifestations of chronic filarial disease.

332 SCLEROSING KERATITIS INDUCED BY *ONCHOCERCA VOLVULUS* ANTIGENS CORRELATES WITH PRODUCTION OF TH2-ASSOCIATED CYTOKINES. Pearlman E*, Lass JH, Diaconu E, Hazlett, Jr FE, Bardenstein DS, and Kazura JW. Division of Geographic Medicine, Department of Medicine; and Department of Ophthalmology, Case Western Reserve University, Cleveland, OH.

Onchocerciasis is a major cause of blindness in the developing world. To examine the role of Th-associated cytokines in corneal pathology due to this helminth, we utilized a murine model in which BALB/c mice are sensitized to soluble *O. volvulus* antigens (OvAg) by four weekly sc immunizations and injected intrastromally with 10μg OvAg one week later. Severity of corneal pathology was scored 0-3 for stromal keratitis and neovascularization. The mean scores for OvAg-immunized animals were 1.7 and 1.8, respectively. No pathology developed in either unimmunized BALB/c mice or immunized, T cell deficient (nu/nu) mice. Keratitis and neovascularizaton induced by OvAg was significantly greater than that induced by similar treatment with the mycobacterial antigen PPD (keratitis and neovascularization scores were 0.3 and 0.4, respectively, $p<0.01$). Stimulation of inguinal lymph node cells (LNC) from OvAg- or PPD-sensitized mice with homologous antigen resulted in equivalent degrees of proliferation. In contrast, LNC from OvAg-sensitized mice showed a Th2-like cytokine profile (10ng/ml IL-4 ; 4.2ng/ml IL-5; <0.05ng/ml IFN-γ), whereas PPD-immunization stimulated a Th1-like cytokine pattern (16ng/ml of IFN-γ; 0.8ng/ml IL-4 ; 0.5ng/ml IL-5). Histological examination of corneas from these mice demonstrated the presence of large numbers of eosinophils in OvAg-, but not PPD-treated animals. Taken together, these data implicate Th2 cytokines and eosinophils in the pathogenesis of *O. volvulus* - mediated corneal disease.

333 REGULATORY CYTOKINES IN THE LYMPHATIC PATHOLOGY OF ATHYMIC MICE INFECTED WITH *BRUGIA MALAYI*. Rao UR*, Zometa CS, Vickery AC, Kwa BH, Nayar JK, and Sutton ET. College of Public Health; College of Medicine, University of South Florida, Tampa, FL; and Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL.

Athymic nude mice parasitized by *Brugia malayi* develop massively dilated lymphatics. Histological examination of lymphatics has shown the presence of numerous mononuclear and giant cells in the lymphatic lumen. Endothelial cells are hyperplastic with microvillar processes on the lumenal border. The low opening pressure of dilated lymphatics suggests that multiplication of these cells may be important. Scanning electron microscopy reveals a perturbed endothelium with closely apposed mononuclear cells. Because cytokines, including those produced by mononuclear cells, are known to influence the proliferation and metabolic activity of endothelial cells, the levels of IL-1, IL-4, IL-6 and TNF were measured, by EIA, in the lymph from parasitized dilated lymphatics. We found that levels of IL-1 (10-1740 pg/ml) were elevated compared to 6 pg/ml in unparasitized mouse serum. Similarly, IL-6 (10-10000 pg/ml) and TNF levels were increased compared to serum from parasitized and unparasitized mice. Levels of IL-4 in lymph were low and comparable to those of mouse serum (7-12 pg/ml). The MTT reduction assay was used to quantify possible direct mitogenic effects of adult worms on human umbilical vein endothelial cells. Results showed that proliferation of endothelial cells was significantly ($P<0.01$-0.001) suppressed by 40-47%. Thus it appears that adult worms exert

their effects upon endothelial cells through a network of cytokines possibly produced by numerous mononuclear cells or giant cells elicited by the parasite.

334 LOCAL TNF, PGE-2 AND HISTAMINE PRODUCTION DURING LIMB EDEMA IN *BRUGIA PAHANGI* INFECTED DOGS. Orton S, Schreuer D, and Hammerberg B*. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

The natural occurring lymphatic filarial parasite of dogs, *Brugia pahangi*, causes clinical manifestations of limb pathology similar to lymphatic filariasis in people. Just as in man there is an apparent familial predisposition to severe limb edema in dogs and selective breeding has resulted in litters where 80% of the offspring manifest acute reversible limb edema beginning at about 8 weeks pi compared to about 10-20% of offspring from randomly bred dogs. Site-of-infection lymph node cells were taken from randomly and selectively bred dogs by biopsies at 4, 8, and 12 weeks pi and cultured for the collection of supernatants. These supernatants were assayed by bioassay or immunoassay for the presence of TNF-α, PGE-2 and histamine. Among 5 offspring of a random bred pair of dogs one developed limb edema that correlated in time with elevated TNF and very high levels of PGE-2. Three of 4 offspring of a selectively bred pair showed limb edema in response to infection, but all 4 dogs had node cells that produced elevated levels of TNF, PGE-2 and histamine. The sampling of node cells at the site of infection before and during limb edema formation permits the monitoring of potential pathogenic cytokine production during disease manifestation. In the results presented here TNF-α is an apparent important contributor to limb edema in lymphatic filariasis.

335 UP-REGULATION OF ENDOTHELIAL CELL ICAM-1 AND E-SELECTIN EXPRESSION BY LOCAL CYTOKINES IN PATIENTS WITH LYMPHATIC FILARIASIS. Freedman DO*, Parker SB, De Almeida AB, Maciel MA, Braga C, Silva MC, and Furtado AF. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL; and Centro des Pesquisas Aggeu Magalhaes, Recife, Pernambuco, Brazil.

We have previously shown endothelial cells to be potentially important immunologic effectors in the localized host inflammatory response to lymphatic-dwelling filarial parasites. Using a microplate based endothelial cell ELISA, the present study assessed the ability of 5-day supernatants [SN] generated from filarial antigen-driven PBMC of patients with *Wuchereria bancrofti* infection to upregulate surface adhesion receptors of the selectin and immunoglobulin superfamilies on lymphatic endothelial cells. When compared to SN from 16 subjects with asymptomatic microfilaremia [MF], filarial ag driven SN from 22 subjects with lymphatic pathology [LP] stimulated significantly ($p<.005$; Mann-Whitney U) more ICAM-1 expression (LP=1.632 Specific OD units[SODU] vs MF=0.982 SODU) on the endothelial cells. Additionally, SN from LP's stimulated significantly ($p<.04$) more surface E-selectin expression when compared to MF's (LP=0.868 SODU vs MF=0.612 SODU). In contrast, stimulation of VCAM expression was not significantly different between the two clinical groups (LP=0.349 SODU vs MF=0.297 SODU). For each adhesion receptor tested, unstimulated control SN run in parallel for each subject showed no difference between LP's and MF's in baseline response. A complex relationship between adhesion receptor expression and SN content of IL-1,IL-4, γ-IFN, and TNF-α was found. The identification of adhesion receptors and ligands that are potential targets for immuno-therapeutic blockade with monoclonal antibodies, soluble receptor analogues, or blocking peptides will open possible new approaches to disease control.

336 INDUCTION OF A *SCHISTOSOMA MANSONI* SERPIN GENE FOLLOWING TRANSFORMATION. Ogundipe JO*, Aman RA, and Blanton RE. Division of Geographic Medicine, Department of Medicine, Case Western Reserve Universitiy, Cleveland OH; and National Museums of Kenya, Institute of Primate Research, Karen, Nairobi, Kenya.

To understand the function of a schistosome serine protease inhibitor (serpin) as it relates to host parasite interaction, an *Schistosoma mansoni* (SM) cDNA library constructed in λ gt 11 was screened with a full length *S. haematobium* (SH) serpin cDNA. Nucleotide sequence analysis of a 1.6 kb cDNA revealed significant sequence similarity only with members of the serpin gene family. Further analysis also showed approximately 73% identity with an SH serpin gene at the 3' end, but abrupt divergence at the 5' end. The putative reactive center of the clone contains Ile as the P1 amino acid. This contrasts with the SH serpin's Phe at its reactive center. This difference suggests that the 2 gene products must also differ significantly in function. Except for heat shock proteins, the levels of most messages do not change during transformation of cercariae to schistosomula. Northern blot hybridization, however, revealed that the message corresponding to the SM serpin is nearly undetectable in cercariae, but is present as a 1.7 kb mRNA, in schistosomula by at least 8 hrs after transformation and is abundant in adult worms. There was cross hybridization to SH adult worm mRNA, but at <1/10th intensity. The timing of transcription indicates that the gene product is at its higest level during the period after the parasite enters the human host. These results coupled with previous studies on inhibition of activation of Hageman factor by crude schistosome lysates and the schistosome's resistance to complement killing 24 hours following trasformation are all consistant with a role for the schistsome serpin in host parasite interaction.

337 THE *SCHISTOSOMA HAEMATOBIUM* SERINE PROTEASE INHIBITOR AT THE HOST-PARASITE INTERFACE. Blanton RE*, Fujioka H, Maeno Y, and Aikawa M. Case Western Reserve University, Department of Medicine and Department of Pathology, Cleveland, OH.

The sequence of a cDNA clone encoding an *Schistosoma haematobium*-specific antigen showed it to be a member the serine protease inhibitor gene family (SHSPI). The target of the gene product is not known, but identifying the location of this product might indicate whether this gene is involved in host-parasite interaction and/or regulation of an endogenous parasite protease. By 2 different methods the SHSPI was shown to be localized and concentrated on the surface of adult worms. Adult worms were fractionated by ultracentrifugation into soluble cytoplasmic and insoluble membrane fractions. On Western blot, the detergent-extracted membrane fraction showed more intense binding of rabbit anti-recombinant SHSPI antibodies than the soluble fraction. To further define whether this reaction was from internal or external membranes, immunofluorescence and immunoelectron microscopy were performed on several developmental stages. Most cercariae showed faint immunofluorescence on the surface and an intense reaction at the tail-body junction or at the poles. Both male and female adults, however, demonstrated very bright fluorescence over their entire surface. These findings were confirmed by immunoelectron microscopy that also showed a lower concentration of the SHSPI located in the body of cercariae and adults. We have been able to demonstrate that a gene product from a member of the serine protease inhibitor gene family is also localized on the tegument of adult worms. Thus the SHSPI is in a position to regulate some host responses such as clotting or complement activation. Tegument is perhaps the parasite's most vital organ as it mediates so many of aspects of parasite survival.

338 A RECOMBINANT *SCHISTOSOMA HAEMATOBIUM*-SPECIFIC ANTIGEN IS PREFERENTIALLY RECOGNIZED BY IgE AND IgG4 IN PATIENTS WITH URINARY SCHISTOSOMIASIS. Li Z*, King CL, Ogundipe JO, and Blanton RE. Case Western Reserve University, Division of Geographic Medicine, Department of Medicine, Cleveland, OH.

Parasite-specific IgE levels are an important predictor of reinfection rates with *Schistosoma haematobium* (SH). Specific antigens responsible for this allergenic response, however, have not previously been identified. A recombinant SH-specific antigen was found to be a surface-associated serine protease inhibitor (serpin). Analysis of this recombinant protein showed that the serpin is associated with elevated IgE and IgG4 responses in SH infections. Sera from 41 Kenya patients with chronic SH infection, 18 patients with *S. mansoni* (SM) infection, 14 patients with *S. japonicum*

infection, 7 cases with other parasitic diseases and 17 healthy individuals were studied by ELISA for IgG, IgG4 and IgE responses. The ELISA positive rates for SH serpin specific IgG, IgG4 and IgE in SH patients were 93% (38/41), 95% (39/41) and 97% (36/37), respectively. In comparison with control groups, the specificity of anti-SH serpin IgG, IgG4 and IgE were 92%, 100% and 95%, respectively. The seropositivity rates were not related to the intensity of infection or the age of the patients. Western blot assay confirmed the results obtained by ELISA, but required the use a much lower dilution of sera. Serpin- specific antibody responses to therapy were measured in 1 patient. Two months after treatment, IgG4 levels fell by 50%, while IgE levels rose by 24 fold. These results suggest that recombinant SH serpin may be a valuable antigen in serodiagnosis and management of urinary schistosomiasis, especially in the areas where both SH and SM are prevalent. Furthermore, the recombinant SH serpin represents one of the antigens responsible for the allergenic responses of typical of protected individuals.

339 EXPRESSION AND PURIFICATION OF *SCHISTOSOMA MANSONI* CANDIDATE VACCINE SURFACE ANTIGEN GP22. Suri P*, Madikizela M, Lee J, Goldberg M, McCray JW, and Knopf PM. Division of Biology and Medicine, Brown University, Providence, RI; and Department of Biology, Morehouse College, Atlanta, GA.

Studies from our laboratory have previously reported on cloning and characterization of a *Schistosoma mansoni* gene, GP22, that encodes for a family of candidate vaccine antigens 18-22 kDa. In order to purify large quantities of GP22 gene products to test their efficacy in vaccination trials, a segment of GP22 (140 amino acids, codons 43-182) was generated by PCR, and cloned into the NdeI/Bam HI sites of pET 15b expression vector system (Novagen). The recombinant protein contains a polyhistidine tag at the amino end to facilitate the purification of induced protein by affinity chromatography using Sepharose 6B charged with nickel ions, and a thrombin cleavage site to eliminate the vector sequences. The recombinant product is inducible, and can be detected by Western immunoblots using monospecific antibodies prepared in rabbits against two different GP22 B-cell peptide epitopes called α and δ. Target protein accumulates mainly as inclusion bodies and was purified from induced cultures under denaturing conditions. The purified recombinant protein migrates on SDS PAGE electrophoresis both as two species of about 30 and 60 kDa, respectively. Currently we are purifying large amounts of this protein to use in vaccination trials.

340 CONSTRUCTION OF A VACCINE AGAINST *SCHISTOSOMA MANSONI* UTILIZING A TANDEMLY-REPEATED PEPTIDE DEMONSTRATING CROSS REACTIVITY WITH THREE PROTECTIVE WORM ANTIGENS. Petzke MM*, McCray, Jr. JW, and Knopf PM. Division of Biology and Medicine, Brown University, Providence, RI

The genomic DNA GP22 encodes a family of 18-22 kDa *Schistosoma mansoni* surface glycoprotein antigens which are uniquely recognized by protective rat antiserum. Sequence analysis of this clone has predicted the presence of four B-cell epitopes (α, β, γ and δ) and a putative T-cell epitope. Monospecific antibodies against a synthetic peptide of the δ epitope cross react with defined schistosome antigens Sm97, Sm28 and Sj26 using ELISA and Western blot analyses. We have isolated a 141-bp EcoR1 restriction fragment of GP22 which encodes 47 amino acids encompassing both the δ epitope and the adjacent putative T-cell epitope. Cloning of this fragment, and multimeric constructs of 2, 4 and 8 tandemly repeated fragments, in the pMAL-c2 expression vector has yielded monomeric, dimeric, tetrameric and octameric subunit proteins linked to a maltose binding protein (MBP) carrier. Recognition of the fusion proteins by anti-δ was demonstrated by Western blot. Rats vaccinated with 50 μg of the purified dimer-MBP protein (D7) produced antibodies which reacted on an ELISA with peptide δ and D7, but not with peptide α. Protection experiments, as well as vaccine trials using the various constructs with or without the MBP carrier, are currently being planned.

341 CHARACTERIZATION OF A *SCHISTOSOMA MANSONI* cDNA CLONE THAT ENCODES PHOSPHOGLYCERATE KINASE, A POTENTIAL VACCINE CANDIDATE. Lee K, Karim A, Shalaby K, and LoVerde P*. Department of Microbiology, State University of New York, Buffalo, NY; and Department of Biochemistry, Ain Shams University, Cairo, Egypt.

Well characterized sera which has conferred passive immunity has identified several cDNA clones upon immunoscreening λ gt11 cDNA libraries. One of the cDNA clones with an insert of 1.3Kb encodes a deduced amino acid sequence that has a high homology to phosphoglycerate kinase (PGK), an enzyme involved in ATP production in the glycolytic pathway. A rescreen of the λgt11 cDNA library resulted in a 1.5Kb cDNA clone that contains the entire coding region of PGK. The coding region was overexpressed in pGEX-2T and the recombinant PGK- *Schistosoma japonicum* glutathione S-transferase (Sj26) fusion protein was purified by glutathione agarose beads and subsequently cleaved with a site specific protease to remove the Sj26 protein. The 45 kDa PGK was found to be enzymatically active and used to immunize mice. The anti-PGK polyclonal antibodies were then purified over a column containing PGK coupled through activated CNBr to sepharose. A western blot using the affinity purified antibodies identified a 45KDa band (native PGK?) in a NP-40 adult worm extract. Ten monoclonal antibodies were produced and will be used in immunofluorescence and immunocytochemical localization studies to identify the cellular location of the antigen in the parasite. Presently, vaccination studies are underway to assess the protective potential of PGK.

342 IMMUNOLOGICAL CHARACTERIZATION OF LEUCINE AMINOPEPITIDASE FROM SCHISTOSOME EGGS. Kastens WA*, Secor WE, and Harn DA. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The granulomatous response to schistosome eggs in host tissues has been shown to be mostly directed against egg glycoproteins. One such glycoprotein, leucine aminopeptidase (sLAP), was purified from the eggs of *Schistosoma mansoni* by detergent extraction of the ultracentrifuge pellet of egg homogenates, FPLC chromatofocussing and electroelution from the polyacrylamide gel. The purified sLAP was found to be strongly bound by acute, chronic and vaccinated mouse sera. sLAP is also recognized by three monoclonal antibodies, each of which recognize distinct oligosaccharide epitopes of *in vitro* lymphoproliferation of spleen cells from acutely infected mice. The cellular immune response to deglycosylated versus native sLAP was further characterized *in vitro* in both acutely and chronically infected mice. The results suggest that sLAP may be one of the egg glycoproteins that stimulate granuloma formation and that differential responses to carbohydrate versus peptide determinants may be involved in immunomodulation during the chronic phase.

343 PARASITE CYTOCHROME C IS IMMUNOGENIC IN FASCIOLIASIS. Del Valle AI* and Hillyer GV. Laboratory of Parasite Immunology and Pathology, Department of Pathology, University of Puerto Rico School of Medicine. San Juan. PR.

Cytochrome C (Cyt C) is a water-soluble heme protein essential for phosphorylative oxidation in organisms ranging from microscopic eukaryotes to mammals. While it has been used extensively to establish evolutionary relationships between species, its immunogenicity is still a topic for discussion. Antibodies from patients infected with *Fasciola hepatica* (Fh) have been shown to recognize Fh adult worm antigens in the molecular weight range of the Cyt C (ca. 13 kDa). An anti-Fh whole worm extract antiserum cross-reacted with rabbit heart Cyt C. Studies were designed to determine production of anti-Cyt C antibodies in Fh infected rabbits. The fluke Cyt C was isolated from adult worms by a combination of column chromatography using Sephadex G-50 and isoelectric focusing using a Rotofor cell with a 5-7 pH ampholyte range. Spectrophotometric analysis of the fluke cytochrome demonstrated the heme protein's distinctive pattern: the Soret γ band at ~410 nm and the

β and α bands in the 500-600 nm region. Monospecific antisera against the Fh Cyt C were developed in mice and a rabbit. The immunoreactivity of the molecule was compared to that of mammalian and fungal species by FAST-ELISA and Western Blot analysis. Antisera obtained from a rabbit infected with Fh recognized a *Saccharomyces cerevisiae* Cyt C by two weeks after infection and still had high absorbance values by 4 weeks of infection. The antibody levels to Cyt C rose in parallel with antibodies to worm extracts. These results clearly establish the immunogenic potential of Fh Cyt C. Its implications in physiology and, perhaps, immune mechanisms remain to be elucidated.

344 A HUMAN SERUM FACTOR ACTIVATES PHOSPHORYLATION OF PUTATIVE SURFACE RECEPTORS IN *SCHISTOSOMA MANSONI*. Wiest PM* and Brautigan DL. International Health Institute, Brown University, Providence, RI; and Division of Biology and Medicine, Brown University, Providence, RI.

Activation of receptor kinases by extracellular ligands leads to receptor and substrate phosphorylation. To identify cell surface receptors in the multicellular parasite *Schistosoma mansoni*, detergent-soluble adult worm proteins were immobilized on wheat-germ agglutinin (WGA)-Sepharose and incubated with [^{32}]P-ATP with and without heat-inactivated human serum. Autophosphorylation of proteins, a characteristic of receptor kinases, was analyzed by 7.5% SDS-PAGE and autoradiography. Serum-dependent phosphorylation was detected in two proteins (74 and 78 kDa) in the parasite's membrane glycoproteins. Phosphorylation was time dependent with maximal phosphorylation achieved by 20 min at 23°C. Human serum also induced autophosphorylation of these two glycoproteins in tegumental extracts (0.2% Triton X-100) from intact worms consistent with their surface localization. Genistein (100 μM), a tyrosine kinase inhibitor, did not block phosphorylation suggesting that these receptor kinases phosphorylate serine/threonine residues. The size of the human serum factor as determined by gel filtration using Fast Performance Liquid Chromatography (SW 300) was 70 to 100 kDa. These data suggest that a high molecular weight human serum factor stimulates autophosphorylation of serine/threonine receptor kinases(s) in *S. mansoni*. These receptors may represent new vaccine targets against schistosomiasis.

345 HUMAN STRONGYLOIDIASIS: AN AUTOPSY STUDY WITH QUANTITATIVE PARASITOLOGY. Haque AK, Schnadig V, Rubin SA, and Smith JH*. Department of Pathology, University of Texas Medical Branch, Galveston, TX; and Department of Radiology, University of Texas Medical Branch, Galveston, TX.

Complete autopsies on seven patients with premortem or post mortem diagnoses of *Strongyloides stercoralis* infection were retrospectively reviewed and numbers of each state of parasites / area of histologic section determined. While the series is small, these data suggest the following pathobiologic features of human strongyloidiasis: 1.) Filariform larvae principally use the lymphatic systems and lymphohematogenous dissemination (intestinal and mesenteric lymphangioles, cisterna chyli, thoracic duct and finally left subclavian vein) to migrate from the intestine to the lungs; 2.) Oviposition is principally concentrated in the proximal jejunum, rather than the duodenum; 3.) Intestinal invasion by filariform larvae is most intense in the distal ileum and right colon; 4.) The finding of adult females, eggs and rhabditiform larvae in sputum and histologic sections of lung indicates a bronchial site of propagation, and 5.) This bronchial site of replication may be more resistant to chemotherapy than the enteric focus. A protocol for prospective study of human strongyloidiasis is proposed.

346 IVERMECTIN VS ALBENDAZOLE IN THE TREATMENT OF STRONGYLOIDIASIS IN ITALIAN PATIENTS. Scaglia M*, Gatti S, Bruno A, Bernuzzi AM, Cevini C, and Gaxotte P. Lab. Clinical Parasitology, Inst.Infectious Diseases, University-IRCCS S. Matteo, Pavia, Italy; and MSD-Chibret, Paris, France.

This study was undertaken to evaluate and compare the efficacy of the oral treatment of uncomplicated strongyloidiasis with ivermectin (IV) vs albendazole (AL). One hundred patients (pts) (57 males, 43 females; mean age: 65.6 yrs, range: 3-94 yrs), affected by *Strongyloides stercoralis* infection, were enrolled in an open trial. The drug schedules were: 400 mg of AL for 3 days (47 pts) and 200 μg/Kg of IV in a single dose (53 pts). Routine parasitological follow-up (coproparasitological exam of fixed stool samples; eosinophil count) was undertaken at 1 and 3 months after therapy. Fifteen pts were excluded (5 AL; 10 IV) from the protocol (10 were lost at follow-up, 5 died during the trial). Of 43 pts treated with IV, 42 (97.7%) were cured; a 2nd treatment with IV, administered to the pt not sterilized, cleared up the infection. Of 42 pts treated with AL, 37 (88.1%) were cured; the remaining 5 pts re-treated with IV showed resolution of the infection. The eosinophil count decreased after therapy (range: 0-46%, mean: 12.8%). Side effects were negligible in both groups. In conclusion, IV treatment was significantly more effective than AL in uncomplicated strongyloidiasis. IV in single oral dose was highly effective and well tolerated in our series of pts. The relationship of dose to efficacy indicated an advantage in comparison with other antihelminthic drugs administered in repeated doses.

347 INTRAOCULAR INFECTION WITH *ALARIA* SP. MESOCERCARIAE: TWO CASES FROM CALIFORNIA]. McDonald HR, Kazacos KR*, Schatz H, and Johnson RN. One Daniel Burnham Court, Suite 210C, San Francisco, CA; and Department of Veterinary Pathobiology, Purdue University, West Lafayette, IN.

Two unrelated Chinese males, 35 and 38 years old, presented for ophthalmologic examination because of unilateral decreased vision. Patient 1 had meandering pigmentary tracks in the retina, healed and active areas of retinitis and a yellow, circular intraretinal mass. Six days later the yellow mass was extending, retracting and bending and was immediately photocoagulated with laser. Morphometric analysis of fundus photographs indicated an approximately 500 by 190 μm worm, identified as a trematode and most likely an *Alaria* sp. mesocercaria based on its size, shape and movement. Patient 2 had vitritis, vasculitis, multiple focal tracks of posterior retinitis, an edematous macula and slightly inflamed optic nerve. His ocular inflammation fluctuated over 11 months, and he developed retinal atrophy, pigmentary clumping, more severe vasculitis, and an epiretinal membrane involving the macula. No etiology was determined and ocular steroids controlled the inflammation. Subsequent examination 21 months after he was first seen revealed a motile, approximately 500 μm parasite encapsulated in the anterior vitreous. The encapsulated worm was removed by vitrectomy surgery and fixed in hot AFA fixative. The worm was stained, mounted, and identified as an *Alaria* sp. mesocercaria, 555 by 190 μm in size. Both patients periodically ate various undercooked or raw oriental dishes, including snails, sushi, octopus, duck and, most significantly, frogs legs, which were considered to be the likely source of infection.

348 *BAYLISASCARIS PROCYONIS* CAUSING NEURAL LARVA MIGRANS IN A CHILD AND DUSN/OCULAR LARVA MIGRANS IN A MAN. Kazacos KR*, Cunningham CK, McMillan JA, Weiner LB, Goldberg MA, Katz B, Boyce WM, and Wozniak EJ. Department Veterinary Pathobiology, Purdue Univ, W. Lafayette, IN; Department Pediatrics, SUNY Health Science Center, Syracuse, NY; Department Neuro-Ophthalmology, California Pacific Med Center, San Francisco, CA; and Department Veterinary Microbiology & Immunology, University of California, Davis, CA.

Larvae of the raccoon ascarid, *Baylisascaris procyonis*, were identified as causing meningoencephalitis in a 13-month-old boy in upstate New York and ocular disease in a 29-year-old man in northern California. The child was admitted with torticollis, right sided gaze preference, nuchal rigidity, inability to sit or stand, and hepatomegaly. He had a leukocytosis, 39% eosinophilia and the CSF had 125 WBC per mm^3 with 60% eosinophils. The patient stabilized following aggressive steroid therapy, but with persistent cortical blindness, right sided hemiparesis and progressive cerebral atrophy.

Thiabendazole and ivermectin therapy were considered equivocal. The California man presented with a history of transient obscurations of vision in the right eye and was diagnosed as having diffuse unilateral subacute neuroretinitis (DUSN). Six weeks later, a large nematode larva was seen in the retina and destroyed by laser photocoagulation. The parasite measured 1,727 by 67 μm and was most compatible with a larva of *B. procyonis*. Both patients were seronegative for *Toxocara* and other parasites and seropositive for *Baylisascaris* by ELISA and/or Western blotting. Infection of the child was linked to the ingestion of soil containing infective *B. procyonis* eggs, near a large raccoon latrine on the family's farm. The man had considerable exposure to raccoons frequenting his rural home, and 8/12 raccoons from the area were positive for *B. procyonis*.

349 CANINE HOOKWORMS IN THE HUMAN GUT. Prociv P*, Croese J, Loukas A, Opdebeeck J, and Fairley S. Department of Parsitology, The University of Queensland, Brisbane, Australia; and Townsville General Hospital, Queensland, Australia.

We have recently found that human eosinophilic enteritis can result from infection with *Ancylostoma caninum*, a common hookworm of dogs. However, not everybody who harbors the parasites develops the disease. We here describe pertinent clinical and laboratory findings in 9 cases, collected over 8 years, from 2 cities with a combined population less than 2 million in the state of Queensland, Australia. Five were diagnosed in a single gastro-enterological practice in Townsville; 3 were referred by surgeons form major hospitals. Three patients were diagnosed during the initial 6 years and 6 presented in the last 2 years. All described potential exposure to infective hookworm larvae. Three had laparotomy for acute abdominal pain, and 6 were colonoscoped, 5 with pain and 1 without symptoms. Six had blood eosinophilia and 5/8 had elevated IgE levels; 6/8 had eosinophilic inflammation of the gut. Only a single hookworm was found in each case, identified as *A. caninum* in 6; in 3, damage to the specimen precluded specific identification, but no patient has visited an area endemic for human hookworms. While all worms were adults, non was sexually mature. Each of the 8 cases tested by ELISA and Western blot showed specific antibody (IgG and/or IgE) responses to the secretions of *A. caninum*. Given its wide distribution, human enteric infections with the parasite are likely to be common but unrecognized. The chief symptom is abdominal pain, sometime acute, but infection may be asymptomatic. The pathology in some cases suggests an underlying type 1 hypersensitivity to secretory antigens.

350 SNAKE BITES BY TAIPANS IN PAPUA NEW GUINEA: SEVERE NEUROTOXICITY AND HAEMOSTATIC DYSFUNCTION; LIMITED EFFICACY OF SPECIFIC ANTIVENOM. Warrell DA*, Lalloo DG, Trevett AJ, Black J, Paul M, Naraqi S, Hutton RA, and Theakston RD. Centre for Tropical Medicine, University of Oxford, UK; University of Papua New Guinea; Royal Free Hospital, London, UK; and Liverpool School of Tropical Medicine, Liverpool, UK.

Taipans (genus *Oxyuranus*) possess the most lethal of all snake venoms. In Australia their bites were almost invariably fatal before the advent of antivenom. Venom components include a presynaptic phospholipase A_2 neurotoxin (taipoxin), a complex calcium channel blocker (taicatoxin) and a prothrombin activator. In Port Moresby General Hospital, Papua New Guinea, we studied 171 patients with proven bites by taipans (*Oxyuranus scutellatus canni*) of whom 135 had systemic envenoming. In 101 patients blood was incoagulable on admission and haemostatic assays were consistent with the activity of the venom procoagulant. Ten patients were thrombocytopenic and 77 showed spontaneous systemic bleeding. Antivenom restored coagulability in almost all patients within 12 hours. ECG abnormalities (transient septal T-wave changes) were found in 29 patients and renal dysfunction in three. 114 developed progressive neurotoxicity; ptosis, external opthalmoplegia, bulbar palsy and respiratory paralysis which required ventilatory support in 50. Unlike patients envenomed by death adders (*Acanthophis* sp) the taipan victims showed no response to anticholinesterases or specfic antivenom. 25 patients developed neurotoxicity after being given antivenom and 48 deteriorated despite this treatment. However, all but 6 patients eventually made a

complete recovery after being ventilatated for 7 to 128 hours We are now exploring the use of ancillary pharmacological agents such as 3,4 diaminopyridine and improved antivenoms to prevent or delay life-threatening neurotoxicity.

351 PATTERN OF ANTI-HEV BY ELISA IN AN EPIDEMIC OF HEPATITIS IN PAKISTAN. Bryan JP*, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH, and Legters LJ. Department of Preventive Medicine, Uniformed Services University of the Health Science, Bethesda, MD; Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD; and Pakistan U.S. Lab for Seroepidemiology, Rawal, Pakistan.

In the spring of 1987, 131 people were admitted to a hospital in Sargodha, Pakistan with non-A, non-B hepatitis. Hepatitis E virus (HEV) was detected previously by immune electron microscopy (IEM) in 10 of these patients. An ELISA employing an antigen expressed in baculovirus from ORF-2 of hepatitis E virus was used to determine the pattern of IgM and IgG anti-HEV in sera collected from hospitalized patients or their contacts on March 20, 1987, and 1 and 20 months later. 122 cases (92%) were confirmed as HEV: IgM anti-HEV was detected in 119 sera obtained before and during hospitalization; two others developed IgG anti-HEV; and in another, HEV was detected in a fecal specimen. Of the remaining 9 hospitalized persons, 7 had normal hepatic enzymes at the time of sera collection (9-30 days before hospitalization in 6), and 2 had IgG but no IgM anti-HEV. IgM anti-HEV was detected in 20/22 specimens obtained up to 2 weeks before hospitalization and in all 18 sera obtained 5-7 weeks after hospitalization. IgG anti-HEV followed a similar pattern. Peak IgM and IgG anti-HEV titers appeared during weeks 2-4 after hospitalization. Twenty months after hospitalization, IgM anti-HEV was not detected in any of 33 patients, but IgG anti-HEV was found in all. IgG anti-HEV in sera at the beginning of the outbreak appeared to be protective; none of the 30 contacts of patients with hepatitis with IgG but no IgM anti-HEV were hospitalized compared with 8 (33%) of 24 who had no IgG or IgM anti-HEV detected ($p = .002$). This ELISA confirms HEV as the etiology of this outbreak, detects IgM anti-HEV as early as 14 days before hospitalization, and detects IgG anti-HEV for at least 20 months after admission.

352 ACUTE FEBRILE ILLNESS IN SOMALIA DURING OPERATION RESTORE HOPE. Magill AJ* and Smoak BL. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; and Department of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC.

Acute febrile illness (AFI) was a major cause of hospitalization in U.S. forces deployed to Somalia during Operation Restore Hope. Between 25 February and 01 May 1993, all patients (U.S. forces only) with a temperature > 100.5°F were entered into an AFI study. Ninety-two patients were evaluated with a daily clinical and epidemiological history, physical examination, complete blood count, and malaria smears. Serum was obtained on admission (early acute), discharge (late acute), and at least one month after admission (convalescent). All cases were arbitrarily divided into 4 categories: dengue or dengue like illness in 45 of 92 (49%), gastroenteritis in 18 of 92 (20%), malaria in 6 of 92 (7%) and miscellaneous in 23 of 92 (25%). Of the 45 suspected arboviral cases, 10 (22%) were classified as dengue based on a strongly positive IgM antibody-capture assay. The cases were sporadic and occurred throughout Somalia. *Shigella* was isolated in 3 cases of gastroenteritis. The malaria cases (3 [*P. falciparum*], 1[*P. vivax*], 1[*P. malariae*] and 1 undetermined) were characterized by low parasitemias and mild clinical presentations. The miscellaneous category included pharyngitis, sinusitis, pneumonia, abdominal pain, mononucleosis, hidradenitis, cellulitis, heat exhaustion and drug hypersensitivity. There were no deaths and no complications due to malaria or any other febrile illness during the 65 day study period. Clinically suspected arboviral infections were the leading cause of febrile admissions during this study period.

353 THE UTILITY OF MAGGOT THERAPY FOR TREATING CHRONIC WOUNDS. Sherman RA*, Wyle F, Vulpe M, Levsen L, and Castillo L. Veterans Affairs Medical Center, Long Beach, CA; and University of California, Irvine, CA.

During the 1930's and 1940's, Maggot therapy (MT) was used regularly in hundreds of North American hospitals for treating bone and deep tissue infections. In 1990, we began evaluating the utility of MT, using *Phaenicia sericata* (Meigen) larvae to treat various non-healing wounds. MT completely debrided most necrotic wounds within one week, which was more rapid than all other non-surgical methods ($p<0.05$). Wound healing rates were greater in patients treated with MT than in patients receiving only conventional dressings; wound surface areas decreased an average of 22%/week in the former group, compared to surface areas which increased, on average, by 13%/week in the conventionally treated group ($p<0.01$). The wounds of 19 patients were closed surgically; 5 received pre-surgical MT debridement. Eight of the 14 wounds which did not receive pre-surgical MT became infected and/or dehisced post-operatively (57%); none of the 5 wounds which received pre-surgical MT became infected post-operatively. Based on this demonstrated utility of MT as an adjunct in the treatment of multiple soft tissue maladies, we believe that MT may be valuable when the use of special surgical, aseptic, or energy-requiring proceedures may not be feasible.

354 DOES BCG WORK? A META-ANALYSIS OF BCG EFFICACY IN PREVENTION OF TUBERCULOSIS. Colditz GA, Brewer TF, Berkey CS, Wilson ME*, Burdick E, Fineberg HV, and Mosteller F. Harvard School of Public Health, Boston MA; and Harvard Medical School, Boston MA.

The recent rise in tuberculosis incidence in many countries has prompted scrutiny of a range of interventions to prevent and control infection. We performed a meta-analysis to answer three questions: 1) Is BCG effective? 2) If so, how effective is it and how confident are we of the results? 3) What factors are associated with better or worse performance of BCG? An extensive review of the literature yielded 26 studies that met our eligibility criteria. We characterized studies by design and gave each a data validity score based on preselected features that included comparability of control and vaccine groups, intensity of surveillance and completeness of follow-up, and methods used to diagnose tuberculosis. To combine and analyze the findings in these studies, we used random effects approaches that take account of heterogeneity among the studies. We found that BCG gave about 50% protection across many study designs and populations. Protective effect was stronger in studies with more definitive diagnoses of tuberculosis. In prospective studies, about 80% of variance between studies could be explained by two key factors: the rate of tuberculosis in the control population (higher protection in studies with higher rate of tuberculosis in the control arm) and geographic latitude (higher efficacy in studies done further from the equator). These results should aid decision making about tubercuolsis control.

355 MACROPHAGE-MEDIATED KILLING OF MICROSPORIDIA *IN VITRO*. Didier ES* and Aldras AM. Tulane Regional Primate Research Center, Covington, LA.

Microsporidia are obligate intracellular protozoan parasites which are increasingly recognized as opportunistic pathogens in AIDS. Microsporidia are capable of surviving and replicating in macrophages due to an absence of lysosomal fusion. In this study, we demonstrate that macrophages can be activated with lipopolysaccharide (LPS) and interferon-γ (IFN-γ) to reduce parasite levels. J774.1 murine macrophage-like cells were incubated with LPS (1.0 ng/ml) and IFN-γ (100 μ/ml) for 24 hrs prior to addition of *Encephalitozoon cuniculi*. Parasite recovery decreased by 56.8% in 48 hrs compared to parasite recovery from cells incubated with medium and microsporidia. Rhesus macaque alveolar macrophages incubated with LPS (100 μg/ml) and IFN-γ(100 μ/ml) for 24 hrs prior to addition of *E. hellem*, resulted in a 78.6% decrease in parasite recovery six days later when compared to cultures incubated with medium and parasites. *E. hellem* recovery from the human

macrophage cell line, THP-1, was reduced by 67.8% from cultures incubated with LPS (1.0 ng/ml) and IFN-γ(100 μ/ml) for 24 hrs prior to addition of parasites for 48 hrs compared with medium control cultures. In all cases, parasite recovery increased when the metabolic inhibitor for synthesis of nitric oxides, NG-monomethyl L-arginine (NMMA), was added to the cultures in the presence of LPS and IFN-γ. These results indicate that macrophages incubated with LPS and IFN-γ can reduce microsporidia levels, *in vitro*, and that nitrogen intermediates may be important mediators in microsporidia destruction.

356 HUMAN CYTOTOXIC T-CELL CLONES SPECIFIC FOR *TOXOPLASMA GONDII* LYSE TACHYZOITE-INFECTED TARGET CELLS. Curiel TJ*, Purner MB, Krug EC, Xiong C, and Berens RL. Division of Infectious Disease, University of Colorado Health Sciences Center, Denver, CO; and Paravax, Inc., Ft. Collins, CO.

Infection with *Toxoplasma gondii* (Tg) is a significant cause of morbidity and mortality in the perinatal setting, and in immunocompromised hosts. Animal models suggest that immunity is primarily cellular, with cytotoxic T-cells (CTL) playing a pivotal role. We are investigating human cellular immunity to Tg with the goal of defining elements of protective immunity suitable for a human vaccine. Lymphocytes from Tg+ donors were stimulated with RH strain Tg tachyzoite antigen *in vitro*, and subcloned at limiting dilution. Cloned cells were propagated by stimulation with IL-2, antigen and irradiated feeder cells approximately every 2 weeks. Lysis is assessed in a standard 51Cr release assay. We have derived human CD4+ CTL clones that specifically lyse autologous Tg-infected target cells. Lysis appears to be HLA class II (DR)-restricted, and inhibited by anti-T-cell antibody. Stimulation with RH strain antigen produced CTL that also recognized target cells infected with C strain tachyzoites, suggesting a degree of cross-reactivity. These CD4+ CTL did not inhibit the infectivity of extracellular tachyzoites, as has been reported for some CD8+ CTL. A limited number of CD8+ Tg-specific human CTL have been generated as well, and are being characterized. Analysis of specific immunogenic Tg proteins is in progress. Characterization of effector cells mediating Tg-specific immunity in humans, and identification of immunogenic proteins may eventuallylead to rational design of a successful human anti-Tg vaccine.

357 CHARACTERIZATION AND PURIFICATION OF *TOXOPLASMA GONDII* EXCRETED-SECRETED ANTIGENS. Frachette MJ, Autheman JM, and Rizvi FS*. Department of Parasite Immunology, Pasteur Mérieux Sérums & Vaccins, Marcy l'Etoile, France.

Natural infection of *Toxoplasma gondii* in immunocompetent individuals induces an acquired resistance to reinfection with the parasite. However, antigens involved in eliciting protective immunity have not been completely defined. We have characterized the excreted-secreted antigens (ESA) of *T. gondii* as candidates for vaccination against toxoplasmosis. By using a battery of monoclonal antibodies raised against these molecules, we identified several antigens among which 27, 35, 39, 43, 63 and 78 kDa molecules were common to the tachyzoite (proliferative form) and the bradyzoite (specific form of chronic infection). We also showed the presence of parasite surface molecules (P22, P30, P35 and P43) in excreted-secreted antigens. The comparison of ESA of RH tachyzoites and Ts4 tachyzoites (temperature-sensitive mutant of RH) showed that two molecules of 29 and 78 kDa were specifically released by RH tachyzoites. Some of the ES antigens were purified by monoclonal antibody affinity chromatography or by ion-exchange chromatography and their role in inducing protective immune response was studied.

358 KT5926: AN AGENT WHICH MAY INTERFERE WITH MYOSIN FUNCTION IN *TOXOPLASMA GONDII*. Schwartzman JD* and Wellehan J. Department of Pathology, Dartmouth Medical School, Hanover, NH.

We have investigated the effect on *Toxoplasma gondii* motility and invasion of KT5926, a potent and selective inhibitor of myosin light chain kinase in several eukaryotic systems. We tested the effect of KT5926 on the motility and intracellular development of *Toxoplasma gondii* within human fibroblasts. 1 μM KT5926 incubated for 30 min. prevented active egress of intracellular *T. gondii*, even following stimulation by calcium ionophore A23187, which rapidly stimulated virtually all intracellular *T. gondii* to exit host cells in the control without KT5926. *T. gondii* which had exited host cells before the inhibitor was applied remained motile, however their ability to invade and grow within new host cells was markedly diminished. Intracellular parasites which had been treated with 1-5 μM KT5926 appeared morphologically unaffected but did not multiply for at least 48 hrs. Removal of the inhibitor and overnight incubation of the intracellular parasites restored the ability of the parasites to respond to calcium ionophore by exiting host cells. Obvious host cell toxicity was evident at concentrations greater than 10 μM. When intracellular parasites were treated with KT5926 and then assayed for their ability invade new host cells and to form plaques on fibroblast monolayers, at 1 μM KT5926 there was 90% inhibition of new cell invasion. The parasites maintained normal levels of uracil incorporation over two hours of drug treatment. KT5926 appears to interfere with a step necessary for initiation of motility and also with host cell invasion and intracellular survival. A KT5926-resistant strain of *T. gondii* has been selected for study of the function of myosin in invasion and motility.

359 COMPARISON OF THE RIBOSOMAL DNA OF VIRULENT AND AVIRULENT STRAINS OF *TOXOPLASMA GONDII*. Nimmo KA and Brindley PJ*. Tropical Health Program, Queensland Institute of Medical Research, Brisbane, Queensland, Australia.

Toxoplasma gondii causes serious morbidity or mortality in the developing fetus and in other immunocompromised individuals including AIDS patients, but apparently not all parasite strains induce these catastrophic consequences. Based on RFLPs at three loci, it was recently proposed that virulent strains of *T. gondii* originated from a single lineage which has remained genetically homogeneous despite being globally widespread, as reported previously. In like fashion, we have observed genomic differences between virulent and avirulent strains of *T. gondii* at other loci. In order to investigate this, we compared the gene structure at one locus, the repetitive ribosomal gene (rRNA), in the virulent RH and avirulent ts-4 strains. (ts-4 is a temperature sensitive mutant derived from the virulent RH strain of *T. gondii*. ts-4 grows *in vitro* at 33°C but not higher temperatures. Vaccination of mice with living ts-4 effectively protects them from challenge infection with the virulent RH strain.) Using pairs of consensus oligonucleotide primers, we amplified fragments from genomic DNA which together comprise the entire rDNA locus of *T. gondii*. The ribosomal gene unit is approx. 8 kb in length. Physical mapping and nucleotide sequence analysis of the fragments revealed differences between ts-4 and RH at this locus. In particular, 1) at least 4 separate point mutations distinguish the 18S rRNA genes of ts-4 and RH; 2) RFLPs obtained by hybridizing EcoR1- or BamH1-digested genomic to the PCR amplified 18S likewise distinguish ts-4 from RH, which suggests the presence of deletions or insertions in the intergenic spacers; and 3) differences in methylation are likely to be present. We anticipate that oligonucleotide primers based on these sequence differences will allow discrimination of avirulent (e.g. ts-4, S48) from virulent parasites, including RH and the Brazilian strains OH3 and S11, in experimental PCR-based diagnosis of toxoplasmosis.

360 EFFICACY TRIAL IN CATS WITH EXPERIMENTAL VACCINE CONTAINING MODIFIED LIVE *TOXOPLASMA GONDII* T-263 STRAIN. Choromanski L*, Freyre A, Fishback J, and Popiel I. Miles Inc., Animal Health Products, Shawnee Mission, KS; University of Kansas Medical Center, Kansas City, KS; University of Kansas Medical Center, Kansas City, KS; and Paravax, Inc., Ft. Collins, CO.

Toxoplasma infection has been reported in many species, but members of the Felidae family are fundamental to transmission of *Toxoplasma gondii* because they excrete resistant oocysts in feces. A feline toxoplasma vaccine that reduce the number of *Toxoplasma* oocysts around human habitations would benefit public health. Previous studies have demonstrated that oral administration to cats of tissue cysts of the oocyst negative mutant strain of *T. gondii* T-263 induces protective immunity and these cats do not shed oocyst if they are reinfected. The purpose of the study reported here was to examine and compare different doses of *Toxoplasma* T-263 bradyzoites for the ability to immunize cats. In the first experiment, groups of cats received two doses, 19 days apart, of *Toxoplasma* T-263 tissue cysts or released bradyzoites and were challenged 47 days later with the wild-type oocyst producing strain of *Toxoplasma*. All cats seroconverted after vaccination and none of them shed *Toxoplasma* oocysts following challenge. In a second experiment, groups of cats received two doses, 3 weeks apart, of 10, 100 or 1000 bradyzoites of *Toxoplasma* T-263 and were challenged 42 days later. Following challenge, one group of cats (n=5) vaccinated with 10 nonpurified bradyzoites shed *Toxoplasma* oocysts. The remaining groups of cats were protected against challenge in the range of 75% to 100%. Thus, orally administered vaccine containing *Toxoplasma* T-263 bradyzoites induced immunity to oocyst shedding.

361 DETECTION OF PATHOGENIC PROTOZOA IN FECAL SPECIMENS FROM URBAN DWELLING DOGS. Jafri HS*, Moorhead AR, Reedy T, Dickerson JW, Wahlquist SP, Schantz PM, and Bryan RT. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA.

Microsporidia are an important cause of chronic diarrhea in AIDS patients. *Cryptosporidium* and *Giardia* are common causes of diarrheal illness in both immunocompetent & immunosuppressed persons; both have been linked to waterborne & child day care associated-outbreaks of diarrhea. Although rural reservoirs for *Cryptosporidium* are established, little is known about the role of urban animals as potential reservoirs for most enteric protozoa. We obtained fecal specimens from dogs from an urban animal control center in Fulton Co., GA. Formalin-fixed fecal specimens were examined for microsporidia with Weber's chromotrope-based stain, and for *Cryptosporidium* and *Giardia* with the Merifluor IFA test. Dogs were mostly of mixed breed (strays or former pets) & ranged in age from <6 wks to >2 yrs. Most appeared healthy & free of obvious disease. Spores of microsporidia were detected in 6/20 (30%) specimens; *Cryptosporidium* oocysts & *Giardia* cysts or trophozoites were detected in 5/49 (10.2%) and 13/49 (26.5%) specimens, respectively. Two (4.1%) of 49 dogs were co-infected with *Cryptosporidium* & *Giardia*. Of the 6 with microsporidia, 1 was co-infected with *Giardia*, and 1 with *Cryptosporidium* and *Giardia*. This report is the first to document microsporidia in canine feces. Moreover, the high number of dogs infected with these human pathogens (microsporidia, *Giardia*, and *Cryptosporidium*) suggests a possible role for dogs in disease transmission.

362 BROAD SPECTRUM PCR-BASED STRATEGY FOR DETECTING *BABESIA* AND *THEILERIA* IN TICK AND VERTEBRATE HOSTS. McLaughlin GL*, Gordon VR, Vodkin MH, Ssenyonga GS, Nanteza E, Rubaire-Akiiki R, Wafula O, Hansen RD, and Kakoma I. Purdue University, West Lafayette, IN; University of Illinois, Urbana, IL; and Makerere University, Kampala, Uganda.

Comparative computer-assisted matching analysis of DNA sequences of ribosomal DNA identified conserved regions characteristic of hemotropic Apicomplexa parasites. Synthetic DNA primers selected from these regions were tested and successfully amplified infected blood containing *Babesia bovis, B. bigemina, B. canis, Plasmodium falciparum, P. vivax, P. malariae,* and *P. knowlesi* species, but did not amplify negative controls (uninfected blood and samples containing *Trypanosoma, Leishmania, Pneumocystis* and *Neisseria* DNA). In evaluations using clinical samples in Uganda, processing with Genereleaser (BioVentures, Murfeesboro, TN) or Isoquick (Microprobe Crop, Garden Grove, CA) gave equivalent results. Amplified DNA of the predicted size was obtained from a *B.*

canis blood sample (one parasite/50 thick smear microscopic fields) and from three engorged female ticks, but not from two non-engorged ticks removed from the infected dog. Positive blood samples of *Theileria parva* were also successfully detected from unprocessed thick smears and from methanol-fixed thin smears. In summary, these primers detect apicomplexan parasites in either vertebrate or arthropod hosts and this presents a broad-spectrum efficient strategy to detect and type hemotropic parasites of the phylum Apicomplexa.

363 DIAGNOSIS OF *NEOSPORUM CANINUM* USING RECOMBINANT TACHYZOITE ANTIGEN. Jenkins MC*, Bjerkas I, and Dubey JP. Protozoan Diseases Laboratory, ARS, USDA, Beltsville, MD; Norwegian College of Veterinary Medicine, Oslo, Norway; Zoonotic Diseases Laboratory, ARS, USDA, Beltsville, MD.

In an effort to develop a diagnostic assay for *Neospora caninum*, cDNA encoding antigenic proteins of *N. caninum* tachyzoites were identified and expressed in *Escherichia coli*. The recombinant clones were identified by screening tachyzoite cDNA libraries with polyclonal sera prepared against three different *N. caninum* native proteins. In previous studies, these native antigens were found to react with convalescent sera but not with pre-immune sera from a wide range of *N. caninum*-infected animals. Immunostaining of nitrocellulose paper impregnated with unpurified recombinant *N. caninum* protein distinguished sera from pre-immune and infected animals. A dot-blot assay using purified recombinant *N. caninum* antigen is now being developed to facilitate diagnosis of this parasite under field conditions.

364 POST KALA-AZAR DERMAL LEISHMANIASIS IN THE SUDAN: A POPULATION BASED STUDY. El-Hassan AM, Zijlstra EE*, Meredith SEO, Ismael A, and Ghalib HW. Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Sudan; Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, The Netherlands; NH Swellengrebel Laboratory of Tropical Hygiene, Royal Tropical Institute, Amsterdam, The Netherlands; Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Sudan; and Department of Microbiology, University of Juba, Sudan.

From 1991-1993 the incidence, clinical presentation, methods of diagnosis including the Polymerase Chain Reaction (PCR) and response to therapy of Post Kala-azar Dermal Leishmaniasis (PKDL) were studied at the community level in a cluster of villages in the endemic area of kala-azar in the Eastern Sudan. In one village of 1400 inhabitants, the cumulative incidence of PKDL was 19 per thousand for 1991 and 25 per thousand for 1992. PKDL followed kala-azar in 56% of cases. In the surrounding villages (total estimated population 12,000) another 89 cases were found. PKDL developed within 2 to 4 weeks following kala-azar in most cases; others developed PKDL while still on treatment of kala-azar. In some cases there was no history of previous kala-azar. The clinical presentation can be variable as will be demonstrated with a number of slides. The differential diagnosis will be discussed. Diagnosis was made on clinical appearance, previous history of kala-azar and conversion in DAT and /or Leishmanin skin test. In selected cases, smears or biopsies were taken. Using the PCR on impression smears of skin biopsies, 3 of 4 were positive; results of PCR in biopsies are being analyzed. In 7 of 13 patients PCR was positive on peripheral blood using a drop of blood on filter paper. Those with severe disease (19) were given treatment with 20 mg/kg Pentostam for 15-30 days; 12 were cured after one course of treatment, 7 needed further treatment. 47 cases were considered to have mild to moderate disease and were not treated; 35 cured spontaneously, 12 had prolonged illness some of whom needed treatment later. PKDL is not uncommon in the Sudan if looked for at field level and may cause severe morbidity. It may be an important factor of disease transmission in between outbreaks.

365 PLACENTAL PATHOLOGY OF CONGENITAL CHAGAS DISEASE FROM INFECTED NEONATES IN COCHABAMBA, BOLIVIA. Lora J*, Schwartz DA, Torrico F, Balderrama F, Moore AC, and Bryan RT. Facultad de Medicina-UMSS, Cochabamba, Bolivia; Programma Nacional de Control de la Enfermedad de Chagas, Cochabamba, Bolivia; Emory University, Atlanta, GA; and Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

In Bolivia estimated infection rates with *Trypanosoma cruzi* vary from 11% in urban to 70% in rural areas. About 35% of school-age children are infected with Chagas' disease in endemic areas. Congenital transmission of *T. cruzi* is an important source of infection, but the mechanism and timing of transplacental transmission remain largely unknown. We studied prospectively 130 *T. cruzi*-seropositive women from Cochabamba, Boliva and describe the pathologic features of placentas from 5 neonates with parasitologically-confirmed congenital Chagas disease. Histopathologic abnormalities were examined by routine histochemistry, and immunohistochemistry with monoclonal antibodies was used to identify specific cell types (ex. macrophages) involved in patterns of reaction and injury. Amastigotes were identified from all 5 placentas, with the extraplacental membranes having the greatest parasite burden. Focal villitis, predominantly lymphocytic, was a common feature. Umbilical cord involvement was also present. Increased numbers of immunologically-confirmed macrophages were present in subamnionic connective tissue and Wharton's jelly, which may provide a mechanism for transplacental transmission. The prominent occurrence of amastigotes in umbilical cord mesenchyme and extraplacental membranes is suggestive of amniotic fluid infection. Studies are in progress to characterize the inflammatory cell phenotypes in these placentas.

366 ENDEMIC KALA-AZAR IN THE SUDAN: DOES PREVIOUS EXPOSURE TO *LEISHMANIA MAJOR* PROTECT AGAINST CHALLENGE WITH *LEISHMANIA DONOVANI*? Zijlstra EE*, El-Hassan AM, Ismael A and Ghalib HW. Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, The Netherlands; Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Sudan; and Department of Microbiology, University of Juba, Sudan.

Kala-azar (visceral leishmaniasis) is endemic in the Sudan, but no longitudinal studies on epidemiology have been conducted. A longitudinal study was performed in the village of Um-Salala (1400 inhabitants) in the endemic area of kala-azar (visceral leishmaniasis) in Eastern Sudan. In 1990, 1991, 1992 and 1993, cases of kala-azar were nil, 2, 14 and 18 and of Post Kala-azar Dermal Leishmaniasis (PKDL) 13, 27, 25 and 35, respectively. The ratio clinical:subclinical cases of kala- azar was higher (1.4:1) than usually reported from endemic areas. From April 1991- April 1992 the cumulative incidence of clinical cases including those diagnosed during the survey and those diagnosed in retrospect on history suggestive of kala- azar and seroconversion in the Direct Agglutination Test (DAT), was 50 per thousand, increasing to 80 per thousand when subclinical cases were also included. Most adults in the village are immigrants from Western Sudan from an area where cutaneous leishmaniasis (CL) caused by *Leishmania major* is common and kala-azar has not been reported since decades. The skin test positivity rate for leishmanin in this group was 91%. Kala-azar only occurred in previously skin test negative indi- viduals who were mostly children, born in Um-Salala where CL does not occur. The distribution of kala-azar cases suggests a possible protective effect of previous exposure to CL (*L. major*) after challenge with *L. donovani*, the causative agent of kala-azar in the Sudan. This finding may be important in the view of the killed *L. major* vaccine that is currently being tested in endemic areas of cutaneous leishmaniasis.

367 APPLICATION OF THE PCR TO DETECTION OF *LEISHMANIA* PARASITES IN FINGER PRICK BLOOD SPOTS ON FILTER PAPER. Meredith SE*, Schoons GJ, Kroon N, Zijlstra EE, Van Eyes GJ, and El-Hassan AM. Department of Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands; Department of Infectious Diseases, Tropical Medicine and AIDS,

AMC, Amsterdam, The Netherlands; Department of Molecular Cell Biology, University of Limburg, Maastricht, The Netherlands; and Leishmania Research Group, Institute of Endemic Diseases, University of Khartoum, Khartuom, Sudan.

Detection, diagnosis and identification of Leishmaniasis may be difficult owing to low numbers of parasites present in clinical samples. The PCR has improved the sensitivity and specificity of diagnosis of several infectious diseases. A *Leishmania* specific PCR assay was developed based on the SSU rRNA genes which amplifies DNA of all *Leishmania* species. Point mutations occurring within the rRNA genes allow differentiation of the Leishmania complexes using primers constructed with the 3' ends complementary to the specific point mutations present in the SSU rRNA genes of the *Leishmania* species. Biopsy material, blood, lesion impressions and blood spots on filter paper can be used in the assay. In a longitudinal study on the incidence rates of VL, subclinical cases and PKDL in an endemic region of Sudan, filter paper blood spots from proven and suspected VL patients, PKDL and control samples from an endemic region in Sudan are being taken. The blood spots were analyzed in the Direct Agglutination Test (DAT) and by PCR and results compared with clinical and parasitological data. The first results indicate that the PCR on blood spots is a simple and sensitive means of detecting active VL; in PKDL patients without previous VL parasites are detectable in the skin.

368 DEVELOPMENT AND EVALUATION OF A CLINICAL PREDICTION RULE FOR AMERICAN CUTANEOUS LEISHMANIASIS IN COLOMBIA. Weigle KA*, Escobar MA, Arias AL, Martinez FR, and Rojas C. Department of Epidemiology, University of North Carolina, Chapel Hill, NC; and Fundacion Centro Internacional de Investigaciones Medicas (CIDEIM), Cali, Colombia.

Neither parasitological nor molecular diagnosis of leishmaniasis is widely available in clinical settings where American cutaneous leishmaniasis (ACL) caused by *Leishmania (V) braziliensis* and *L. (V) panamensis* is endemic. Therefore four clinical prediction rules for ACL were developed which incorporated physical exam findings (clinical rule), physical exam and leishmanin skin test (LST) (clinical- LST rule), physical exam and historical information (clinical-historical rule), or physical exam, historical information and LST (clinical-historical-LST rule). One hundred parasitologically diagnosed ACL cases and 38 cases of chronic skin lesions of other etiologies comprised the derivation set. The validation set consisted of 124 ACL cases and 35 patients with lesions of other etiologies. Components of each rule were selected by bivariate analysis, then stepwise logistic regession. Sensitivity, specificity and efficiency were calculated for each score threshold, the threshold with the lowest error rate was selected for each rule. When these rules were applied to the validity set the sensitivity, specificity and efficiency relative to traditional laboratory diagnosis were respectively: clinical 93%, 31%, 79%; clinical-LST 90%, 73%, 85.9%; clinical- historical 97%, 51%, 87%; clinical-historical-LST 92%, 70%, 87%. Inclusion of LST skin test consistently improved the specificity of the rules. Should a given clinical setting warrant optimizing either sensitivity or specificity alone the rule thresholds can be adjusted. These and other prediction rules, once evaluated in other settings, could be incorporated into the surveillance or screening components of leishmaniasis control programs.

369 LACK OF SYNERGY BETWEEN INTERFERON-γ AND GLUCANTIME IN TREATING CUTANEOUS LEISHMANIASIS IN GUATEMALA. Arana BA, Navin TR*, Arana FE, Berman JD, and Rosenkaimer F. Universidad del Valle de Guatemala, Guatemala City, Guatemala; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Walter Reed Army Institute of Research, Washington, DC; and Boehringer Ingelheim, Ingelheim am Rhein, Germany.

Interferon-γ may be beneficial when added to pentavalent antimonials in the treatment of visceral leishmaniasis. To test whether it acts synergistically with antimonials in the treatment of cutaneous leishmaniasis, we conducted a randomized, double-blind clinical trial among 63 Guatemalans with

parasitologically confirmed cutaneous leishmaniasis. The three treatment groups were Glucantime (20 mg antimony per kg body weight i.v.) for 5 days with 5 daily subcutaneous injections of 0.2 mg recombinant interferon-γ; the same regimen of Glucantime without interferon-γ; and the same daily dose of Glucantime for 10 days. All patients completed their prescribed course of therapy. Of 20 patients who received Glucantime plus interferon-γ and have completed 13 weeks of follow-up, 15 (75%) were clinically and parasitologically cured. Of 18 patients who received just Glucantime for 5 days, 15 (83%) were cured, and 19 (90%) of 21 patients who received 10 days of Glucantime have responded clinically and parasitologically by 13 weeks. Although interferon-γ was not synergistic with Glucantime under these conditions, the finding that 90% of patients responded to 10 days of Glucantime suggests that the currently recommended 20-day course may be unnecessary and that shortened courses of antimonials should be tested for cutaneous leishmaniasis in other geographic areas.

370 TREATMENT OF CUTANEOUS LEISHMANIASIS WITH A SHORT COURSE OF PENTAMIDINE. Soto J*, Grogl M, and Berman J. Bogota Military Hospital, Bogota, Colombia; and Walter Reed Army Institute of Research, Washington, DC.

Pentamidine isethionate, 2 mg/kg/every other day (qod) intramuscularly, was evaluated for Colombian cutaneous leishmaniasis. When this dose was administered every other day for 7 injections, 23/24 patients were cured (96%). In comparison, 21/23 patients randomized to the positive control treatment, Glucantime (10 mg antimony BID/kg/day intramuscularly for 20 days), were cured (93%), and 8/22 patients randomized to no treatment (negative controls) were cured (36%). In further experiments, 2 mg pentamidine/kg/qod for only 4 injections cured 32/38 patients (84%). A higher daily dose, 3 mg/kg, qod for only 4 injections cured 49/51 (96%). The toxicity of the three pentamidine regimens with respect to headache, myalgias, abdominal pains, bitter taste, hypotension, and hypoglycemia was:

2 mg/kg/qod for 7 injections:
30% of patients had 3.25 side effects each (4 side effects were severe).
2 mg/kg/qod for 4 injections:
55% of patients had 1.7 side effects (3 moderate).
3 mg/kg/qod for 4 injections:
64% of patients had 1.5 side effects (5 moderate).

We propose that the regimen of 3 mg pentamidine/kg/qod for 4 injections is the optimal pentamidine regimen in terms of efficacy and toxicity for Colombian cutaneous leishmaniasis, and is competitive with standard Glucantime therapy, especially when length of treatment is considered.

371 EFFICACY OF 28-DAY AND 40-DAY REGIMENS OF SODIUM STIBOGLUCONATE (PENTOSTAM) IN THE TREATMENT OF MUCOSAL LEISHMANIASIS. Franke ED*, Llanos A, Echevarria J, Cruz ME, Campos P, Tovar AA, Lucas CM, and Berman JD. Naval Medical Research Institute Detachment, Lima, Peru; "Alexander von Humboldt" Institute of Tropical Medicine, Cayetano Heredia University, Lima, Peru; Hospital Regional del Cusco, Cusco, Peru; and Walter Reed Army Institute of Research, Washington, DC.

The efficacy and toxicity of two regimens of antimony, 28 and 40 days of 20 mg antimony/kg/day, were compared in the treatment of culture-positive mucosal leishmaniasis involving more than one anatomic site. Forty consecutive eligible Peruvian patients with infiltrative or ulcerative mucosal disease of the lips, nose, palate-uvula-pharynx, or larynx-epiglottis were randomized to receive either 28 days (P28) or 40 days (P40) of Pentostam. Treatment was prematurely terminated due to thrombocytopenia in 3 patients and 2 patients did not complete 6 months of follow up. At one month post-treatment, 13% (2/16) of the P28 patients and 16% (3/19) of the P40 patients no longer had

infiltrates or ulcers and were considered initially cured. During a further eleven months of follow-up, infiltrated lesions healed in 8 more P28 patients and in 10 more P40 patients. The cure rate after 12 months of follow-up was therefore 63% for both groups (10/16 in the P28 group and 12/19 in the P40 group). The total of 13 patients who had infiltrates or ulcers at the 9-12 month follow-up were considered failures. All 7 patients (3 in the P28 group and 4 in the P40 group) whose lesions were culture positive for *Leishmania* at some point in the 12 months after treatment, and who were thereby parasitologic failures, were also clinical failures. Since the cure rates did not differ between the two treatment regimens, there is no therapeutic advantage to increasing the length of treatment with Pentostam to 40-days in patients with mucosal leishmaniasis involving more than one anatomic site.

372 OPEN FIELD TRIAL OF SHORT COURSE LIPOSOMAL AMPHOTERICIN B IN COMPLICATED VISCERAL LEISHMANIASIS. Seaman J, Wilkinson R*, Boer C, de Wilde E, Sondorp HE, and Davidson RN. MSF (Holland), Nairobi, Kenya; and Department of Tropical Medicine, St. Mary's Hospital Medical School, Northwick Park Hospital, Harrow, UK.

In late 1988 a Medecins Sans Frontieres (MSF)-Holland team working in Khartoum, Sudan reported a large number of cases of Visceral Leishmaniasis (VL) amongst displaced peoples of the Nuer tribe fleeing the civil war and famine in the Western Upper Nile region of Southern Sudan. Subsequently the epidemic was confirmed by another MSF group working in the field. It has been established that at least 50,000, of a total of less than 500,000, people have died of VL with mortality in some villages exceeding 50%. The mortality untreated is 70%. Two treatment centres have been established at which over 15000 cases have so far been treated. Mortality treated is now less than 10%. Chemotherapeutic management is routinely with 20 mg/kg/day Sodium Stibogluconate (Sb^v) i.m. for 30 days, or Sb^v plus Aminosidine 15 mg/kg/day for 17 days which are equally effective. Some patients however relapse or are unresponsive despite both these regimes. Liposomal Amphotericin B (AmBisome, Vestar, CA, USA) is known to be highly effective and relatively non-toxic in VL. An open field trial of 9-15 mg/kg AmBisome in total as 3 doses on days 0,3, and 10 was therefore instituted. A 'rescue' regime of higher dose AmBisome was included in the protocol. To date (25/5/93) 13 patients have been enrolled into this trial all of whom had drug resistant VL or were at high risk of dying. Of seven patients with complete data to date, 6 have been clinically and parasitologically cured with one death. There has been improvement in the clinical parameters of spleen size, liver Size and haemoglobin. Complete data of this trial and the adverse circumstances in which it has been conducted will be presented.

373 SHORT COURSE LIPOSOMAL AMPHOTERICIN B ('AMBISOME') IN PATIENTS WITH MEDITERRANEAN VISCERAL LEISHMANIASIS. Davidson RN*, DiMartino L, Gradoni L, and Giacchino R. Infection and Tropical Medicine, St Marys Hospital Medical School, Northwick Park Hospital, Harrow United Kingdom; Division Pediatrics, Ospedale Pausilipon, Naples, Italy; Istitute Superiore Sanita, Laboratori di Parasitologica, Rome, Italy; and Department of Infectious Diseases, Istituto Gaslini, Genoa, Italy.

Amphotericin B is a powerful antileishmanial drug. Incorporation into liposomes makes amB less toxic as well as targeting drug to macrophage-rich tissues such as spleen, liver, bone marrow. This makes liposomal amphotericin B (L-AmB) an ideal choice for treatment of visceral leishmaniasis (VL). L-AmB was uniformly successful in the treatment of 20 immunocompetent patients with Mediterranean VL, used at a dose of 1-1.8 mg/kg/d x 21 d or 3 mg/kg/d x 10 d. A series of dose-reducing trials have been conducted to ascertain the minimum dose/duration/hospitalization which L-AmB treatment of VL will allow. 6 children aged 14 mo - 9 yrs and 4 adults received L-AmB 4 mg/kg/d on days 1,2,3,4,5 and 10, a total dose of 24 mg/kg. 12 children aged 7 mo - 15 yrs and 5 adults received L-AmB 3 mg/kg/d on days 1,2,3,4,5 and 10, a total dose of 18 mg/kg. Adverse events were confined to mild transient elevation of BUN/creatinine in some patients. All patients have been

clinically/parasitologically cured, and are under follow up. 3 children and 2 adults have been treated with L-AmB 3 mg/kg/d on days 1,2,3,4 and 10, a total of 15 mg/kg. L-AmB enables short course and outpatient treatment of immunocompetent patients with VL, and has great advantages over current pentavalent antimonial regimens.

374 AN INVESTIGATION OF THE POSSIBLE ROLE OF BACTEREMIA IN CEREBRAL MALARIA IN CHILDREN IN LAGOS, NIGERIA. Alabi SA*, Prada JJ, Ajayi-Obe, Prieto I, Omonigbehin EA, Sodeinde O, Lehman L, and Kremsner PG. National Institute for Medical Research, Lagos, Nigeria; Landesinstitut fur Tropenmedizin, Berlin, Germany; Lagos University Teaching Hospital, Lagos, Nigeria; and Universidad Complutense de Madrid, Spain.

In Nigeria and most developing countries, malaria is a leading cause of childhood mortality and morbidity. It kills one out of every 20 children before the age of 5 years in rural Africa, and claims some one million lives annually (WHO). The high mortality rate is attributed to complications such as cerebral malaria (CM) by *Plasmodium falciparum*. However, factors that may predispose to CM are poorly understood. Between October 1991 and June 1992, we investigated the incidence of bacteremia in children (<10 yrs) with CM at the the Lagos University Teaching Hospital (LUTH), Nigeria. Bacterial isolates were also examined for some virulence properties such as hemolysin production, invasiveness, resistance to antibiotics, and plasmid profiles; at the Inst. for Trop. Med., Berlin and the Univ. of Madrid, Spain. Of 62 children studied, 8 (13%) had bacteremia, 3 with *Ps. aeruginosa*, 2 with *S. typhi*, and 1 each with *E. coli*, *Ser. marcescens*, or *Ent. aglomerans*. Four of the 8 isolates produced hemolysin, 5 had plasmids and were resistant to tetracycline, ampicillin or chloramphenicol. There was no hybridization with the invasive gene probe. Two (25%) of the 8 children with bacteremia died while 11 (20.4%) of the 54 without bacteremia also died. Many of our isolates possessed virulence properties, though there was no significant association between bacteremia and death of children ($P>0.05$) probably due to adequate management of cases. The need to suspect bacteremia in children with severe malaria and in whom fever persists despite correct antimalarial treatment is emphasized.

375 INTRACRANIAL PRESSURE MONITORING IN KENYAN CHILDREN WITH CEREBRAL MALARIA. Newton CR*, Kirkham FJ, Sowume A, Waruiru C, Mwangi I, Murphy S, and Marsh K. Kenya Medical Research Institute, Kilfi Kenya; and Department of Neurosciences, Institute of Child Health, London, UK.

Intracranial hypertension (IH) may produce a poor outcome in African children with cerebral malaria (CM) by reducing cerebral perfusion pressure (CPP) or causing transtentorial herniation. We monitored intracranial pressure (ICP) with a Camino fiberoptic measuring system in 8 children with CM (WHO definition). CT scans of the brain were performed on removal of the catheter. Opening ICP did not predict maximum ICP. All children had ICP>10 mmHg, the maximum ICP was between 13 and 98 mmHg. Two children with a maximum ICP>40 mmHg and minimum CPP<40 mmHg, had severe brain swelling with generalized hypodensity on the CT scans and recovered consciousness with severe sequelae. Of the 3 children with a maximum ICP<40 mmHg and a minimum CPP>40 mmHg, 2 had brain swelling on CT scans and all recovered without neurological sequelae. The other 3 children with maximum ICP<20 mmHg and minimum CPP>60 mmHg had normal scans and did not have any sequelae. Mannitol (0.5g/Kg) reduced ICP in all cases in which it was given, but in the children with severe ICP, it did not control ICP even if given 2-4 hourly. ICP may be raised for prolonged periods in CM, mannitol controls moderate IH, but severe IH appears to be associated with poor outcome despite treatment.

376 MEASUREMENT OF CEREBRAL BLOOD FLOW IN AFRICAN CHILDREN WITH CEREBRAL MALARIA. Marsh K, Newton CR*, Edwards DA, Sowume A, Cope M, and Kirkham FJ. Kenya Medical Research Institute, Kilfi Kenya; Department of Paediatrics & Neonatal Medicine, Royal

Postgraduate Medical School, London, UK; Department of Medical Physics & Bioengineering, University College, London, UK; Department of Neurosciences, Institute of Child Health, London, UK.

Studies of cerebral malaria (CM) have been impeded by the lack of cerebral blood flow (CBF) data. We have used near infrared spectroscopy (NIRS) to measure CBF in Kenyan children with CM. The method uses a small change in arterial oxygen saturation (SaO_2), induced by altering the inspired oxygen fraction to cause an increase in cerebral HbO_2 concentration ($DHbO_2$) which represents an accumulation of the tracer. The quantity of the tracer introduced is given by the time integral of the change in SaO_2 ($DSaO_2$). The concentration of $DHbO_2$ can be quantified using NIRS, and the $DSaO_2$ by pulse oximeter. Thus, provided cerebral oxygen extraction is constant and the measurement is made in less than the cerebral transit time, then from the Fick principle:

$$CBF = K[DHbO_2] / Hb.\int_0^t (DSaO_2)dt$$

where K is a constant that reflects the molecular weight of hemoglobin and cerebral tissue density. 25 measurements of CBF were made in 8 children who fulfilled the WHO criteria for CM. CBF ranged from 15.1-65.0 (median 41.4) ml/100 g brain tissue/min. The highest CBF were measured in 2 children with the worst outcome. These preliminary data suggest that NIRS can be used to provide repeated, non-invasive measurements of CBF in children with CM.

377 ELEVATED TRANSFERRIN SATURATIONS ARE ASSOCIATED WITH DEEP AND PROLONGED COMA IN CHILDREN WITH CEREBRAL MALARIA. Fernandes NF, Thuma PE, Biemba G, Zulu S, Parry D, Brittenham GM, and Gordeuk VR*. Department of Medicine, MetroHealth Medical Center, Case Western Reserve University School of Medicine, Cleveland, OH; Department of Pediatrics, Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, PA; and Macha Mission Hospital, Choma, Zambia.

The pathophysiology of cerebral malaria may include lipid peroxidant damage to cellular and subcellular membranes of the brain by iron-generated free radicals. To determine if elevated circulating iron levels are found in children with deep coma and prolonged recovery of consciousness, we measured serum transferrin saturations in 28 Zambian children who presented with strictly defined cerebral malaria. The mean ± SD transferrin saturation was 37 ± 30% in 19 children with deep coma (scored 0-2 on the Blantyre coma scale) compared to 19 ± 10% in 9 children with light coma (scored 3-4) ($P = 0.026$). The subjects were enrolled in a trial comparing 72-hour infusions of desferrioxamine B or placebo in addition to standard therapy with quinine and Fansidar. Duration of coma during experimental therapy was significantly correlated with transferrin saturation ($P<0.0005$) and with treatment ($P=0.04$). Children were categorized as to whether or not transferrin saturations were > 43%, 3 SD above the normal mean value for age. After adjustment for type of treatment, duration of coma was 68 ± 10 hours in five children with markedly elevated transferrin saturations, mean 79%, compared to 34 ± 23 hours in 23 children with the lower saturations, mean 18% ($P=0.004$). We conclude that elevated transferrin saturations are found at presentation in children with deep coma and prolonged duration of coma, and may represent an impaired ability to safely handle iron released during hemolysis.

378 ACUTE INFLAMMATORY CYTOKINE RESPONSES IN SERUM AND CEREBROSPINAL FLUID (CSF) AMONG INDONESIANS WITH CEREBRAL MALARIA OR SEVERE COMPLICATED MALARIA. Richie TL*, Harianto PN, Tjitra E, Solihin A, Basri H, Punjabi NH, Marwoto H, Alwi-Datau E, Larasati RP, Pudjoprawoto N, Hoffman SL, and O'Hanley PD. Naval Medical Research Unit #2 Jakarta, Indonesia; Department of Internal Medicine, University of Sam Ratulangi, North Sulawesi, Indonesia; National Institute of Health Research and Development, Jakarta, Indonesia; Subdivision of Immunology, Medical Faculty, University of Sam Ratulangi, North Sulawesi,

Indonesia; Naval Medical Research Institute, Bethesda, Maryland MD; and Departments of Medicine and Microbiology, Stanford University, Stanford, CA.

Recent studies of malaria-infected foreign travellers and individuals residing in malarious areas suggest that clinical severity is associated with elevated systemic levels of inflammatory cytokines (interleukin[IL]-1, IL-6, and tumor necrosis factor [TNF]). TNF and IL-6 serum levels are correlated with mortality for patients with cerebral malaria (CM). We have begun characterization of acute inflammatory cytokine responses in malaria-infected patients in order to devise new strategies for modulating the morbidity and mortality of CM patients. To date, we have carefully monitored the clinical condition of 21 confirmed or suspected CM patients and 6 patients with complicated severe (>1 end-organ damaged) malaria without neurological manifestions. All acutely-ill patients received parenteral quinine dihydrochloride followed by oral quinine sulfate to complete a full 7 day course. Serum specimens were collected from each patient prior to initiation of therapy and longitudinally during the hospital course. Serum and some paired plasma specimens were assessed for acute inflammatory cytokines by immunoassays. In addition, the majority of patients had CSF collected and evaluated for IL-1, IL-6, and TNF levels. Results indicate that TNF and IL-6 were variably detected in the CSF of CM patients: 4 of 13 patients showed measurable levels of TNF (mean 2.6 pg/ml, range 1.4 to 5.1) and 12 of 12 patients showed measurable levels of IL-6 (mean 59 pg/ml, range 4 to 320). One patient out of 13 had detectable IL-1· in the CSF (2.5 pg/ml). TNF and IL-6 were present in the serum and plasma of nearly all CM and severely infected malaria patients and correlated with mortality. TNF ranged from 0 to 200 pg/ml, IL-6 from 7 to 318 pg/ml. Mean serum levels were 45 pg/ml (TNF) and 112 pg/ml (IL-6) for cerebral malaria patients and 70 pg/ml (TNF) and 173 pg/ml (IL-6) for patients with severe, non-cerebral malaria. TNF and IL-6 levels were higher in those CM patients who died (67 pg/ml and 135 pg/ml, respectively) than in those who survived (22 pg/ml and 87 pg/ml, respectively). As a general rule, TNF serum levels remained high or rose higher than initial levels in patients who died, but fell rapidly in patients who survived. In contrast, in many surviving patients, IL-6 levels rose initially and then fell gradually over several days. IL-1· was detectable at low levels in about half the patients and showed no obvious relationship with outcome. Our preliminary conclusion is that some inflammatory cytokine responses, in particular TNF, correlate with disease severity in malaria-infected patients, but do not correlate specifically with neurological (as opposed to other severe) manifestations.

379 METABOLIC DISORDERS, TNF-α, IFN-γ AND SOLUBLE ELAM AND ICAM-1 RECEPTORS IN SEVERE AND CEREBRAL MALARIA IN AFRICAN ADULTS. Deloron P*, Niyongabo T, Dumont N, Astagneau P, Ndarugirire F, Muhirwa G, Ndayiragije A, Brelivet JC, Aubry P, and Peyron F. INSERM Unité 13, Paris, France; Kamenge Hospital, Bujumbura, Burundi; and Fac of Medicine, Lyon, France.

48 adult patients presenting with cerebral (CM, n=31) or severe (SM, n=17) malaria were enroled at the Kamenge hospital, Bujumbura, Burundi. SM was defined from WHO criteria as parasite count ≥100,000/µl, blood creatinine>265 µmol/l, hemoglobin<5g/l, and/or bilirubin>50 mmol/l (No patients was hypoglycemic). Blood was obtained before quinine infusion for routine hematology and biochemistry, and measurement of plasma levels of TNF-α, IFN-γ, as well as soluble endothelial receptors sELAM and sICAM-1. Mean age (29.1 yrs), temperature (39.4°C), blood levels of hemoglobin (11.7 g/100ml), creatinine (172.4 µmol/l), bilirubin (60.3 µmol/l), and glucose (6.9 mmol/l) were similar in CM and SM groups (all p>.1). Mean parasite counts were higher in SM than in CM (116,192 vs. 11,920, p=.003). Levels of TNF-α, IFN-γ, and sICAM-1 were similarly elevated in CM and SM groups (267 pg/ml, 79 pg/ml, and 387 ng/ml; all p>.2). Levels of sELAM were higher in SM than in CM (433 vs. 191 ng/ml, p=.003) and correlated to parasite density (r=+.44; p=.002). After adjustement on parasitemia, levels of sELAM remained higher in SM than in SM (p=.03). All 7 patients who died were CM. In CM, fatal cases had higher levels of creatinine (p=.004), bilirubin (p=.02), and sELAM (p=.02) than non fatal cases. In a multivariate model, only the level of creatinine remain significantly higher in fatal than in non fatal CM (p=.26). In these African adults, blood levels of TNF-α, IFN-γ,

sELAM and sICAM-1 appear not to be related by their own in the development of neurological manifestations nor in the outcome. The main pronostic factor of mortality is the level of creatinine.

380 HYPERBILIRUBINAEMIA IN PATIENTS WITH SEVERE MALARIA IN NORTHERN SULAWESI, INDONESIA. Harianto PN*, Tenda-Moeis E, and Thomas TL. Department of Internal Medicine, University of Sam Ratulangi, Gunung Wenang Hospital, Manado, Indonesia; and Departmente of Tropical Medicine, U.S. Naval Medical Research Unit #2, Jakarta, Indonesia.

Hyperbilirubinemia (jaundice) is a common presentation of severe malaria in adults. Measurements of liver function tests, hepatic blood flow and liver biopsy specimens have not clarified the pathophysiology underlying liver function abnormalities in severe malaria. A study of 111 cases of severe malaria was conducted in North Sulawesi, Indonesia. There were 51 cases (46%) with bilirubin greater than 3 mg% (group A); 14 cases (13%) with bilirubin 2 - 3 mg% (group B); 18 cases (16%) with bilirubin 1.2 - 2 mg% (group C); and 28 cases (25%) with normal serum bilirubin (< 1.2 mg%) (group D). The mortality of these groups was 33%, 29%, 17% and 11% respectively. The range of serum bilirubin in group A was 3.1 - 36.4 mg%; mean bilirubin was 11.4 mg%. The ratio of direct to indirect bilirubin was less than one in only nine cases, suggesting evidence of hemolysis. The SGOT ranged from 6 to 226 μ/L (normal < 18), mean 54.6 μ/L; SGPT ranged from 5 to 154 μ/L (normal < 22), mean 40 μ/L; yGT ranged from 4.5 to 218 μ/L (normal 6 - 28), mean 48.5 μ/L; alkaline phosphatase ranged from 88 to 506 μ/L (normal 60 - 170), mean 197 μ/L. The level of bilirubin correlated positively with the degree of parasitemia. None of these cases developed hepatic failure. Hypoglycemia was more common in group A compared with groups B and C (25% versus 14% and 16%). Elevated serum creatinine (> 2.0 mg%) was most common in group A (53%), compared with groups B (36%), C (28%) and D (26%). The patients were treated with quinine dihydrochloride 1500 to 2000 mg per day intravenously followed by oral quinine sulfate when the patients became conscious. Abnormal liver function, in particular hyperbilirubinemia, is an important parameter in severe malaria. Hyperbilirubinemia is correlated with hypoglycemia, elevated creatinine, and increased mortality.

381 SUPPORTIVE THERAPY OF FALCIPARUM MALARIA WITH PENTOXIFYLLINE: A PROSPECTIVE RANDOMIZED STUDY. Hemmer CJ*, Hort G, Chiwakata C, Kern P, Nawroth PP, and Dietrich M. Department of Medicine, Bernhard-Nocht-Institute for Tropical Medicine, Hamberg, Germany; and Heidelberg University Medical School, Heidelberg, Germany.

Human falciparum malaria is associated with elevated serum levels of Tumor Necrosis Factor α (TNFα). In murine malaria (*Plasmodium berghei*), fatal cerebral complications are prevented by neutralization of TNFα by antibodies or by suppression of TNFα production by pentoxifylline (POF). Therefore we tested in a prospective randomized study, whether administration of POF (Verum) in addition to antiparasitic therapy leads to lower TNFα levels than antiparasitic therapy without POF (Placebo), and whether this effect is associated with clinical benefit for POF. Fifty-three patients with falciparum malaria received either 20 mg/kg POF in 150 mL of saline over 24 hours, or 150 mL of saline without POF, both for 5 days. During the course of the study, POF infusions had to be discontinued because of nausea (after 1 to 3 days) in 11 of 26 patients receiving POF, while only 4 of 27 patients receiving Placebo requested premature termination of the study medication. This difference was significant ($p<0.05$). Even though the POF doses administered in this study were quite high, TNFα levels tended to be higher in the POF group than in the placebo group (not signif.). In addition, POF was associated with higher plasma levels of neutrophil elastase than Placebo ($p<0.01$). No differences were observed in lethality. POF also did not affect parasitemia, hemoglobin, lactate dehydrogenase, creatinine, Thrombin-Antithrombin III complexes and neopterin. The protocol required termination of the study, if significant differences concerning clinical outcome, side effects, or important laboratory parameters became evident. Since POF had significantly more side effects

than placebo, and since statistically it was not expected that continued recruitment would reveal beneficial effects for POF, the study had to be discontinued. POF does not effectively suppress the production of TNFα in falciparum malaria. No clinical benefit is derived from this drug. In contrary, elevated elastase levels might even suggest potentially deleterious effects. Therefore, POF should not be used as a supportive therapy in falciparum malaria.

382 IN-HOSPITAL MORTALITY AND MORBIDITY DUE TO MALARIA-ASSOCIATED SEVERE ANEMIA IN TWO AREAS IN MALAWI WITH DIFFERENT MALARIA TRANSMISSION PATTERNS. Slutsker L*, Taylor TE, Wirima JJ, and Steketee RW. Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA; College of Osteopathic Medicine, Michigan State University, East Lansing, MI; and University of Malawi, College of Medicine, Blantyre, Malawi.

It has been suggested that malaria-associated severe anemia (MASA, parasitemia and hematocrit <15%), is more frequent in areas with year-round as opposed to seasonal malaria transmission. From 1990-92 in Malawi, we determined prospectively the contribution of MASA to malaria morbidity and mortality among all children admitted to Mangochi District Hospital (MDH, year-round transmission) and Queen Elizabeth Central Hospital (QECH, seasonal transmission). Admission age, hemoglobin/hematocrit, and malaria thick smears were collected; in-hospital survival or death was recorded. The prevalence of MASA was 8.5% at MDH compared with 5.2% at QECH ($P<0.05$). Infants at MDH were twice as likely to have MASA as those at QECH. Parasite density was not related to the risk of MASA at MDH, but was at QECH. A similar proportion of all deaths were malaria-related at MDH (17.5%) and QECH (20.4%); however, MASA accounted for 54% of malaria-related deaths at MDH compared with 32% at QECH. The contribution of MASA to morbidity and mortality was greater in the year-round transmission area. Malaria case management strategies that focus on fever but not on MASA may have limited impact on reducing malaria-related mortality.

383 HEALTH IMPACT OF *PLASMODIUM FALCIPARUM* AMONG HOSPITALIZED PEDIATRIC PATIENTS. Zucker JR*, Ruebush TK, Olango C, Were JB, and Campbell CC. Malaria Branch, Centers for Disease Control, Atlanta, GA; Siaya District Hospital, Siaya, Kenya; and Kenya Medical Research Institute, Kenya.

The disease manifestations of *Plasmodium falciparum* (Pf) infection were studied in hospitalized children in Nyanza Province, western Kenya. Children < 5 years of age admitted to Siaya Hospital from November 1, 1991 through February 15, 1993 were evaluated by history and physical examination, admission hemoglobin (Hb), blood smear for *Plasmodium*, blood culture and lumbar puncture (LP) when indicated, and outcome of hospitalization. Of the 3338 patients admitted, 1610 (48%) had fever (>37.5°C), 576 (17.3%) had Hb <5.0 g/dl, and 40 (1.2%) had coma or inability to localize pain (CP). Of febrile children, 1100 (68.3%) had Pf, compared with 953 (60.9%) in afebrile children. Of 55 (1.6%) children who had hyperparasitemia (HP) (>10^6 asexual parasites/mm^3), 34 (61.8%) were febrile. In children with Hb <5.0 g/dl, 83% (478) were parasitemic. Of the children with CP, 26 (65%) were parasitemic and only 1 (3.8%) child had HP. Case fatality rates for Pf-associated febrile illness, HP, anemia, and CP disease were 5.9% (65 deaths), 5.5% (3 deaths), 14.9% (86 deaths), and 42.3% (11 deaths), respectively. Temperature elevation does not accurately identify children at risk of severe malaria. Strategies must be developed to provide for improved recognition of parasitemia and malaria-associated anemia.

384 A COMPARATIVE STUDY OF ECDYSTEROID TITERS AND METABOLISM IN EMBRYOS OF TWO TICK SPECIES. Dotson EM*, Connat JL, and Diehl PA. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, Georgia; Departement de Zoologie et de

Biologie Animale, Universite de Geneve, Geneve, Switzerland; and Institute de Zoologie, Universite de Neuchatel, Neuchatel, Switzerland.

Ecdysteroids, found in numerous invertebrates, have been shown to play important roles in cuticle production and reproduction in many ectoparasites including ticks. To determine the titer and role of ecdysteroids in tick embryos, we studied the fluctuations of the ecdysteroids throughout development and correlated these with embryonic events. Metabolism of radiolabelled hormones was also monitored *in vivo* and *in vitro*. Techniques used included radioimmunoassay, high performance liquid chromatography, transmission electron microscopy, mass spectrometry and tissue culture. In embryos of the ixodid tick *Amblyomma hebraeum*, the incorporated free ecdysteroids appear to be inactivated between the 15th and 30th days of development by formation of apolar ester conjugates and 3α epimers of these conjugates. The incorporated hormones probably play a role in events that take place before day 15; i.e. meiotic reinitiation or formation of embryonic cuticles A and B, formed by day 8. Although no distinct peaks of ecdysteroids were detected, a few data points were higher around day 44 during larval epicuticle production. In the argasid tick *Ornithodoros moubata* the ecdysteroid conjugates found in the newly-laid eggs do not appear to be hydrolyzed to release the free hormone. The high titers of 20-hydroxyecdysone and ecdysone correlated with larval epicuticle production at the end of embryonic development appear to be produced by *de novo* synthesis.

385 THE PYRUVATE DEHYDROGENASE COMPLEX FROM ANAEROBIC PARASITIC HELMINTHS. Klingbeil M*, Summers M, Sidawy E, and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

Pyruvate dehydrogenase complex (PDC) isolated from anaerobic mitochondria of the parasitic nematode, *Ascaris suum*, is unique among eukaryotes and is modified to function under the reducing conditions present in the host gut. In contrast to complexes from yeast and other eukaryotes, PDC purified from *A. suum* lacks protein X and contains 43 and 45 kDa proteins of unknown function. To gain insight into the function of these proteins, PDH_a kinase was isolated from the purified ascarid PDC. The ascarid PDH_a kinase has a specific activity of 1-3 nmol/min/mg in the intact complex and was much less active than the corresponding mammalian enzyme (10-12 nmol/min/mg). Resolution of the purified PDC in 1.2 M NaCl by chromatography on Sephacryl S-400 yields two major peaks. Peak I contains dihydrolipoyl transacetylase (E2), dihydrolipoyl dehydrogenase (E3), the 43 and 45 kDa proteins, and all of the PDH_a kinase activity. Based on 2D gel electrophoresis and amino terminal sequencing, it appears that the 43 kDa band contains at least two distinct comigrating proteins (phosphorylated E1α, pI 6.05, and additional proteins which run as a smear between pH 6.8 and 7.7). Treatment of Peak I with p-hydroxymercuriphenyl sulfonate precipitates most of the E2 and the 43 and 45 kDa proteins, leaving a supernatant fraction containing E3, minor bands between 40 and 45 kDa and most of the PDH_a kinase activity. These data suggest that PDH_a kinase activity is not associated with the major proteins at 43 and 45 kDa.

386 THE PYRUVATE DEHYDROGENASE COMPLEX FROM ANAEROBIC PARASITIC HELMINTHS. Diaz F* and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

The subunit composition of the pyruvate dehydrogenase complex (PDC) isolated from anaerobic muscle of the parasitic nematode, *Ascaris suum*, differs significantly from PDCs isolated from other eukaryotes. For example, it lacks protein X and contains 43 and 45 kDa subunits of unknown function. To gain insight into these differences, the PDC was purified to apparent homogeneity from the equine nematode, *Parascaris equorum*, and the canine cestode, *Dipylidium caninum*. The *P. equorum* PDC was similar in subunit composition and regulatory properties to the PDC isolated from *A. suum*. Only dihydrolipoyl transacetylase (E2) was acetylated during incubation in [2-^{14}C]pyruvate indicating that protein X is not present. The *P. equorum* PDC contained two distinct dihydrolipoyl

dehydrogenase (E3) bands on SDS-PAGE, with apparent M_rs of 55 and 53.5 kDa. The amino terminal sequence of both proteins is SSGNEVDLVVIGSGPGGYVAAIKAAQLGMK, suggesting that the smaller band may have arisen by proteolysis, although the ratio of the two bands is constant in different preparations. In contrast, the subunit composition of the *D. caninum* PDC was significantly different from that of *A. suum* and contained four major bands after separation by SDS-PAGE of 59, 58, 49, 36 kDa, which correspond to E3, E2 and the α and β subunits of pyruvate dehydrogenase. The 43 and the 45 kDa subunits were absent. This subunit composition much more closely resembles that of the *S. cerevisiae* PDC. Acetylation with [2-^{14}C]pyruvate yielded a major acetylated band of 58 kDa and a minor band of 60 kDa which comigrated with E3, suggesting that the *D. caninum* PDC contained protein X. These results suggest that the modification in the subunit composition of the *A. suum* PDC is restricted to the nematodes and not due to alterations associated with anaerobic metabolism in general.

387 PROTEASE RELEASE COINCIDES WITH RE-ACTIVATION OF INFECTIVE HOOKWORM LARVAE. Hawdon JM, Perregaux MA, and Hotez PJ. Medical Helminthology Laboratory, Yale University School of Medicine, New Haven, CT.

The third-stage infective larva (L3) of hookworms is a developmentally arrested, non-feeding life history stage that resumes development in response to a signal encountered during infection of the definitive host. L3 of the canine hookworm *Ancylostoma caninum* resume feeding *in vitro* when incubated at 37° C in tissue culture medium containing a low molecular weight filtrate of canine serum and S-methyl glutathione. Excretory/secretory (ES) products released during this activation contain a protease of 38-41 kDa, as detected by gelatin substrate non-denaturing gel electrophoresis. Non-activated L3 failed to release the protease. SDS-PAGE indicated that the enzyme is a major component of ES products. O-phenanthroline (1 mM) completely inhibited protease activity and *in vitro* feeding, but not exsheathment, indicating that the enzyme is a metalloprotease that may function in feeding and larval re-activation during infection.

388 PRELIMINARY CHARACTERIZATION OF NEMATODE BOMBESIN/GASTRIN-RELEASING PEPTIDE BINDING SITES. Huntington MK*, Thompson DP, Geary TG, Mackenzie CD, and Williams JF. Department of Microbiology, Michigan State University, East Lansing, MI; Animal Health Discovery Research, The Upjohn Company, Kalamazoo, MI; and Department of Pathology, Michigan State University, East Lansing, MI.

We have previously reported evidence of bombesin-like peptides (BLPs) and their binding sites in nematodes. Approximately 500 pg bombesin equivalents per gram extract are present in free living, luminal dwelling, and tissue dwelling nematodes, localized to the hypodermocuticular junction. BLP binding sites have been found in the hypodermis and body wall musculature of *Ascaris suum*, and exhibit a K_D of 2.8 nM and a B_{max} of 1 fmol/mg protein in our preparations. This report expands the receptor localization to filariae, and begins to address questions related to the physiological function of helminth BLPs. Utilizing biotinylated bombesin with a fluorescent-labelled avidin, we have found binding to the hypodermal region of *Dirofilaria immitis* and *Onchocerca volvulus* . This binding is displaced by 1 uM of unlabelled peptide and is consistent with the results obtained in *A. suum*. The application of semipurifed nematode extract containing the putative BLP(s) to *Xenopus* oocytes that express murine bombesin/gastrin-releasing peptide receptor elicits a concentration-dependent depolarization of the oocyte membrane. Furthermore, when excised longitudinal muscle segments of *A. suum* are exposed to bombesin, tonotropic effects are recorded. These data suggest that nematode BLPs may be involved in regulating membrane ionic conduction. Further characterization of this peptide/receptor system in nematodes will yield a greater understanding of the "neuroendocrine" physiology of these invertebrates, and may uncover potential targets for novel anthelmintics.

389 VOLATILE FATTY ACID EFFLUX FROM ISOLATED SEGMENTS OF *ASCARIS SUUM* BODY WALL. Blair KL*, Ho NF, Barsuhn CL, Geary TG, and Thompson DP. Animal Health Therapeutics, The Upjohn Company, Kalamazoo, MI; and Drug Delivery Systems Research, The Upjohn Company, Kalamazoo, MI.

Recent studies demonstrated that *Ascaris suum* excretes volatile fatty acids (VFAs) across the cuticle. Based on preliminary studies, VFA excretion by intact *A. suum* is Na^+-independent and insensitive to agents that inhibit their transport in vertebrates. The biochemical basis for these potentially important host-parasite differences are unknown. The kinetics and directionality of VFA excretion by isolated body wall (cuticle, hypodermis and muscle) of *A. suum* was characterized. VFA levels within freshly dissected segments were at near equilibrium concentrations with respect to VFA concentrations of the psedoceolomic fluid. Throughout the course of incubations in a two-chamber diffusion cell, VFA levels on the muscle side were greater than those on the cuticle side. Like the intact animal, the isolated body wall acidifies neutral media and alkalinizes acidic media. The dominant VFAs excreted at early time points (2-4 hr) were 2-methyl butyric and 2-methyl valeric acids. By 24 hr, however, acetic and propionic acids were the dominant VFAs. These results suggest that during short term incubations at least (≤ 6hr), isolated segments of body wall provide a useful model for investigating the factors that affect VFA synthesis and excretion by *A. suum*.

390 APPARENT INVOLVEMENT OF A PROTON GRADIENT IN THE MITOCHONDRIAL ENERGY-LINKED TRANSHYDROGENATION OF ADULT *HYMENOLEPIS DIMINUTA*. Park JP* and Fioravanti CF. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH.

The mitochondrial, inner membrane-associated transhydrogenase of adult *Hymenolepis diminuta* catalyzes a reversible hydride transfer between reduced and oxidized pyridine nucleotide; i.e., an NADPH:NAD and NADH:NADP transhydrogenation. Recently, it was demonstrated that the NADH:NADP reaction occurs in either a non-energy linked or energy-linked mode, with the latter resulting from energization of the system by either electron transport utilization of NADH or the hydrolysis of ATP via Mg^{2+}-ATPase, as described previously. Using *H. diminuta* submitochondrial particles as the source of transhydrogenase activity, the potential involvement of a proton gradient in the ATP-dependent, energy-linked reaction was assessed by the use of three protonophores, i.e., carbonylcyanide 3-chlorophenylhydrazone (CCCP), carbonyl-cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) and niclosamide. These assessments were made in the presence of rotenone to preclude NADH utilization by the electron transport chain. At a concentration of 2 μM, both CCCP and FCCP markedly inhibited ATP-dependent, energy-linked transhydrogenation by about 70% and 80%, respectively. Moreover, at a concentration of 1 μM, the anticestodal agent, niclosamide, inhibited the energy-linked reaction by about 60%. These data are consistent with the notion that the ATP-dependent, energy-linked transhydrogenation between NADH and NADP is driven by a proton gradient that is established by ATP hydrolysis. In turn, protonophoric disruption of the gradient effectively inhibits the energy-linked reaction.

391 CATALYSIS OF PYRIDINE NUCLEOTIDE TRANSHYDROGENASE ACTIVITY BY ADULT *HYMENOLEPIS DIMINUTA* SUBMITOCHONDRIAL PARTICLES. Whitmore MM* and Fioravanti CF. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH.

The mitochondrial, pyridine nucleotide transhydrogenase of adult *Hymenolepis diminuta* engages in hydride transfer between NADPH and NAD. As such, this inner membrane-associated enzyme serves a vital metabolic function in the supply of NADH needed for operation of the cestode anaerobic electron transport system. Employing *H. diminuta* submitochondrial particles as the source of enzyme activity and acetylpyridine NAD (AcPyAD) as the hydride acceptor, the transhydrogenase

system was evaluated with respect to NADPH-dependent NAD reduction. At the optimal pH of 6.5 and at saturating substrate concentrations, NADPH-dependent AcPyAD reduction occurred at a rate of about 0.40 μmol/min/mg protein. The K_m for NADPH and AcPyAD was 66 μM and 35 μM, respectively. Whereas NADP inhibited NADPH utilization competitively, it resulted in an apparent mixed inhibition in terms of AcPyAD reduction. By comparison, NAD acted as an apparent competitive and noncompetitive inhibitor with respect to AcPyAD reduction and NADPH utilization. In general, 5'-AMP simulated NAD and 2'-AMP simulated NADP in inhibiting the transhydrogenase-dependent catalysis of AcPyAD reduction. Interestingly, palmitoyl-CoA competitively inhibited NADPH oxidation while seemingly causing a noncompetitive inhibition in terms of AcPyAD reduction. In turn, these data suggest that the transhydrogenase contains two substrate binding sites, i.e., an NADP(H) and NAD(H) site, that would be subject to inhibition in a site-specific fashion. Moreover, the effect of palmitoyl-CoA suggests that the NADP(H) binding site has a hydrophobic character.

392 MITOCHONDRIAL NADH:NAD TRANSHYDROGENATION AND THE LIPOAMIDE DEHYDROGENASE OF ADULT *HYMENOLEPIS DIMINUTA*. Walker DJ* and Fioravanti CF. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH.

Adult *Hymenolepis diminuta* mitochondria catalyze both an NADPH:NAD and NADH:NAD transhydrogenase reaction. The former is the product of an inner membrane-associated, pyridine nucleotide transhydrogenase that is essential to the anaerobic energetics of the cestode. As one of its functions, the NADPH:NAD transhydrogenase catalyzes hydride transfer from intramitochondrial NADPH, generated via malate utilization, to NAD, producing the NADH required for electron transport. Our initial findings suggested that the NADH:NAD reaction is the product of at least two membrane-associated enzymes viz., lipoamide dehydrogenase and NADH dehydrogenase. In this regard, an association of NADH:NAD transhydrogenation activity with the purified, mitochondrial lipoamide dehydrogenase of adult *Ascaris suum* was previously reported. As part of a detailed study of mitochondrial transhydrogenations in adult *H. diminuta*, its lipoamide dehydrogenase was isolated and characterized. The lipoamide dehydrogenase liberated by sonication of mitochondrial membranes was used as starting material. Heat fractionation and subsequent ion exchange, gel filtration and hydroxylapatite chromatographies resulted in a 150-fold purification of the cestode lipoamide dehydrogenase. The enzyme is a 93 kDa homodimer with a monomeric M_r of 47 kDa. Indeed, the purified enzyme catalyzes NADH:NAD transhydrogenation with a ratio of lipoamide dehydrogenase to NADH:NAD activity of about 5:1. The two activities differ in terms of pH optima (lipoamide dehydrogenase, 6.5; NADH:NAD, 7.5) and in the greater sensitivity of the lipoamide dehydrogenase activity to divalent cation inhibition. Preliminary peptide sequence analysis suggests a high degree of correlation between the *H. diminuta* and mammalian lipoamide dehydrogenases.

393 MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE OF cDNA CLONES ENCODING THE 2-METHYL BRANCHED CHAIN ENOYL CoA REDUCTASE FROM *ASCARIS SUUM*. Duran E*, Johnson K, Wheelock M, Komuniecki PR, and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

The 2-methyl branched-chain enoyl CoA reductase plays a pivotal role in the reversal of β-oxidation operating in anaerobic mitochondria of the parasitic nematode, *Ascaris suum*. An affinity purified polyclonal antiserum against the reductase was used to screen a cDNA library constructed in λgt11 with poly(A)$^+$ RNA from adult *A. suum* muscle. A 1.2 kb partial cDNA was isolated and sequenced. Additional sequence at the 5' end was determined by the Rapid Amplification of cDNA Ends procedure. Sequence analysis of the cDNAs revealed the 22 nucleotide trans-spliced leader sequence characteristic of many nematode mRNAs, an open reading frame of 1236 nucleotides and a 3'-untranslated sequence of 109 nucleotides including a short poly(A) tail 14 nucleotides from a polyadenylation signal (AATAAA). The open reading frame encoded a 396-amino acid sequence (M_r

43,046) including a 16 amino acid leader peptide. Two dimensional gel electrophoresis of the purified reductase yielded multiple spots with two distinct but overlapping amino-terminal sequences. Both sequences overlapped with the sequence predicted from the mRNA and one of the sequences was identical to the predicted sequence. Comparison of the ascarid sequence with that of mammalian acyl CoA dehydrogenases revealed a high degree of sequence identity, suggesting that these enzymes may have evolved from a common ancestral gene even though the ascarid enzyme functions as a reductase not as a dehydrogenase. Immunoblotting of *A. suum* larval stages and adult tissues suggested that the reductase was only found in anaerobic muscle. Northern blotting using the partial cDNA revealed a hybridization band of about 1.5 kb and also suggested that the enzyme was tissue-specific and developmentally regulated.

394 NEUTRALIZING ANTIBODIES INDUCE A CIRCUMSPOROZOITE-LIKE REACTION WITH *CRYPTOSPORIDIUM PARVUM* SPOROZOITES. Riggs MW*, Sterling CR, Stone AL, Westhof NC and Bentley DL. Department of Veterinary Science, University of Arizona, Tucson, AZ.

Cryptosporidiosis is a diarrheal disease for which effective therapy and prophylaxis are needed, and occurs in calves and humans throughout the world. We produced hyperimmune bovine colostral antibody against whole *Cryptosporidium parvum*, and panels of monoclonal antibodies (mAbs) against defined, immunoaffinity-isolated, neutralization-sensitive zoite antigens. Antibodies had highly significant *in vitro* neutralizing activity against isolated *C. parvum* sporozoites, and *in vivo* therapeutic efficacy in oocyst-challenged neonatal BALB/c mice, or persistently infected adult SCID mice. Upon incubation with mAb 3E2-A8 or hyperimmune colostral antibody, sporozoites underwent distinct morphologic changes observed by DIC microscopy, which were further characterized by immunoelectron microscopy. The changes consisted of progressive formation and eventual shedding of membranous sporozoite surface antigen-antibody complexes, similar to the malarial circumsporozoite reaction. Infectivity of sporozoites having undergone this reaction was neutralized. mAb 3E2-A8 recognized an epitope common to multiple sporozoite glycoproteins ranging from 50 to >200 kDa. One or more of the antigens defined by neutralizing mAb 3E2-A8, and corecognized by neutralizing colostral antibody, may be mechanistically involved in the circumsporozoite-like reaction. These antigens can be evaluated as subunit immunogens to produce well characterized polyclonal antibody for control of cryptosporidiosis, or as targets for mAb-based immunotherapy. Further, neutralizing antibodies should facilitate studies on the circumsporozoite-like reaction, mechanisms of antibody-mediated neutralization, and the molecular pathogenesis of cryptosporidiosis.

395 CLONING OF THE GENE FOR P68, A *CRYPTOSPORIDIUM PARVUM* SPOROZOITE PROTEIN THAT IS THE TARGET OF PROTECTIVE ANTIBODY *IN VITRO*. Petersen C*, Barnes DA, Lewis S, and Doyle PS. University of California, San Francisco, San Francisco General Hospital, San Francisco, CA; and ImmuCell Corp., Portland, ME.

Cryptosporidium parvum causes severe gastrointestinal disease in immunocompromised persons and self-limited disease in immunocompetent hosts. Attempts to identify agents for chemotherapy and immunotherapy have increased as the AIDS epidemic has spread, but no effective therapy has been identified. We have studied proteins on the surface and in the apical complexes of the sporozoite, one of the infective stages which exist free in the gut, in order to identify molecules which may be important for invasion and intracellular development and which may be targets for immunotherapeutic or chemotherapeutic intervention in cryptosporidiosis. Two *Cryptosporidium* λ-gt11 genomic expression libraries were previously screened with anti-*Cryptosporidium* polyclonal antibodies in order to identify immunogenic proteins. A clone comprising an 870 bp fragment encoding a portion of a 68 kD sporozoite protein was identified. Sequences analysis of the 870 bp segment suggested that the cloned protein showed homology to metal binding proteins. P68 was localized to the anterior end of sporozoites in the region of the apical complex organelles. Anti-

recombinant protein antibody, raised in mice, significantly inhibited *Cryptosporidium* invasion and development in MDCK cells relative controls.

396 *CRYPTOSPORIDIUM* INFECTION AND ATTEMPTED CONTROL OF TRANSMISSION IN A DAIRY HERD. Zajac AM*, Holland RJ, and Moore GA. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg VA; and College of Veterinary Medicine, Michigan State University, East Lansing MI.

Following the diagnosis of severe cryptosporidiosis in calves, the presence of *Cryptosporidium* was monitored in a dairy herd for approximately 1 year. Fecal samples were collected from 42 heifer calves at 1, 4, 8 and 12 days after birth (AB) and examined for oocysts by a modified Sheather's sugar flotation test. Thirty-five heifers were sampled daily for 2 weeks AB, then every 2 weeks until 8 weeks AB and then monthly for 1 year. While an 86% prevalence of infection was detected in heifers sampled 4 times, the parasite was found in 100% of heifers sampled daily for 2 weeks. An effort was also made to interrupt transmission of the parasite. Heifer calves were routinely removed from their dams at less than 1 day AB, placed in barn stalls and moved to hutches several days later. Management was altered by placing 6 calves in stalls unused for 6 months (Group A), putting 5 calves directly into hutches (Group B) and placing 5 calves into hutches at a site never used for calf rearing (Groups C). Calves in all groups became infected but the age at patency increased and the severity of infection appeared to be reduced in Groups B and C. These results suggest that all calves will be infected when *Cryptosporidium* is present in a dairy herd, but that management changes can reduce severity of infection.

397 EXPRESSION OF GLCNAC/GALNAC TRANSFERASE ACTIVITIES DURING ENCYSTATION OF *GIARDIA LAMBLIA*. Das S* and Gillin FD. Department of Pathology, UCSD-Medical Center, San Diego, CA.

Although the cyst wall is responsible for the resistance of *Giardia* to hypotonic lysis and disinfection, little is known about its components. On the basis of WGA binding, Ward et. al. reported chitin, a polymer of N-acetylglucosamine (GlcNAc), as a major component of the cyst wall. Disputing the presence of chitin, however, Jarroll et. al. showed galactosamine (GalN) or N-acetylgalactosamine (GalNAc) to be a major cyst constituent. To determine if GlcNAc and GalNAc transferase (GT) activities are involved during cyst wall biosynthesis, we measured incorporation of radioactive GlcNAc or GalNAc from UDP-GlcNAc or UDP-GalNAc into alcohol precipitable material during *in vitro* encystation of *G. lamblia*. GT activities were 10-15 times higher in encysting cells compared to trophozoites and were equally distributed in the pellet (P-GT) and the high speed (100,000 x g) supernatant (S-GT) fractions. We then asked whether a single enzyme was responsible for transfer of both GlcNAc and GalNAc. Incorporation by both P-GT and S-GT was dependent on Mn^{2+}, and inhibited by sugar nucleotides and UDP. Moreover, each individual GT incorporated GlcNAc and GalNAc with similar kinetic constants (K_m and V_{max}), suggesting broad substrate specificites. However S-GT had significantly lower K_ms and higher V_{max} for both sugars compared to P-GT. Isolation and charaterization of the macromolecular (>30 kDa) products revealed other key differences.S-GT incorporated GlcNAc and GalNAc into products that were not digested by proteinases or chitinase. In contrast, P-GT incorporated GlcNAc and GalNAc into glycoproteins, since the products were digested by proteinases. Interestingly, incorporated GlcNAc was cleaved by chitinase, releasing chitotriose, chitobiose and GlcNAc, while incorporated GalNAc was not removed from the product. These studies support the existence of at least 2 regulated glycosyl transferases in encysting *G. lamblia*, and suggest that GlcNAc and GalNAc are incorporated into both glycoproteins and proteinase-insensitive macromolecules.

398 DEFECTIVE CYST WALL ANTIGEN EXPRESSION AND TRANSPORT AND BILE SALT UPTAKE BY AN ENCYSTATION DEFICIENT SUBLINE OF *GIARDIA LAMBLIA*. Reiner DS*, Hetsko ML, Das S, Ward HD, McCaffery M, and Gillin FD. Department of Pathology, UCSD Medical Center, San Diego, CA; Division of Geographic Medicine and Infectious Diseases, Tufts University School of Medicine, Boston, MA; and Division of Cellular and Molecular Medicine, UCSD, San Diego, CA.

Despite the importance of cysts for the transmission of giardiasis, little is known about the process by which *Giardia lamblia* trophozoites differentiate into this resistant form. We have induced the complete life cycle of *G. lamblia in vitro* for the first time and have found that encystation entails the expression of a number of new antigens, as well as formation of a novel class of large Encystation Specific Secretory Vesicles (ESV) that transport stage-specific proteins to the nascent cyst wall. The monoclonal antibody GCSA-1, which was raised against purified cyst walls, recognizes protein species of ~25-46 kDa that are regulated by individual encystation stimuli (exposure to bile at alkaline pH). The GCSA-1 epitope is maximally expressed after ~14 hr of encystation and localizes to the interior, but not the membrane of the ESV by frozen section immunoelectron microscopy. To further understand the process of encystation, we compared two sublines of strain WB that differ in their ability to encyst *in vitro*. Water resistant cysts were not detected in subline A6 under conditions in which subline C6 formed ~2 X 10^5 cysts ml^{-1}. Moreover, subline A6 did not form ESV efficiently or detectably express antigens recognized by mAb GCSA-1 or by polyclonal anti-cyst sera. In other studies, we have shown that trophozoites take up bile salts by a novel active pathway that is similar to ileal bile salt transport in the host. Interestingly, uptake of the bile salt taurocholate by subline A6 was reduced 10 to 20 fold, compared with that of C6, although transport by both strains was sodium-dependent and regulated by bile salt starvation. This suggests that the decrease in bile salt transport by A6 may be related to its block in encystation.

399 THE CYSTEINE PROTEASE OF GIARDIA LAMBLIA IS A TARGET FOR STRUCTURE-BASED DRUG DESIGN. Ward WL* and McKerrow JH. Department of Anatomic Pathology, University of California, VAMC, San Francisco, CA.

Proteolytic enzymes are known to be key enzymes in the life cycle of many parasites. We are therefore investigating the cysteine protease of *Giardia lamblia*. We are specifically interested in the sequence and structure of the protease, its subcellular location and function, and whether or not inhibition of the protease will disrupt the life cycle of *G. lamblia*. A fragment of the gene was amplified by PCR using degenerate primers designed around conserved motifs flanking the active sites of other parasite proteases. This fragment was used as a probe to locate the intact gene in a genomic library. The resulting sequence shows marked homology to other parasite proteases, and displays features characteristic to giardia, including a presumptive TATAA box and S.D.-like box, lack of introns, and a stop codon shortly followed by AGTPuAAPyr. Studies of the structure, function and molecular evolution of the protein encoded by this gene are now underway. Using Z-F-R-MNA, a specific fluorescent substrate, we localized the cysteine protease to peripheral vacuoles, which release their contents during excystation of giardia. We have also shown that synthetic fluoromethylketones inhibit this protease in both cytoplasmic extracts and in cultures. The inhibitors do not significantly interfere with replication or encystation, suggesting that the primary role of the cysteine protease is in excystation.

400 AN ADULT MOUSE MODEL FOR *GIARDIA LAMBLIA*. Byrd LG, Conrad JT, and Nash TE*. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD.

To study the effect of antigenic variation on host immunity, nine isolates of *G. lamblia* were inoculated (400,000 trphozoites *per os)* into adult (4wk old) C3H/HeJ mice. Only the H7 clone of isolate GS (characterized by the presence of a 57-kDa viable surface antigen (VSP) recognized by the

monoclonal antibody G10/4) gave a viable infection as determined by microscopic examination of cultured intestinal contents. As few as 500 trophozoites could establish an active infection in 4 wk old c3H/HeJ mice, however 500,000 trophozoites gave more consistent infections. Three patterns of infection were observed when 4 wk old mice were inculated with GS-H7. DBA/2N mice resolved the infection after 2 wk. BALB/cAn and C3H/HeJ mice remained infected with small numbers of trophozoites up to 9 wk post inoculation (PI) and preliminary data suggest that C3H/HeJ mice remain infected even 27 wk PI. The immuno-incompetent SCID and nu/bg/xid mice also remained infected, however the number of trophozoites recovered was 10 fold greater than the number of trophozoites recovered from BALB/c or C3H/HeJ. The 57kDa VSP was present on 90-100% of the trophozoites recovered from BALB/c and C3H/HeJ 1 wk PI whereas 2 wk PI, the percentage of G10/4 (+) trophozoites fell to <10%. In contrast, trophozoites recovered from SCID mice were always 90-100% G10/4(+), even 10 wk PI. This mouse model may provide a means of discerning the differences in the immune responses responsible for these three infection patterns and may provide information on the function of antigenic variation and the role the immune system may play in the shift of surface antigens.

401 ISOLATION OF ADHESION DEFICIENT *GIARDIA LAMBLIA* CLONES WITH A REDUCED ABILITY TO ESTABLISH INFECTION IN MONGOLIAN GERBILS. Hernandez-Sanchez J and Ortega-Pierres MG. Department of Genetics and Molecular Biology, Center for Research and Advanced Studies IPN, Mexico, DF, Mexico.

A common factor in pathogenic mechanisms of giardiasis is the attachment of trophozoite to intestinal epithelial cells. This event is crucial to both initial colonization and maintenance of infection. Several factors have been proposed to participate in this event, among them are surface antigens. Thus, to analyze the role of such molecules on parasite attachment and pathogenicity we have selected clones deficient in adhesion to MDCK cells and characterized them. Clones C5 (wild), C6 (spontaneously deficient in adhesion) and C7 (isolated by mutagenesis with nitroso guanidine) were obtained from WB strain. Clones C6 and C7 showed a lower adhesion as compared with clone C5 and WB. Analysis of total antigens (TA) and surface labelled components (SLC) by One- and Two-dimensional electrophoresis showed a 200 kD component only in clone C5 and strain WB. A specific monoclonal antibody to this surface antigen reacted highly with clone C5 and and strain WB, but not with clones C6 and C7. This MAb inhibited significantly the adhesion of WB and C5 to MDCK cells but not adherence of clones C6 and C7. Clone C5 was also significantly more infective in Mongolian gerbils than clones C6 and C7.These results suggest that 200 kD antigen is involved in the attachment of trophozoites to MDCK cells and that deficiency on adhesion correlates with inability of trophozoites to colonize the epithelium.

402 QUANTITATION OF *GIARDIA* CYSTS AND *CRYPTOSPORIDIUM* OOCYSTS IN FECAL SAMPLES BY A DIRECT IMMUNOFLUORESCENCE ASSAY. Xiao L* and Herd RP. Department of Veterinary Preventive Medicine, The Ohio State Uinversity, Columbus, OH.

The lack of simple and sensitive quantitative tests has impeded epidemiological studies on *Cryptosporidium* and *Giardia* infections. A quantitative direct immunofluorescence assay (IFA) was developed for the detection of these two parasites in fecal samples, using an IFA kit from Meridian Diagnostics, Cincinnati, OH. Recovery rates of the IFA for *Cryptosporidium* oocysts in calf feces seeded with 1,000, 10,000, 100,000 and 1,000,000 oocysts were 14.8, 40.8, 84.2 and 78.2%, respectively. Coefficients of variation were high when numbers of oocysts in feces were low, but low when numbers of oocysts in feces were high. Nevertheless, IFA detected infection in all seeded samples. Recovery rates of the IFA for *Giardia* cysts in feces seeded with 1,000, 10,000 and 100,000 cysts per gram were 76.4, 96.9 and 89.6%, respectively. Coefficients of variation were 7.4-22.1%. By comparison, recovery rates of *Giardia* cyst by sucrose gradient flotation were 20.5, 51.2 and 42.9%, respectively. Cysts per gram obtained by sucrose gradient flotation with samples from infected livestock were only 49.1-

54.8% of those obtained by the IFA. Zinc sulfate flotation detected only 36.4% of infections when cysts per gram were <1,000. This IFA should be useful in epidemiological and control studies of these two parasites.

403 IL-12 PRODUCTION IN RESPONSE TO *LEISHMANIA MAJOR* BY RESISTANT AND SUSCEPTIBLE STRAINS OF MICE. Vieira LQ*, Wysocka M, Scharton TM, Afonso LC, Trinchieri G, and Scott P. Department of Pathobiology, University of Pennsylvania, Philadelphia, PA; and The Wistar Institute, Philadelphia, PA.

IL-12 induces interferon-γ production by T and NK cells and promotes differentiation of Th1 subsets *in vitro*. Since resistance to *Leishmania major* in mice is dependent upon the differentiation of Th1 cells, we investigated the capacity of cells from resistant (C3H/HeN) and susceptible (BALB/c) mice to produce IL-12 following infection. Similar levels of IL-12 were produced by both mouse strains 2 days after infection. In contrast, by 14 days resistant mice produced more IL-12 than susceptible animals. Since macrophages are a source of IL-12, the capacity of bone marrow-derived (BM) macrophages from both C3H/HeN and BALB/c mice to produce this cytokine was investigated. When stimulated *in vitro* with *Staphylococcus aureus*, a potent stimulator of IL-12 production, BM macrophages from both C3H/HeN and BALB/c mice produced IL-12 when infected *in vitro* with IL-12. However, BM macrophages of either strain of mouse produced negligible levels of IL-12 when infected *in vitro* with *L. major* in the peritoneal cavity, we were able to trigger IL-12 production consistently. Furthermore, *in vitro* infection of peritoneal macrophages also stimulated production of IL-12, although levels of IL-12 obtained with *L. major* infection were notably lower than those obtained with *S. aureus* stimulation. Taken together, these results suggest that in murine leishmaniasis IL-12 is being produced by the infected macrophage. They also suggest that the susceptibility of BALB/c mice to *L. major* is not related to an intrinsic inability to make IL-12, but rather may be related to the inhibition of IL-12 production as the infection progresses.

404 648 LEISHMANIA INFECTION OF HUMAN MONOCYTES IN VITRO INDUCES PRODUCTION OF TGFβ. Kanesa-thasan N*, Danielpour D, and Nacy C. Walter Reed Army Institute of Research, Washington, DC; and National Cancer Institute, National Institutes of Health, Bethesda, MD.

Mononuclear phagocytes constitutively release transforming growth factor β [TGFβ], a multipotent cytokine capable of suppressing antimicrobial effector activities. Infection of murine macrophages with the obligate intracellular parasite Leishmania enhances production of TGFβ. We show that infection of human PBMC with Leishmania major amastigotes induces a rapid 2-fold increase in production of TGFβ; augmented production results from monocytic populations, not lymphocytes. Replication of parasites within monocytes promotes greater release of TGFβ: production increases with time in culture and increased numbers of infective parasites, but not with addition of heat killed amastigotes. Ingestion of latex beads, zymosan, or killed gram negative bacteria *Franciscella tularensis* also fails to sustain an increase in monocyte TGFβ production. However, infection of monocytes with another intracellular parasite, *Toxoplasma gondii*, results in levels of production of TGFβ intermediate to those found in *Leishmania* infections. These results suggest that *Leishmania* parasites induce a rapid and specific TGFβ response in host monocyte/macrophages.

405 FAB FRAGMENTS OF THE MONOCLONAL ANTIBODY 4A4 TO *TRYPANOSOMA CRUZI* GP 83 NEUTRALIZE TRYPOMASTIGOTE BINDING AND ENTRY AND CONFER IMMUNOPROTECTION. Villalta F*, Smith C, Ruiz-Ruano A, Johnston D, and Lima MF. Division

of Biomedical Sciences, Meharry Medical College, Nashville, TN; and Department of Microbiology, Meharry Medical College, Nashville, TN.

An understanding of how *Trypanosoma cruzi* binds and enters mammalian cells is critical to the development of molecular means of intervention. The monoclonal antibody 4A4 (Mab 4A4) recognizes an epitope on the gp 83 of trypomastigotes required for trypomastigote attachment to heart myoblast cells. We investigated whether the monovalent Fab fragments of Mab 4A4 could neutralize trypomastigote infectivity *in vitro* and could transfer passive immunoprotection in Balb/c mice. Removal of N-linked oligosaccharides from the gp 83 abolished the recognition of this GPI-membrane anchored molecule by the Mab 4A4. Removal of N-linked oligosaccharides from ^{125}I labeled gp 83 also reduces its specific binding to heart myoblasts, indicating that a carbohydrate moiety is involved in *T. cruzi* recognition by heart cells. Monovalent Fab fragments of the Mab 4A4 strongly inhibit trypomastigote binding and prevent trypomastigote entry into heart myoblasts in a concentration dependent manner. Administration of these Fab fragments i.v. into Balb/c mice 2 hours before i.v. challenge with blood trypomastigotes followed by three i.p. doses of these Fab fragments resulted in a dramatic reduction in the levels of parasitemia. After 27 days, the mortality of the mice receiving irrelevant Fab fragments was 100%, whereas 0% mortality was observed in the group of mice receiving the Fab fragments of the Mab 4A4. These results indicate that the Mab 4A4 recognizes an immunoprotective sugar epitope on the trypomastigote cell adhesion molecule (gp 83) and neutralizes *T. cruzi* infectivity *in vitro* and *in vivo*. This sugar epitope may be part of an experimental molecular vaccine against *T. cruzi* infection.

406 *TRYPANOSOMA CRUZI* INFECTION AND IMMUNUNIZATION IN CLASS I AND CLASS II MHC DEFICIENT MICE. Tarleton RL*, Postan M, Grusby M, and Glimcher L. Department of Zoology, University of Georgia, Athens, GA; and Department of Cancer Biology, Harvard School of Public Health, Boston, MA.

We have previously shown that mice require both $CD4^+$ and $CD8^+$ T lymphocytes to survive acute infection with *Trypanosoma cruzi*. Mice depleted of either T cell subpopulation by antibody treatment, or gene knockout mice which lack $CD8^+$ T cells fail to control the parasitemia and succumb early in the acute phase of infection. In this study we have made use of class I, class II and class I and II MHC knockout mice to further characterize the role of $CD4^+$ and $CD8^+$ T cells in immunity to *T. cruzi* infection. As expected, class II^{--} mice are highly susceptible to *T. cruzi* infection, and have parasitemias and times of death similar to the class II^{--} mice. In addition, and like the class II^{--} mice, class II^{--} mice exhibit essentially no inflammatory response in the heart and skeletal muscle despite the presence of large numbers of parasite-containing pseudocysts. Unlike their class II^{--} counterparts, class II^{--} mice fail to generate high levels of IFN-γ in the serum, or increased IFN-γ production *in vitro* and have lower levels of IFN-γ mRNA in their splenic lymphocytes. In an attempt to generate chronically infected mice of the class II^{--} phenotype, we have infected mice with low virulence strains of *T. cruzi* or have immunized these mice with 8-methoxypsoralen-treated parasites prior to challenge with virulent *T. cruzi*. This immunization results in lower parasitemia and slightly increased longevity but no survival of greater than 60 days. These studies confirm the importance of both $CD4^+$ and $CD8^+$ T cells in immunity to experimental *T. cruzi* infection and suggest that stimulation of the $CD8^+$ T cell compartment may be essential for effective vaccination.

407 DIFFERENTIAL INFECTIVITY OF HUMAN MONOCYTES BY AMASTIGOTES AND PROMASTIGOTES OF *LEISHMANIA MAJOR* BASED ON FLOW CYTOMETRIC ANLAYSIS. Leiby DA*, McMahon-Pratt D, and Toro LA. Transmissible Diseases, American Red Cross, Rockville, MD; Epidemiology & Public Health, Yale University School of Medicine, New Haven, CT; and Bacterial Diseases, Walter Reed Army Institute of Research, Washington, DC.

In previous studies we described the development of techniques to identify monocytes infected with promastigotes of Leishmania major by flow cytometry (FACS). The present study was designed to compare the relative infectivity of monocytes by amastigotes and promastigotes of *Leishmania major* using FACS. Elutriated monocytes were infected *in vitro* with stationary phase promastigotes or amastigotes obtained from the footpads of *Leishmania*-infected scid mice. Scid mice were used as a source of amastigotes to eliminate background problems previously attributed to murine IgG associated with immunocompetent mice. Infected monocytes were analyzed by FACS at 24 hr postinfection as described previously. Monocytes infected with amastigotes or promastigotes demonstrated extensive intracellular staining specific for *L. major* following permeabilization. Promastigote-infected monocytes, however, also stained positively for *L. major* in the absence of permeabilization, indicating the presence of *Leishmania*-antigens associated with the cell membrane. Surface antigens were not detected on amastigote- infected monocytes under any conditions. When promastigote- infected monocytes were cultured for an additional 5 days, surface antigens of *L. major* were absent. At present, the source of the membrane associated antigen in promastigote-infected monocytes is under investigation, but it may be indicative of microbicidal activity present during infection with promastigotes that is absent with amastigotes.

408 THE ROLE OF THE *LEISHMANIA* SURFACE PROTEASE-gp63 IN COMPLEMENT FIXATION AND CELL ADHESION. Brittingham A*, Morrison CJ, McMaster RM, and Mosser DM. Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA; and Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

The major surface protein of *Leishmania* is a 63 kd glycoprotein (gp63), which has been implicated in the establishment of infection. It has been shown to be an acceptor site for complement deposition. It has also been suggested to mediate the direct attachment of the promastigote to its host cell via the macrophage complement receptor type 3, Mac-1 (CD11/18b). Finally, gp63 has been implicated in the proteolytic degradation of complement components. A CHO cell line transfected with pNUTgp63 and expressing gp63 on its surface was used to study the role of leishmania gp63 in cell adhesion and complement fixation. We demonstrate that gp63 expressing cells do not bind directly to purified Mac-1 in the absence of exogenous complement. In the presence of fresh non-immune serum, however, these cells do bind avidly to purified Mac-1. C3 fixation to gp63 transfected cells was studied by flow cytometry and immunofluorescent staining for C3 and C3 fragments. Complement fixation to gp63 transfected cells occurs in non-immune serum in the presence of EGTA/Mg^{++}. We have begun studies to examine the role of gp63 in regulating complement activation on these cells. Studies are underway to measure the form of C3 and the kinetics of C3 deposition on the surface of gp63 expressing cells.

409 T HELPER CELL CYTOKINE PROFILES AND B CELL IG ISOTYPE RESPONSES TO THE TRYPANOSOME VSG MOLECULE SUGGEST ALTERNATIVE CLASS SWITCH REGULATORY MECHANISMS. Schopf LR*, Schieifer KW, and Mansfield JM. Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI.

T helper cell responses to the trypanosome variant surface glycoprotein (VSG) molecule recently have been characterized in this laboratory. B10.BR mice infected with *Trypanosoma brucei rhodesiense* clones LouTat 1 and 1.5 mount a VSG type specific response that is mediated primarily by the Th1 cell subset in a $I\text{-}A^k$ restricted manner; the predominant cytokines produced are IFNγ and IL-2 but not IL-4. Since infected mice mount a significant T-dependent B cell response to VSG determinants, we examined such mice for the isotypes of VSG specific IgG produced in the context of the VSG specific Th1 cell responses. Isotype profiles of antibody to the VSG of LouTat 1 and 1.5 trypanosomes showed that IgG_1 and IgG_3 were the predominant responses; while some mice made detectable amounts of IgG_{2b} there was no evidence for a VSG specific IgG_{2a} response. These findings

were unexpected since the T helper cell responses predicted a predominant IgG_{2a} response. Our results suggest, therefore, that clonal stimulation, maturation and Ig class switching of B cells in trypanosomiasis may be mediated by non-classical combinations of cytokines or by unrelated mechanisms.

410 COMPARATIVE COMPLEMENT SELECTION IN BACTERIA CAN BE USED TO SCREEN FOR SELECTIVE INHIBITORS OF A PURINE SALVAGE ENZYME OF *PLASMODIUM FALCIPARUM*. Eakin AE*, Serrano AE, and Craig SP. Department of Biochemistry, University of Puerto Rico School of Medicine, San Juan, PR; and Department of Microbiology, University of Puerto Rico School of Medicine, San Juan, PR.

Escherichia coli strain SØ609, provided by Per Nygaard, was used to select for activity of recombinant human and malarial hypoxanthine phosphoribosyltransferases (hprt) and to screen for substrate analogues as selective inhibitors of these enzymes. In the initial screens, 61 purine analogues were tested under conditions where growth of the recombinant bacteria required complementation by activity of either the human or malarial hprt. Control bacteria either were grown on non-selective media or were transformed with sham plasmids. After overnight culture, the radii for the zones of inhibition of bacterial growth around impregnated discs revealed that 9 of the compounds were selective against the the malarial hprt, as compared to the human enzyme. Eight of these compounds possessed a related moiety at the 6 position of the purine base. This is consistent with a report that the 6 position plays a major role in enzyme activity in the hprt of *Plasmodium lophurae*, as described previously. Furthermore, two of the selective compounds identified in the recombinant screens (6-thio guanine and 6-mercaptopurine) previously were identified as "potent competitive inhibitors" of the malarial hprt., as described previously. These results strongly indicate that the recombinant approach identifies lead compounds that are selective inhibitors of hprt's. We are currently working to generate quantitative data (i.e. IC_{50}'s and K_i's) to support the results from the recombinant screens. Kinetic constants will provide estimates for minimum concentrations of the compounds needed for testing the compounds as inhibitors of the growth of *P. falciparum in vitro*.

411 EVALUATION OF THE ANTIMALARIAL ACTIVITY OF WR250417 (PS-15) USING THE *IN VITRO - IN VIVO* MODEL. Rieckmann KH*, Yeo AE, Edstein MD, Jacobus DP, and Canfield CJ. Army Malaria Research Unit, Ingleburn, Australia; Jacobus Pharmaceutical Company, Inc, Princeton, NJ; and Pharmaceutical Systems Inc, Gaithersburg, MD.

WR250417 (also known as PS-15), N-(3-(2,4,5-trichlorophenoxy- propyloxy)-N'(1-methylethyl)-imidocarbonimidic diamide hydrochloride, is a new, orally-active,dihydrofolic acid reductase (DHFR) inhibitor. It is metabolized *in vivo* to WR99210, a triazine inhibitor of DHFR which was shown to be extremely active against multidrug-resistant strains of *Plasmodium falciparum* more than 20 years ago. However, further development of WR99210 was abandoned because of severe gastro-intestinal symptoms associated with its use. Following the development of WR250417, studies were initiated to compare the antimalarial activity of serum specimens collected from 10 *Saimiri* monkeys that had received a single oral dose (30mg/kg) of either WR250417 or WR99210. The antimalarial activity was determined by observing the highest serum dilution inhibiting the *in vitro* growth of an isolate of *P. falciparum* obtained from continuous culture, relating this to the minimum inhibitory concentration of WR 250417 and WR99210 for that isolate, and converting the results into drug concentration equivalents of WR99210 (WR99210 is at least 100 times more active than WR250417 *in vitro*). The bioassay results showed that monkeys receiving WR250417 had very high serum antimalarial activity, presumably due to the rapid conversion of WR250417 to WR99210 after absorption from the gastrointestinal tract, and that effective antimalarial concentrations persisted beyond 48 hours after its administration. On the other hand, oral administration of WR99210 resulted in very low serum concentrations of the drug, probably associated with poor absorption. Estimation of serum concentrations of WR250417 and WR99210 by high performance liquid chromatography confirmed

the bioassay results. These findings indicate that WR250417 (PS-15), used in combination with other antimalarials, may eventually become a very useful drug for the treatment of multidrug-resistant falciparum malaria.

412 NEW, ANTIMALARIAL TRICYCLIC 1,2,4-TRIOXANES; PRECLINICAL *IN VIVO* EVALUATIONS. Posner GH*, Oh CH, Webster HK, and Rossan RN. Department of Chemistry, The Johns Hopkins University, Baltimore, MD; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Gorgas Memorial Laboratory, Panama, Panama.

The endoperoxide sesquiterpene lactone, artemisinin, and its semi-synthetic derivatives are becoming increasingly important as clinical antimalarial drugs with impressive activity against multidrug resistant (MDR) forms of *Plasmodium falciparum* (PF). Total synthesis of artemisinin is a complex and expensive process. Recently, we designed and synthesized a series of simple, tricyclic 1,2,4-trioxanes structurally related to artemisinin. Several of these compounds proved to be extraordinarily potent against PF *in vitro*. Now we have concluded initial preclinical studies with two of these synthetic trioxanes and have compared them to arteether (ethyl ether of artemisinin) using *Aotus* monkeys infected with MDR PF. Trioxanes WR 279137 (12 and 48mg/kg), WR 279138 (12 and 48 mg/kg) and arteether (48 mg/kg) were administered i.m. in three 12 hourly doses to *Aotus lemurinus lemurinis* (Panamanian owl monkeys) infected with Vietnam Smith/RE PF, and these monkeys were monitored for parasitemia. WR 279137 at 12 mg/kg cleared parasitemia in two monkeys but recrudescence occurred in one animal. Treatment of the recrudescent infection with 48 mg/kg was curative. The infections in two monkeys treated initially with 48 mg/kg were cured (four month follow-up). WR 279138 produced a similar outcome: 12 mg/kg suppressed parasitemia in two monkeys but was not curative; however, 48 mg/kg cured all four monkeys treated. No evidence of acute toxicity was noted. Arteether (48 mg/kg) cured the infections in both monkeys treated. These observations show these two synthetic trioxanes to be as effective as arteether against MDR PF in the *Aotus* monkey.

413 DIFFERENTIAL NEUROTOXICITY OF ARTEMISININ ANALOGS IN AN *IN VIVO* MODEL. Brewer TG*, Petras JM, Peggins JO, Li Q, Lin AJ, Sperry M, Figueroa L, Aguilar A, and Schuster BG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research Washington, DC; and Division of Neuroscience, Walter Reed Army Institute of Research, Washington, DC.

Several artemisinin (qinghaosu-QHS) derivatives are in use or development as antimalarial drugs. Two of these, arteether (AE) and artemether (AM) have been shown to cause a distinctive neurotoxicity in dogs and rats after multiple I.M. doses of 12.5 or 25.0 mg/kg/day for 7-14 days. In order to assess the potential for neurotoxicity of other analogs, we used the above-noted *in vivo* multiple dose model with administration of daily molar-equivalent doses by parenteral or oral gavage routes. Vehicle control, artesunate (AS), and artelinic (AL) acid were given at 15.0 and 30.0 mg/kg and 34.25 and 68.5 mg/kg, respectively, by both IM and PO routes (n = 4 each) for 14 days. Bioavailability was confirmed at similar doses for all drugs/routes by analysis of plasma drug levels using HPLC with reductive electrochemical detection. Dihydroartemisinin (DQHS), which is a common metabolite of AE, AM, AS and AL, was formulated for IM administration at isomolar doses of 11 and 23 mg/kg/day. Stained serial sections from perfusion-fixed brains of treated and control animals were compared to similar sections of AE and AM treated animals which were scored for cytopathology on a 0 to +4 ordinal scale. Compared to AE and AM, AL and AS had significantly less injury while DQHS showed a substantial increase in both the rate and severity of neuropathic lesions. The reasons of these striking differences are not known but could be due to differences in drug lipid solubility, chemical structural differences resulting in different receptor interaction, or differences in metabolism but are likely due to increased time exposure to DQHS at the neural tissue target.

414 IN VITRO NEUROTOXICITY OF ARTEMISININ ANALOGS. Wesche DL*, DeCoster M, Tortella F, and Brewer TG. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Division of Neuroscience, Walter Reed Army Institute of Research, Washington, DC.

Neurotoxicity of arteether (AE) and artemether (AM) has been previously reported in dogs and rats. Degenerative lesions characterized by hyalinization and loss of Nissl substance in a discrete regional brainstem distribution are suggestive of a specific neuronal target, but it is unclear whether the neuronal injury is a result of drug (or metabolite) interaction with membrane bound neurotransmitter receptors, intracellular enzymes, or intracellular organelles. We evaluated several *in vitro* models in order to further elucidate possible mechanism(s) of neurotoxicity which included: primary fetal rat neuronal cell cultures, fetal rat primary astrocyte cultures, and transformed neuronal cultures (rat-derived neuroblastoma NG108 and mouse-derived neuroblastoma Neuro-2a). Endpoints of toxicity were LDH release and/or radiolabelled-leucine uptake, respectively after incubation in presence of drug (or control) at graded concentrations from 10^{-10}M to 10^{-4} M. Initial results indicate that neurotoxicity is dose and time dependent for compounds tested. Acute exposure (45 min) to drug results in delayed, but not immediate, cell toxicity. Comparisons of relative potencies in NG108 cells indicate that both the sesquiterpene lactone and endoperoxide are necessary but not the sole determinants of activity; substitution at positions 9 and 10 of the artemisinin (QHS) backbone influences the degree of toxicity; and, dihydroqinghaosu (DQHS), a metabolite common to all QHS analogs currently being developed for clinical use, is the most potent of all analogs tested. Furthermore, DQHS is toxic to all three neuronal cell lines but not to fetal rat primary astrocytes. These results are consistent with a specific neuronal target. Studies are underway using these models to attempt to identify a causative molecular mechanism for neurotoxicity of these drugs.

415 PHARMACOKINETICS OF SODIUM ARTESUNATE AFTER IM AND IV ADMINISTRATION. Benakis A*, Paris M, Plessas C, Hien TT, Waller D, and White NJ. Department of Pharmacology, University Medical Center, Geneva, Switzerland; Center for Tropical Diseases, Cho Quan Hospital, Ho Chi Minh City, Viet Nam; and Wellcome-Mahidol University of Oxford Tropical Medicine Research Programme, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Artemisinin (ART) and its derivatives are a promising new group of anti-malarial drugs. Artemisinin is relatively insoluble and so a number of derivatives have been developed, in particular Artesunic acid (ARTES) a hemisuccinate of 12-hydroxyartemisinin. Sodium salt is water-soluble, which permits i.m. and i.v. administration. It has been reported that Dihydroartemisinin (DHART) is the major metabolite in plasma and it has been suggested that it is the active principle, while the Na ARTES is the prodrug. The aim of the present study was to establish the pharmacokinetic parameters of Na ARTES and DHART in patients with severe malaria. The study was conducted in 12 Vietnamese patients with severe falciparum malaria (6 for the i.v. and 6 for the i.m. administration, age 18 to 36 years) who received 2 mg/kg of Na ARTES (Guilin No 2, Pharmaceutical Factory, Guangxi, P. R. China). All responded well to treatment. Quantification of the two compounds Na ARTES and DHART was performed by HPLC with electrochemical detection in reductive mode. The detection limit was 10 ng/ml of plasma for both compounds. After i.m. administration, the C_{max} value was 0.51 μg/ml for Na-ARTES and 0.39 μg/ml for DHART. After 90 min, the concentration were respectively 0.11 μg/ml and 0.20 μg/ml. The pharmacokinetic parameters were as follows: Na ARTES: constant rate of biotransformation: 12.75±4.67 h^{-1}; half life of biotransformation: 0.055±0.021 h; constant rate of elimination: 1.42 ±0.12^{-1}; half life of elimination: 0.49±0.04 h. ForDHRT: constant of apparent rate of biosynthesis: 3.86±1.29 h^{-1}, half life of biosynthesis: 0.19±0.05 h; constant rate of elimination: 0.44±0.04 h^{-1}; half life of elimination: 1.59±0.15 h. After i.v. administration of Na ARTES the DHART values: C_{max} 2.64 μg/ml for Na ARTES and 2.02 μg/ml for DHART. At 90 min the Na ARTES was not detectable and DHART was found to be 0.19 μg/ml.

416 PRIMAQUINE ADJUNCT TO 28 DAY EVALUATION OF HALOFANTRINE VS. CHLOROQUINE FOR THERAPY OF MALARIA IN PEOPLE REMAINING EXPOSED TO INFECTION IN IRIAN JAYA. Fryauff DJ*, Baird JK, Basri H, Bangs MJ, Wiady I, Purnomo, Masbar S, Tjitra E, and Hoffman SL. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Institute of Research of Infectious Diseases, Jakarta, Indonesia; and Naval Medical Research Institute, Bethesda, MD.

Reinfection and relapse among residents of endemic areas confounds analyses of the efficacy of blood schizonticidal antimalarial agents. We employed primaquine therapy and prophylaxis in a 28 day post-therapeutic evaluation of halofantrine versus chloroquine for treatment of vivax or falciparum malaria among non-immune transmigrants from Java living in the hyperendemic Arso region of Irian Jaya, Indonesia. The study began in January 1993 and continues to October 1993. As of May 1993, 28 subjects had completed the evaluation. All of these subjects were experiencing their first parasitemia since arriving in Irian Jaya. They were randomized as to therapy with chloroquine (25 mg/kg in 3 doses over 48 hours) or halofantrine (24 mg/kg in 3 equal doses at 6 hour intervals). One day after beginning blood schizonticidal therapy, all subjects began a regimen of 0.5 mg/kg primaquine daily for 14 days, followed by the same dose every other day for 14 more days. The table below summarizes the data available in May 1993:

Recrudescences During 28 Days Follow-Up

	Halofantrine	Chloroquine
P. falciparum	0/7	8/9
P. vivax	0/9	0/3

This study illustrates the utility of the primaquine adjunct in the conduct of 28 day post-therapeutic evaluations of blood schizonticidal agents in endemic areas. The preliminary data show halofantrine may be highly efficacious against chloroquine- resistant *P. falciparum*, and the drug also seems efficacious against *P. vivax*.

417 CAUSAL PROPHYLAXIS USING PRIMAQUINE IN NON-IMMUNE TRANSMIGRANTS IN THE ARSO REGION OF IRIAN JAYA, INDONESIA. Baird JK*, Fryauff DJ, Basri H, Bangs MJ, Wiady I, Purnomo, Masbar S, Richie T, Tjitra E, Subianto B, Jones TR, and Hoffman SL. U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia; Institute of Research of Infectious Diseases, Jakarta, Indonesia; Provincial Health Service Jayapura, Irian Jaya, Indonesia; and Naval Medical Research Institute, Bethesda, MD.

A trial of causal prophylaxis by primaquine was conducted using transmigrants from Java who just arrived in Irian Jaya. The incidence rate among 45 males taking 0.5 mg primaquine base per kg body weight every other day between December 1992 and April 1993 was measured and compared to the same among 54 other newly arrived males taking supervised weekly chloroquine prophylaxis (5 mg base/kg bw) in the same village over the same period. A total of 35 infections occurred in this cohort of 99 transmigrants followed for 1473 person weeks: 30 among the people taking chloroquine (18 *P. falciparum*, 12 *P. vivax*) and 5 among those taking primaquine (4 *P. falciparum*, 1 *P. vivax*). The incidence density ratio (IDR) for infection in the face of chloroquine vs. primaquine for prophylaxis was 3.96 for *P. falciparum* ($P<0.015$) and 10.56 for *P. vivax* ($P<0.012$). Primaquine was better tolerated than chloroquine; the IDR for weekly survey of physical complaints not associated with parasitemia was 5.08 ($P<0.00001$) for chloroquine versus primaquine. The IDRs for headache (5.86, $P<0.00001$), stomach ache (6.97, $P<0.005$) and malaise (14.6, $P<0.002$) largely accounted for this difference. Liver function tests and cell blood counts were normal among people taking primaquine. Causal prophylaxis using primaquine was more efficacious and better tolerated than standard chloroquine prophylaxis among non-immune transmigrants living in the Arso region of Irian Jaya, Indonesia.

418 EXTENDED DOSE HALOFANTRINE FOR DRUG-RESISTANT FALCIPARUM MALARIA. Watt G *, Loesuttiviboon L, Jongsakul K, Shanks GD, Ohrt C, Karnasuta C, Schuster B, Fleckenstein L. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Surasinghanat Army Hospital, Aranyaprathet, Thailand; Army Malaria Research Unit, Ingleburn, Australia; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Mefloquine is rapidly losing efficacy against Thai strains of *Plasmodium falciparum* and conventional doses of halofantrine are ineffective. We therefore used pharmacokinetic simulation to design an extended dose halofantrine regimen and tested it in 26 soldiers stationed along theThai-Cambodian border. Halofantrine was given after meals as 3 doses of 500 mg each at 4h intervals on the first day followed by 500 mg daily for 6 days (total dose 4.5 g). Twenty-six soldiers treated with quinine-tetracycline served as controls. There were no significant differences in efficacy between halofantrine and quinine ($p>0.1$) as assessed by cure rate (92% vs 85%), mean parasite clearance time (82h vs 81h) or mean fever clearance time (93h vs 99h). Halofantrine was better tolerated than quinine. The side effects score was lower (2 vs 11; $p<0.001$), there were less days on which side effects occurred (2.0 vs. 5.5 days; $p<0.001$), and fewer patients had adverse effects on every treatment day (4% vs 42%; $p<0.01$). High dose halofantrine is as effective and better tolerated than quinine-tetracycline for multidrug-resistant falciparum malaria.

419 MALARIA IN U.S. ARMY SOLDIERS DURING AND AFTER OPERATION RESTORE HOPE. Smoak BL*, Defraites RF, Magill AJ, and Wellde BT. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington DC; and Department of Immunology, Walter Reed Army Institute of Research, Washington DC.

Mefloquine was used for malaria chemoprophylaxis in all Army personnel except aviators and persons for whom it was contraindicated. Primaquine was not universally recommended for terminal prophylaxis because the threat of infection with *Plasmodium vivax* was considered low. Compliance with chemoprophylaxis during Operation Restore Hope was good; only 10 cases occurred in Army troops. From February to early June 1993, 62 soldiers who recently returned from Somalia were treated for malaria. All but 7 were stationed at Fort Drum, NY. Complete laboratory, clinical, and epidemiological data are available for the initial 32 cases that occurred at Ft Drum. Based on thick/thin smears, 27 cases had infections with *P. vivax*, 3 with *P. falciparum*, and two had mixed infections. All were men aged 18 to 37 years. Most of the cases (84%) had spent time in the Jubba river valley, although 5 soldiers had not and had been in various towns and cities throughout Somalia. The soldiers were ill an average of 4 days prior to seeking medical help. Symptoms began an average of 60 days after leaving Somalia and 39 days after the last reported dose of mefloquine. All cases were treated with oral medications, but 2 cases with *P. falciparum* infections had hematocrits of 20 and one had pulmonary edema. As a result of the large proportion of *P. vivax* infections, primaquine is now recommended for all returning troops.

420 MALARIA CIRCUMSPOROZOITE PROTEIN BINDS TO HEPARAN SULFATE PROTEOGLYCANS ON THE SURFACE OF HEPATOCYTES. Frevert U*, Sinnis P, Cerami C, Shreffler W, Takacs B, and Nussenzweig V. Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY; Michael Heidelberger Division of Immunology, Department of Pathology, New York University Medical Center, New York, NY; and F. Hoffmann-La Roche Ltd., Basel, Switzerland.

Minutes after transmission by infected mosquitoes, malaria sporozoites enter the host's hepatocytes. The specific interaction between the region II-plus of the circumsporozoite protein (CS) and heparan

sulfate proteoglycans (HSPG) on the basolateral surface of hepatocytes may provide an explanation for the selectivity and speed of the host cell invasion. The CS/HSPG interaction can be abolished by previous treatmentof the hepatocytes with heparitinase, but not with chondroitinase ABC. Correspondingly, heparin, but not chondroitin sulfate inhibits the binding suggesting that the interaction is mediated by the glycosaminoglycan (GAG) chains of the proteoglycan. Besides in the liver, CS binding sites are found only in the granules of connective tissue mast cells, which contain heparin, and on certain basolateral membranes and laminae rarae of seleted basement membranes in the kidney,which are rich in heparan sulfate. These locations, however, are not available for sporozoites travelling in the blood circulation. The purified CS-binding proteoglycan from HepG2 cells has a molecular weight of 400-700 kDa. It is tightly associated with the plasma membrane of HepG2 cells and can be released by mild trypsin treatment. The CS receptors share these properties with the syndecan family of integral membrane proteoglycans.

421 THE IN VITRO DEVELOPMENT OF *PLASMODIUM YOELII* EXOERYTHROCYTIC FORMS IN HUMAN HEPATOMA CELL LINES. Calvo Calle JM*, Moreno A, Frevert U, and Nardin E. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

The study of exoerythrocytic stages of the rodent malaria parasite, *Plasmodium yoelii*, and the human malaria *P. falciparum*, has been hampered by the lack of an *in vitro* culture system. Unlike *P. berghei*, sporozoites of *P. yoelii* and *P. falciparum* invade but do not develop in the Hep-G2 human hepatoma cell line. To define an *in vitro* system for the growth of exoerythrocytic forms (EEF) of *P. yoelii*, we used a panel of human hepatoma cell lines to study sporozoite invasion and EEF development. As earlier described, the Hep-G2 cell line supported the growth of *P. berghei* and *P. vivax* EEF, but not *P. yoelii* or *P. falciparum*. In contrast, two cell lines, HuH1 and HuH2 were found to support not only low numbers of EEF of these malaria species, but also*P. yoelii* sporozoite invasion and EEF development. Preliminary data suggest that *P. vivax* and *P. falciparum* EEF also grow in these two lines. Mice injected with *P. yoelii* EEF cultures developed patent parasitemias showing that infective EEF were produced in the hepatoma cells. Electron microscopy was used to compare the development of *P. yoelii* EEF in hepatoma cells and in murine primary hepatocytes. The ability to grow EEF of *P. yoelii* and *P. falciparum in vitro* will facilitate the identification of protective EEF antigens for inclusion in vaccines aimed at the pre-erythrocytic stages of the malaria parasites.

422 CEREBRAL MALARIA IN MICE: VASCULAR ADHESION OF RBC AND MICRORHEOLOGIC CHANGES. Kaul DK, Nagel RL, and Shear HL*. Division of Hematology, Albert Einstein College of Medicine-Montefiore Hospital, New York, NY.

We have studied the lethal strain of *Plasmodium yoelii* 17X (Py17XL), originally described by Yoeli and Hargreaves in 1974, to understand the microcirculatory changes during cerebral malaria. This model more closely resembles human cerebral malaria than the *P. berghei* ANKA infection because it shows little, if any, inflammation in the brain. Mice were injected intraperitoneally with 5x105 parasitized erythrocytes. When the mice reached approximately 30% parasitemia, they were studied *in vivo* using the cremaster muscle preparation. Under video monitors, red cell adherence was observed in small diameter venules in the cremaster muscle. In addition, the parasitized animal demonstrated reduction of venular red cell velocities compared with uninfected controls. When washed red cells from infected mice were infused into the rat mesocecum preparation, there was again significant adhesion of these cells to the venular endothelium. This was accompanied by a higher peripheral resistance. Light and electron microscopy of the brain and the cremaster muscle showed close association of infected erythrocytes to the endothelium of small venules. These observations closely mimic the cytoadherence *P. falciparum*-infected red cells t venular endothelium. The *P. yoelii* 17XL model should allow detailed analysis of the molecular mechanisms involved in the adhesion of malaria-infected erythrocytes to the endothelium.

423 IN VITRO CYTOADHERENCE OF *PLASMODIUM FRAGILE* TO BRAIN RHESUS ENDOTHELIAL CELLS. Krogstad DJ*, Krogstad FM, Didier PJ, Malmstrom SL, Collins WE, and Aikawa M. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; Tulane Regional Primate Research Center, Covington, LA; Centers for Disease Control, Atlanta, GA; and Case Western Reserve University School of Medicine, Cleveland, OH.

Plasmodium fragile undergoes deep vascular schizogony in the rhesus monkey, and produces altered consciousness and central nervous system pathology similar to human cerebral malaria. These studies were performed to determine whether cytoadherence of *P. fragile* occurred with rhesus brain endothelial cells. Rhesus brain endothelial cells were obtained by mechanical sieving and collagenase digestion of post-mortem material, followed by plating in Medium 199 with 20% Fetal Bovine Serum containing endothelial cell growth factor and heparin (the 1B195 primary cell culture). The assay was performed by incubating 2% suspensions of rhesus red cells (at parasitemias of 5-7%) in equal parts of RPMI-1640 and Medium 199 plus 20% Fetal Bovine Serum with the endothelial cell monolayers for 90 minutes at 37o in glass slide chambers. After rinsing with Hanks' Balanced Salt Solution and fixation with 0.1% buffered formalin, more than 90% of adherent cells were parasitized ($p < 0.001$). These results indicate that *P. fragile* adheres to rhesus brain endothelial cells *in vitro*. They suggest that this cytoadherence is an *in vitro* correlate of the phenomena responsible for cerebral malaria *in vivo*, and that it should permit the testing of potential interventions under direct observation *in vitro*.

424 MAINTENANCE OF *PLASMODIUM FALCIPARUM* IN THE PERITONEAL CAVITY OF NOD SCID MICE. Moore JM*, Shultz LD, and Rajan TV. Departments of Pathology and Microbiology, University of Connecticut Health Center, CT; and Jackson Laboratory, Bar Harbor, ME.

Detail investigation of the interaction between the malarial parasite and the host immune system have been hampered by the absence of suitable animal models. The human malarial parasites have an obligate requirement for human red blood cells (hRBC) for growth. A suitable animal model must therefore be capable of tolerating the presence of hRBC for prolonged periods of time. In attempting to develop the SCID mouse as a model for human malaria, we were faced with two obstacles. When hRBC were injected into SCID mice, we found that they were cleared quantitatively and rapidly. Secondly, mouse serum appeared to be extremely toxic to human malarial parasites. To overcome the first obstacle, we determine whether all strains of mice homozygous for the scid mutation were as efficient at clearing red cells as the C.B.-17 scid/scid strain. Of various strains tested, the NOD SCID mouse was the best, in that human red cells could be detected in circulation for as long as one week following intraperitoneal (ip) injection. To overcome the second obstacle, we have gradually adapted *Plasmodium falciparum* cultures to grow in increasing amounts of mouse plasma or ascites fluid. We have recently injected cultures of *P. falciparum* that have been adapted to grow in 90% RPMI 1640 + 3% human serum + 7% mouse ascites fluid. Mice were also given supplemental ip injections of human red blood cells daily. Circulating human red blood cells containing *P. falciparum* ring, schizont and gametocyte stages were found in circulation for as long as nine days following intraperitoneal injection.

425 EFFECT OF ERYTHROCYTE MEMBRANE ON THE EXTRACELLULAR DEVELOPMENT OF THE ERYTHROCYTIC CYCLE OF *PLASMODIUM FALCIPARUM*. Williams JH, Gill GS, and Trager W. The Rockefeller University New York, NY.

Plasmodium falciparum is an important pathogenic intracellular protozoan. We previously reported the extracellular development of the erythrocytic cycle of *Plasmodium falciparum*. The initial

development of merozoites to rings was fairly high (10%-30%). However, the number of parasites that developed to late -stage trophozoites and early schizonts was too low (1% of initial merozoites) for cultivation and continued study. We supplemented the extracellular medium by increasing the erythrocyte membrane content (cell membrane, cytoskeleton and surface proteins) to increase the number of late-stage parasites. This was accomplished by lysing erythrocytes with hypotonic phosphate buffer and washing the ghosts with potassium-RPMI-10% serum to produce membranes that were placed in a 60% erythrocyte suspension. This suspension was used to prepare the red cell sonicate-ATP-pyruvate medium previously reported. Merozoites were suspended in supplemented red cell sonicate and mixed with Matrigel to form a soft gel. The gel was overlaid with the membrane supplemented red cell sonicate-ATP-pyruvate medium. The medium was replaced and the gels sampled as previously described. The membrane supplemented sonicate medium did not have a significant effect at 12 hours; at 36 hours however it showed a 50%-100% increase in late-stage trophozoites and early schizonts over the control unsupplemented medium. These data suggest components of the erythrocyte ghost cells are necessary for late-stage development.

426 CA^{2+} AND H^+ HOMEOSTASIS IN *PLASMODIUM FALCIPARUM*. Mikkelsen RB* and Dyer M. Departments of Radiation Oncology and Microbiology/Immunology, Medical College of Virginia, Richmond, VA.

Using digitized video-intensified fluorescence microscopy and the fluorescent dyes, BCECF and fura-2, the cytosolic pH and free $[Ca^{2+}]$, respectively, of both the erythrocyte and parasite compartments of *Plasmodium falciparum* infected erythrocytes were determined. Parasite cultures were synchronized by sorbitol lysis. *In situ* calibration of dye fluorescence with pH or $[Ca^{2+}]$ utilized nigericin and ionomycin to equilibrate transmembrane ion gradients. The average parasite cytosolic pH increased from 6.8 at early rings to 7.1 with trophozoites and schizonts. The erythrocyte cytosolic pH remained constant at 7.1. With free parasites isolated by N_2-cavitation, recovery from nigericin or NH_4Cl induced acidification of parasite cytosol was completely dependent on the presence of Na^+ and blocked by amiloride analogues that inhibit Na^+:H^+ exchange. Anion channel inhibitors and the ATPase inhibitor, orthovanadate, were without effect. Ca^{2+} measurements revealed a relatively constant free $[Ca^{2+}]$ = 50-100 nM in the erythrocyte cytosol and 150-200 nM in the parasite cytosol throughout parasite maturation. On-going studies are investigating regulatory mechanisms for parasite cytosolic free $[Ca^{2+}]$.

427 SEROLOGICAL STUDY ON HUMAN CYSTICERCOSIS IN BALI. Theis J *, Goldsmith RS, Flisser A, Koss J, Chioino C, Plancarte A, Segura A, Widjana D, and Sutisna P. University of California, San Francisco, CA; Universidad Nacional Automa, Mexico City, Mexico; Arizona State University, Scottsdale, AZ; and Universitas Udayana, Bali, Indonesia.

Taenia solium and *T. saginata* infections in humans are occasionally reported in Bali. The three stool surveys for taenia eggs and proglottides conducted during the past 15 years indicated positive rates of 3, 5 and 23%, respectively, with an apparent predominance of *T. saginata* infections. Only rarely, however, has the diagnosis of cysticercosis been confirmed radiologically or parasitologically. This study was undertaken to determine the prevalence of antibody in patients with epilepsy and among persons tested in a serosurvey conducted in four regions of the island. The serologic test was the highly specific and sensitive immunoelectrotransfer western blot assay (AF). 55% of the positive sera reacted to glycoprotein 50, 31% to GP 42, 6% to GP 18, and 1% to GP 13, 14, or 21. Of the 75 patients with epilepsy, 14% were seropositive. The four regions in the survey were: low/wet (274 persons), high (>500 m)/wet (181), low/dry (217), and high/dry (74). Of the 746 persons (<1 to >60 yrs, 45% male) tested, 13% were positive. Antibody prevalence was uniformly distributed in the four regions and was similar regardless of age class, gender, or urban-rural status. Preliminary medical-anthropologic evaluation indicates that both raw pork and beef are eaten frequently in Bali, both as domestic and ceremonial dishes, and that pigs in rural areas commonly roam freely. Thus, taeniasis

solium infection and cysticercosis appear to be endemic and widespread in Bali and to have a significant impact on human health.

428 AN EPIDEMIOLOGICAL STUDY OF *TAENIA SOLIUM* TAENIASIS AND CYSTICERCOSIS IN TWO GUATEMALAN COMMUNITIES. Garcia-Noval J, Fletes C, Moreno E, Mencos F, de Mata F, Torres R, Allan JC*, and Craig PS. Facultad de Medicina, Universidad de San Carlos, Guatemala; Hospital General, Instituto Guatemalteco de Seguridad Social, Guatemala; Hospital San Juan de Dios, Guatemala; and Department of Biological Sciences, Salford University, Salford, UK.

Taenia solium taeniasis/cysticercosis is endemic in Guatemala. Although neurocysticercosis is increasingly being diagnosed in neurological wards there have been no studies conducted on this parasite in rural areas of the country. The current study involved two communities in the Department of Juitiapa, Quesada and El Jocote. These were selected as they were very close together but differed markedly in socioeconomic terms. For instance 39% (446/1137) of individuals in El Jocote had no proper toilet, in Quesada this figure was 4.8% (57/1197). Sera were analyzed by Western blot against purified *T. solium* specific glycoprotein antigens. 29.5% of individuals in El Jocote and 12% in Quesada were seropositive although in the latter village analysis is still underway. In both communities around 4.5% of the population were epileptic, as detected by a detailed questionnaire. 80% of CT scans on these individuals show some abnormality with calcifications suggestive of neurocysticercotic lesions in 48% (30/61) of the individuals tested thus far. Intestinal taeniasis was diagnosed by microscopy and by a dipstick dot ELISA for *Taenia* coproantigens. 2.9% (28/955) of individuals in El Jocote and 1% (9/860) in Quesada were infected. After chemotherapy 26 worms were identified as *T. solium* and 1 as *T. saginata*. Pigs were maintained in both villages. 14% (14/100) were positive for *T. solium* cysts in the tongue in El Jocote and 4% (1/25) in Quesada. Risk factors for taeniasis and cysticercosis will be identified. For instance females are significantly more likely to be seropositive or infected with intestinal taeniasis. *T. solium* is a significant parasitic infection in rural Guatemala.

430 LONG-TERM FOLLOWUP OF ALBENDAZOLE TREATMENT OF HYDATID DISEASE. Nahmias J*, Goldsmith RS, Soibelman M, El-On J. University of California, San Francisco, CA; and Ben Gurion University of the Negev, Israel.

In short-term followup studies of hydatid disease treated with albendazole, many patients (10-43% for liver cysts) have shown radiologic regression or disappearance of their cysts. As yet, however, there have been few followup studies that extended to 2 years or longer. We report on 59 patients (ages 8 to 63) followed for a minimum of 2 years (range, 2-5 years). Diagnosis was by cyst imaging (maximum size: liver, 10 cm; lung, 3 cm) plus one or more positive serologic tests. The albendazole dosage was 800 mg/d in 2 divided doses (8 persons ages 8 to 14 years were treated with 20 mg/kg/d) given as 4 sequential 30-day courses, with 14-day intervals between courses. The findings for 51 patients with liver cysts (16 had multiple cysts) were: 45% cured (all cysts disappeared), 12% possibly cured (>50% contraction, complete consolidation; unchanged after 12 additional months), 31% improved, and 12% no change. Of 11 patients with lung cysts (4 had multiple cysts; 5 also had liver cysts): 72% cured, 9% improved, and 18% no change. No patient worsened during treatment, albendazole was well tolerated except for marked transaminase elevations in one patient, and there were no recurrences during followup. These findings indicate that medical treatment alone is often successful. The time required to recognize cyst changes was longer than previously described: The mean time from the end of treatment to first detection of liver cyst changes was 9.2 months (range, 3-36 months); for lung cysts, 13.4 months (range, 3-24 months). The mean time from end of treatment to first detection of cure for both groups of patients was 22.5 months (range, 12-60 months).

431 EVALUATION OF AN IMMUNOASSAY FOR THE SEROLOGICAL DIAGNOSIS OF CYSTICERCOSIS IN HUMANS. Rosenblatt JE*, Sloan LM, and Schneider SK. Division of Clinical Microbiology, The Mayo Clinic, Rochester, MN.

We evaluated a commercially available (LMD Laboratories, Carlsbad, CA) enzyme linked immunoassay (ELISA) for the detection of antibodies in serum to the cysticerci of *Taenia solium*. This qualitative screen is a rapid test performed in microtiter wells coated with porcine cyst fluid as the antigen and employs Protein A-peroxidase and TMB as an indicator system. The ELISA was performed on 242 serum sample; 198 from a pool of normal healthy individuals and 44 from patients in whom the diagnosis of cysticercosis was suspected. ELISA results from these patient serums were compared with those obtained by a reference laboratory (CDC) using an immunoblot assay. Ten of the 44 patient serums were positive by both immonoblot assay and ELISA while 30 were negative by both. Two "false positive" and two "false negative" ELISA results were noted. No clinical evidence of cysticercosis could be found in the histories of any of these four patients; thus the two "false negatives" may actually be "true" negatives. Nine of 198 normal healthy serums were also found to be "falsely positive". The ELISA rapid, easy-to-read test with a specificity of 94%, sensitivity of 83%, and positive predictive value of 83% when compared to the immunoblot assay. The sensitivity would improve to 100% if the two questionable ELISA "false negatives" were eliminated.

432 IMMUNOBLOT SURVEYS SHOW *TAENIA SOLIUM* CYSTICERCOSIS TO BE A SIGNIFICANT GLOBAL PUBLIC HEALTH PROBLEM. Tsang VC*, Pilcher JB, Singhal BS, Gilman R, Wei GZ, Garcia F, Roman G, Geerts S, Asch H, Lee RV, Schantz PM, and Bryan RT. Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

The immunoblot assay (EITB), which detects cysticercosis-specific antibodies to seven glycoprotein antigens, has been employed extensively, under field conditions, for five years. The specificity for this test remained at 100%, sensitivity for cases with more than 2 cysts is 97%, and 1 cyst is 60% -80%. This assay has been proven to be an important, practical, and useful alternative to radiology (CT) and magnetic imaging for the diagnosis of cysticercosis. As a survey tool, it was invaluable in establishing the seriousness of this significant public health problem. Examples of several international surveys are summarized:

Communities	% EITB +	No Surveyed
a Amazon delta	0	130
b Bolivia (rural)	28	159
c Bolivia (pigs)	38.9	193
d Peru (rural)	11	371
e Peru (urban)	0	377
f Peru (pigs)	33	133
g Mexico (2 rural comm)	4.8-10.8	2,525
Neurological patients		
a P.R. China (Beijing)	44	198
b India (Bombay)	32	107
c Rwanda (epileptic)	21	34
d Mexico (epileptic)	11	271
e Peru (epileptic)	19	578

Survey data from Peru, China, and India will be discussed in detail to illustrate the extent of cysticercosis as a public health problem.

433 FAMILY CLUSTERING OF NEUROCYSTICERCOSIS, SAN PABLO DEL LAGO, ECUADOR. Cruz I*, Cruz M, Canelos P, Schantz PM, and Roman G. Ecuadorean Academy of Neurosciences, Quito, Ecuador; Parasitic Diseases Branch, National Center For Infectious Diseases, CDC, Atlanta, GA; and Neuroepidemiology Branch, National Institute of Neurologic Diseases and Stroke, NIH, Bethesda, MD.

To test the hypothesis that intrafamilial dissemination of *Taenia solium* eggs from carriers among family members or household employees may be a more important source of infection than other possible direct or indirect routes in the endemic environment, 80 household contacts of 23 persons with symptomatic neurocysticercosis confirmed by computed tomography (CT), were investigated in an Andean community. All were subjected to a complete medical questionnaire, a physical and neurological examination, intracerebral CT imaging, and stool examination. CT images compatible with neurocysticercosis were detected in 18 (22.5%) of asymptomatic family members. Thirteen (56%) of the 23 families had more than one member infected with cerebral cysticercosis; in ten families (43%) there was a clinical history of tapeworm expulsion in at least one member, but stool examinations failed to reveal any current *Taenia* infections. Among 118 household members of randomly selected families from the same community without a member with symptomatic neurocysticercosis, intracerebral CT images compatible with cysticercosis were found in 8 (6.8%) persons (OR 3.99, CI 1.53- 10.7, P<.005). Household contact with a *Taenia* carrier appears to be an important risk factor for neurocysticercosis in endemic communities.

434 CYSTICERCOSIS SEROPREVALENCE IN AN ORTHODOX JEWISH COMMUNITY IN NEW YORK CITY. Moore AC*, Lutwick LT, Schantz PM, Pilcher J, Wilson M, Fried J, Ware D, Haichou X, Hyon S, Chapnick EK, Abter EI, and Grossman JR. Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; and Maimonides Medical Center, Brooklyn, NY.

Neurocysticerosis cases were identified in 1991 in four unrelated families in an orthodox Jewish community in New York City. Transmission was linked to tapeworm-infected housekeepers who had emigrated from Latin American countries where *Taenia solium* is endemic. To evaluate the extent of and risks for locally-acquired cysticercosis, a seroprevalence survey was conducted on approximately 8% of the community. The immunoblot assay for cysticercosis antibodies in 1789 persons from 612 families was positive in 1.3%. All 23 seropositive individuals were asymptomatic; further evaluation by intracerebral neuroimaging is in progress. Seropositivity was associated with female sex (relative risk = 2.45, p<.05) but not with age, household size, or travel history. Three seropositive persons were clustered in one family. Results show that exposure in this community is unexpectedly high. Practices contributing to exposure risk include employment of immigrant housekeepers (82% of all households; 92% of households with seropositivity) and high employee turnover (5.9 per household per 5 years). Further studies are needed to define tapeworm carriage rates and criteria for pre-employment screening of domestic workers from *T. solium*-endemic areas.

435 A TAENIACIDAL DOSE OF PRAZIQUANTEL PROVOKED NEUROLOGICAL SYMPTOMS IN A PERSON WITH PREVIOUSLY UNDIAGNOSED NEUROCYSTICERCOSIS. Flisser A*, Madrazo I, Plancarte A, Schantz P, Allan J, Craig P, and Sarti E. Facultad de Medicina, UNAM, Mexico D.F., Mexico; Hospital de Especialidades, CMN Siglo XXI, IMSS, Mexico D.F., Mexico; Parasitic Diseases Branch, DPD, NCID, CDC, Atlanta, GA; Department of Biological Sciences, University of Salford, United Kingdom; and Direccion de Epidemiologia, Secretariat of Health, Mexico D.F., Mexico.

We report a case of neurocysticercosis that became symptomatic after the administration of praziquantel (pzq) for mass treatment of taeniasis during an intervention trial for the control of *Taenia solium*. Fourteen out of 2452 persons who received pzq at 5 mg/kg body weight developed headaches; five of these, whose headaches lasted 6 to 10 days were examined by MR brain scans.

Many small enhancing images of viable cysticerci were seen in the MR scan of one person, a 15-year-old girl, who girl reported having had recurrent mild headaches for 5 years. Her mother had taeniasis as demonstrated by stool examination, a positive fecal antigen assay, and identification of *T. solium* in her feces following pzq treatment. The girl was given pzq at 50 mg/kg body weight for 15 days with corticosteroids. A repeat MR scan, performed 6 months post treatment, revealed that all images of cysticerci, except one, had disappeared. During treatment her headaches ceased and she remains well. In conclusion, we recommend close surveillance for possible adverse reactions in populations receiving mass taeniacidal treatment because praziquantel, even at a dose 150 times less than recommended for treatment of cysticercosis, was capable of inducing neurologic symptoms in a previously undiagnosed case of neurocysticercosis.

436 INTERLEUKIN-1 IN *BIOMPHALARIA GLABRATA*: INCREASED HEMOCYTE SUPEROXIDE PRODUCTION AND DECREASED OUTPUT OF *SCHISTOSOMA MANSONI* CERCARIAE. Connors VA, De Buron IC, and Granath, Jr. WO*. Division of Biological Sciences, University of Montana, Missoula, MT.

As we previously reported, *Biomphalaria glabrata* plasma contained an interleukin-1 (IL-1)-like factor which was detected by immunoassay and bioassay procedures. *Schistosoma mansoni*-resistant strains (10-R2, 13-16-R1) of *B. glabrata* were found to maintain elevated levels of this IL-1-like factor following infection with the parasite whereas there were no changes in IL-1-like activity in schistosome-susceptible (M-line) snails. Also, recombinant human IL-1 (rhIL-1) was found to prime superoxide production by hemocytes of 13-16-R1 but not M-line *B. glabrata*. We report here that rhIL-1 also primes superoxide production in hemocytes from resistant 10-R2 snails. Further, the effect of rhIL-1 on hemocytes from resistant snails was abrogated by both competitive inhibition with a human interleukin-1 receptor antagonist protein and inactivation of rhIL- 1 by heat treatment. The effect of rhIL-1 *in vivo* was examined by injecting schistosome-susceptible (M-line) snails with the cytokine prior to infection with miracidia of *S. mansoni*. Results indicated that a marked, prolonged and significant decrease in cercarial output, relative to sham injected controls, occurred. Since injection with foreign proteins does not affect cercarial output, these data indicate that in *B. glabrata*, cytokines, particularly IL-1, may play a significant role in resistance of these snails to infection with schistosomes.

437 *FASCIOLOIDES MAGNA* INTERMEDIATE SNAIL HOSTS: HABITAT PREFERENCES AND INFECTION PARAMETERS. Laursen JR* and Stromberg BE. University of Wisconsin-Madison, Madison, Wisconsin; University of Minnesota, Saint Paul, MI.

The large American liver fluke, *Fascioloides magna*, is enzootic in cervids and Lymnaeid snails in specific scattered regions of North America. The species of intermediate hosts and their habitat preferences, as well as infection parameters in these snails need to be determined to understand regional fluke transmission dynamics. Although several species of Lymnaeid snails are present within the fluke's range in Minnesota, only three were found in St. Croix State Park where *F. magna* is common in deer. Snails were collected during repeated sampling of 25 sites in 4 aquatic habitat types throughout the open water seasons of 1990 and 1991. Two of the three snail species were infected with *F. magna*. *Lymnaea caperata* (n=1734) were most numerous in woodland ponds, but were also found in shallow marshes, and lotic habitats. Infected *L. caperata* were recovered from woodland ponds, and shallow marshes. *Lymnaea modicella* (n=218) were most common at the mud-line in deep marsh/lake habitats, but they were also recovered from the three previously described habitats. Infected *L . modicella* were recovered from a single deep marsh/lake site. The mean prevalence of infection in any habitat type was less than 2% in either snail species. Infected snails were not recovered from flowing water sites. None of 307 *L. exilis* recovered from marsh habitats were infected. *Lymnaea caperata* could over-winter with infection and shed cercariae in early spring. Snails shed cercariae throughout the open water season. Changes in snail numbers and infection

parameters correlated with available moisture levels, and agreed with yearly changes in the prevalence of flukes in deer.

438 DETECTION OF *FASCIOLA HEPATICA*-INFECTED INTERMEDIATE HOSTS WITH AN OLIGONUCLEOTIDE-BASED ASSAY. Rognlie MC*, Maika KL, and Knapp SE. Veterinary Molecular Biology, Montana State University, Bozeman, MT.

A clear understanding of transmission of *Fasciola hepatica* is hampered by the difficulty of finding fluke-infected snails. We have therefore developed a sensitive assay to detect *F. hepatica* infection in individual snails. The assay makes use of a 28-base oligonucleotide probe that is complementary to a variable sequence region of *F. hepatica* small subunit ribosomal RNA. The region of fluke RNA containing the probe site is amplified from purified total RNA using fluke-specific primers with the Reverse Transcriptase-Polymerase Chain Reaction. Amplified products are detected by electrophoresis and positive results are confirmed on a Southern blot using the radiolabeled oligonucleotide probe. The assay will detect femtogram levels of purified fluke RNA in 5 μg of purified *Pseudosuccinea columella* snail RNA added as background. Additionally, the assay is at least 90% reliable and does not produce false positives. The parasite is detectable in the snail immediately after attachment of the miracidia and throughout the infection period. The assay is being used to study vector suitability and to gather seasonal transmission data. Current and future applications of the assay will be presented.

439 RECOMMENDATIONS FOR THE SELECTION OF CONTROL AGENTS FOR THE BIOLOGICAL CONTROL OF AQUATIC VECTOR ORGANISMS. Hofkin BV*. Department of Biology, University of New Mexico, Albuquerque, NM.

In its 1987 recommendations regarding the use of biological control against water-borne pathogens, the World Health Organization lists the life history characteristics that a predator should possess in order to serve as an effective control agent. These characteristics such as a short generation time relative to that of the target organism and a high degree of prey specificity, are those same features associated with successful predators and parasitoids in "classical biological control" developed primarily for the control of terrestrial pests. But because aquatic predators with such features are rare it has been argued that competitors have more promise as control agents of medically relevant vectors such as trematode-transmitting snails. It is argued here that because predation interactions are fundamentally different in aquatic habitats, the WHO recommendations are not valid for aquatic vectors such as snails and mosquito larvae. Long-lived generalist predators such as crayfish in the case of snails, and *Gambusia* in the case of mosquito larvae are more likely to bring about desired control. Furthermore, not all habitats are equally amendable to biological control and recommendations are presented here describing the type of habitat in which such control is most likely to be safe and effective.

440 SATELLITE THERMAL INFRARED IMAGERY AND THE DISTRIBUTION OF SCHISTOSOMIASIS IN THE NILE DELTA OF EGYPT. Malone JB*, Huh OK, Fehler DP, Wilson PA, and Elmagdoub AI. Veterinary Microbiology & Parasitology, Louisiana State University, Baton Rouge, LA; Coastal Studies Institute and Landscape Architecture, Louisiana State University, Baton Rouge, LA; and Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Day-night pairs of NOAA satellite thermal infrared images from the AVHRR were processed to produce temperature maximum (Tmax), temperature minimum (Tmin) and temperature difference (dT) maps of the lower Nile river valley. Image subsets of the Nile delta for dT on 16 AUG 90 and 14 FEB 91 were analyzed. Values of dT at specific locations were derived using the median of 5 X 5 pixels (28 Km2) centered on the latitude and longitude of 41 survey sites listed in 1937, 1983 and 1990

surveys of the Nile Delta. A Spearman correlation coefficient matrix revealed a significant inverse relationship on both imagery dates between median dT values and ranked data on prevalence of *S. mansoni* in 1937 and 1983 surveys. Results suggest that lower dT values reflect wetter hydrologic regimes that are more suitable for *S. mansoni*. Similar analysis for *S. haematobium* revealed a positive relationship of dT and prevalence in 1937. Results suggest that 1) AVHRR dT maps reflect hydrological variables that can be used as a predictor of environnmental risk of schistosomiasis on a regional scale, and 2) risk assessment by environmental satellites may be an important component of geographic information system (GIS) models currently being developed for national schistosomiasis control programs.

441 GASTROPOD INTERNAL DEFENSE AND TREMATODE PARASITE SURVIVAL. Adema CM*. Department Biology, University of New Mexico, Albuquerque, NM.

Trematode-gastropod compatibility is a specific phenomenon considered to be determined mainly at an immunobiological level. To better understand this interaction, cytotoxic characteristics of gastropod hemocytes (primary defense cells) were investigated using kinetic, cytochemical and functional assays. Apart from phagocytosis and encapsulation, hemocytes have at their disposal several cytotoxicity effector mechanisms. Reactive oxygen intermediates are released upon hemocyte activation in a respiratory burst-like fashion. Currently, the presence of lysosomal enzymes, cytolytic activity, likely nitric oxide, and other defense components have also been demonstrated in gastropods. Although effective separately, synergistic interactions of these effector systems enhance cytotoxicity. Intra-molluscan stages of compatible trematodes are vulnerable to cytotoxic effector components *in vitro*. Possible strategies for parasite survival *in vitro* will be discussed.

442 PARASITIC CASTRATION BY *SCHISTOSOMA MANSONI* DOES NOT PREVENT INFECTED *BIOMPHALARIA GLABRATA* SNAILS FROM ACTING AS MALES. Cooper LA* and Lewis FA. Biomedical Research Institute, Rockville, MD.

In many snail/trematode systems infection of the snail intermediate host results in a reduction or complete cessation of egg production. This parasite mediated castration has been well documented for the snail *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*. Because these snails are hermaphrodites, they are capable of reproducing either as females, by laying eggs, or as males by cross fertilizing another individual. Accordingly, we carried out an experiment designed to test the hypotheses that infected snails which were unable to act as females could still contribute to the genetic makeup of an interbreeding snail population by acting as males. Forty-eight juvenile pigmented *B. glabrata* snails were exposed to the NMRI strain of *S. mansoni*. By day 35 post exposure, 29 were shedding cercariae. Of these, 7 were completely castrated and never laid eggs while the remaining 22 were partially castrated and produced only a few eggs. By using the pigment markers in progeny snails to monitor cross-fertilization, it was found that 5 of the completely castrated, 9 of the partially castrated, and 21 of 29 uninfected controls were able to inseminate uninfected albino partners. In all cases, pigmented progeny began to appear within a few days of cross-fertilization, and for 6-8 weeks afterwards all of the progeny from the albino parents were heterozygous for pigmentation. Two of the infected snails could act as males for as long as eleven weeks after becoming completely castrated. These results show that infection of *B. glabrata* by *S. mansoni* does not prevent castrated snails from contributing to the genetic makeup of future generations. Therefore, castrated snails may help to maintain the genes which encode for susceptibility to parasite infection even within hyperendemic transmission foci.

443 *SCHISTOSOMA MANSONI*: INFLUENCE OF LARVAL INFECTION ON POLYPEPTIDE SYNTHESIS AND TRANSLATABLE RNA POOLS IN SNAIL CEREBRAL GANGLIA. Bai G* and Yoshino TP. University of Wisconsin-Madison, Madison, Wisconsin.

To elucidate the mechanisms by which larval *Schistosoma mansoni* infection inhibits the reproductive function of its intermediate host, *Biomphalaria glabrata*, we examined the effects of larval infection on polypeptide synthesis and translatable RNA pools in cerebral ganglia (CG) of *B. glabrata*. At 14, 21, and 28 days post-infection (PI), CGs were removed from infected and control snails. To study the effects of larval infection on polypeptide synthesis, CGs were pulse-chased with [^{14}C] amino acids. The culture supernatants and CG homogenates were analyzed by SDS-PAGE/fluorography. Comparison of translatable RNA pools was conducted by RNA extraction, *in vitro* translation, SDS-PAGE and fluorography. Preliminary results showed: (1) A 30 kDa polypeptide was consistently enhanced at 14, 21, and 28 days PI, while an additional 45 kDa polypeptide was selectively enhanced at 28 days PI. Sporocyst stages predominate at 14 and 21 days PI, whereas at day 28 PI, sporocysts begin to release cercariae. It is hypothesized that sporocysts may be responsible for stimulating synthesis of the 30 kDa polypeptide, while the 45 kDa molecule is in response to the cercarial stages. (2) No differences were found in translatable RNA pools between infected and control groups, although there was a significant increase in quantities of total RNA in CG of infected snails as compared to uninfected snails at 14 days PI. This result suggests that larval infection is exerting a subtle influence on translation, posttranslational processing or protein secretion, rather than transcription or posttranscriptional RNA processing.

444 FEASIBILITY OF COMMUNITY FINANCING FOR MOLLUSCICIDING: SOME RESULTS FROM A SCHISTOSOMIASIS ENDEMIC REGION OF NORTHERN CAMEROON. Khan MM*, Greer GJ, Hewett BS, and Cline BL. Department of Health Systems Management and International Health and Development, Tulane University, New Orleans, LA; Department of Tropical Medicine, Tulane University, New Orleans, LA; and Department of Anthropology, Washington State University, Pullman, WA.

Schistosomiasis remains an endemic disease of the world with about 200 million infected individuals. This study estimates the cost of molluscicide in a developing region and examines community's willingness and ability to pay for this intervention. Molluscicide is often considered too costly to be adopted by a poor community. In northern Cameroon the cost of the program was found to be less than 0.1 percent of total income of the community. If the perceived benefits of schistosomiasis control are higher than the costs, the program should be implementable through community financing. The cost paid for treating schistosomiasis, corrected for the probability of contracting the disease, can be used as a proxy for benefits obtained due to the prevention of the infection. In northern Cameroon, the cost of treating schistosomiasis exceeds 2,500 FCFA per case which is much higher than the cost of molluscicide estimated at 500 FCFA per household per year. Given the income distribution pattern, most of the households in this region should be able to pay for molluscicide. However, ability to pay does not imply household's willingness to pay. The community will be willing to participate in this type of snail control program if a majority of households have at least one infected individual. Excluding the low-intensity infection cases, who may not be aware of the presence of the infection, the overall prevalence rate required for community participation should be in between 14 to 20 percent. In many villages in Northern Cameroon, the prevalence rates are found to be higher than 20% and therefore, community financing of molluscicide should be feasible.

445 AN UNUSUAL HUMORAL RESPONSE OF *BIOMPHALARIA GLABRATA* TO INFECTION WITH *ECHINOSTOMA PARAENSEI*: CHARACTERIZATION AND FUNCTIONAL STUDIES. Hertel LA* and Loker ES. Department of Biology, University of New Mexico, Albuquerque, NM.

Previous studies have revealed acellular particulate matter (PM) in the hemolymph of M line *Biomphalaria glabrata* snails following infection with the trematode *Echinostoma paraensei*. It is not present in corresponding nonexposed snails. Further examination of the particulate matter was

undertaken to determine its nature and function. SDS PAGE and Western blot analysis of pelleted PM revealed the presence of 2 prominent bands at 200 and 80-120 kDa. Both bands correspond to material that has previously been shown to be lectins. To determine whether PM formation was influenced by parasite excretory/secretory products (ESP), concentrated ESP from primary *E. paraensei* sporocysts was mixed in equal volumes with culture medium, or plasma from control or infected snails. Aliquots of these mixtures were examined either by time-lapse photography or directly 5, 30, 60, 120 and 240 min after mixing. PM was not seen in either medium or plasma from control snails. Fine granular material was present after 5 min in the plasma from infected snails, that coalesced into clumps of PM over the 2hr period. To determine whether PM formation removes parasite products that inter- fere with hemocyte function, equal volumes of medium or concentrated ESP were mixed with medium or plasma from control or infected snails. After 2h equal volumes of particle-free supernatant from each preparation were mixed with freshly collected M line hemolymph. The percentage of round and spread cells was scored blindly for each sample using hemocytometers. Preliminary results indicated that PM formation significantly reduced the amounts of parasite products that interfere with hemocyte spreading.

446 IMPACT OF A PILOT DRACUNCULIASIS ELIMINATION PROGRAM AFTER THREE YEARS OF INTERVENTION IN TWO PROVINCES OF BURKINA FASO. Ouedraogo JB*, Hutin YJ, Yameogo G, Soula G, Fabre-Teste B, Hien R, and Guigemde TR. Centre Muraz (O.C.C.G.E.),Bobo-Dioulasso, Burkina Faso; Service d'Epidemiologie, de Statistique et d'Information Sanitaire du Secretariat General de l'O.C.C.G.E., Bobo-Dioulasso, Burkina Faso; and Direction de la Medecine Preventive, Ministere de la Sante, Ouagadougou, Burkina Faso.

As a first step of the national dracunculiasis elimination program, a pilot project was initiated in 1989 in two provinces of Burkina Faso (Bam and Oubritenga). The project was based upon sanitary education, filters distribution, and epidemiological surveillance. Primary health workers (PHW) conducted these activities in villages, and were supervised by nurses and sanitary authorities at the district level. Data from the surveillance system (1989-1992) were analysed, and a cluster sample of villages reported as endemic by PHW in 1992 was visited in early 1993 to evaluate the impact of the project. The number of filters distributed between 1989 and 1992 was 17470 in Bam (170520 population in 1989) and 28963 in Oubritenga (321665 population in 1989). Dracunculiasis annual incidence fell from 0.68% in 1989 to 0.30% in 1992 in Bam (a reduction of 55.4%) and from 0.95% to 0.26% during the same time period in Oubritenga (a reduction of 72%). In villages that were endemic in 1992, 54.8% (95% C.I. 45-64.6) of compounds in Bam and 60% (95% C.I. 49-71) in Oubritenga were using filters when going out in the fields during the rainy season. 52.7% (95% C.I. 43-62) of the compound chiefs in Bam and 62.4% (95% C.I. 55-70) in Oubritenga identified dirty water as the cause of guinea worm disease. PHW can successfully lead dracunculiasis elimination activities under the supervision of health staff, and such programs can achieve significant reduction in dracunculiasis incidence. Improvement could be sought through PHW motivation and better supervision.

447 USE OF GEOGRAPHIC POSITIONING TECHNOLOGY IN IVERMECTIN DISTRIBUTION ACTIVITIES IN GUATEMALA. Richards F*, Bennett P, Lujan R, Zea R, Castro J, Gonzalez C, and Klein R. Division of Parasitic Diseases, Centers for Disease Control and Prevention; Universidad del Valle de Guatemala; and Guatemalan Ministry of Public Health and Social Welfare; The River Blindness Foundation.

Accurate maps of disease-endemic areas are an extremely useful tools for managers of control programs. Detailed maps exist for most developed countries but may be difficult to obtain for project areas in the developing world. We report our experience using geographic information software (GIS) and hand-held global positioning systems (GPS) to aid in repetitive ivermectin delivery to 149 communities with endemic onchocerciasis in Guatemala. Technical problems included the lack of available electronic maps and determining the coordinates of the communities in the project area.

To solve the first problem, we created our own digitized maps by merging information from available paper maps of the areas. Government statistics, gazetteers, and 1:50,000-scale maps were used to identify target communities. These sources identified coordinates of 73% of settlements. Field reconnaissance using GPS was required to locate the remaining communities. Repetitive GPS readings (n=33), measured at known coordinates over a 1-month period, were accurate within a 300-meter radius. The maps we created were of greatest benefit when large printouts were provided to the field teams. These printouts improved communication between management and field workers. Regional mapping centers could aid in the creation of GIS-legible data bases and custom thematic maps for use in disease control programs.

448 EVALUATION OF ALTERNATE METHODS OF RAPID ASSESSMENT OF EPIDEMICITY OF *ONCHOCERCA VOLVULUS* IN COMUNITIES IN SOUTHERN CAMEROON. Kollo B*, Mather FJ, and Cline BL. Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA; and Department of Biostatistics and Epidemiology, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

The recently expanded use of ivermectin for large scale onchocerciasis treatment programs highlighted the pressing need for noninvasive approaches for assigning control priority to endemic communities. The standard diagnostic method (skin snips) carries unacceptable risks of transmission of agents such as HIV and HBV, and is relatively costly and time consuming. We evaluated potential alternate diagnostic indicators of onchocerciasis for usefulness as tools for rapid, safe, low cost assessment of onchocerciasis endemnicity in communities. The study was conducted in the Dja et Lobo Division, South Province, Cameroon, a tropical forest region with a very high prevalence of infection. Thirty-two study villages were selected, stratified by expected level of encemnicity, and a total of 846 adult (20 years and over) male residents of these communities were examined according to a defined protocol. For each study subject the number of nodules, and the presence of economic blindness and leopard skin were noted, as well as degree of skin excoriations. Further information was collected to quantify the severity of pruritus, and DEC patch test reactivity was recorded at 24 and 48 hours. Urine specimens were examined for microfilaruria. Two skin snips from the iliac crest served as the reference standard for each subject. Leopard skin and nodules showed the strongest correlation with both the skin snip prevalence and the microfilarial density (CMFL). We selected a >20% rate of nodules or a ≥20% rate of leopard skin as the most appropriate decision rule for assigning a community to high priority for control, which corresponds to >90% skin snip prevalence in adult males. While this decision rule should not be applied to regions with "savannah onchocerciasis", we believe the methodology can and should be used to determine decisions rules appropriate for areas with different dynamics of transmission and clinical expression of onchocerciasis.

449 LARGE SCALE APPLICATION OF *ONCHOCERCA VOLVULUS* DNA PROBE BASED TECHNOLOGY BY THE ONCHOCERCIASIS CONTROL PROGRAMME OF WEST AFRICA. Toe L, Merriweather A* and Unnasch TR. Insecticide Research Unit, Onchocerciasis Control Programme, Bouake 01, Cote d'Ivoire; and Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

The recent development of strain and species specific DNA probes for *Onchocerca volvulus*, together with the ability to utilize these probes to classify all lifecycle stages of the parasite has offered the opportunity of utilizing this technology to monitor the activities of onchocerciasis control programs. In March of 1992, a laboratory was established by the Onchocerciasis Control Programme of West Africa (OCP) to utilize the strain and species specific probes for this purpose. During 1992, this laboratory succeeded in classifying 868 parasite samples using the strain and species specific DNA probes. These samples included the majority of the larvae identified in infected *Simulium damnosum* s.l. found by mass dissection of approximately 200,000 blackflies collected throughout the

OCP area. The results of this study have been used to evaluate the effect that animal filariae have had on the estimation of the annual transmission potential (ATP) for *O. volvulus* in the OCP control area. Animal filariae were found to effect the estimation of the ATP in all regions of the programme. The effect was most pronounced in the Northwest of the OCP control area, where the majority of infected flies were found to be carrying animal filariae. Strain specific DNA probes were also used to demonstrate that the severe and less severe strains of the parasite co-exist in several areas along the southern and eastern borders of the control area. These probes have also been used to demonstrate the presence of a mixed strain infection in an individual residing in an area in which the two strains are co-endemic. To our knowledge, this represents the first routine application of a PCR based DNA probe assay to identify a parasitic infection by a laboratory situated in an endemic area. The success of this laboratory demonstrates that PCR based DNA probe technologies may be practically applied by laboratories based in areas endemic for parasitic infections, and that these techniques may be used to provide information useful to disease control programs.

451 THE CULTURAL CONTEXT AND CONTROL OF "FOREST" ONCHOCERCIASIS IN SOUTH PROVINCE, CAMEROON. Hewlett B*, Cline BL, and Kollo B. Department of Anthropology, Washington State University, Pullman, WA; and Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, LA.

This paper examines cultural practices and beliefs associated with "forest" onchocerciasis and other filarial infections in two rural Camaroonian ethic groups - Fang farmers and Baka (pygmy) foragers. Qualitative and quantitative methods were utilized to assess the community members' disease recognition, cause, signs and symptoms, and prognosis of the disease. The aims of the study were to: 1) elucidate behaviors which enhance or diminish health status relative to onchocerciasis and related filarial infections, 2) assist in making culturally appropriate decisions about the implementaion and long term maintenance of an ivermectin distribution program, and 3) maximize integration of the ivermectin distribution program into the primary health care system. The Fang and Baka had extensive knowledge of onchocerciasis and other filarial infections. All filarial infections were referred to as *minak* while onchocerciasis was caled "illness of the Dja" – the Dja being the major river in the area and the primary breeding ground for blackflies. The primary symptom of *minak* was itching while the primary sign of "illness of the Dja" was leopard skin. Fang and Baka cultural practices are summarized and utilized to make recommendations for the development of culturally sensitive and appropriate health educaiton messages, and to identify potential problems health workers may encounter in an ivermectin distribution program.

452 POLYMERASE CHAIN REACTION BASED DIAGNOSIS OF *ONCHOCERCA VOLVULUS* INFECTION: IMPROVED DETECTION OF ACTIVE INFECTION. Nutman TB*, Zimmerman PA, Aruajo E, Elson LH, Phadke P, Kubofcik J, and Guderian RH. Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD; and National Center of Tropical Medicine-Quito Extension, Hospital Vozandes, Quito, Ecuador.

Definitive diagnosis of *Onchocerca volvulus* (Ov) infection requires the identification of the parasite in either the skin or in subcutaneous nodules. More recently, recombinant diagnostic antigens (Ag) have been used to overcome the poor sensitivity of the parasitological approach. However, these antibody based assays cannot distinguish between past infection and current infection; nor can they be used to assess efficacy of chemotherapy in that antibodies to these recombinant Ag remain elevated following micro- and macrofilaricidal treatment. To assess the efficacy and utility of a polymerase chain reaction (PCR)-based diagnostic for Ov infection, skin snip examination was performed in 94 individuals from an Ov-endemic region of Ecuador and compared, in a blinded fashion, with a non-radioactive PCR assay based on the O-150 repeat sequence. Of the 60 patients found to be microfilaria (MF) positive on skin snip examination, all were found to be positive in the PCR-based assay (100% sensitivity). In addition, 13 of the 34 found to be MF negative on skin snips were found to be infected

based on the PCR assay. In a second study of 28 amocarzine treated MF+ patients studied 3 months after treatment (at a time when they were MF-), 16 (57%) were positive in the PCR-based assay. Of these 16, 14 (88%) became MF+ when re-assessed parasitologically 3 months later. These data suggest that this PCR-based assay is significantly more sensitive than current methods and that it overcomes the deficiencies of both parasitogic and serologic methods in detecting active Ov infection. As this technique has now been placed in an ELISA format, its use in the areas where Ov is endemic is undergoing field-testing.

453 A SURVEY OF KNOWLEDGE, ATTITUDES, AND PERCEPTIONS (KAPS) OF LYMPHATIC FILARIASIS AMONG RESIDENTS IN LEOGANE, HAITI. Eberhard ML*, Walker EM, Addiss DG, and Lammie PJ. Division of Parasitic Diseases, National Center for Infectious Diseases, Center for Diseaase Control, Atlanta, GA.

The success of efforts to control infectious diseases depends, in large measure, on community awareness and understanding of the disease. To assess the KAPs of filariasis, we interviewed 82 residents of a disease endemic area. Fewer than 50% of residents had heard of filariasis and only 5% knew that it was transmitted by mosquitoes. In contrast, all persons had heard of hydrocele and elephantiasis. Hydrocele was thought to be caused by a blow to the testis (66%) or gas (31%), while walking barefoot on soil or water (36%) or ceremonial powder (26%) were commonly given as causes of elephantiasis. Of 82 respondents, 73 and 13 thought that hydrocele could be treated through surgery or a "drug", respectively, whereas 29 and 24 believed that either surgery or a "drug" could be used to treat elephantiasis. Hydrocele and elephantiasis ranked 2nd and 3rd, respectively, behind AIDS as perceived health problems, most likely because residents believed that treatment for malaria, intestinal worms, anemia, and diarrhea was easily obtained. Trends in responses by age, sex, and symptomatology were noticed, but none were statistically significant except that symptomatic persons were more likely to have sought treatment than asymptomatic persons ($p=0.0006$). The results of this survey indicate that for control efforts to be successful, community awareness of the causes, relationship between infection and disease outcome, and goals of treatment must be heightened through education campaigns.

454 ENVIRONMENTAL FACTORS INFLUENCING THE DISTRIBUTION OF LYMPHATIC FILARIASIS IN THE NILE DELTA. Faris R*, Ramzy RM, Emira HA, and Gad AM. Center for Research and Training on Vectors of Disease, Ain Shams University, Cairo, Egypt.

Two villages in Gharbya governate with similar populations (circa 3500) were studied to identify environmental factors that may account for the focal distribution of bancroftian filariasis in the Nile Delta. A random sample of 10% of the houses of each village were studied. All household members were examined for evidence of clinical filariasis and night blood was tested for microfilariae and filarial antigen. Prevalence rates of microfilaremia and antigenemia were 4.3% and 6.3% in village A and 0% and 0% in village B. Outdoor mosquito species and habitats were similar in the two villages (mainly *Cx. pipiens*). Compared to the nonendemic village, village A had a significantly higher crowding index (people/house), fewer houses using indoor insecticides or employing sanitary waste disposal (keeping garbage in covered containers for regular pickup) and more houses facing vacant areas, with standing water indoors, or with indoor animal sheds ($P < 0.01$ for each of these factors by Chi square). Crowding, nonuse of insecticides, and lack of sanitary waste disposal were all significantly associated with filariasis when infected and uninfected households within village A were compared. We conclude that enviromental factors may strongly influence the distribution of filariasis between and within villages in Egypt.

455 AGE-SPECIFIC PREVALENCE OF *WUCHERERIA BANCROFTI* ANTIGENEMIA IN A HAITIAN POPULATION. Lammie PJ*, Eberhard ML, Dickerson JW, Walker EM, and Hightower

AW. Division of Parasitic Diseases, Parasitic Diseases Branch, Centers for Disease Control, Atlanta, GA.

Filarial antigen detection assays may serve as a useful adjunct to blood exams for community-based surveys, in terms of both the diagnostic and epidemiologic information provided. In this study, the Og4C3 antigen detection assay was used to analyze the distribution of *Wuchereria bancrofti* antigenemia in a Haitian population. Nocturnal blood surveys were performed in the community of Belloc; a 20 µl blood film was prepared and 100 µl of blood was collected as a source of serum for the antigen detection assay. Of 247 people surveyed to date (age 1 - 75 yrs), 74 (30%) were microfilaremic (MF+). Using 15 µl of serum diluted 1:10 for the assay, all of the MF+ individuals were antigen positive (Ag+). Parallel studies indicated that assay sensitivity was maintained using serum obtained from daytime bleedings. The prevalence of antigenemia in MF- residents was 27.7%. The overall antigenemia of 49.4% contrasted with that of a neighboring community located 1 km away where the prevalence of microfilaremia and antigenemia were 6.2% and 23.7%, respectively. Consistent with our previous observations in clinic-based populations, antigenemia in persons with elephantiasis was significantly lower (10%) than in other MF- individuals. The age-specific prevalence of antigenemia increased from 36.8% in the 0-4 yr age group to 77.8% in those 50 yr and older. The distribution of circulating antigen levels showed no evidence for a decrease with age. If confirmed in further surveys, these results may carry significant implications about the relationships between infection, immunity, and disease.

456 *WUCHERERIA BANCROFTI* PCR FOR THE DETECTION OF INFECTIVE L_3 LARVAE IN POOLS OF MOSQUITO HEADS. Chanteau S*, Luquiaud P, Failloux AB, Plichart C, Ung A, Lardeux F, and Williams SA. Institut Louis Malarde, Tahiti; Centre ORSTOM, Tahiti; and Smith College, Northampton, MA.

The assessment of the impact of a filariasis control programme is based on the determination of parasitemia in humans and of the proportion of infectious mosquitoes. This implies the dissection of thousands of mosquitoes and use of morphological criteria to identify the larval stage and the species involved. Species-specific PCR assays are available, but to date none of them are stage-specific. We have used the species-specific *Wuchereria bancrofti Ssp* I repeat PCR to detect a single infective L_3 larva from pools of 50 mosquito heads. Even though the use of heads will underestimate the infectious rate, it is useful because there are no stages other than infective L_3 larvae found in the head. A simple and effective method, combining freeze/boiling and silica particle extraction was developed to release filarial DNA free of PCR inhibitors. As little as 1/1000 L_3 (about 0.1pg of filarial DNA) can be detected by ethidium bromide staining of PCR products run on agarose gels. We have generated a semi-quantitative PCR assay to estimate the number of infective L_3 larvae in mosquito pools, based upon the signals obtained using control pools inoculated with increasing numbers of L_3 larvae. For field applications, various methods (EDTA and drying) to preserve mosquitoes for later PCR processing and the use of lyophilized PCR mixes have been evaluated. Continued improvement of these DNA technologies will enable the application of this assay in real field conditions. This assay will be compared to conventional dissection methods in a community pilot study on ivermectin in French Polynesia.

457 DETECTION OF *WUCHERERIA BANCROFTI* CIRCULATING ("FREE") DNA IN BLOOD AND PLASMA USING THE *SSP* I PCR SYSTEM. Zhong M*, Williams SA, McCarthy J,and Ottesen E. Department of Biological Sciences, Smith College, Northampton, MA; and Laboratory of Parasite Diseases, National Institutes Health, Bethesda, MD.

Last year we presented data on a sensitive *Wuchereria*-specific PCR assay developed based on a new repeat DNA family (the *Ssp* I repeat) isolated from *W. bancrofti*. The sensitivity and specificity of this PCR system indicates its potential for ultra-sensitive detection in field collected samples and should

have a great impact on epidemiological studies and diagnosis. Since *W. bancrofti* in most endemic areas is nocturnally periodic or subperiodic, the classic diagnostic methods are inconvenient because night blood collection is required. To address this problem, we investigated the detection of circulating DNA released in patients' blood. The most likely source of this DNA is from parasites which are killed by the host immune system. Blood and plasma samples collected from *W. bancrofti* infected individuals in Mauke, Cook Islands, were screened for the presence of "free" parasite DNA. When each blood sample was screened with the *Ssp* I PCR assay, 25 of the 28 samples (89%) were PCR positive. Even samples from patients with very low numbers of microfilariae (e.g. one sample with 1 mf/ml, one sample with 2 mf/ml, one sample with 3 mf/ml, one sample with 5 mf/ml, and two samples with 8 mf/ml) repeated tested PCR positive. Because only 50 ul of each blood sample was screened per assay, it is likely that the positive signals detected came from "free" parasite DNA and not from microfilariae. Positive PCR results were also obtained from two patients with "cryptic" infections and from "microfilariae-free" filtered blood and serum samples. These data further support the premise that the *Ssp* I PCR assay can detect circulating parasite DNA.

458 SURFACE COAT AND EPICUTICLE ANTIGENS FROM LARVAE OF THE PARASITIC NEMATODE TOXOCARA CANIS. Gems DH and Maizels RM*. Wellcome Research Centre for Parasitic Infections, Department of BIology, Imperial College, London, UK.

Second-stage larvae of the canine ascarid *Toxocara canis* can infect human and other paratenic hosts, entering into a state of arrested development. This diapause can last for as long as 9 years *in vivo*, during which time the parasite effectively evades host immunity. One mechanism to promote parasite survival appears to be a glycocalyx or surface coat which can be rapidly shed if attacked by antibodies or leukocytes. Using PCR techniques with spliced leader and oligo-dT primers, we have cloned and sequenced full length cDNAs for two proteins which appear to be associated with this coat. One clone encodes a small polypeptide of 177 amino acids, which we believe to be the core protein of TES-120, a heavily O-glycosylated major component of the surface coat. Sequence analysis reveals three domains: a signal peptide, a central domain with 74% serine/threonine domain, and a cysteine-rich domain. The organisation of the molecule is thus very similar to that of mammalian membrane-associated mucins. The second protein is likely to be TES-32, which is known by immunoelectron microscopy to be associated with the larval epicuticle. This is a N-linked glycoprotein with a polypeptide of 26 kDa, in agreement with the full sequence we have isolated. This clone has homology with mammalian and yeast phosphatidylethanolamine binding proteins, but most interestingly contains two cysteine domains homologous to TES-120. This offers the possibility that TES-32 is an anchor protein for the surface coat mucin. Both clones are highly abundant in parasite cDNA, and both TES-120 and TES-32 proteins are similarly overexpressed. The surface coat is therefore a major metabolic investment by the parasite, perhaps because it plays an essential role in warding off host immune attack.

459 BRONCHOALVEOLAR LAVAGE EOSINOPHILS FROM MICE INFECTED WITH *TOXOCARA CANIS* DO NOT EXPRESS Fc RECEPTORS FOR IgE. Kayes SG*, Hester RB, Finkelman FD, and Jones RE. Department of Structural and Cellular Biology, University of South Alabama College of Medicine, Mobile, AL; Department of Microbiology and Immunology, University of South Alabama College of Medicine, Mobile, AL; and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.

Tissue-invasive nematode parasites often elicit dramatic increases in the number of circulating eosinophils and a concurrent rise in serum IgE levels. It is thought that parasites opsonized with anti-parasite IgE molecules are bound by eosinophils expressing Fc receptors for this isotype which then leads to eosinophil degranulation and parasite killing. To analyze eosinophil Fc receptor expression we carried out a flow cytometric study of bronchoalveolar lavage (BAL) eosinophils from mice infected with *Toxocara canis*. Spleen cells, from both normal mice and mice infected for 14 days

with *T. canis*, expressed CD23 (type II Fc ε receptor) and bound soluble IgE (sIgE). In contrast, BAL eosinophils from mice 14 days PI neither bound sIgE nor expressed the IgE Fc receptor. Infected mice had >500-fold increase in IgE by 14 days PI. Culture of BAL eosinophils with exogenous IgE mAb or IL-4 plus IL-5 failed to induce either the expression of CD23 or IgE binding. In contrast to BAL cells, culture of unfractionated normal spleen cells with IL-4 induced these cells to express more CD23. BAL eosinophils expressed the Fc γ receptor II and bound IgG but did not bind IgM or IgA. It is concluded that mouse BAL eosinophils exposed to high levels of IgE neither express CD23 nor bind IgE in contrast to rat and human eosinophils.

460 ALLATOSTATIN-IMMUNOREACTIVE NEUROSECRETORY CELLS IN THE SYNGANGLION OF THE TICK *DERMACENTOR VARIABILIS* (ACARI: IXODIDAE). Zhu XX* and Oliver JH. Institute of Arthropodology & Parasitology, Georgia Southern University, Statesboro, GA.

Allatostatins are a family of insect neuropeptides that are produced from brain and regulate the synthesis of juvenile hormone by the corpora allata. Using immunocytochemistry based on a monoclonal antibody against allatostatin I and horseradish peroxidase-diaminobenzidine reaction, we have demonstrated the presence of immunoreactive neurosecretory cells in the synganglion (the tick "brain") of *Dermacentor variabilis* unfed, virgin females. These cells are located in the protocerebral, cheliceral, stomodeal and opisthosomal regions of the synganglion. Strongly immunoreactive granules are accumulated in the subperineurial areas of cheliceral and opisthosomal ganglia, suggesting that an allatostatin-like substance may be stored in these regions. The wide distribution of allatostatin-immunoreactive cells in the synganglion of *D. variabilis* provides evidence that the allatostatin-like substance in this tick species may have multiple functions.

461 IDENTIFICATION AND CHARACTERIZATION OF MENINGEAL WORM ANTIGENS. Neumann NF*, Samuel WM, and Belosevic M. Departments of Zoology and Immunology, University of Alberta, Edmonton, Alberta, Canada.

We studied the antigens of adult and third stage larvae of the meningeal worm, *Parelaphostrongylus tenuis*, in an attempt to identify potential serodiagnostic molecules for this important infection of wild and domesticated ungulates. Soluble extracts of *P. tenuis* were separated by SDS-PAGE and two-dimensional electrophoresis and analyzed by immunoblotting using purified rabbit anti-*P. tenuis* immunoglobulin G(LgG). Out of more than 75 molecules, 7 antigens from adult worms, 4 molecules in the 170-120 kD range (pl 6 to 6.6) two molecules of 55 kD (pl 5.6 and 5.8) and one molecule of 13 kD, and two antigens from the third state larvae of 25-30 kD (pl 6.3) and 13 kD, characterized the meningeal worm compared to three other nematodes, *Dictyocaulus vivparus*, *Protostrongylus baudini* and *Trichinella spiralis*. The serum from elk experimentally infected with *P. tenuis* recognized a similar profile of the meningeal worm antigens when compared to the serum from immunized rabbits. Several antigens were identified to have serodiagnostic potential. In addition, third stage larvae recognized similar antigens, indicating that relatively low number of parasites will induce a significant anti-*P. tenuis* antibody response in elk. This research has set the foundation for the development of a serodiagnostic test for meningeal worm infection in wild and recently domesticated elk and other ungulates.

462 *TRICHURIS SUIS* (WHIPWORM) INFECTION IN PIGS EXACERBATES PRE-EXISTING PORCINE INTESTINAL ADENOMATOSIS. Mansfield LS, Urban JF, and Hill DE. Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Biosystematics Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

Mucohemorrhagic enteropathies of the intestinal tract have complex and interrelated etiologies that have profound economic impact in the swine industry. A new form of Porcine Intestinal Adenomatosis (PIA) was observed in weanling pigs with naturally-acquired and experimentally-induced infections of *Trichuris suis*. Prior to infection with the parasite, pigs exhibited 1 cm diameter nodular adenomatous lesions restricted to the colon. After experimental infection with the whipworm, PIA lesions were exacerbated, leading to significant inflammatory cell infiltration, secondary bacterial infection and destruction of the nodular cystic wall allowing escape of disease producing organisms to surrounding tissues, even though whipworms were not directly associated with the lesions. PIA has been previously reported as a disease of the small intestinal mucosa of pigs caused by a *Campylobacter* spp.. Here we report similar epithelial cell proliferative changes in a colonic nodular form from which *Campylobacter* spp. were isolated. Additionally, infiltration of inflammatory cells and secondary bacterial species resulting from *T. suis* infection suggest a new pathogenic mechanism for the severe disease associated with necrotic proliferative enteritis in pigs.

463 CHARACTERIZATION OF A NOVEL 3,6-DIDEOXYHEXOSE AND ITS ROLE IN DEFINING THE ANTIGENICITY OF IMMUNODOMINANT *TRICHINELLA SPIRALIS* GLYCOPROTEINS. Wisnewski N *, Zeidner NS, McNeil M, Grieve RB, and Wassom DL. Paravax, Inc., Fort Collins, CO; and Departments of Pathology and Microbiology, Colorado State University, Fort Collins, CO.

The monosaccharide composition of an affinity-purified family of stage-specific, antigenically-related *Trichinella spiralis* larval glycoproteins (TSL-1) was determined by gas chromatography/mass spectrometry (GC/MS). The TSL-1 glycosyl composition was unusual in that fucose accounted for 36 molar percent of the glycosyl residues, and a 3,6-dideoxyhexose was identified, accounting for 24 molar percent of the glycosyl residues. Previously, 3,6-dideoxyhexoses have been found only in some bacterial lipopolysaccharides and in ascaroside alcohols of *Ascaris* eggs. This *Trichinella* sugar has been identified as a 3,6-dideoxyarabinohexose, the same as found in *Ascaris* eggs. However, the absolute configuration of the TSL-1 sugar is D (tyvelose), not L (ascarylose). Methylation analysis indicated that the TSL-1 tyvelose was present entirely as non-reducing terminal residues. The TSL-1 antigens, affinity purifed from L1 with the carbohydrate epitope-specific monoclonal antibody Tsp 130, evoke a protective immune response to *T. spiralis*. To determine whether this novel glycosyl residue, the first 3,6-dideoxyhexose to be found on a glycoprotein, plays a role in the immunodominance of the TSL-1 antigens, a competitive ELISA was developed to measure the inhibition of monoclonal antibody binding by chemically synthesized tyvelose. Utilizing Tsp 130 at 50% binding capacity to crude larval homogenate, tyvelose inhibited Tsp 130 binding to solid-phase antigen by 25-61% at concentrations of tyvelose ranging from 0.1-16 μg/ml. These results suggest that tyvelose may play an essential role in determining the precise epitope of the immunodominant antigen of *T. spiralis*. L1.

464 IL4 REGULATES PROTECTIVE IMMUITY TO *TRICHINELLA SPIRALIS*. Urban JF*, Gamble HR, Madden KB, Katona IM, and Finkelman FD. Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Departments. of Pediatrics and Medicine, Uniformed Services Univ. of the Health Sciences, Bethesda, MD.

Gastrointestinal (GI) nematode parasites elicit strong Th2 responses and increased production of IL4 which stimulates IgE synthesis and affects mucosal mastocytosis. A role for IL4 in immunity to *Trichuris muris* and *Heligmosomoides polygyrus* was demonstrated when mAbs that block the IL4 receptor (R) inhibited protective immunity in mice either infected for the first time with *T. muris*, or exposed to a challenge infection with *H. polygyrus*. In contrast, mice genetically resistant to *T. spiralis* were reported to express immune responses typical of strong Th1 activation. To examine further this apparent discrepancy in host immunity to different GI nematodes, BALB/c mice were injected with mAbs against IL4R, IL5, or INFγ, and inoculated with infective *T. spiralis* larvae; muscle larvae (ML) were recovered 1 mo later to determine efficacy. Mice treated with anti-IL4R had 3 times more ML

than infected controls, while the number of ML in mice treated with anti-IL5 or anti-IFNγ was similar to controls. This protocol was repeated in previously infected mice injected with mAbs at the time of a challenge inoculation; the results were similar. In addition, injection of exogenous IL4 into mice during a primary infection significantly reduced adult newborn larval production *in vitro*, and the *in vivo* ML burden recovered 30 days after inoculation. It appears that a general role for IL4 in protection against GI nematodes must be considered.

465 THE HOOKWORM ANTICOAGULANT: A NOVEL TISSUE FACTOR PATHWAY INHIBITOR. Cappello M, Vlasuk GP, Hawdon JM, and Hotez PJ. Departments of Pediatrics and Epidemiology & Public Health, Yale University School of Medicine; and Corvas International, Inc.

Human hookworm infection, which causes chronic gastrointestinal blood loss, is the leading cause of anemia in the developing world. While it has been postulated for nearly a century that the adult worm facilitates blood feeding through the secretion of an anticoagulant, the exact mechanism by which hookworms interfere with host hemostasis has not been identified. Soluble protein extracts from homogenates of adult *Ancylostoma* hookworms were found to prolong both prothrombin (PT) and partial thromboplastin (PTT) clotting times. In addition, hookworm protein inhibited the activity of purified clotting factor Xa as measured by chromogenic substrate assay. No inhibition of purified thrombin, another serine protease in the common pathway of coagulation, was observed. The *Ancylostoma* anticoagulant, purified using ion-exchange chromatography, a factor Xa affinity column, and gel filtration, respectively, has an apparent MW of 6500 Da. In addition to displaying anti-Xa activity, the hookworm anticoagulant is also a potent inhibitor of the VIIa/tissue factor complex. We used the polymerase chain reaction (PCR) with oligonucleotide primers constructed from primary amino acid sequence of the purified protein to amplify a 100 bp gene product from an adult Ancylostoma cDNA library. Cloning and sequencing of the PCR product has revealed that this gene encodes for a protein which has significant homology to many Kunitz-type serine protease inhibitors, including human Tissue Factor Pathway Inhibitor. The hookworm anticoagulant, the first invertebrate extrinsic pathway inhibitor, may have clinical use as a therapeutic antihemostatic agent. In addition, vaccine therapy directed at the anticoagulant represents a potential strategy aimed at reducing hookworm associated blood loss and anemia.

466 CUTICULAR ANTIGENS OF THE EQUINE NEMATODE *STRONGYLUS VULGARIS*. Philpott MS and Klei TR*. Department of Veterinary Microbiology & Parasitology, School of Vet Medicine, Louisiana State University, Baton Rouge, LA.

Strongylus vulgaris causes severe mesenteric arteritis which is often fatal in naive horses. Reduction (>80%) in parasite burden and protection from disease can be achieved by oral administration of irradiated L3 larvae, suggesting an effective immune response can be generated to antigens present by this stage of the parasite. To define antigens on the surface of *S. vulgaris* L3 larvae, a panel of monoclonal antibodies were prepared which bind to molecules exposed on the surface of the worm. All of the monoclonals are specific for *Strongylus vulgaris* and did not bind to *Strongylus vulgaris* L4 or to L3 or L4 of the related species, *S. edantatus*. The molecules recognized by the antibodies fall into two groups: those present immediately after exsheathment of the larvae which are lost within two days in culture and molecules present on the L3 larvae which are expressed until the molt to the fourth stage larvae. Some antigens could be removed from the surface of the worm by treatment with the detergent CTAB, while others could not. Immunoprecipitation studies are currently in progress to determine the electrophoretic mobilities of CTAB extracted molecules.

467 AN ULTRASTRUCTURAL STUDY OF *EIMERIA* DEVELOPMENT IN UNIMMUNIZED AND FOREIGN HOST IMMUNIZED CHICKENS. Danforth HD* and Augustine PC. Protozoan Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

An ultrastructural study was done to compare the intracellular development of *Eimeria tenella* in unimmunized chickens and chickens that were foreign host immunized (FHI) by repeated oocyst inoculations of the turkey coccidium *E. adenoides*. Cellular infiltration of lymphocytes and heterophils was seen as early as 24 hr post challenge (PC) in the ceca of the FHI chickens. Sporozoite and meront stages were seen within the heterophils of FHI birds at 48 to 96 hr PC. No such cellular infiltration was seen in unimmunized birds until 72 hr PC, and no parasites were seen in the heterophils. Asexual and sexual development were delayed in the FHI birds when compared to unimmunized birds so that few mature sexual stages were seen in the FHI birds by 144 hr PC. In addition, the infected host cells of the FHI birds showed greater ultrastructural degeneration at 72-96 hr PC than host cells in unimmunized birds. Parasites within the degenerating host cells of the FHI chickens had increased vacuolization and abnormal budding of merozoites. These results identify some of the characteristics of an E. tenella challenge infection in FHI chickens. The effects include early infiltration of lymphoid and nonlymphoid cells in the challenged ceca, a delay in parasite development and an increase in host cell degeneration.

468 Quantitative genetic changes at the β-tubulin genes in *Haemonchus contortus* associated with resistance to benzimidazoles. Beech RN*, Prichard RK, and Scott ME. Institute of Parasitology, McGill University, Quebec, Canada.

Resistance to benzimidazoles (BZ) in *Haemonchus contortus* has been linked to changes in β-tubulin. Analysis of DNA from a mixture of many individuals has shown qualitative changes in the β-tubulin genes from resistant strains. Using the polymerase chain reaction it is possible to amplify sufficient DNA from single adult *H. contortus* to quantify allelic variation at each of the two β-tubulin loci. In an effort to characterize alleles associated with resistance, the genotypes (with respect to the two β-tubulin genes) of individual adult *H. contortus* from one susceptible and two BZ resistant strains have been determined. In the susceptible strain both loci were highly polymorphic. In a cambendazole resistant strain, derived from the susceptible strain, one locus was found to be almost entirely homozygous for one of the alleles present in the susceptible strain. The second locus, although showing a reduced variability, still retained several alleles. A similar pattern was observed in an independently derived thiabendazole resistant strain. Surprisingly, the frequency of the alleles associated with BZ resistance approached 50% in the susceptible strain. These results suggest that both β-tubulin loci play a role in BZ resistance, selection with different benzimidazoles results in similar changes in the *H. contortus* population and it may be practical to develop DNA based markers to identify BZ resistance in field strains of *H. contortus*.

469 CONTROL OF PERINATAL TRANSMISSION OF *TOXOCARA CANIS* WITH MILBEMYCIN OXIME IN DOGS. Stewart VA*, Hepler DI, and Grieve RB. Department of Pathology, Colorado State University, Fort Collins, CO; and CIBA-GEIGY Animal Health, CIBA-GEIGY Corporation, Greensboro, NC.

Toxocara canis, the intestinal roundworm of dogs and the chief causative organism of visceral larva migrans in humans, is relatively easy to treat as an adult. However, this parasite is difficult to eradicate from dog populations and from ecosystems because of its ability to persist in a larval stage in the tissues of preparturient dogs and paratenic hosts. Prenatal transmission remains an important source of infection even in litters with good veterinary care. Milbemycin oxime, administered orally to the pregnant and lactating bitch, has been evaluated for its efficacy against transplacental and perinatal transmission of *T. canis*. Both 0.5-1.0 mg/kg twice weekly and 1.5-3.0 mg/kg once weekly from day 35 of pregnancy until weaning have been found to reduce transplacental transmission and to be >99% effective in producing pups that are free of worms at weaning. Preventing the release of

vast numbers of eggs prior to the first veterinary visit by the puppy offers clear public health advantages.

470 IMMUNOGENICITY OF MULTIPLE ANTIGEN PEPTIDES (MAPS) CONTAINING *PLASMODIUM VIVAX* CS EPITOPES. de Herrera MA*, Escobar P, De Plata C, Corradin G, and and Herrera S. School of Health, Universidada del Valle, Cali, Colombia; and Institute of Biochemistry, University of Lausanne, Lausanne, Switzerland.

We have previously described four T cell epitopes recognized by humans on the *Plasmodium vivax* CS protein. One of them is located inside the central repetitive domain and contains a sequence recognized by a protective monoclonal antibody. This peptide was used as a B cell epitope in this study. Another T cell epitope was on the animo terminus and the other two on the carboxyl region. A total of 8 Multiple Antigen Peptides (MAPs) were constructed, containing different molar ratios of some of these epitopes. Four MAPs (I-IV) were assembled using different combinations of T/B cell epitopes, another two (VI, VII) containing only T cell epitopes and the other two (V, VII) contained the B cell epitope and a tetanus toxin derived universal T cell epitope (tt-30). The immunogenicity of these MAPs was tested in BALB/c mice and in *Aotus lemurinus* monkeys. Cellular immune response was evaluated by *in vitro* proliferation assays and antibody production by ELISA test. In addition γ IFN induction by the MAPs was measured by an ELISA test. The MAP V containing the B cell epitope (p11) and the tt-30 epitopes and the MAP IV containing P11 and one of the T cell epitopes p25 showed to be the most immunogenic, in both mice and monkeys. The remaining MAPs were able to induce variable stimulation indexes and antibodies titers. Two of the MAPs containing only T cell epitopes (VI and VII) and aimed to be controls, also induced antibody titers in monkeys indicating that their sequence could, in addition, contain B cell epitopes. The antibodies induced by some of these MAPs were also able to recognize the native CS molecule on the *P. vivax* sporozoite in an IFA test. detailed results and potential usefulness of these constructs as malaria vaccine candidates will be discussed.

471 IMMUNOGENICITY OF A MAP SYSTEM CONTAINING B AND T HELPER REPEAT EPITOPES OF *PLASMODIUM FALCIPARUM* CS PROTEIN IN ALUM: POSSIBLE VACCINE APLICATION. De Oliveira GA*, Clavijo P, Nussenzweig RS, and Nardin EH. Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, NY.

The first trials with a synthetic vaccine based on the repeats of *P. falciparum* CS protein, $(NANP)_3$-TT, induced low titers of antibody inmost of the volunteers. Titers were dose dependent but the use of larger amounts of vaccine was precluded by carrier toxicity. Multiple antigen peptides (MAPs) provide high epitope density and do not require carriers. MAPs containing 4 copies of the 5'repeat T cell epitope (T1)and the 3'repeat B epitope, $(T1B)_4$, induce very high anti-sporozoite antibody titers in mice immunized with MAPs in Freund's. Adjuvants are a limiting factor in the design of human vaccines and, only aluminum hydroxide (Alum) is currently approved for human use. We found that, differently from other MAPs containing $(NANP)_3$ and non-repeat Tepitopes, greater than 70% of the $(T1B)_4$ MAPs bound to alum. C57Bl/10mice, immunized i.p. or s.c. with two or three doses of $(T1B)_4$/alum,had anti-sporozoite antibody titers comparable to those obtained with Freund's. The kinetics of the response differed with the two adjuvants. A low anti-sporozoite antibody response, induced by a single injection of sporozoites, can be greatly enhanced by administration of $(T1B)_4$ in alum. An effective booster was also observed 3 months after sporozoite inoculation, raising the possibility of using a MAP-based vaccine to increase the antibody levels of people living in malaria endemic areas.

472 IMMUNOGENICITY OF A MULTIPLE ANTIGEN PEPTIDE CIRCUMSPOROZOITE PROTEIN MALARIA VACCINE. Church P*, Corradin G, Hunter RL, and Hoffman SL. Malaria Program,

Naval Medical Research Institute, Rockville, MD; Department of Biochemistry, University of Lausanne, Epalinges, Switzerland; and Department of Pathology, Emory University, Atlanta, GA.

Efforts at inducing protection by active immunization with vaccines designed to produce antibodies against the repeat region of the *Plasmodium falciparum* circumsporozoite (CS) protein have shown limited success. A lack of effect may be due, in part, to a failure to induce adequate quality or quantity of antibodies against NANP, the immunodominant B cell epitope found on the repeat region of the *P. falciparum* CS protein. To formulate a more immunogenic vaccine we synthesized a 4 branched multiple antigen peptide (MAP4) with each arm bearing one copy each of P_2 and P_{30}, described previously as universal T helper epitopes from tetanus toxin, and six copies of NANP. After two doses of 50 μg of antigen, administered either intramuscularly or subcutaneously in Freund's adjuvant, B10.Br mice develop high antibody titers in an ELISA (absorbance at 410nm equal to 0.5 at serum dilutions of 1:24,000 or greater) against MAP4 $(NANP)_{10}$. Immunofluorescence assays (IFA) against air dried sporozoites with the same sera react at dilutions of 1:24000. To attempt to induce similar responses using an adjuvant suitable for human use additional studies were performed using a nonionic block copolymer. Antibody levels by ELISA or IFA equaled or exceeded results with Freund's adjuvant when the block copolymer was administered with squalene as a water in oil emulsion or with 20 μg of detoxified lipid A. This peptide is now undergoing further evaluation for development for human use.

473 PROTECTION AGAINST *PLASMODIUM YOELII* BY CS PROTEIN MULTIPLE ANTIGEN PEPTIDE-INDUCED POLYCLONAL ANTIBODIES. Wang R*, Charoenvit Y, Porrozzi R, Mellouk S, Sedegah M, Corradin GP, Hunter RL, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD; Dpto. Ultraestrutura e Biol. Cel. IOC-FIOCRUZ, Rio de Janeiro, Brazil; Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland; and Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA.

Passive transfer of Mabs against the *Plasmodium yoelii* circumsporozoite protein (PyCSP) protects mice against sporozoite-induced infection. Active immunization of mice with synthetic peptide, recombinant protein, and live vector vaccines has been shown to induce high levels of antibodies to the repeat region of the PyCSP, but has yet to protect against parasitemia, perhaps due to inadequate antibody levels. To induce higher antibody levels against sporozoites, we constructed a branched chain polymer that included B cell epitopes (4 copies of the PyCSP tandem repeat, QGPGAP) and T helper epitopes (2 universal tetanus toxin epitopes). These multiple antigen peptides (MAPs) were given with Freund's adjuvants, TiterMAX, a nonionic block copolymer P1004 and monophosphoryl lipid A (P1004/MPL), or P1004/MPL and Lipofectin (P1004/MPL/lip). Two or three vaccine doses induced antibody titers to sporozoites of 20,000 (Freund's, TiterMAX) and 10,000 (P1004/MPL, P1004/MPL/lip). In contrast passive immunization with a protective Mab or with 3 doses of irradiated sporozoites produced titers of 1280. Mice were challenged 2 weeks after the third immunization with 100 sporozoites; 78% of TiterMAX, 60% of Freund's, 50% of P1004/MPL, and 40% of P1004/MPL/lip immunized mice were protected. All controls challenged with 40 sporozoites became parasitemic. Protection correlated with antibody titer by ELISA and inhibition of liver stage development assay. This first successful protection of mice against sporozoite challenge using a synthetic pepide vaccine designed to induce protective antibodies against the PyCSP lays the foundation for human trials with an analogous vaccine.

474 EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF A FALCIPARUM SPOROZOITE VACCINE. Migasena S, Kyle DE*, Khusmith S, Singhasivanon P, Suntharasamai P, Srisuriya P, Pavanand K, Wongsrichanalai C, Viravan C, Cohen J, Ballou WR, Webster HK, Chongsuphajaisiddhi T, and Gordon DM. Department of Immunology and Parasitology, AFRIMS, Bangkok, Thailand; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand;

Department of Immunology, WRAIR, Washington, DC; and SmithKline Beecham Biologicals, Rixensart, Belgium.

A clinical study was conducted in Thailand to evaluate the safety and immunogenicity of a recombinantly produced *Plasmodium falciparum* circumsporozoite protein (CSP) based vaccine candidate. The RTS,S vaccine has the hepatitis B virus envelope protein as a carrier for B-cell and T-cell epitopes from the central repeat region and the carboxy terminal region of CSP. The vaccine formulation contained 50 μg/ml of RTS,S, 500 μg/ml of alum, and 50 μg/ml of 3-deacylated monophosphoryl lipid A (3D-MPL). A total of 30 adult Thai male volunteers, 15 malaria naive volunteers at Mahidol Vaccine Trial Centre and 15 malaria experienced individuals from Chantaburi Province, received two doses of vaccine (0 and 8 weeks). Each dose of vaccine was well tolerated in both groups; the major reaction noted was tenderness at the site of infection one and two days after each dose. Peak antibody levels to the repeat region of CSP were seen at two weeks after the first dose in both groups, but no boosting was observed after the second dose. In contrast, antibodies to the carboxy terminal region of CSP increased after each dose. Interestingly, 5 of 14 malaria naive and 7 of 15 malaria experienced volunteers developed cytotoxic T-lymphocyte activity against a T-cell epitope in the carboxy terminal region of CSP. The results of this study indicate that the RTS,S/alum/3D-MPL vaccine was safe and immunogenic in adult Thai males and when compared to results of a similar trial in American volunteers, suggest that ethnic differences may play a role in the immune response to this investigational malaria vaccine.

475 SAFETY, IMMUNOGENICITY AND EFFICACY OF LIPOSOME-ENCAPSULATED *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE ANTIGEN ADJUVANTED WITH ALUM AND LIPID A. Heppner DG*, Gordon DM, Gross M, Trofa AF, McKinney DA, Alving CR, Owens R, Sylvester DR, Porter TG, Theisen T, Sadoff JM, and Ballou WR. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC; SmithKline Beecham Pharmaceuticals, King of Prussia, PA; and Department of Enteric Infections, Walter Reed Army Institute of Research, Washington, DC.

Seventeen malaria naive adult male volunteers are participating in a Phase I/IIa study designed to evaluate the safety, immunogenicity, and efficacy of a recombinantly produced *Plasmodium falciparum* subunit candidate vaccine, NS1RLF, encapsulated in liposomes with alum and lipid A. NS1RLF contains important B and T cell epitopes found in the flanking regions of the *P. falciparum* circumsporozoite protein (CSP), but is devoid of repeat amino acid sequences from the central region of the CSP. Two groups of volunteers have begun immunizations on a 0, 2, and 6 month schedule with vaccine formulations containing approximately 200 ug of NS1RLF, 500 ug of alum and either low dose lipid A (100 μg, N=8), or high dose lipid A (1000 μg, N=9). After two doses, both formulations were well tolerated, and immunogenic as measured by plate ELISA using a related capture antigen, GP195RLF, and by the development of anti-sporozoite antibodies, as determined in an immunofluorescence assay. Although variations in individual anti-GP195RLF titers were noted, the high dose lipid A recipients developed higher geometric mean titers than did the lower dose group. Volunteers will receive a third dose of vaccine and undergo experimental challenge via the bites of five malaria infected mosquitoes. Details of humoral and cellular immune responses to NS1RLF and vaccine efficacy will be discussed.

476 COMBINED USE OF RECOMBINANT INFLUENZA VIRUS AND RECOMBINANT VACCINIA VIRUS INDUCES CD8+ T CELL-MEDIATED PROTECTIVE IMMUNITY AGAINST MALARIA. Rodrigues M*, Li S, Rodriguez D, Rodriguez JR, Esteban M, Palese P, Nussenzweig RS, and Zavala F. Department of Medical and Molecular Parasitology, New York University, New York, NY; Department of Microbiology, Mount Sinai School of Medicine, New York, NY; and Department of Biochemistry, State University of New York, New York, NY.

We evaluated the immunogenic properties of recombinant vaccinia and influenza viruses expressing the cytotoxic epitope of the *Plasmodium yoelii* CS protein. We demonstrated for the first time, that as described for recombinant vaccinia viruses, recombinant influenza is a most efficient vector for the induction of class I MHC restricted CD8+ T cells against foreign epitopes. Immunization of mice with recombinant influenza followed by a booster with recombinant vaccinia virus induced protective immunity against sporozoite induced malaria. The sequence of immunization is crucial, since priming with recombinant vaccinia followed by recombinant influenza, failed to induce protective immunity. The protection induced by immunization with these live carriers is mostly mediated by CD8+ T cells, since treatment of immune mice with anti-CD8 antibodies abolishes the anti-malaria immunity. We evaluated several recombinant vaccinia and influenza viruses expressing different portions of the CS protein, inserted in different regions of the respective viral genome. Although all these constructs are capable of inducing anti-CS CD8+ T cells, some appear to be more efficient at inducing protective immunity.

477 SAFETY AND IMMUNOGENICITY OF ALUM-ADJUVANTED SPf66 PRODUCED IN THE UNITED STATES UNDER CGMP STANDARDS. Gordon DM*, Sadoff JC, Heppner DG, Klotz FW, Seguin MC, Duffy PE, Krzych U, and Ballou WR. Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.

The synthetic *Plasmodium falciparum* peptide/polymer antigen SPf66 has been manufactured in the United States and an alum-adjuvanted vaccine formulation produced according to cGMP standards. Ten malaria-naive adult men and women have received a total of 29 doses of the WRAIR alum-adjuvanted SPf66 (clinical lot #13301). This clinical lot has been well tolerated with the most commonly noted side effects being mild tenderness, erythema, and induration at the site of injection. Vaccine immunogenicity was determined by a standard ELISA using SPf66 as the capture antigen. Volunteers were scored as seroconverters if the mean absorbance of their specific post-immune sera, run in triplicate, exceeded the group mean pre-immune absorbance by three standard deviations. Six of the nine volunteers (66.7%) who received a total of three doses of alum-adjuvanted SPf66 seroconverted. The one volunteer who received only two doses of vaccine failed to develop any measurable antibody response. Immunofluorescence assays using blood stage parasites have been uniformly negative. The one volunteer with the highest anti-SPf66 antibody response, as measured by ELISA, demonstrated a weakly positive response against an 83kD antigen by western blot analysis using blood stage parasite extracts derived from the CAMP strain of *P. falciparum*. Preliminary experiments demonstrated that eight of the nine volunteers developed T cells capabale of recognizing SPf66 upon *in vitro* exposure in T cell proliferative assays.

478 PROTECTION IN *PLASMODIUM YOELII* MALARIA IS ASSOCIATED WITH AN ANAMNESTIC IgG2a ANTIBODY RESPONSE. Kidd MR*, Lal AA, and Hunter RL. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease, Atlanta, GA; and Department of Pathology, Emory University, Atlanta GA.

Experiments were conducted to determine the optimal regimen for inducing prolonged immunity against lethal *Plasmodium yoelii* malaria using whole killed parasite antigen and to evaluate the nature of the protective immune response. Mice were immunized with *P. yoelii* antigen in two adjuvants: copolymer P1004 with detoxified RaLPS in saline or the same materials in a squalane-in-water emulsion. Vaccines using both adjuvants produced protection following immunization with the whole killed parasites. 100% of mice immunized with 100 μg of antigen and boosted twice with 50 μg survived a challenge that killed 93% of control animals. Protection persisted for 9 months. Passive immunization with serum from immunized and challenged mice protected at least as well as active immunization. In further studies, a single immunization with 100 μg protected 75% of the animals from dying. A dose response study showed that immunization with as little as 12 μg

followed by a 5 μg boost protected 4 out of 6 animals. Some of the protected animals had very low antibody titers prior to challenge. However, protection was associated with priming of animals for IgG2a antibody response as measured by IFA. These data support the hypothesis that protection can be mediated by antibody of the IgG2a isotype.

479 A 12 KDA FRAGMENT OF THE *PLASMODIUM YOELII YOELII* 17XL MEROZOITE SURFACE PROTEIN MSP-1, EXPRESSED IN *ESCHERICHIA COLI*, PRODUCES PROTECTIVE IMMUNITY IN MICE. Daly TM* and Long CA. Department of Microbiology and Immunology, Hahnemann University, Philadelphia, PA.

The merozoite surface protein-1 (MSP-1) is a major focus of efforts to develop a vaccine against the erythrocytic stages of malaria, since it has been shown to induce significant protection against virulent challenge infection in both rodent and primate experimental models. In addition, passive transfer of the monoclonal antibody mAb 302, which binds to the cysteine-rich, carboxyl-terminal region of the *Plasmodium yoelii yoelii* (*P.y.y.*) 17XL MSP-1, provided dramatic protection against a lethal *P.y.y.* 17XL infection in a murine model. We have demonstrated that this cysteine-rich, carboxyl-terminal region of the *P.y.y.* 17XL MSP-1 can be expressed in a native conformation as a glutathione S-transferase fusion protein (GST-PYC1) in *Escherichia coli*. Following immunization with the GST-PYC1 fusion protein in Ribi adjuvant, both inbred and outbred mice were completely or partially protected against challenge infection with homologous malarial parasites. A truncated construct, GST-PYC2, and modified isolation procedures have produced a fusion protein which provided complete protection in inbred mice when administered in Ribi adjuvant over a range of protein concentrations. In addition, different adjuvants have been shown to influence significantly the level of protection generated by immunization with the GST-PYC2 fusion protein. Further studies to assess the contribution of cellular and humoral immunity to the protection observed *in vivo* are in progress.

480 EXPOSURE TO*PLASMODIUM FALCIPARUM* HIGHLY INCREASES THE PROTECTIVE CAPACITY OF MALARIA RECOMBINANT PROTEINS. Herrera S, De Herrera MA, Zapata C, Renjifo G, Gonzalez M, and Schoenfeld HJ*. Certa U. School of Health, Universidad del Valle, Cali, Colombia; and Pharma Research Technology, F. Hoffman-LaRoche Ltd, Basel, Switzerland.

The conserved recombinant fragment 190L of the *Plasmodium falciparum* MSP-1 antigen induces partial protection of *Aotus* monkeys when it is hybridized to a promiscuous T cell epitope (CS.T3 from the CS protein). Immunization of these monkeys for three times with the recombinant 190L.CS.T3 induced high specific antibody titers as well as production of levels of γ-IFN levels. The same immunization protocol used with *P. falciparum* rp 41 (aldolase) did not induce protection. 190L.CS.T3 was prepared and purified following the standards of Good Manufacturing Practice (GMP) and used for *Aotus* protection experiments. Two groups of *Aotus* monkeys were immunized 4 times using as adjuvant either Freund's adjuvants (CFA/IFA) or Immuno-stimulating-complexes (ISCOMS) and one group used as control was immunized with rp-41. Monkeys of the first two groups were less protected than in previous experiments but the third group delayed the development of parasitemia. In a second challenge 10 months late, the Aotus from the 3 immunized groups spontaneously controlled the infection after a very low peak of parasitemia. We discuss here the importance of boosting of the immune response with the native proteins, the possible role of g-IFN in protection and the importance of these results in the evaluation of malaria vaccines.

481 REGULATION OF *PLASMODIUM FALCIPARUM* MSP-1 T CELL EPITOPE RECOGNITION BY THE MAJOR HISTOCOMPABILITY COMPLEX. Chang SP*, Hashimoto AK, Kanda P, and Hui GS. Department of Tropical Medicine & Medical Microbiology, John A. Burns School of Medicine,

Honolulu, HI; and Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX.

The *Plasmodium falciparum* merozoite surface antigen MSP-1 is under development as a blood stage malaria vaccine. In previous studies we have shown that when mouse strains possessing different MHC genes are immunized with MSP-1, all strains produce anti-MSP-1 antibodies but antisera of only a few strains inhibit *in vitro* parasite growth. A recombinant baculovirus polypeptide (BVp42) based on the C-terminal fragment of the *P. falciparum* MSP-1 protein also induces the production of antibodies which inhibit *in vitro* parasite growth. In the present study, we immunized several strains of congenic mice sharing the C57BL/10 genetic background but differing in H-2 haplotype with BVp42 to determine whether this antigen would prime T cells utilizing various MHC restriction elements. BVp42-primed mouse T cells proliferated when restimulated in culture with BVp42, whole parasite extracts and purified, parasite MSP-1. While T cell responsiveness to the complete BVp42 polypeptide was observed for all strains examined, BVp42-primed T cells from different strains recognized different sets of MSP-1-based synthetic peptides. These differences in T cell epitope recognition are consistent with differences in B cell epitope recognition exhibited by various H-2 haplotypes, and suggest an association between MSP-1 T helper cell and B cell specificity.

482 NATURAL HISTORY OF HIV-1 INFECTION IN A COHORT OF FIFTY-FOUR SEROPOSITIVE, FILIPINO PROSTITUTES. Perrault JG*, Manaloto CR, Caringal LT, Santiago EG, Basaca-Sevilla V, Hayes CG, and Anthony RL. U.S. Naval Medical Research Unit No. 2, Manila Detachment, Republic of the Philippines.

A prospective follow-up study on the progression of HIV infection was carried out on a cohort of 54 Filipino prostitutes. When applying the 1993 CDC AIDS Classification Standards, the cumulative probability of developing AIDS was 52.9% within 5 years and 73.8% within 6 years after seroconversion for HIV-1 antibodies. The cumulative probability of death was 52.1% within 6.5 years following seroconversion and 52.7% within 1.5 years after the diagnosis of AIDS. Although 43 AIDS-indicating clinical conditions were observed, a CD4+ cell count below 200/mm3 was the initial AIDS-indicating condition in more than 50% of the patients. It is quite possible, however, that these low CD4+ cell counts are being exacerbated by the immunosuppressive effect of chronic parasitic infections which are commonplace in this population (i.e. ascariasis, trichuriasis, amoebiasis, giardiasis and hookworm). *Mycobacterium tuberculosis* and/or unspeciated acid fast bacilli, presumed to be *M. tuberculosis*, and *Pneumocystis carinii* pneumonia were the first AIDS-indicating conditions in the other patients. The time of onset of AIDS was not significantly different whether based upon the 1993 AIDS Classification System, Clinical Category C infection or CD4+ cell counts. This congruency suggests that Category C conditions can be used as the primary AIDS-indicating event in developing countries when a flow cytometry facility is not available. Few AIDS cases would be missed. However, if the 1987 CDC Classification Standards had been applied, only 6 of the 28 AIDS cases would have been classified as AIDS and only 9 would have progressed to AIDS at any point in this study. The addition of pulmonary tuberculosis and a CD4+ count of <200/mm3 to the list of AIDS-indicator conditions in the revised system accounted for the difference.

483 LONGITUDINAL STUDIES OF BABIES BORN TO HIV-1 SEROPOSITIVE FILIPINO PROSTITUTES; DIAGNOSTIC DILEMMAS. Manaloto CR*, Caringal LT, Hayes CG, Perrault JG, Santiago EV, Gonzales VL, and Anthony RL. U.S. Naval Medical Research Unit No. 2 Detachment Republic of the Philippines; and San Lazaro Hospital, Manila, Republic of the Philippines.

Fifteen term infants born to 12 HIV-1 seropositive Filipino prostitutes have been monitored for signs of HIV-1 infection for a mean of 37.1 months (range 4-68 months; 95% CI = 25.7-48.5). Twelve babies were followed since birth; 3 others entered the study at 8, 9 and 10 months of age respectively. Serum was tested for HIV antibodies and HIV antigen, mononuclear cells were cultured for virus isolation

and comprehensive physical examinations were performed quarterly. Of the 12 babies studied since birth, 11 remained Western blot positive for the first six months of age. The 12th case had reverted to indeterminant within 3 months. After one year, five were still positive, five were indeterminant and two were negative. Two of the three babies who were first seen at >6mos of age were indeterminant on the initial examination; one converted to positive over the next six months. Of the 10 babies who have now been monitored for >18 months, four remain negative, two are confirmed positive with positive lymphocyte cultures for HIV and four remain indeterminant by Western blot. These indeterminant Western blots (no reactivity with p24 and gp41 and/or gp 120/160) have now persisted for 31-60 months (mean 50.6; 95% CI = 41.4-59.7). Although a definitive diagnosis cannot be made on the basis of these laboratory findings, clinical observations (PGL, thrush, anergy etc.) are highly indicative of an HIV infection. It is suspected that the broad spectrum of bacterial and parasitic diseases (i.e., ascariasis, trichuriasis, giardiasis and amoebiasis) which afflict most of these babies contributes to both the AIDS like symptoms as well as to the inability to mount a measurable HIV antibody response.

484 HIV-2 IN INDIA. Banerjee K*. National Institute of Virology, Pune, India.

The existence of HIV-1 in India has been established as early as in 1985. With the serosurveys used HIV-1 ELISA and Western blot (WB) kits relatively high prevalence of seropositivity was seen in western India followed by southern India. In the north part of India the prevalence was low. During the serosurveys a number of sera were found indeterminate in WB. These sera were tested by HIV-1+2 ELISA and Liatek (Line immunoassay kit which could detect both HIV-1+2). A number of such sera were positive for HIV-2 and HIV-1+2(1). Following this about 250 sera each from the two high risk groups viz. patients attending STD clinics and female sex workers collected from 1989 to 1992 were tested by HIV-1 ELISA, HIV-1+2 ELISA, HIV-1 WB and Liatek. In all 1000 sera from STD patients and 1008 sera from female sex workers were tested. When tested by HIV-1 ELISA, between the two risk groups, 70 and 259 sera respectively were positive for HIV-1. HIV-1 WB showed 62 and 241 positives. The HIV-1+2 ELISA showed 102 positives from STD group and 342 were positive from the prostitute group. The line immunoassay showed 60, and 231 HIV-1 positives respectively in two high risk groups. Two and 23 sera respectively were positive both for HIV-1 and 2. For HIV-2, in 5 and 22 (total 27 cases) cases respectively were positive in the two groups. The HIV-2 positives were from Bombay, Pune, Kolhapur, Miraj. It seems therefore the HIV-2 is well spread under western India and possibly making inroads through ports in southern India as well.

485 GENETIC ANALYSIS AND MOLECULAR PHYLOGENY OF PRIMATE T-CELL LYMPHOTROPIC VIRUS TYPE I. Song KJ*, Nerurkar VR, Saitou N, and Yanagihara R. Laboratory of Central Nervous System Studies, NINDS, National Institutes of Health, Bethesda, MD; and Laboratory of Evolutionary Genetics, NIG, Mishima, Japan.

Type C retroviruses, designated simian T-cell lymphotropic virus type I (STLV-I), have been isolated from several genera of Old World monkeys and apes, but not from New World monkeys and prosimians. To determine the genomic diversity and molecular evolution of STLV-I and to clarify their genetic relationship to human T-cell lymphotropic virus type I (HTLV-I), we enzymatically amplified, then directly sequenced selected regions of the *gag, pol, env* and *pX* genes of STLV-I strains from Asia and Africa. STLV-I strains from Japanese macaques, which exhibited sequence similarities ranging from 98.5% to 99.8% among themselves, diverged by 12.9% to 13.3% from an STLV-I strain from a naturally infected rhesus macaque, by 9.7% to 11.2% from STLV-I strains from Africa and by 8.8% to 11.2% from cosmopolitan and Melanesian HTLV-I strains. By contrast, the interspecies nucleotide sequence similarity among the African STLV-I strains was remarkably high, ranging from 96.9% to 97.4%, and they diverged by only 2.2% to 2.8% from the HTLV-I strain EL from equatorial Zaire. Dendograms based on these sequences indicated that STLV-I and HTLV-I strains formed four major geographically dependent clusters: the first comprised of STLV-I from southern Asia; the

second of STLV-I strains from Japan and Indonesia; the third of HTLV-I strains from Melanesia; and the fourth of STLV-I strains from Africa and representative cosmopolitan strains of HTLV-I. Our sequence and phylogenetic analyses are consistent with an archaic presence of STLV-I in Asia, probably predating macaque speciation, with subsequent independent virus evolution in Asia and Africa.

486 HTLV-I FROM IRANIAN-BORN MASHHADI JEWS WITH SPASTIC MYELOPATHY: GENETIC AND PHYLOGENETIC LINK WITH VIRUS STRAINS FROM JAPAN AND INDIA. Nerurkar VR*, Song KJ, Achiron A, Melland RR, Hamiel O, Shohat B, Melamed E, and Yanagihara R. Laboratory of Central Nervous System Studies, NINDS, NIH, Bethesda, MD, U.S.A.; Beilinson Medical Center, Petah-Tiqva, Israel; Sheba Medical Center, Tel-Hashomer, Israel; and Weizmann Institute of Science, Rehovot, Israel.

Human T-cell lymphotropic virus type I (HTLV-I)-caused diseases, namely HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATLL), have been identified recently in the Middle East. To determine the molecular epidemiology and evolution of HTLV-I in the Middle East, we amplified by PCR, then directly sequenced 856 nucleotides spanning selected regions of the HTLV-I IgagI, IpolI, IenvI and ItaxI genes in DNA extracted from uncultured peripheral blood mononuclear cells, obtained from six Iranian-born Mashhadi Jews, four with spastic myelopathy and two asymptomatic carriers. Alignment and comparison of these gene sequences with those of cosmopolitan and Melanesian strains of HTLV-I indicated that the virus strains from Iranian-born Mashhadi Jews were 99.8% to 100% identical among themselves and 98.5% to 98.7% homologous to the Japanese HTLV-I prototype ATK. No frame shifts, insertions, deletions or disease-specific base changes were found in the regions sequenced. Dendrograms based on 856 nucleotides indicated that the HTLV-I strains from Iranian-born Mashhadi Jews clustered with those from Japan, southern India and the Caribbean, suggesting a common origin or source of infection with subsequent parallel evolution in these geographically distant and culturally disparate populations. Further molecular genetic studies of other HTLV-I strains from the Middle East and the Indian subcontinent, and of STLV-I isolates from nonhuman primates from Eurasia, may provide additional insights into the emergence and early dissemination of these type C lymphotropic retroviruses.

487 PREVALANCE OF HEPATITIS E AMONG NON-A, NON-B PATIENTS IN THE KATHMANDU VALLEY, NEPAL. Innis BL, Myint KS, Clayson ET*, Narupiti S, Mongkolsirichaikul D, Manomuth C, and Shrestha MP. Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; and Teku Hospital, Kathmandu, Nepal.

In the Kathmandu Valley of Nepal, sporadic cases of non-A, non-B hepatitis are common, with peak incidence occurring during the monsoon season. To determine the proportion of acute non-A, non-B hepatitis in Kathmandu attributable to infection with the hepatitis E virus (HEV), we evaluated sera and stool of 79 non-A, non-B hepatitis patients from June 1988 through August 1992. Their ages ranged from 14 to 74 (median 25); there were 45 males and 34 females. The number of patients examined per year ranged from 10 to 21. We defined a case as hepatitis E if anti-HEV antibodies were detected in acute sera using a fluorescent antibody blocking assay (FABA) or, if HEV RNA was detected in stool by reverse transcription polymerase chain reaction (RT-PCR). Anti-HEV antibodies were detected in 17 of 79 (22%) cases; whereas, of the 30 cases examined to date, HEV RNA was detected in 24 (80%) of the cases, indicating that HEV accounts for a large percentage of acute non-A, non-B hepatitis cases in Kathmandu. HEV excretion was detected in stools collected from 1 to 15 days of onset of symptoms, suggesting that RT-PCR is a valuable diagnostic tool. Also in 10 cases, HEV RNA was detected but anti-HEV antibodies were not detected, suggesting that the RT-PCR is a more sensitive diagnostic assay than the FABA, or that a variant strain of HEV circulates in Nepal; both of these hypotheses are being examined.

488 MOLECULAR CHARACTERIZATION OF CARRIER RABIES VIRUSES. Warner CK* and Fekadu M. Viral & Rickettsial Zoonoses Branch, CDC, Atlanta, GA.

Rabies viruses isolated from rabies carrier dogs were characterized by restriction enzyme analysis. The viral genomes were examined with enzymes that recognized 39 restriction sites representing 663 bases or 5.5% of the genome. Viral isolates from a carrier dog that was experimentally infected with one of the carrier viruses were also examined. These isolates were shed in the saliva of the infected dog at intervals after recovery from rabies infection. Virus was also isolated from the tonsil of this dog at necropsy. The relative differences in the isolates were compared using the PAUP program. All the viral isolates from the single dog were more closely related than the individual carrier isolates were to each other. None of the viral isolates were identical to any of the other isolates in this analysis. The virus strains were also compared with CVS, with Pasteur virus, and with a street rabies virus isolated in the area. The carrier isolates were most closely related to the street strain from the area and distantly related to CVS and Pasteur.

489 IDENTIFICATION OF GLOBAL RESERVOIRS OF DOG RABIES. Smith JS*, Orciari LA and Yager PA. Viral & Rickettsial Zoonoses Branch, Centers for Disease Control, Atlanta, GA.

When virus isolates are characterized by antigenic typing with monoclonal antibodies, reservoirs of rabies virus in various wild animal species in different areas of the world often contain widely differing variants. In contrast, virus isolates originating in areas where rabies is enzootic in dogs show only limited antigenic diversity. Antigenic identity in dog rabies virus samples may have arisen through a convergence of amino acid changes during adaptation of different wild animal variants to dogs (i.e., biological fitness) or alternatively, may indicate a global reservoir of dog rabies which arose from a common source (i.e., multiple introduction of the same variant). We sequenced a 200 nucleotide region of the nucleoprotein of rabies virus samples from more than 200 isolates collected in reservoirs for rabies in sylvatic and domestic species on 6 continents. Phylogenetic analysis of nucleotide sequences, case surveillance, and historical records all support the emergence of rabies in dogs and some wild canids in many parts of the world as a consequence of the transport of infected domestic dogs during European colonization in the 17th and 18th centuries.

490 EPIZOOTIC RACCOON RABIES IN NEW ENGLAND: ENVIRONMENTAL ASSOCIATIONS OF EXPANDING DISTRIBUTION AND HUMAN RISK. Wilson ML*, Cartter ML, and Cooper GH. Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, CT; and Epidemiology Section, Department of Health Services, State of Connecticut, Hartford, CT.

An estimated $300 million is spent annually in the U.S. to prevent rabies, with another $15 million expended and immeasurable emotional suffering endured during post-exposure treatment of some 18,000-20,000Americans. Most such exposures involve indirect contact with rabid wild vertebrates through pets. Epizootic rabies in raccoons recently emerged in the northeastern U.S., and now is expanding at an alarming rate. As part of a larger project on the epidemiology of rabies there, we studied ecological factors associated with transmission and risk of human exposure. Various vertebrates from Connecticut were tested for rabies virus antigen during 1991-1993 to monitor the spread of infection. Species-specific prevalences confirmed that most transmission occurred among raccoons. Temporal analyses suggested that the rate of transmission was similar throughout the region, while the spatial pattern of spread was not uniform. Various ecological factors were associated with evidence of transmission. We also analysed environmental and social characteristics of presumed human exposure.These initial observations are discussed as they relate to raccoon infectivity and immunity, to proposals for wildlife vaccination programs, and ultimately to reducing human risk.

491 FAILURE OF PRAZIQUANTEL THERAPY IN CHRONICALLY INFECTED PATIENTS WITH *SCHISTOSOMA HAEMATOBIUM* IS ASSOCIATED WITH ELEVATED PARASITE-SPECIFIC IgG4. Medhat A*, Nafeh M, Shata T, Mohamed S, Shehata M, Helmy A, Saad M, Strickland GT, and King CL. Department of Microbiology, Assiut University, Assiut, Egypt; Department of Tropical Medicine, Assiut University, Assiut, Egypt; Department of Epidemiology and Preventive Medicine, University pf Maryland, Baltimore, MD; and Division of Geographic Medicine, Case Western Reserve University, Cleveland, OH.

The present study is an ongoing prospective trial to examine the effects of praziquantel therapy on the morbidity and immune responses in patients with chronic *Schistosoma haematobium* infections in Upper Egypt. In one arm of the study, patients received 40 mg/kg of praziquantel in a single oral dose (n=100) and were examined at 1 (n=72) and 3 (n=39) months post-treatment for the presence of viable ova in a single urine examination. Patients with viable ova at 1 mo post-treatment (37.5%) were retreated with praziquantel and by 3 mos after initial therapy 15.7% continued to excrete living eggs. Because praziquantel may act synergistically with anti-schistosome antibodies, we examined whether treatment failures after repeated praziquantel therapy may be related to impaired humoral responses to the parasite. Adult worm (SWAP)-specific IgE and IgG4 antibodies were measured in sera of patients who responded (n=33; RES) and failed (n=6; NONR) treatment by ELISA. Intensity of infection was similar between the two groups (RES; geometric mean [GM]=9.5 ova/10ml urine vs NONR, GM=11.8 ova/10ml). Both groups were comparable with respect to age, sex and water contact. SWAP specific IgE levels were identical between the two groups (RES, GM=0.17 μg/ml; NONR, GM=0.1 μg/ml), whereas SWAP specific IgG4 levels tended to be higher among NONR (GM=330 μg/ml) compared to RES (GM=90 μg/ml, p=0.09). These results suggest that failure to eliminate ova excretion in some schistosome infected individuals with praziquantel treatment may be associated with development of blocking antibodies (IgG4).

492 SEROLOGIC DETECTION OF *SCHISTOSOMA MANSONI* USING CP1, AN ADULT WORM ANTIGEN. Chappell CL* and Newman PT. Department of Family Medicine, Baylor College of Medicine, Houston, Texas.

Despite ongoing efforts, schistosomiasis remains a major parasitic infection in many countries of the world. Currently, infection is identified by the microscopic detection of parasite ova in fecal smears, a time-consuming and relatively insensitive method. An ELISA-based assay could increase detection sensitivity, while allowing many samples to be run concurrently. We have explored the use of a worm-specific protein, CP1, as a serodiagnostic antigen. CP1 is a cysteine proteinase involved in parasite nutrient acquisition and is subsequently released into host circulation. The sensitivity of the anti-CP1 response was evaluated using *Schistosoma mansoni*-infected mice challenged with various cercarial doses. Infections with 150, 100, 50 or 10 cercaria were followed from 4-30 weeks. In addition, a group of mice receiving 50 cercaria were treated with praziquantel. Mice were considered infected if worms were recovered or if there was a significant increase in liver and splenic weights. Sera from these mice were tested for anti-CP1 IgM, IgG and IgE over the course of the infection. Positive antibody titers were found with all levels of challenge infection as early as 2-4 weeks after infection, i.e. during the prepatent period. The kinetics of the IgM and IgG responses were related to the intensity of infection; while IgE response showed no dose dependency. Following praziquantel treatment, liver and splenic weights returned to normal and worm recovery was greatly reduced, but not always eliminated. IgM and IgG titers began to fall significantly 6 weeks after treatment; however, none returned to pre-infection levels. Thus, the anti-CP1 response can be used to detect light, as well as heavy, infections early in the course of the disease and are significantly decreased following treatment.

493 SCHISTOMIASIS OF EXPATRIATES AND VISITORS TO MALAWI: SEROLOGIC DIAGNOSIS AND SPECIATION. Pilcher JB*, Tsang VC, Noh J, Al-Sherbiny MM, Wilson M, Ware DA, Cetron MS, Addis DG, and Chitsulo L. PDB, NCID, Centers for Disease Control and Prevention, Atlanta, GA; Ministry of Health, Malawi; and Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

Unexpectedly high seroprevalence of schistosomiasis among visitors and CDC field station staff near Lake Malawi, an area previously noted as "schistosomiasis-free", prompted us to survey of 958 expatiates and visitors. Diagnosis of 302 cases of schistosomiasis was made by screening all serum samples with a Falcon based enzyme- linked immunosorbent assay (FAST-ELISA) and to confirm and speciate with the enzyme-linked immunoelectrotransfer blot (EITB). Both of these assays utilize microsomal antigens from the three major human *Schistosoma* species. The sensitivity and specificity of these assays are in the range of 98% to 99%. Our survey data are summarized as follows: FAST-ELISA positive for *S. mansoni* &/or *S. haematobium* = 353/958 FAST-ELISA positive for *S. japonicum* = 32/138 EITB confirmed for *S. mansoni* alone = 88 EITB confirmed for *S. haematobium* alone = 278 EITB confirmed for *S. japonicum* alone = 3 EITB confirmed for *S. mansoni* + *S. haematobium* = 60. The data indicate the presence of a significant risk of schistosomiasis in the vicinity of Lake Malawi.

494 PRESENCE OF *BULINUS GLOBOSUS*, INTERMEDIATE HOST OF *SCHISTOSOMA HAEMATOBIUM*, IN LAKE MALAWI, AFRICA: A CHANGING PERSPECTIVE ON TRANSMISSION. Sullivan JJ*, Cetron MS, Chitsulo L, Nakhate W, and Addiss DG. Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA; and Community Health Sciences Unit, Ministry of Health, Lilongwe, Malawi.

In mid-April, 1993, a preliminary snail survey was conducted on the southern shore of Lake Malawi in the areas of Salima, Nkopola and Cape Maclear as part of a study of schistosomiasis prevalence in groups which use the lake for recreation. In the Salima area, 25 *Bulinus globosus* were collected from three temporary pools (2-3 m diameter) and seven snails were found in a reed bed in a small lagoon immediately behind the lake shore. None of these snails shed cercariae. However, a large population of *B. globosus* was found on the lake shore at Maldeco village south of Nkopola. The snails were found in reed beds on both sides of Maldeco village; this finding represents the first report of this snail on the lake shore itself. Of 317 snails collected in this site, one was positive for *Schistosoma hematobium* by shedding. No schistosome infections were found in 75 snails examined by crushing. All snails collected in Maldeco and in the Salima area were >5 mm in length indicating that snails were no longer breeding and may signify a dry season decline. Although *B. globosus* was not found along the shore of Cape Maclear, several specimens were collected in an agricultural area behind Chembe, the largest village on the Cape. The finding of *B. globosus* along the shore of Lake Malawi, particularly in Maldeco, indicates that suitable shore habitats exist for *B. globosus* and the position that Lake Malawi is free of schistosomiasis is no longer tenable.

495 SCHISTOSOMIASIS TRANSMISSION ALONG THE SHORES OF LAKE MALAWI: AN EPIDEMIOLOGICAL INVESTIGATION OF RESIDENT EXPATRIATES AND VISITORS OF MALAWI. Cetron MS*, Chitsulo L, Addiss DG, Sullivan JJ, Pilcher JB, and Tsang VC. Division of Parasitic Diseases, NCID, Centers for Disease Control, Atlanta, GA; Community Health Sciences Unit, Ministry of Health, Lilongwe, Malawi.

In 1992 two Peace Corps volunteers (PCVs) contracted central nervous system schistosomiasis due to *Schistosoma hematobium* infection, presumably as a consequence of recreational water exposure at a resort area on Lake Malawi. Lake Malawi has been widely considered free of schistosomiasis. To determine the risk for acquiring schistosomiasis in Lake Malawi, a cohort of 1000 resident expatriates and visitors to Malawi were questioned about their fresh water contact, recreational water activities,

and medical history. Blood specimens for anti-schistosomal antibodies (ASA) were collected to determine the seroprevalence of *S. hematobium* (SH) and *S. mansoni* (SM) using FAST-ELISA and immunoblot analyses. The expatriate population included 99 U.S PCVs, 65 U.S. State Dept.employees, 151 other U.S. citizens and 643 non-U.S. foreign nationals. Among expatriates, 97% (930)had water exposure at resort areas on Lake Malawi; 47%(440) reported Lake Malawi as their only source of water contact in a schistosomiasis-endemic area. The prevalence of ASA was 32% for both the entire cohort (306/958) and those whose reported water exposure was limited to Lake Malawi (141/440). Among the latter 141 seropositive expatriates, 96% (135) had antibodies to SH, 19% (27) to SM, and 15% (21) to both SH and SM. Epidemiologic data indicate that *S. hematobium* is endemic among expatriates in Malawi and recreational water contact on Lake Malawi is a likely source of schistosomiasis transmission.

496 SERODIAGNOSIS OF SCHISTOSOMIASIS IN KENYA USING SCHISTOSOME EGG ANTIGENS IN ELISA. Doenhoff MJ*, Butterworth AE, Hayes RJ, Tricker K, Ouma J, and Koech D. School of Biological Sciences, University of Wales, Bangor, UK.; Department of Pathology, University of Cambridge, Cambridge, UK.; Department of Epidemiology & Population Sciences, London School of Hygiene & Tropical Medicine, London, UK.; Department of Epidemiology and Social Research, Christie Hospital, Manchester, UK; Division of Vector-Borne Diseases, Nairobi, Kenya; and Kenya Medical Research Institute, Nairobi, Kenya.

As yet no single immunodiagnostic test for schistosomiasis is in widespread use, but two possibilities exist: detection of either circulating antigens or specific antibody. In pursuit of the latter course, the serodiagnostic potential of schistosome egg antigens has been further tested in Kenya. Blood samples were obtained from 4 different areas: (i) non- endemic control area, (ii) area endemic for *Schistosoma mansoni* only, (iii) area with *S. haematobium* only, and (iv) mixed infection area. Three antigens were applied in conventional ELISA: (a) crude soluble *S. mansoni* egg antigens (Sm-SEA), (b) partially-purified fraction of Sm-SEA containing two cationic antigens (CEF6), and (c) crude soluble *S. bovis* egg antigens (Sb-SEA). Results on sensitivity and specificity of the antigens in ELISA will be summarized, and possible reasons for the poor immunological specificity encountered in the endemic areas discussed. The *S. haematobium* bloods reacted more intensely against Sb-SEA than Sm-SEA, and the combined use of Sm-SEA, CEF6 and Sb-SEA enabled discrimination between endemic infections of *S. haematobium* and *S. mansoni*. After chemotherapy of *S. mansoni* infection, specific antibody reactivity against CEF6 declined more rapidly than antibody reactivity against Sm-SEA. The results indicate that methods for detecting egg-specific antibodies can have epidemiological and serodiagnostic value in schistosome endemic areas.

497 THE IMPACT OF SCHISTOSOMIASIS ON NUTRITIONAL STATUS OF BRAZILIAN CHILDREN. Parraga IM*, Assis AO, Reis MG, Prado MS, King CH, Barreto ML, and Blanton RE. Case Western Reserve University, Cleveland, OH; Federal University of Bahia, Salvador, Brazil; and Oswaldo Cruz Foundation, Salvador, Brazil.

Light or moderate intensity infection with *Schistosoma mansoni* may contribute to nutritional deficiencies, and therefore affect 90% of those infected. We report on the first stage of a randomized, placebo controlled, double-blind study of the effects of treatment for *S. mansoni* on growth and development in Brazilian school children. Anthropometric measurements and fecal examination were performed on 563 *S. mansoni* infected children and age and sex matched controls between the ages of 7 and 14.99. The community as a whole exhibited chronic malnutrition with linear stunting in 54% of the population. Infected children, however, were significantly smaller in both height (ht) and weight (wt) than uninfected children ($p<0.05$). Statistical differences were due to greater disparity between infected and uninfected females (ht $p<0.002$, wt $p<0.007$), despite their generally better level of nutrition than males. While infected males were shorter and weighed less than controls, these differences were not significant. Female growth and development was negatively associated with

intensity of infection, since those heavily infected were more malnourished than those with light or no infection (ht $p<0.01$, wt $p<.02$). Those moderately infected were intermediate in their deficits and, thus, not significantly different from any group. Though the prevalence of anemia was low for the level of malnutrition in the community, there was a trend toward lower hematocrits with increasing intensity of infection. The direct relationship between infection and nutritional status will be determined by measurement of treated and untreated children after 1 year and correlated with changes in serum Zn, Fe, transferrin receptor and TNF.

498 LOCAL PERCEPTIONS OF THE CLINICAL FEATURES OF SCHISTOSOMIASIS IN EGYPT AND THEIR IMPACT ON TREATMENT SEEKING BEHAVIOR. Mehanna S*, Winch PJ, El-Katsha S, and Watts S. Social Research Center, American University in Cairo, Cairo, Egypt; and Department of International Health, The Johns Hopkins University, School of Hygiene & Public Health, Baltimore, MD.

The primary method of control of schistosomiasis in Egypt is through passive chemotherapy, in which people who suspect they have the disease are encouraged to go to their local health unit to be tested and treated. If people are unable to recognize the symptoms of schistosomiasis, this strategy may fail. This paper presents data on local perceptions of schistosomiasis from three distinct regions of Egypt: Middle Egypt, the Nile Delta and an area recently reclaimed from the desert. Regardless of whether infection with *Schistosoma haematobium* is common or extremely rare in a given area, it was found that people associate schistosomiasis with urinary symptoms. Reasons for this situation include 1) the fact that until recently *S. haematobium* was extremely common in the Nile Delta; 2) the symptoms of schistosomiasis mansoni are neither distinctive nor specific and therefore do not make much of an impression on people; 3) hematuria due to renal calculi is extremely common and may be attributed to urinary schistosomiasis; and 4) health education often fails to stress the difference between the two forms of the disease. New health education strategies therefore are needed, which should include a focus on training for the staff of village health units.

499 WORKING WITH LOCAL PEOPLE AND HEALTH UNIT STAFF TO IMPROVE SCHISTOSOMIASIS CONTROL: A PARTICIPATORY RESEARCH PROJECT IN MENOUFIA GOVERNORATE, EGYPT. El-Katsha S*, Watts S, Khairy A, and El-Sebaie O. Social Research Center, American University in Cairo, Cairo, Egypt; and High Institute of Public Health, Alexandria, Egypt.

This paper will present a community-based approach to the control of schistosomiasis in two villages in the Nile Delta. The first phase of the project consisted of an in-depth investigation of the social, cultural and ecological setting in which schistosomiasis transmission was occurring in the villages. This included focus group discussions with villagers and assessment of the functioning of the health unit. Further data collection evolved through the establishment of a collaborative relationship with the staff of a local health unit and other government institutions. Their ability to evaluate local environmental and health conditions and take action in response to the findings was enhanced through training and practice in stool and urine examination, malacological surveys, water quality investigations and assessment of people's educational needs with respect to hygienic practices. This method of conducting research leaves behind an infrastructure for understanding and using the results of the study. For example, local clinic staff are developing strategies for dealing with the fact that many people focus on urinary symptoms in relation to schistosomiasis, even though *S. mansoni* accounts for the vast majority of cases of the disease in the two villages.

500 DETECTION OF *BRUGIA MALAYI* MICROFILARIAL DNA IN INDONESIAN BLOOD SAMPLES USING THE POLYMERASE CHAIN REACTION. Lizotte MR and Williams SA*. Department of Biological Sciences, Smith College, Northampton, MA.

There is need for sensitive, rapid, species-specific diagnosis of *Brugia* filarial parasites as traditional methods are tedious and time consuming with little guarantee of species-specificity. A PCR-based assay was developed using the *Hha* I family of highly repeated DNA sequences from *Brugia*. The assay was tested in a field study in Indonesia. 124 human blood samples were collected including 30 endemic normal samples, 65 microfilariae positive samples, and 29 non-endemic normal samples. The blood samples were screened using the traditional filtration microscopy method as the "gold standard" to which the PCR assay was compared. The samples were digested with Proteinase K, extracted with phenol and chloroform, and dialyzed. Five microliters of the dialyzed product were used in PCR reactions using *Hha* I PCR primers. The results of the field study showed that the PCR assay correctly identified all of the microfilariae positive samples as PCR positive, and all of the non-endemic normal samples as PCR negative. Additionally, all of the endemic normal samples were identified as PCR negative, except for four PCR positive samples. It is likely that these four cases are either very low level infections or cryptic infections, and that in these four cases circulating DNA released from dead filariae was detected. These results indicate that the *Hha* I PCR detection system is rapid, species-specific and sensitive. Additional advantages of this system are the reliable diagnosis of low level infections and the likely detection of circulating parasite DNA. Further studies will be undertaken to confirm detection of circulating DNA.

501 EFFECT OF IVERMECTIN PROPHYLAXIS ON ANTIBODY RESPONSES TO *ONCHOCERCA VOLVULUS* RECOMBINANT ANTIGENS IN EXPERIMENTALLY INFECTED CHIMPANZEES. Chandrashekar R, van Swinderen B, Taylor HR, and Weil GJ. Washington University School of Medicine, St. Louis, MO; and Victoria Eye and Ear Hospital, East Melbourne, Australia.

Antibody responses to recombinant *Onchocerca volvulus* antigens OC3.6 and OC9.3 were studied in experimentally infected chimpanzees. Sera from 3 groups of 6 animals were tested by ELISA with recombinants expressed as GST fusion proteins. Groups I and II were treated with 200 μg/kg ivermectin on the day of L_3 injection or on day 28, respectively. Group III were untreated controls. As previously reported, ivermectin prophylaxis was successful in group I only; 1 of 6 animals in that group developed a patent infection vs. 4 of 6 in each of the other groups. Antibodies to OC3.6 developed during the prepatent period in all three groups. In contrast, antibodies to OC9.3 were usually first detected around the time of onset of patency. Several animals had early antibody responses to OC9.3, but these animal subsequently failed to develop MF patency. Only 1 of 6 animals in group I produced antibodies to OC9.3 while all 12 animals in groups II and III developed antibodies to this antigen. These results suggest that seropositivity to OC3.6 is a reliable marker of early infection with *O. volvulus* regardless of the later outcome of infection. Antibodies to OC9.3 usually indicates the presence of mature infections, and this antigen may be more useful than OC3.6 for monitoring trials of prophylactic drugs or vaccines.

502 ANALYSIS OF GENETIC DIVERSITY IN *DIROFILARIA IMMITIS* BY PCR WITH ARBITRARY PRIMERS. van Swinderin B*, Berg D, and Weil GJ. Washington University School of Medicine, St. Louis, MO.

Arbitrary primer PCR is a powerful new technique for analysis of genetic diversity. Genomic DNA of *Dirofilaria immitis* was amplified in separate reactions with three primers and products were analyzed by agarose gel electrophoresis. Identical patterns were obtained with DNA from microfilariae, male and female worms. Patterns obtained with DNA from *Brugia malayi, Onchocerca volvulus, A. viteae,* and *D. corynoides* showed no similarity to those obtained with *D. immitis*. Results obtained with an inbred strain of *D. immitis* (maintained by Dr. John McCall for about 12 generations over 25 years) were compared to those obtained with 75 outbred worms from six locations in the U.S. and Italy. *D. immitis* patterns were all very similar, but minor polymorphisms were common. A total of 29 patterns was observed. As expected, inbred worms were less diverse than

outbred worms; 82% of 32 inbred worms had identical patterns, and only 4 patterns were observed in these worms. By comparison, 15 patterns were observed in 25 outbred worms from Missouri, and the most common pattern was present in only 16% of these worms. Most polymorphisms were observed in worms from all locations, but one was only present in Italian worms. Arbitrary primer PCR appears to be a promising method for analyzing intraspecific diversity in nematodes.

503 DEVELOPMENT OF A POLYMERASE CHAIN REACTION (PCR) TEST TO SURVEY FOR VECTORS OF DOG HEARTWORM, *DIROFILARIA IMMITIS*. Scoles GA*. Vector Biology Laboratory, Department of Biological Science, University of Notre Dame, Notre Dame, IN.

Incrimination of *Dirofilaria immitis* vectors is inherently difficult. Because vector-stage parasite prevalence is low, large numbers of mosquitoes must be dissected to obtain meaningful data. Identification of filariid species from vector stages can be problematic; there are several species of mosquito borne filariids that could be mistaken for *D. immitis*. The Polymerase Chain Reaction (PCR) provides a rapid and sensitive alternative to dissection of mosquitoes and simplifies the problem of filariid identification. PCR can amplify parasite DNA out of a background of vector/host DNA in crude preparations or purified DNA isolates. Mosquitoes can be processed individually or in pools. Both *D. immitis*-specific and nematode-specific PCR primers have been designed to aid in detection and identification of parasites. Species-specific primers were designed from a *D. immitis* surface antigen gene. Parasites were detected in individual mosquitoes; from pools of one infected mosquito in 10; from mosquitoes dried for 48 hrs. and from blood of an infected dog. There was no amplification when these primers were tested against mermithid nematodes or against *Onchocerca lienalis*. Group-specific primers designed from mtDNA sequences and the rRNA genes of *Caenorhabditis elegans* and *Ascaris suum* have been used to amplify DNA from *D. immitis* and various other filariid species. Amplified products of the group-specific primers from different species can be distinguished using size variation or restriction fragment lengths.

504 LOCALIZATION OF LECTINE BINDING ON EXTRACELLULARLY MELANIZED MICROFILARIAE OF *BRUGIA MALAYI* (NEMATODA:FILARIOIDEA) IN *ANOPHELES QUADRIMACULATUS*. Nayar JK*, Chikilian ML, Mikarts LL, Knight JW, and Bradley TJ. Florida Medical Entomology Laboratory, IFAS-University of Florida, Vero Beach, FL; and Department of Ecology and Evolutionary Biology, University of California, Irvine, CA.

Binding patterns of Fluorescein isothiocyanate (FITC)-conjugated and gold-conjugated lectins on extracellularly melanized sheaths and microfilariae of subperiodic *Brugia malayi*, isolated from the abdominal hemocoel of *Anopheles quadrimaculatus*, were examined using both light and electron microscopy. All five FITC-conjugated lectins tested (*Helix pomatia*, Peanut, Wheat germ, Lentil and Concacavalin A) bound strongly to the fibrous material accompanying the melanin deposits on the surface of microfilariae and sheaths. Significant inhibition of FITC-lectin binding occurred when lectins were preincubated with their complementary carbohydrates. *Helix pomatia* binding was totally inhibited by N-Acetyl δ-glucosamine and N-Acetyl-δ-galactosamine. Other lectins showed partial inhibition, such as Peanut by galactose and lactose, WGA by N-Acetylneuraminic acid, and Con A by Methyl α-(D)-mannopyranoside. Lentil binding was not inhibited by any of its complementary carbohydrates. The three gold-conjugated lectins (*Helix pomatia*, Peanut and Con A) bound moderately to the fibrous material accompanying the melanin deposits on the microfilarial sheaths, and the sheathed and exsheathed microfilariae isolated within 24 hr post-infection. *Helix pomatia* and Peanut lectin also bound strongly to the remnants of lysed plasmatocytes found in the proximity of the melanized structures isolated 72 hr post-infection. Con A binding to the plasmatocytes was weak. These results indicate that the fibrous material accompanying melanin deposits and the membranes of plasmatocytes contain glycoconjugates with exposed carbohydrates moieties. Glycoconjugates with similar lectin binding properties are found on the dense cytoplasmic material accompanying the intracellularly melanized L_1 larvae of *B. malayi* in *An. quadrimaculatus*.

505 ORIENTATION OF *ONCHOCERCA LIENALIS* MICROFILARIA WITHIN *SIMULIUM VITTATUM*. Lehmann T*, Cupp EW, Cupp MS. Department of Entomology, University of Arizona, Tucson, AZ.

Although the sites of development of filariid nematodes in their intermediate host are well known, the means by which they locate these anatomical targets have remained enigmatic. To clarify the orientation of *Onchocerca lienalis* microfilariae (mf) migrating toward the thoracic muscles of *Simulium vittatum*, a laboratory host, the following points were investigated: 1. Can mf travel passively to the thorax - drifting by hemolymph currents? 2. Do mf move "randomly" in the fly's body? or, 3. Do they use information to direct themselves toward the thorax and/or to recognize this target? If the third proposition is true, what types of cues are involved? The results suggest that arrival of mf to the thorax requires active migration and mf cannot rely on hemolymph currents, as dead mf inoculated into the abdomen remained there, while live mf (inoculated similarly) accumulated in the thorax. However, only 60% of the total number of mf were in the thorax. Thus, the proportion of mf that successfully arrive in the thorax is not high enough to rule out "random" movement. Neither could the distribution of mf (inoculated into the center of the abdomen), in cross sections (240 µm thick) at various time points (until 24h) pi falsify the "random" movement hypothesis. Direct inoculation into the thorax was followed by arrival of most mf in the abdomen and 24h pi the migration success of mf inoculated into the thorax was similar to that of mf inoculated into the posterior abdomen. The hypothesis that mf inherently differ in their navigation skills was rejected because mf that were collected from thoraces (ie., successful migrators) 2h pi and re inoculated to other flies had migration success similar to mf collected from flies' abdomens (ie., unsuccessful migrators) and as uninjected controls. In contrast to these results, *in vitro* experiments showed strong evidence that mf distinguished between the fly's thorax and abdomen. Microfilariae mixed in a Matrigel gel accumulated in live pieces of thorax reaching a density 2.5 times higher than in pieces of abdomen (3-5h post exposure). This difference was observed despite the larger volume and surface area of the pieces of abdomen compared with those of thorax. Tissue extracts made in saline and Schneider's tissue culture medium were not attractive to mf, neither were thoraces of dead flies (either heat [55°C]- or frozen-killed [-70°C]). The difference between mf behavior *in vivo* and *in vitro* suggests that mf inoculated into *S. vittatum* cannot fully express their navigational potential.

506 INFECTIVITY AND NORMAL DEVELOPMENT OF THIRD STAGE *BRUGIA MALAYI* MAINTAINED *IN VITRO*. Yates JA*, Schmitz KA, Nelson FK, and Rajan TV. Oakland University, Department of Biological Sciences, Rochester, MI; and University of Connecticut Health Center, Farmington, CT.

Shipment of L3s to laboratories lacking insectary facilities has been accomplished by transporting live infected vectors or L3s cryopreserved in liquid nitrogen. Our objective was to find culture conditions for transporting L3s that would promote survival of *Brugia malayi* larvae without altering their capacity to infect susceptible hosts. In order to evaluate the effect of culture temperatures on infectivity, fresh L3s were divided into groups which were either immediately injected into jirds (infectivity control) or incubated for 24, 48, or 120 hours in Ham's F12 in tightly sealed tubes kept horizontally at either 0°C, 20°C, or 37°C, before they were injected into jirds. Necropsies were performed on the jirds 120 days after injection to recover and count adult worms. Levels of microfilaremia were also determined. Adult worms were fixed so that mean length and diameter measurements of males and females in the experimental groups could be compared. We found that L3s held overnight at 0°C, although apparently viable, were unable to survive in jirds. However, larvae kept at 20°C and 37°C produced patent infections with adult worms in normal locations. There was no statistical difference in mean worm recoveries or size of worms from jirds injected with freshly harvested L3s and jirds injected with larvae that were kept overnight at 20°C or 37°C. When cultured L3s were shipped from Michigan to Connecticut by overnight air courier, along with infected

live mosquitos, they were 99% viable upon arrival. Cultured L3s produced patent infections in C.B-17-scid/scid mice with worm recoveries comparable to those observed in mice injected with L3s freshly harvested from shipped mosquitos.

507 VISUALIZATION OF *WUCHERERIA* AND *BRUGIA* LARVAL STAGES IN INTACT MOSQUITOS. Green DF* and Yates JA. Oakland University, Department of Biological Sciences, Rochester, MI.

Over the past several decades epidemiological data from insect vectors typically has been obtained by mass dissection (crushing) or by dissection of individual specimens. The former is quick and easy but gives no information on distribution of infection, larval location, or presence of early developmental stages; while the latter is very tedious and time consuming which usually prohibits the examination of large numbers of insects. We describe a new technique which can provide data similar to that obtained by individual dissection, including calculation of infection and infective rates. This technique is easy enough to accommodate large numbers of insects. Brief incubation of ethanol fixed mosquitos in sodium hypochlorite to reduce cuticular pigmentation followed by incubation in an organic solvent to remove lipids, allowed microscopic visualization of filarial larvae within the abdomen, thorax, head, and proboscis of *Brugia*-infected *Aedes aegypti* from our laboratory and *Wuchereria*-infected *Anopheles punctulatus* from Papua New Guinea. After evaluation, infected mosquitos could be removed for further study. We compared the classical techniques to our technique using *Ae. aegypti* infected by feeding on jirds with *B. malayi* microfilaremias. Three random groups of 125 mosquitos each were fixed in ethanol or crushed for evaluation 14 days after infection. Comparisons of the infective rate, total number of infective stage larvae (L3s) observed, and locations of L3s showed that this new technique was comparable to the established methods. In mosquitos fixed earlier in infection, first stage and second stage larvae were also readily seen within the cleared flight muscles of the thorax.

508 CHRONIC HEARTWORM INFECTION DEPRESSES ENDOTHELIUM-DEPENDENT RELAXATION OF THE IN VITRO CANINE PULMONARY ARTERY. Mupanomunda M*, Williams JF, and Kaiser L. Department of Physiology Michigan State University, East Lansing MI; and Department of Microbiology and Public Health Michigan State University, East Lansing MI.

Our working hypothesis is that filarial parasites release biologically active factors that circulate and alter mammalian cell function. *Dirofilaria immitis*, the canine heartworm, depresses endothelium-dependent relaxation of the *in vitro* pulmonary artery, suggesting that filarial factors may be responsible for decreased exercise tolerance seen in dogs with heartworm infection. Filarial factors could also influence constriction. The present study was done to determine if chronic infection with heatworm *in vivo* alters consrictor responses of the *in vitro* pulmonary artery. Rings of pulmonary artery from control and heartworm infected dogs were suspended in tissue baths filled with warm oxygenated physiologic salt solution, and connected to force transducers for meaasurement of changes in isometric tension. Dose responses to norepinephrine (NE), sertonin (5HT), histamine, PGF2a and the thromboxane analogue U44069 were done. Constriction responses to PGF2a and U44069 were significantly depressed in pulmonary artery rings from heartworm infected dogs when compared to control. However, constrictions to NE, 5HT and histamine were not different. These results suggest the *D. immitis* specifically alters constriction responses to thromboxane and U44069; perhaps, filarial arachidonic acid metabolites interact with thromboxane receptors in the pulmonary artery of heartworm infected dogs. Circulating filarial factors may prove important in the pathogenesis of both human and animal filariasis, and, thus, could represent a novel site for pharmacologic intervention in these diseases.

509 CHARACTERIZATION OF THE CYTOCHROME B GENE AND MITOCHONDRIAL GENOME OF *ONCHOCERCA VOLVULUS*. Keddie EM*, and Unnasch TR. Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL; and Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL.

Many parasitic helminths are believed to utilize both aerobic and anaerobic respiratory pathways during their life cycles. Our lab is specifically interested in the electron transport system of the human parasitic nematode *Onchocerca volvulus* . As an initial step in the study of mitochondrial genes, a portion of the *O. volvulus* cytochrome b gene has been cloned and sequenced. Conserved primers derived from the cytochrome b sequences of *A. suum* and *C. elegans* were used to amplify a 773bp product from *O. volvulus* genomic DNA. PCR reactions using human genomic DNA as template and the same primers amplified no discernable products. The *O. volvulus* PCR product encodes an open reading frame with no evidence of post-transcriptional modification. The *O. volvulus* PCR product nucleotide sequence has 68.2% and 69.3% homology to similar sections of *A. suum* and *C. elegans*, respectively. The predicted amino acid sequence of the PCR product has 68% similarity to both the *A. suum* and *C. elegans* amino acid sequences. When compared to the similar section of human cytochrome b, the *O. volvulus* PCR product has 49.5% homology in nucleotide sequence and 49% similarity in amino acid sequence. The PCR product hybridized to identical 16kd bands on a Southern blot of *O. volvulus* genomic DNA digested with three different enzymes, but not to similarly prepared human DNA. Southern blots of ethidium bromide treated and untreated uncut *O. volvulus* genomic DNA imply a circular nature for the 16kb band, suggesting it encodes the mitochondrial genome for *O. volvulus*.

510 IMMUNE RESPONSE TO OV 18 A RECOMBINANT MOLECULE OF *ONCHOCERCA VOLVULUS*. Bradley JE*, Tree TI, Gillespie AJ, Elson L, Guderian RH, and Nutman TB. Department of Biology, Imperial College of Science and Technology, London, UK; Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD; and Department of Clinical Investigation, Hospital Vozandes, Quito, Ecuador.

A cDNA clone encoding an 18kd *Onchocerca volvulus* molecule was isolated on the basis of it being specifically recognised by onchocerciasis sera. The original aim was to use such antigens to develop a specific immunodiagnostic test for this parasite but such antigens also have potential as vaccines. We have therefore continued to characterise this molecule and use seroepidemiological studies to investigate whether there is any correlation with immunity. It has been expressed and full length sequence obtained (Ov 18). Sequence analysis has shown homology to a predicted gene from *C. elegans* but the function of this molecule remains unknown. Antibody was raised to the recombinant protein and used to determine the stage and species specificity and localization of the native molecule. It is present in L3 and adult parasites and preliminary evidence indicates that it is secreted. Southern blot analysis indicated that it is a single copy gene and analogous molecules are present in other filarial nematodes. Although conservation is seen at the DNA level, antibody responses from individuals infected with other parasitic nematodes do not recognise Ov 18. Partial epitope mapping has shown the presence of at least two antibody binding and one T cell epitope on the molecule. The predominant antibody subclass induced to this molecule in infected humans is IgG4 although both IgG1 and IgE are also produced. The potential of this molecule as a correlate of immunity has been investigated using age profile studies and groups of well characterised patients from a hyperendemic area of Ecuador. The results of these studies will be presented.

511 ROLE OF THE FILARIAL RAN/TC4/SPI1 HOMOLOG IN THE DEVELOPMENTAL MATURATION OF *BRUGIA MALAYI* MICROFILARIAE. Dissanayake S*, Xu M, Chen GH, Wang SH, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Guizhou Provincial Institute of Parasitic Diseases, Guiyang, The People's Republic of China.

We have previously reported the cloning of a Ran/TC4/Spi1 homolog from *Brugia malayi* and *Onchocerca volvulus* . This *ras*-like protein is not detectable in immature (< 2 day old) *B. malayi* microfilariae, but it is present in "old" microfilariae. Expression of the molecule is stimulated when microfilariae are cultured *in vitro* in the presence of murine epidermal growth factor (EGF). In contrast to its mammalian homolog, filarial Ran appears to be membrane-bound, because solubilization of the molecule requires > 6 mM deoxycholate. The apparent molecular weight of immunoreactive filarial Ran varies in different life cycle stages of the parasite. Based on SDS-PAGE and Western blotting with specific rabbit antisera, native microfilarial Ran is ~ 24 kDa, comparable to the size predicted from the cDNA sequence. In L3 stage larvae, however, the 24 kDa molecule is not detectable and immunoreactive protein is ~ 35 kDa. Trace amounts of the 35 kDa molecule appear in L1/L2 stage larvae, which do not appear to contain the 24 kDa protein. Exsheathment of microfilariae induced with 20 mM $CaCl_2$ is not sufficient to trigger the 24 kDa to 35 kDa shift in immunoreactive protein. In adult male worms, immunoreactivity is associated with moieties of ~200 and 80 kDa. The function of filarial Ran remains unknown, but the homologous molecule is believed to play a role in maintaining a coordinated cell cycle in mammalian cells. If the same is true for filarial worms, the increased expression of filarial Ran in response to EGF may be a possible mechanism of adaptation of the parasite to its environment.

512 CLONING OF THE *ONCHOCERCA VOLVULUS* HOMOLOGUE OF PROLYL-4-HYDROXYLASE, AN EXZYME INVOLVED IN THE BIOSYNTHESIS OF COLLAGEN. Wilson W*, Unnasch TR. Division of Geographic Medicine, Department of Medicine, University of Alabamsa at Birmingham, Birmingham, AL.

The cuticle of *Onchocerca volvulus* is believed to consist of a structure of highly crosslinked collagen and chitin. Activities involved in the biosynthesis of collagen therefore present a potential target for immuno- and chemotherapeutic attack against the parasite. Previous studies in our laboratory have indicated that 6-8 polypeptides produced by *in vitro* translation of adult *O. volvulus* mRNA were recognized by a rabbit inoculated with viable *O. volvulus* L3. Exhaustive screening of available adult cDNA libraries has resulted in the isolation of 6 classes of cDNA clones producing recombinant proteins recognized by this antiserum. DNA sequence analysis of the final member of this class of clones, designated (1)RAL-6, demonstrated that this cDNA exhibited a 75% identity to a multifunctional protein previously characterized only in vertebrate organisms. This protein acts as the β subunit of prolyl 4-hydroxylase, the enzyme responsible of the oxidation of proline to 4-hydroxyproline in nascent collagen polypeptides. The protein also contains a steroid hormone binding activity, as well as acting as a protein disulfide isomerase, an activity which catalyzed the formation of disulfide bonds in nascent proteins. Genomic Southern blot analysis indicated that the gene encoding (1)RAL-6 is present as a single copy in the *O. volvulus* genome. The cDNA encoded by (1)RAL-6 has been subcloned into both the pMAL and pGEX expression systems, and the recombinant protein purified by affinity chromatography. (1)RAL-6 purified recombinant antigen has been used to prepare a polyclonal antibody for identification and localization of the native antigen, as well as to explore the antigenicity of this molecule in individuals with *O. volvulus* infection. These studies will help to further assist in determining the physiological role that the protein encoded by (1)RAL-6 plays in *O. volvulus*, as well as in determining the potential that this antigen may play in the development of a vaccine against *O. volvulus* infection.

513 CLONING AND CHARACTERIZATION OF CDNA CLONES ENCODING MEMBERS OF THE STEROID RECEPTOR SUPERFAMILY FROM *ONCHOCERCA VOLVULUS*. Yates R* and Unnasch TR. Division of Geographic Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

Molting in parasitic nematodes is an essential parasite function not shared by the host. This process therefore represents a potential avenue for immunotherapeutic and chemotherapeutic attack. Several indirect lines of evidence suggest that in nematodes, as in insects, the molting process is controlled by changes in the level of steroid hormones, such as ecdysone. The central mediator in the action of these hormones is the steroid hormone receptor. In an attempt to identify steroid receptor homologues in *Onchocerca volvulus*, genomic Southern blots were probed with a degenerate oligonucleotide derived from a conserved portion of the DNA binding domain of the *Drosophila melanogaster* ecdysone receptor. The results of this experiment suggested that *O. volvulus* contained 2-3 genes which hybridized to this oligonucleotide. Screening of cDNA libraries derived from adult*O. volvulus* mRNA has resulted in the isolation of two cDNA clone families, which encode two distinct members of the steroid receptor superfamily. Both clones encode a steroid receptor DNA binding region containing two zinc finger moieties. One clone demonstrates the highest degree of similarity to the thyroid receptor subfamily of steroid receptors of which the *Drosophila* ecdysone receptor is also a member, while the second appears to belong to the belongs to the ultraspiricle/retinoid receptor subfamily. A cDNA clone containing a putative full length open reading frame has been obtained for the *O. volvulus* receptor of the ultraspiricle subfamily. Southern blot analysis indicates that this cDNA is encoded by a single copy gene. Genomic analysis indicates that this gene contains at least three introns. The cloning of cDNAs encoding steroid receptors from *O. volvulus* will allow the role that steroids play in the development of parasitic nematodes to be further evaluated.

514 CLONING AND CHARACTERIZATION OF THE *BRUGIA MALAYI* HOMOLOG OF RIBOSOMAL PROTEIN S15. Chen GH*, Wang SH, Xu M, Dissanayake S, and Piessens WF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; and Guizhou Provincial Institute of Parasitic Diseases, Guiyang, The People's Republic of China.

A *Brugia malayi* cDNA clone in λgt11 coding for a predicted protein of 16 kDa was identified as homologous to ribosomal protein S15. At the nucleotide level, the *B. malayi* molecule is 99 % identical to the *Wuchereria bancrofti* and *B. pahangi* homologs, but has only 66-69 % homology to the corresponding mammalian genes. However, the predicted amino acid sequence is 87-94 % homologous to the corresponding proteins in yeast, *Drosophila* and mammals. The complete open reading frame was expressed as a fusion product with maltose-binding protein in the pMal vector. The fusion protein was used to raise specific rabbit antisera. On Western blots, rabbit antisera to the fusion protein react with a 16 kDa molecule that is present in extracts of microfilariae, adult worms and L3 larvae. In contrast to the *B. pahangi* product, which is reported to be recognized by sera from Haitian patients with bancroftian filariasis, the *B. malayi* molecule is not recognized by sera from microfilaremic or amicrofilaremic subjects with bancroftian filariasis from Sri Lanka and the People's Republic of China.

515 *BRUGIA MALAYI* CONTAINS A TRANSLATION PRODUCT DERIVED FROM DNA SEQUENCES HOMOLOGOUS TO MAMMALIAN LINES AND RETROVIRAL REVERSE TRANSCRIPTASES. Wang SH*, Chen GH, Xu M, Dissanayake S, Piessens WF, Araunje AC, and de Souza W. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA; Guizhou Provincial Institute of Parasitic Diseases, Guiyang, The People's Republic of China; and Instituto de Biofisica Carlos Chagas Filho, Rio de Janeiro, Brazil.

LINEs are repetitive DNAs in mammalian genomes. LINE sequences are highly transcribed, but no unequivocal LINE-encoded translation product has been identified so far. We made the chance discovery that three cDNA clones derived from polyA-selected mRNA from the filarial parasite, *Brugia malayi*, contain inserts with striking sequence homology to the second open reading frame of LINEs. The deduced consensus amino acid sequence of the product encoded by clones RTH1 (572 bp), RTH5 (1182 bp) and the first open reading frame (ORF) of clone RTH11 (1942 bp) has >77 % homology to a putative protein encoded by a region in the second ORF of LINEs with homology to viral reverse

transcriptases. Of 39 amino acids that are conserved among 8 viral reverse transciptases, 24 are present in the filarial molecule. The characteristic FXDD motif of reverse transcriptases and five other highly conserved motifs are also present in the filarial molecule. The filarial DNA insert in clone RTH1 was subcloned in pMal, and the parasite product was expressed as a fusion product with maltose binding protein. Rabbit antisera raised against the RTH1 fusion protein specifically recognize native filarial proteins of 120 and 60 kDa in extracts of *B. malayi* microfilariae and adult worms plus a 100 kDa molecule in adult worm extracts. These proteins are not present in extract of gerbil leukocytes, a possible contaminant of the parasite extracts. Immunohistology suggests that the filarial LINE product is preferentially expressed in subcuticular areas of adult worms.

516 IMMUNOHISTOCHEMICAL LOCALIZATION OF ANTIGENIC PROTEINS IN THE TISSUES OF *BRUGIA MALAYI* LARVAE AND ADULTS. Hale TJ*, Rajan TV, Peralta ME, and Yates JA. Oakland University, Department of Biological Sciences, Rochester, MI; and University of Connecticut Health Center, Farmington, CT.

We have previously described four antigenic recombinant *B. malayi* proteins. Our goal in the current study was to identify the worm tissues containing the epitopes present in our recombinant myosin, collagen, paramyosin, and heat shock protein 70. We prepared rabbit antisera to the recombinant proteins for use in indirect immunofluorescence and immuno-gold studies of third and fourth stage larvae (L3s and L4s) and adult worms. Immunofluorescence of live worms showed that collagen epitopes were present on the surface of L3s but consistently absent from the surface of the other stages. In order to substantiate this finding we incubated fresh L3s in highly purified collagenase. Surface fluorescence was completely ablated after 3 hours of collagenase treatment. None of the other antisera were detected at the surface of live worms. In cryosections, collagen was found extensively in the extracellular basal laminae associated with the somatic musculature, and alimentary tract in all stages. In L4 and adult worms collagen epitopes were associated with the basal laminae of male and female reproductive systems. Myosin and paramyosin were localized within the cells of longitudinal muscles and the esophagus but absent from the intestine in all life stages. Myosin and paramyosin were detected in the walls of male and female reproductive tracts in L4s and adults. Interestingly, HSP-70 could not be detected in sections of fresh L3s. However, when L3s were cultured at 37 C for 24 hours before fixation, intracellular HSP-70 was detected as bright fluorescence throughout the hypodermis and cytoplasmic portion of somatic musculature. Efforts to extend these findings with immuno-gold TEM are in progress.

517 CLONING OF AN EARLY IMMUNODOMINANT FILARIAL ANTIGEN: A NOVEL MEMBER OF THE *BRUGIA MALAYI* MYOSIN HEAVY CHAIN FAMILY. Li BW*, Hoppe PE, and Weil GJ. Washington University School of Medicine, St. Louis, MO.

A *Brugia malayi* cDNA clone expression library was screened with serum pools from vaccinated (irradiated L3) and infected jirds to select clones that expressed potentially protective recombinant antigens that were preferentially recognized by sera from vaccinated animals. Bmmyo-2, the largest of a group of related clones, was studied in detail. Jirds produced strong antibody responses to the protein product of Bmmyo-2, Bmmhc-B, as early as one month after vacination with irradiated larvae. Antibody response to Bmmhc-B in infected jirds were weaker than those of vaccinated jirds, and they developed somewhat later. Antibodies produced to Bmmhc-B were reactive with a 200 kDa native *B. malayi* antigen by imunoblot. Sequence analysis of the 1454 bp cDNA insert of Bmmyo-2 showed that it codes for a portion of the rod region of a novel *B. malayi* myosin heavy chain. The deduced amino acid sequence of Bmmyo-2 is 74.6% indetical with that of the corresponding region of *Caenorhabditis elegans* myosin heavy chain B but only 64.6% identical with that of a recently described *B. malayi* myosin heavy chain, Bmmhc-A.

518 DEVELOPMENT OF NOVEL COMPOUNDS FOR THE TREATMENT OF ONCHOCERCIASIS. Strote G*, Bonow I, v. Stenglin E, Wywiol A, and Attah S. Bernhard Nocht Institute for Tropical Medicine, Hamburg, F.R.G.; and Onchocerciasis Chemotherapy Research Centre, Hohoe, Ghana.

Mass treatment of onchocerciasis is presently based on the microfilaricide ivermectin. Since this is not satisfactory the development of a safe macrofilaricidal drug, eliminating the long-lived adult worms, has high priority. To identify new leads for the treatment compounds were used with known activity against other parasitic diseases and those that were active against filarial parasites in model infections. More than 50 antiparasitic agents representing six chemical entities were tested for their efficacy on the adult target organism *Onchocerca volvulus* in an *in vitro* system. The compounds, supplied by WHO's Macrofil Chemotherapy Project, were tested at a drug level of 10 μM with additional experiments at a drug level of 1μM when compounds were active at the higher level. Adult filariae were isolated from excised onchocercomata and the viability of the parasites was assessed by biological and biochemical parameters. Derivatives of amodiaquine as the phenylguanidines of Warner-Lambert/Parke-Davis, thiadiazole and its derivatives, acetylpyridine thiosemicarbazones and amidines as well as cadaverine-related compounds were tested in this system. Most compounds inhibited worm motility, but only some of these reduced the metabolic activity of the worms to a level comparable to that shown by heat-killed worms. Several thiadiazole derivatives and many phenylguanidines turned out to be potent inhibitors of adult *O. volvulus* viability. The phenylguanidines and thiadiazoles acted in a dose-dependant manner. For the phenylguanidines a study of structure-activity relationship was made: the most active derivatives possessed common structural elements. Screening of known and novel compounds proved this *in vitro* system to be suitable for the identification of active and inactive antifilarial drugs. The most active macrofilaricidal compounds in this screen have been selected for further preclinical development.

519 IMIDAZOPYRIDINES: A NEW CLASS OF POTENTIAL ANTIFILARIAL DRUGS IDENTIFIED. Kinnamon KE*, Engle RR, Sundberg RJ, McCall JW, and Dzimianski MT. Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC; and Department of Chemistry, University of Virginia, Charlottesville, VA; and Department of Parasitology, College of Veterinary Medicine, University of Georgia, Athens, GA.

The nematode worms, *Onchocerca volvulus, Wuchereria bancrofti, Brugia malayi* and *B. timori*, are among the most important disease producing parasites of man. Diseases produced by them, i.e., onchocerciasis and lymphatic filariasis place one billion people at risk in 75 countries and cause more than 100 million cases of disease per year. Parasite targets have been both the adult and microfilarial forms. Drugs used widely which affect the adult stages of the parasite are suramin, antimony, and arsenic-bearing compounds. The microfilaricidal drug most extensively used is diethylcarbamazine (DEC). All exhibit significant toxicity. The recently introduced drug, ivermectin, promises more effective management of the microfilaremia; however, better microfilaricidal drugs are urgently needed. A chemical series, imidazopyridines, not previously reported to have antifilarial activity, has been identified. Compounds were evaluated for microfilaricidal activity against both *Brugia pahangi*, ("lymphatic filariasis form"), and *Acanthocheilonema viteae*, ("onchocerciasis form"), in male Mongolian jirds (*Meriones unguiculatus*). The new structures identified have activity against both filarial forms and represent a nucleus around which chemical analogs are being synthesized. One or more of these may lead to a therapeutic agent which can provide relief to the millions of individuals affected by these diseases.

520 LONG TERM EFFECT OF TWO SINGLE DOSES OF IVERMECTIN ON MICROFILAREMIA (MF) OF BANCROFTIAN FILARIASIS IN EGYPT. Youssef FG*, Hassanein SH, Safwat M, Rida M,

Fouad R, and Cummings CE. Basic Science Division, U.S. Naval Medical Research Unit No.3, Cairo, Egypt; Health Units, Qalyubia Governorate, Egypt.

Successful treatment of bancroftian filariasis in endemic villages in Egypt with two single doses (100 μg/kg) of ivermectin given 3 months apart has been previously reported. We now report long term MF suppression using this ivermectin regimen in an identical population. One hundred sixty-one male patients aged 15-55 years had pretreatment mean MF density of 461.7 MF/ml (range: 14-2869 MF/ml), as measured by membrane filtration of 1 ml venous blood collected at 2100-2400 hours. Before the second dose (3 months after the first dose), 50 (31%) had completely cleared, and the remaining 111 had a mean MF density of 3.6%of pretreatment level. Follow-up of 155, 146, and 127 patients at 3, 6,and 9 months after the second dose indicated complete MF clearance in 60%, 45%, and 47% of patients. The MF densities in those still infected were 1.7%, 3.2% and 6.1% of the pretreatment level. These results show that ivermectin in 2 doses (100 μg/kg) 3 months apart cleared up to 60% of patients completely and reduced MF densities to <10% of pretreatment levels. Suppression of MF may reduce the potential for parasite acquisition by the vector, therefore reducing transmission of lymphatic filariasis in endemic areas.

521 MASS CHEMOPROPHYLAXIS OF LYMPHATIC FILARIASIS WITH SINGLE DOSES OF IVERMECTIN IN A POLYNESIAN VILLAGE. EFFICACY AND ADVERSE REACTIONS. Nguyen NL, Moulia-Pelat JP, Glaziou P*, Plichart R, Lardeux F, Martin PM, and Cartel JL. Institut Territorial de Recherches Medicales Louis Malarde, Tahiti, French Polynesia.

In French Polynesia, where lymphatic filariasis due to *Wuchereria bancrofti* var. pacifica is endemic, several therapeutic trials suggested that semi annual single doses of ivermectin 100 mcg/kg could be an interesting alternative strategy as compared to DEC. Consequently, in April 1991, a mass chemoprophylaxis program based on this strategy was implemented in Opoa, a Polynesian village (population = 935). Six months after the first treatment, the microfilaremia (mf) recurrence was about 34 % of the initial one, but no significant reduction of this recurrence was observed after the 2nd and the 3rd treatment (respectively 21.7 and 31.3 %). Moreover, there was no reduction of the mf carriers prevalence (21 %; n = 595) after 3 successive mass treatments. Therefore, the dosage was raised to 400 mg/kg for the 4th round, since this higher dosage proved safe and effective from other trials. This high dosage did not induce more adverse reactions nor worse acceptability than that of 100 mcg/kg. Six months after the 4th mass treament, the mf recurrence was 10 % and the mf prevalence 15 %. Further results are required to determine whether this strategy is effectively adequate for a filariasis control program.

522 EVALUATION OF A TWICE WEEKLY APPLICATION OF 1% NICLOSAMIDE LOTION IN PREVENTING *SCHISTOSOMA HAEMATOBIUM* RE-INFECTION. Abu-Elyazeed RR*, Podgore JK, Mansour NS, Youssef FG, Gere JA, and Hibbs RG. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt; and U.S. Army Medical Material Development Activity, Ft. Detrick, MD.

A randomized double-blind trial was conducted to assess the efficacy of twice-weekly application of 1% niclosamide lotion to prevent *S. haematobium* re-infection. Farmers in Fayoum, Egypt, aged 18 to 40 were treated to cure their *S. haematobium* infection then randomly assigned to self-apply niclosamide or placebo lotion to limbs and trunk. Subjects were exposed to infested water during irrigation from April to October 92. Three hundred fifty subjects did not excrete *S. haematobium* ova during the first 4 months of lotion application and completed the trial, 169 (48.3%) in the niclosamide group and 181 (51.7%) in the placebo group. The niclosamide group was comparable to the placebo group in age, total water contact (102 vs 109 hrs), reported lotion application compliance (93.5% vs 90.6%) and reported water contact beyond lotion application areas (5.3% vs 3.3%). The re-infection rates were 30.8% in the niclosamide and 28.2% in the placebo groups. Thus, twice weekly application of 1% niclosamide lotion did not prevent re-infection. Previous studies in monkeys showed that

niclosamide was effective in preventing infection with *S. haematobium* and *S. mansoni* when applied every other day. In humans, niclosamide applied daily only to limbs gave protection against *S. mansoni*. A more rigorous application regimen may be indicated.

523 FIELD TRIAL OF A 1% NICLOSAMIDE LOTION, TOPICALLY APPLIED FOR THE PREVENTION OF *SCHISTOSOMA MANSONI* INFECTION IN BRAZILIAN SCHOOL CHILDREN. Dietze R, Alencar F, Cerutti C, Bendet I, Pang L, Miller R *, and Gere J. USA Medical Research Unit-Brazil, American Consulate Rio de Janeiro, Brazil; Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC; and US Army Medical Development Activity, Ft. Detrick, Frederick, MD.

We describe the results of a randomized placebo-controlled, double-blind field study of 1% niclosamide topical lotion (TAP) applied twice a week to 300 Brazilian school children. The objective was to assess the safety and eficacy of TAP in preventing schistosomiasis re-infection. The study was conducted in Bahia, Brazil for a period of 12 months. Subjects with positive stool examinaitons for schistosoma eggs were first treated with praziquantel and oxamniquine. TAP was applied under investigator supervision. Stool specimens (three consecutive day collections) were examined monthly. Four slides from each day's sample were examined for schistosoma eggs using formalin-ethyl aceteate sedimentation method. All participants that had stools containing vaible (as determined by hatching, *Schistosoma mansoni* eggs were counted as prophylactic failures. Overall, compliance was excellent, with only 32 children dropping out of the study. No adverse reactions were seen. The code for the double-blind study will be broken prior to the meeting and the final results presented.

524 HEPATOSPLENIC *SCHISTOSOMA MANSONI* INFECTION IS INFREQUENTLY DIAGNOSED IN NORTHERN NIGERIA. Newsome F *. Columbia University College of Physicians and Surgeons, Harlem Hospital Center, New York, NY.

It has been reported that the African-Brazilian has a greater resistance to the development of severe *Schistosoma mansoni* infection (hepatosplenic schistosomiasis with portal hypertension) than the white Brazilian. It has been hypothesized that blacks have a genetic resistance to the severe hepatic form of the infection because of long exposure and adaptation to the parasite in Africa. Studies in northern Egypt, where severe hepatosplenic schistosomiasis is common, have demonstrated an association between the presence of genetically determined HLA antigens (HLA-A1 and B5) and schistosoma hepatosplenomegaly. The morbidity caused by *S. mansoni* infection in subSaharan Africa is poorly documented. In order to examine the hypothesis that blacks have resistance to severe hepatosplenic schistosomiasis, a retrospective study was performed of the frequency of hepatic schistosomiasis encountered on an adult (14 yrs. or more) medical unit of the University Jos Teaching Hospital in an endemic area (19% overall prevalence) in northern Nigeria. From 1982 through 1988 there were 1,826 admissions to the unit. Forty-seven received a liver biopsy for the evaluation of hepatomegaly, 5 of which were compatible with hepatic schistosomiasis. There were 38 patients including eight with ascites and/or hepatosplenomegaly, who had positive stools but who did not have liver biopsy. It is concluded that in the endemic, subSaharan region of Africa studied here, hepatosplenic *S. mansoni* infection is uncommonly diagnosed.

525 RECOMBINANT ANTIGENS FOR THE PREPATENT IMMUNODIAGNOSTIC OF SCHISTOSOMIASIS MANSONI. Oliveira GC* and Kemp WM. Department of Biology, Texas A&M University, College Station, TX.

Schistosomiasis afflicts millions of individuals today. The diagnostic of the disease is based upon the observation of eggs in stool samples. This method is not very sensitive, and in addition, the eggs are

the pathological agent of schistosomiasis. The objective of this work is to identify recombinant proteins that can be utilized in the detection of infection before the onset of egg production. A *Schistosoma mansoni* adult worm cDNA library was screened with serum collected from mice after four weeks of infection. Seven positive clones were identified and purified. The insert sizes varied from 290 to 1,600 bp. Following *in vivo* excision, all clones were partially or entirely sequenced. A search in the GeneBank and EMBL databases indicated that clone SmAct1 displayed almost 80% homology to chicken type five cytoplasmic actin. Another clone, SmAct2, was obtained by screening the cDNA library with a probe prepared from the SmACt1 insert. SmAct1 and SmAct2 displayed 82% homology at overlapping regions. Southern blot analysis indicated the presence of at least two actin genes per haploid *S. mansoni* genome. Restriction analysis of five actin clones was consistent with the existence of three different actin genes. Computer analysis of clone 2SAP2 indicated that it contains EF-hand Ca^{++} binding, and protein kinase motifs. Expression of clone 2SAP2 was obtained in the pFlag vector, yielding a recombinant product of 12 kDa. ELISA and dot-blot experiments demonstrated that normal mouse serum had a low level of reactivity against extract of cells expressing 2SAP2, while 8 week, and 4 week infection sera had significantly higher levels of reactivity.

526 EXPRESSION OF THE *SCHISTOSOMA MANSONI* EGF RECEPTOR HOMOLOGUE AND ALTERNATIVELY SPLICED VARIANTS IN INSECT CELLS. Ramachandran H* and Shoemaker CB. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

A full-length cDNA encoding the *Schistosoma mansoni* homologue of epidermal growth factor receptor (SER) has been cloned and is predicted to encode a protein of 200kD. In addition to the full-length SER cDNA, three variant cDNA classes of SER have been identified (classes 2, 4 and 5). The predicted protein products encoded by the cDNAs share identity with SER at the amino terminus but diverge into different short C-termini. Subsequent genomic cloning and sequencing studies have demonstrated that the variant transcripts are generated from a single gene by the process of alternate splicing. Northern blot and PCR analyses have indicated that both, SER and the variant class 2 SER transcripts are present at comparable levels in adult worms, whereas only SER transcripts are found in cercariae. Class 4 and 5 variant transcripts have also been detected though at lower levels, in adult worms. Class 1, 2 and 4 SER proteins have recently been expressed in insect cells using a baculovirus expression system, and antisera generated against the amino terminal region of SER have been shown to recognize these proteins. Functional studies are currently under way to determine if the tyrosine kinase domain of SER is active. Preliminary results suggest that the full-length SER protein is tyrosine phosphorylated in insect cells. Immunolocalization studies are also being conducted in order to locate the SER proteins within the parasite. Although no function can yet be assigned to the variant classes of SER, one hypothesis is that they may act to interfere with the host's immune response against the full-length protein.

527 CLONING AND CHARACTERIZATION OF *CLONORCHIS SINENSIS* TROPOMYOSIN cDNA. Sohnn EJ, Choi WS, and Hong SJ*. Departments of Parasitology and Anatomy, Gyeong-Sang National University College of Medicine, Chinju, Korea.

In effort to get a potent immunoreagent for serodiagnosis of clonorchiasis we constructed λ ZAP II cDNA library with mRNA from adult Chinese liver fluke, *Clonorchis sinensis*. Among fusion proteins, recombinant tropomyosin of *Schistosoma mansoni* appeared to be very specific in serodiagnosis of schistosomiasis mansoni. Homologous probe was prepared with PCR amplification by using degenerated oligonucleotides which were synthesized upon 5 conserved regions of amino acid sequences of tropomyosins reported previously. This probe had an open reading frame (ORF) of 190 amino acids showing 86.3% homology to *S. mansoni* tropomyosin (SmTm). cDNA library was screened with this probe and 2 clones (CsTm1 & 2) were purified. Clone CsTm1, 1.3 kb in size, contained whole information of tropomyosin including 5'-uncoding region and 3' poly α tail. An

ORF of 204 amino acids starting from first methionine showed 86.3% homology to SmTm and 52.0% to *Trichostrongylus colubriformis* tropomyosins. Gene product of CsTm1 is being expressed in bacteria and further characterization will be presented.

528 IN VITRO CULTURING OF ADULT *SCHISTOSOMA MANSONI* USING SERUM-FREE MEDIA AND DIALYSIS BAGS. Hancock K, Tsang VC, and Call JL*. Parasitic Disease Branch, NCID, Centers for Disease Control and Prevention, Atlanta GA.

Antigenic excretory/secretory (E/S) products from *Schistosoma mansoni* are potentially important for the development of antibodies and diagnostic assays to detect circulating antigens in schistosomiasis. The E/S products to be used as antigen for this development must, by necessity, be free of exogenous contamination. The ability to extend serum-free *in vitro* culture of adult worms is, therefore, essential. Adult worms were perfused from mice, washed in phosphate buffered saline, and placed in sterile dialysis bags at 50 worms/ml of serum-free RPMI with antibiotics, with approximately 300 worms/bag. We successfully cultured adult worms for 14 days with serum-free RPMI within dialysis bags, surrounded by RPMI supplemented with 10% fetal calf serum. At day 7 days E/S medium was removed and replaced with fresh medium. The E/S product was harvested again at day 14. Silver stained polyacrylamide gels and immunoblot showed E/S products from dialysis bags to be less complex then those from static cultures, where worms were in contact with serum-free medium alone. Survival and motility of the worms appears to be much enhanced when using dialysis bags. Presumably, death of worms in the static culture contributed somatic proteins to the medium. This technique may be useful in deriving E/S products from other bloodborne helminths.

529 CHEMOKINETIC BEHAVIOR IN CERCARIAE OF *SCHISTOSOMA MANSONI*. THE ROLE OF LINOLEIC ACID IN BOTH ATTRACTION AND PENETRATION RESPONSE. Shiff CJ* and Graczyk TK. Department of Immunology and Infectious Diseases, The Johns Hopkins University, Baltimore, MD.

Use of the cercariae trap has raised the question about the detection of chemical stimuli by free swimming cercariae. It has been suggested that schistosome cercariae respond only to the tactile stimulus by exploration, creeping and penetration. However, when the trap is used in confined spaces, higher numbers of cercariae are trapped than would be expected if the organisms were randomly distributed in the water. This could be explained if cercariae exhibited some form of chemical response which caused aggregation near the source of stimulus. We have demonstrated in a choice chamber that cercariae migrate toward a source of linoleic acid. The response appears to be a chemoklinokinesis which results in formation of a cloud of organisms that then drift towards the stimulus, but which leaves numbers of outliers. These can be observed in counts made from the choice chamber. Cercariae which closely approach the source of stimulus will attempt to penetrate the base of the vessel (substrate) but this appears to be a dose graded effect which at low levels of stimulus is reversible, but at higher levels produces an irreversible penetration response.

530 SCHISTOSOMIASIS IS A RISK FACTOR ASSOCIATED WITH A HIGH SEROPREVALENCE OF HEPATITIS C VIRUS INFECTION IN EGYPTIAN BLOOD DONORS. Darwish M, Raouf T, Rushdy P, Constantine N, Rao M, and Edelman R. Ain Shams University Faculty of Medicine, Cairo, Egypt; Manchiet El Bakrey Hospital, Cairo, Egypt; National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; and Departments of Pathology and Medicine and the Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD.

We performed serological tests for hepatitis C virus infection on sera obtained from 163 volunteer blood donors seen at one Cairo hospital. We found HCV infection in 36 donors (22%) measured by

2nd generation EIA; 22 (63%) of these positive sera were reactive in a 2nd generation Recombinant Immunoblot Assay (RIBA-2), and another 12 (34%) showed an indeterminate reaction. Overall, 13.6% (95% confidence interval [CI]=8.3%-18.9%) of these Egyptian blood donors were confirmed serologically to be infected with HCV. Of several demographic variables and medical risk factors examined, the serologically confirmed (RIBA-2 reactive) donors were significantly older than non-reactive donors, and the age adjusted risk of being HCV-positive was significantly greater in individuals residing outside Cairo. A knowledge of having received injections, of having a history of schistosomiasis, or of having concomitant hepatitis B surface antigen or antibody were significantly associated with an increased risk of HCV- seropositivity; however, after adjusting for confounding demographic factors, only schistosomiasis (odds ratio=8.9, 95% CI=2.35-33.52) was significantly associated with HCV infection. The HCV seropositive rate of 13.6% among Egyptians is 5- to 35- fold higher than that reported from volunteer blood donors in other countries. Blood banks not testing for HCV should include a history of schistosomiasis in their exclusion criteria used for routine screening of blood donors. The mechanism for the association of schistosomiasis with HCV is under investigation.

531 *SCHISTOSOMA HAEMATOBIUM* AND *S. MANSONI* EGG DISTRIBUTION AND HISTOPATHOLOGICAL CHANGES DUE TO SINGLE SEX OR CROSS SPECIFIC INFECTIONS IN HAMSTERS. Khalil SB*, Mansour NS, Ishak EA, and Hibbs RG. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt; and Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

Histopathological changes, due to infection with one sex of *Schistosoma haematobium* or with two sexes each from a different species, have not been reported. Hamsters infected with various cercarial combinations were sacrificed 17 weeks post infection. In hamsters infected with *S. haematobium* female worms, eggs were deposited only in the host's liver. These eggs (some containing miracidia) were remarkably small with thin wall shells. The cross-mating of male *S. haematobium* and female *S. mansoni* produced typical *S. mansoni* eggs in the large intestine, liver, lungs and even in the urinary bladder. *S. haematobium* eggs from the reciprocal cross were found in the small and large intestines, liver, lungs but not in the urinary bladder. In the 2-way cross specific infections, deposited eggs contained miracidia and granulomatous reactions surrounding the eggs were comparable with that in homologous bisexual infections. Unlike the usual granuloma, lesions around the eggs from *S. haematobium* female single sex infection were hypocellular and formed predominately of lymphocytes and few macrophages. It is conceivably to find such patterns of infections in man due to ecological and epidemiological factors which will be discussed. Such infections may confound the species specific pathology and diagnosis.

532 ULTRASOUND ASSESSMENT OF PRAZIQUANTEL THERAPY OF PATIENTS INFECTED WITH *SCHISTOSOMA HAEMATOBIUM*. Nafeh M, Naser AM*, Shata MT, Ibrahim S, and Strickland GT. Faculty of Medicine, Assuit University, Assuit, Egypt; and International Health Program, University of Maryland, Baltimore, MD.

The changes in the morbidity of urinary the urinary tract were studied kin 153 patients with active urinary schistosomiasis following paraziquantel therapy using abdominal ultrasonography. The mean age of the patients was 15.5 years with a range of 5-58 years and 90.2% were males and 9.8% females.. Urinary bladder wall thickening was present in 60.8%, localized thickening in 51.6%, irregularities in 70.6% and polyps in 7.8% of the patients. Back pressure changes of the right kidney were minimal in 15.7%, mild in 1.3%, moderate in 0.79% and severe in 0.7% whcle back pressure changes of the left kidney were minimal in 13.7%, mild in 3.3% and moderate in 0.7% of the patients.

533 *ECHINOCOCCUS GRANULOSIS* PIG STRAIN FROM POLAND HAS A LOW INFECTIVITY TO HUMANS. Pawlowski Z*, Mrozewicz B, Stefaniak J, Schantz P, Wilson M, Eckert J, Jacquier P,

Haremski T, Nowosielski J, and Zieta B. University School of Medicine, Poznan, Poland; Parasitic Diseases Branch, NCID, CDC, Atlanta, GA; Institute of Parasitology, Zurich, Switzerland; and National Medical and Veterinary Services, Leszno, Poland.

In Poland *Echinococcus granulosus* in pigs appears to be a distinct strain; low infectivity to humans has been suspected but remains to be confirmed. In the area around Leszno, Poland, data on echinococcosis in local dogs and pigs were collected. Human residents of these farms were examined by sonography (651 cases) and serology (219 cases plus 250 controls). Examination of 24,896 pigs (mean age of 7 months) at slaughter revealed 1,623 (6.5%) of pigs infected with *E. granulosus* cysts. Infected pigs originated from 468 of 3,854 pig farms (12.1%). Of 16 randomly selected local dogs, *E. granulosus* adult worms were confirmed at necropsy of the intestine in four. Not a single case of echinococcosis was diagnosed at the local hospital during the last 5 years. Space- occupying lesions were diagnosed by sonography in 6 people living in "infected" farms (5 had a lesion in liver and one in kidney); however, none of the lesions were characteristic of echinococcal cysts. Of 219 people examined serologically, 35% demonstrated low titers of specific antibodies in ELISA and 47% in an IHA test, in comparison to 4.4% in both assays in 250 local control sera. Immunoblot examination in 39 persons with low positive titers was negative. Epidemiologic and clinical investigations in *E. granulosus* pig strain endemic foci in Poland provided evidence for low infectivity of this strain to humans living in that area.

534 ORAL IVERMECTIN, AN ALTERNATIVE FOR TREATING SCABIES INFESTATION. Glaziou P*, Cartel JL, Alzieu P, Briot C, Moulia-Pelat JP, and Martin PM. Institut Territorial de Recherches Medicales Louis Malarde, French Polynesia; and Direction de la Sante Publique, Papeete, French Polynesia.

A randomized investigator-blinded trial of oral ivermectin 100 mcg/kg single dose vs. benzyl benzoate 10% application in the treatment of scabies infestation, was conducted in 1992 in French Polynesia. In total, 44 patients aged 5-56 years were included in the study: 23 in the group ivermectin (IVER) and 21 in the group benzyl benzoate (BB). Rates of recovery were measured at day 7, 14 and 30. At day 30 after treatment, the cumulative recovery rates were 70% (16/23) in the group IVER, and 48% (10/21) in the group BB, 95% confidence intervals 51-87% and 29-70% respectively. The rates of recovery were greater in the group IVER at day 7, 14 and 30, but the overall difference was not statistically significant. The rather low recovery rate in the group treated with benzyl benzoate (48%) can be attributed to the reluctance of a population living under tropical temperatures to use a local application according to the instructions, and to keep the body unwashed for a long enough period. Our results show that oral ivermectin could be a valuable and welcome alternative to benzyl benzoate local treatment, when both treatments are used under field conditions.

535 DOG HOOKWORM: A CAUSE OF EOSINOPHILIC ENTERITIS IN HUMANS. Schantz PM*, Khoshoo V, Loukas A, and Prociv P. Parasitic Diseases Branch, NCID, Centers for Disease Control, Atlanta, GA; Childrens Hospital, New Orleans, LA; and Department of Parasitology, University of Queensland, Queensland, Australia.

We describe a case of eosinophilic enteritis involving a 7-year-old girl, presumptively caused by the dog hookworm,*Ancylostoma caninum*. She presented with bright red blood per rectum, but was otherwise asymptomatic. Colonoscopy revealed multiple, small rectal ulcerations and smooth nodules throughout the colon and 10- 15 cm of terminal ileum examined. Histologically, the affected mucosa contained an intense eosinophilic infiltration (>80 per high power field). Her peripheral eosinophil count and serum IgE were elevated. Stool examinations for ova and parasites in feces were negative on two occasions. Recognizing the similarity of her illness to*A. caninum* infection reported commonly in adults in Australia, we sought serologic confirmation. Her serum tested positive for antibodies to excretory-secretory antigens of *A. caninum* by both ELISA and Western blot

assays. After two courses of mebendazole the patient became asymptomatic. Based on the results of clinical, histologic, and endoscopic evaluation, the patient's response to specific anthelmintic treatment, the positive serologic tests for *A. caninum* antibodies, and household exposure to a dog with documented *A. caninum* infection, we believe this case of eosinophilic enteritis was caused by infection with the dog hookworm. This case provides strong presumptive evidence that *A. caninum* causes enteric disease in humans in the United States.

536 ASCARIASIS PNEUMONITIS A POTENTIALLY FATAL COMPLICATION IN SMOKE INHALATION INJURY. Heggers JP*, Muller MJ, and Herndon DN. Department of Surgery/Microbiology, University of Texas Medical Branch, Galveston, TX; and Shriners Burns Institute, Galveston, TX.

Ascaris pneumonitis in areas of endemic infestation is considered a benign condition. Smoke inhalation with any burn injury can be potentially fatal. A heavy infestation of *Ascaris* could further exacerbate the smoke-induced lung injury. After ingested eggs hatch in the small intestine, the larvae penetrate the mucosa and invade the blood stream and are then carried to the lungs. The larvae break out into the alveolar spaces as they are too large to cross the capillary bed and are carried up the bronchial tree and eventually swallowed. This study describes three cases of Ascaris infection in thermally-injured children. While the burns were <30% total body surface area, two patients who were injured in the same fire had further complication of smoke inhalation. This necessitated sophisticated therapy in order to promote survival. All patients were treated initially with Vermox. The one case without smoke inhalation did not develop pneumonitis and was discharged with no complications whereas the two with smoke inhalation developed severe pneumonitis. One patient was placed on ECMO and did not receive a full course of the Vermox treatment. This patient expired after several weeks of ECMO treatment. The third patient received a full course of Vermox, slowly recovered, and went home. Supportive therapy only is recommended during the lung migration phase of *Ascaris* life cycle. We feel that continuation of chemotherapy (Vermox) would have been beneficial in the second, and fatal, case. *Ascaris*-induced lung injury superimposed upon severe smoke-induced lung injury may have had an additive effect that precipitated severe, unrecoverable respiratory failure.

537 ACUTE PARASITIC INFECTIONS AS A CAUSE OF FEVER OF UNKNOWN ORIGIN (FUO). Farid Z, Hibbs RG*, Kamal M, Mousa M, Karam M, and Shaheen H. U.S. Naval Medical Research Unit No. Three, Cairo, Egypt; and Abbassia Fever Hospital, Cairo, Egypt.

During 1991-1992, 285 patients were referred to the Abbassia Fever Hospital for investigation of fever undiagnosed for over 3 weeks. Acute parasitic infection with eosinophilia was the most common cause of fever, diagnosed in 57 patients. Immunoserology was useful in the diagnosis of these obscure infections, and included Counterimmuno-electrophoresis (CIEP) and the Enzyme-linked immunosorbent assay(ELISA). The CIEP and ELISA diagnosed 25 patients with acute *Fasciola hepatica* infection and 30 with acute *Schistosoma mansoni* infection, 1 with acute *Ascaris lumbricoides* and 1 with *Trichinella spiralis*. Ultrasonography supported the diagnosis and demonstrated abnormalities caused by the *Fasciola* worm in the liver and biliary system. Clinically, acute fascioliasis and acute schistosomiasis present a similar clinical picture: prolonged fever with hepatomegaly, eosinophilia and anemia. The acute phase of the infection was controlled by prednisone; acute schistosomiasis was treated successfully with a single dose of praziquantel 75mg/kg divided into 3 equal parts given in 1 day, and acute fascioliasis was successfully treated with bithionol 50mg/kg daily for 10 days. Acute parasitic infections are important causes of FUO in Egypt, frequently present with eosinophilia, and can be diagnosed by immunoserology and ultrasonography.

538 INTESTINAL PARASITISM AND NECATORIASIS IN AMAZONAS, VENEZUELA. Garrido E*, Hernandez A, Slovanovic S, Nunez M, and Petralanda I. CAICET, Puerto Ayacucho, Amazonas, Venezuela.

We examined the prevalence of gastrointestinal parasites (GIP) in 1200 residents of Amazonas belonging to different ethnic groups (Yanomami, Piaroa, Gohajibo and Criollo). Polyparasitism was common, the average being 5.4 species/person. 74 % of all individuals had at least 3 GIP species and as many as 11 were observed in some persons. Aggregation of protozoan parasites (*Endolimax nana, Entamoeba coli, Giardia intestinalis, Iodamoeba butschli, Chilomastix mesnilii*) as well as of parasitic helminths (*Ascaris lumbricoides,* hookworms, *Strongyloides stercoralis, Trichuris trichiura*) was observed. The most frequent associations were *E. coli/Ch. mesnilii* (25 %), *Ch. mesnilii/A. lumbicoides* (21 %), *S. stercoralis/ Ch. mesnilii* (19 %), and *A. lumbicoides/E. coli* (18 %), with a strict hierarchy in the frequency of infection by some GIP species. Analysis of eggs by the Harada-Mori technique indicated that *Necator americanus* is the only human hookworm species in Amazonas, with a prevalence of up to 35 %. Behnke' golden baby hamster technique was used to generate L3/L4 stages of *Necator* to produce antigens. Average recovery of live L4 from the lungs was 71 - 84 % at day 6 post infection. Somatic and excretory-secretory (ES) antigens of L3 and L4 were used to assess antibody responses in individuals grouped according to their GIP status. The highest levels of antibodies to L4 were seen in persons without *Necator* infections (OD: 0.86 ± 0.15), the lowest in individuals with active *Necator* infection (OD: 0.20 ± 0.20) regardless of their infection status with other GIP.

539 EVALUATION OF TWO IMMUNOASSAYS FOR THE SEROLOGICAL DIAGNOSIS OF TOXOCARIASIS IN HUMANS. Sloan LM* and Rosenblatt JE. Division of Clinical Microbiology, The Mayo Clinic, Rochester, MN.

We evaluated two commercially produced enzyme-linked immunoassays (ELISA) for the detection in serum of antibodies to *Toxocara canis* and compared the results with those from an ELISA performed at a reference laboratory (CDC). Both commercial ELISAs employ strips of micro-wells coated with *Toxocara* excretory/secretory antigens but one (LMD Laboratories, Carlsbad CA) uses Protein A-HRP and TMB as an indicator system, and the other (Affinity Products, Crissier, Switzerland) uses an anti-human IgG-alkaline phosphatase conjugate. A total of 71 serum samples were tested: 13 were from normal healthy humans and 58 were from patients with suspected toxocariasis. All 13 serums from normals were negative by the three different ELISAs. 26, 23, and 22 of the 58 "suspect" serums were positve by the CDC (titre 1:32 or >), LMD (titre 1:100 or >), and Affinity (titre 1:200 or >) ELISAs, respectively. In relation to the CDC results, both the LMD and Affinity tests had one false positive result, which was the same specimen. There were four Affinity and three LMD false negative results (same three for both tests). This study indicates that these two commercial ELISAs provide very similar results and that in relation to the reference CDC method, they both have a specificity of 97% and have sensitivities of 88% (LMD) and 85% (Affinity). The availability of these rapid and simple tests should contribute significantly to the laboratory diagnosis of toxocariasis.

540 COMPARATIVE MORPHOLOGY OF *TAENIA ASIATICA* SP.N. AND *TAENIA SAGINATA* 1782 GOEZE. Eom KS* and Rim HJ. Department of Parasitology, College of Medicine, Chungbuk National University, Cheongju, Chungbuk; and Department of Parasitology, College of Medicine, Korea University, Seoul, Korea.

Among taeniid tapeworms infecting humans, *Taenia solium* Linnaeus 1758 and *Taenia saginata* Goeze 1782 have already been known. Most recently another kind of human taeniid tapeworm *Taenia asiatica* sp.n., known to distribute in Asian countries, has also been reported by the present authors. To make differential diagnosis between the species, a comparative study was conducted by light and scanning electron microscopy using the adult worms collected from two volunteers

experimentally infected each with Korean origin *T. asiatica* and African origin *T. saginata.* As results, *T. asiatica* was different morphologically from *T. saginata* on the following points; 1. presence of unarmed rostellum on the scolex of adult, 2. large number of 'uterine twigs' (57-99, mean 81.4, n=18) and the twigs/branches ratio (4.4, n=18) in the gravid proglottids, 3. prominence of 'posterior protuberance' in the gravid proglottids, and 4. wartlike formations on the external surface of the bladder worm (*Cysticercus viscerotropica*). On the other hand, *T. saginata* was characterized by 1. absence of rostellum, 2. small number of uterine twigs (40-56, mean 50.4, n=12) and ratio (2.3, n=12), 3. less prominent 'posterior protuberance', and 4. rugae on the bladder wall of C. bovis. Testes numbers, cirrus pouch extensions, ovaries, vitelline glands, vaginal openings and sphincters, uterine branches and eggs, sizes of metacestodes, rudimentary hooklets of metacestodes showed no or minor differences. Conclusively we believe this differential diagnostic keys will be useful in all the endemic areas including many tropical Asian countries.

541 FURTHER CHARACTERIZATION OF *TAENIA SOLIUM* (CYSTICERCOSIS) GLYCOPROTEIN ANTIGEN. Hyon SS, Pilcher JB*, and Tsang VC. Parasitic Diseases Branch, NCID, Centers for Disease Control and Prevention, Atlanta, GA.

An immunoblot assay was recently developed for the serodiagnosis of human *Taenia solium* (cysticercosis). After 4 years of field and laboratory application (>15,000 tests), this assay is 100% specific and 95% sensitive. The assay uses lentil-lectin, affinity-purified glycoprotein antigens (GP 50, GP 42-39, GP 24, GP 21, GP 18, GP 14, AND GP 13). To further characterize the antigen, various enzyme digestions and chemical treatments were performed. All fractions were then analyzed by SDS polyacrylamide electrophoresis and immunoblot. Results are summarized below.

TREATMENT	EFFECT OF TREATMENT
$NaIO_4$	No specific M.W. changes, Possible decrease in antigenicity
Dithiothreitol	Destroys or seriously decreases antigenicity of all antigens except GP21
N-Glycanase	M.W. changes in GP 50 and GP 42-39, Sharpens all bands
Neuraminadase	No Changes
O-Glycanase	No Changes
V-8	Destroys antigenicity of GP 21
Proteinase K	Destroys antigenicity of all antigens
Trypsin	Destroys antigenicity of all antigens

The above data suggest that antigenic activity is protein dependent and carbohydrate moieties do not contribute to this activity. These results suggest the feasibility of recombinant cloning of all antigens.

542 NEUROCYSTICERCOSIS IN HOUSTON, TEXAS: REPORT OF 112 CASES. White, Jr. AC*, Shandera WX, Armstrong R, Diaz P, and Tan J. Baylor College of Medicine, Houston, TX.

To define the clinical presentation and response to treatment of neurocysticercosis (NCC), we reviewed the medical records of 112 patients with NCC seen at Ben Taub General Hospital between 1985 and 1991. One hundred and four patients were originally from Latin America, 2 from Asia, and 5 from the U.S. (4 of whom had travelled to Mexico). Symptoms and presentation correlated with radiologic classification. Among 22 (19.6%) with only calcifications on CT scan, 17 (77%) presented with seizures and 3 with psychiatric disturbances. Among 72 with active parenchymal disease, 61 (85%) presented with seizures and 89% had seizures at some point in their course. All available imaging studies for this group revealed evidence of inflammation. Twelve patients (10.7%) had

ventricular disease and presented with increased intracranial pressure (5), headache (3), meningismus (2), and seizures (2). Five cases with NCC of the basilar cisterns presented with seizures (3), meningitis (1), and diplopia (1). Neuroimaging studies were the most useful diagnostic study. Among 73 patients were treated with praziquantel (PZQ), 12 required repeated courses of therapy. Repeated therapy was associated with abnormal CSF profile. Eleven of 12 who were retreated had received corticosteroids prior to PZQ ($p = 0.08$). Eleven patients required CSF diverting procedures; 7 required shunt revision. Shunt revisions appeared to be less common in those treated with PZQ. NCC is a common parasitic disease among hispanic immigrants. Presentation correlates wih the location of cysts. Current therapy is suboptimal with the frequent requirement for repeated courses, surgical intervention, and shunt revision.

543 *TRICHINELLA SPIRALIS*: PREPARATION OF MONOCLONAL ANTIBODIES, LOCALIZATION OF TARGET ANTIGEN, APPLICATION OF COMPETETIVE ELISA, AND IMMUNOCHEMICAL ANALYSIS OF THREE KINDS OF ANTIGENS. Heping Y *, Ruiyuan F, Rengang Z, Li L, and Saoliang H. Department of Parasitology, West China University of Medical Sciences, Chengdu, Sichuan, The People's Republic of China.

A hybridoma was produced by fusion of SP2/0 myeloma cells and immune BALB/C spleen cells. The Monoclonal Antibody (McAb) positive rate was 16.6%. Eight strains were of IgG1, one was of IgG2a, three were of IgM. Three of them which could specifically recognize the L1 antigens fraction were used as probes to localize the target antigen in sections of L1. They were found by indirect immunoperoxidase test to react strongly with cuticle and stichsome and weakly to the lining of the gut. The strain 2G8 ascites titer was as high as $1:1.6 \times 10^7$. The strain only reacted with L1 antigens of *Trichinella spiralis*, it failed to react with adults or newborn larval antigens, or other six helminths antigens by ELISA. 46 and 53 kDa of excretory-secretory antigens (ES) were recognized by 2G8 using Western-Blot test. Its target antigens localized in the stichsome and cuticle of L1 of *T. spiralis*. A McAb competitive ELISA was developed with the 2G8. 20 sera from rabbits infected with T. spiralis were tested. 35% (7/20) were positive on day 17 post infection, 100% were positive on day 31. No cross reaction was found with 29 sera from rabbits infected with other kinds of helminths. The dynamic antibody titer of sera from five infected rabbits were observed with this method for 142 days. The results showed that the pattern of change of antibody may be helpful to judge the state of infection and the method can be considered a sensitive and specific immunological diagnostic method for *T. spiralis* infection. Newborn larvae(NBL) were obtained by culturing adults of *T. spiralis*. They were maintained in tissue culture flasks containing M199 media and incubated in C02 incubator at 37 C for 15-20 hours. NBL were collected by passing media through filtration, centrifugation and repeated wash with NS. The results showed that this method was effective in terms of the activity of adults, NBL and the amount of NBL gained. ES were collected by harvesting the media in which L1 had been cultured for 24 hours. Membrane antigens (MA) of L1 were prepared by using the non-ionic detergent Triton X-114. The soluble antigens of NBL showed in SDS-PAGE and silver staining at least 40 bands of proteins. 28 bands of Glycoproteins, 9 bands of lipoproteins were revealed as similar gels were stained by the periodic acid, silver and Nile's blue staining. Six specific bands were recognized when rabbit polyclonal antibodies were used. All of then are glycoproteins. The MW were 41.5, 40, 29.5, 25, 18, and 11.5 kDa. The components of the antigens showed to be species specific and NBL stage specific. Sera from 34 patients with trichinellosis were test by ELISA using ES antigens. 32 of them were positive. 82 normal human sera were negative. No cross reaction was found in sera from patients with ascariasis and cysticercosis, but cross reaction was found in sera from patients with schistosomiasis and paragonimiasis. The MW of ES were between 96-17.6 kDa determined by SDS-PAGE and silver staining. The chief bands were 40, 48, 55 kDa. The sera from patients with trichinellosis recognized 26 and 48 kDa proteins, but the sera from patients with trichinellosis, schistosomiasis and paragonimiasis also recognized 21 and 32 kDa bands by Western-Blot. It was suggested that the 48 kDa protein is a potential useful protein for diagnosis of human trichinellosis. Analysis of MA by SDS-PAGE and silver staining showed at least 16 bands. The chief bands were 98, 90, 48, 32 and 28 kDa. There was no cross reaction by ELISA with sera from rabbits infected with

Toxocara canis, S. japonicum, P. skrjabini and *C. sinensis*. The specificity was higher than those of excretory-secretory antigens.

544 HUMAN INFECTION WITH CANINE HOOKWORMS: A COMMON CAUSE OF OBSCURE ABDOMINAL PAIN? Prociv P*, Croese J, Loukas A, and Opdebeeck J. Department of Parsitology, The University of Queensland, Brisbane, Australia; and Townsville General Hospital, Queensland, Australia.

We have found recently that human eosinophilic enteritis (EE) is relatively common in northeastern Australia and, in some cases, infection with the dog hookworm, *Ancylostoma caninum* has been clearly implicated. Assuming that disease results from an allergic reaction to hookworm secretions, we have developed diagnostic ELISAs and Western blots to detect IgG and IgE antibodies against the ES antigens of *A. caninum*. We then used this serology in 3 study groups of patients from Townsville, in north Queensland: Group A1 - 42 with EE; Group A2 - 105 with unexplained abdominal pain and blood eosinophilia ((PE), but insufficient evidence for a diagnosis of EE; Group A3 - 84 with unexplained abdominal pain without blood eosinophilia. Control groups included 40 Townsville patients with diagnosed painful abdominal conditions, 100 blood donors from Townsville and another 100 from Tasmania, where *A. caninum* is not found, and 4 patients with enteric *A. caninum* infection. The study groups included a higher proportion of dog owners than controls. The sexes were equally represented, except in group A3, which unavoidedly included patients with irritable bowel syndrome, and therefore, more females. The IgG-ELISA tested positive in 71% group A1, 67% group A2, and 30% group A3, cf. 8% controls. Further, ELISA reading were higher in patients with chronic or recurrent symptoms than those with an acute episode, and declined in convalescence, especially after treatment with mebendazole. The Western blot, performed in only small numbers of cases, proved positive in all 22 study group and hookworm-infected patients examined, cf. 10% of 40 controls. We conclude that cryptic infection with canine hookworms accounts for not only any cases of EE in north Queensland, but also a significant proportion of patients with unexplained abdominal pain and blood eosinophilia (PE), and even some cases of obscure pain without eosinophilia. This study need to be extended to other human populations which are exposed to canine hookworm infection.

545 RESOLUTION OF A CRYPTIC *TAENIA* SPECIES FROM *LEPUS AMERICANUS*. Call JL*. Parasitic Diseases Branch, NCID, Centers for Disease Control and Prevention, Atlanta GA.

Using rostellar hook morphology, cysticerci from *Sylvilagus* spp. are morphologically indistinguishable from cysticerci from *Lepus americanus* and are identified as *Taenia pisiformis*. However, comparison of allelic frequencies from 10 allozymes of cysticerci from *Sylvilagus* spp. (n=6) and cysticerci from *L. americanus* (n=7) yields a genetic identity (I) (Nei, 1978) of 0.058, indicating that they are not genetically close. Species level identification based on morphology is therefore inaccurate, and cysticerci from *L. americanus* represents a morphologically cryptic species. The cysticerci from *L. americanus* compared to *T. serialis* (n=6) coenuri from *L. californicus* have an I of 0.842. Demonstration of a cysticerci-forming *Taenia* spp. from *L. americanus*, which has allozyme patterns more closely related to coerui-forming *T. serialis*, but not *T. pisiformis*, may be relevant to the taxonomic importance of asexual reproductive abilities among tapeworms.

546 EFFECTS OF EXOGENOUS PROGESTERONE ON INFECTIONS OF *HAEMONCHUS CONTORTUS*. Fleming MW*. Helminthic Diseases Laboratory, Agricultural Research Service, USDA, Beltsville, MD.

Periparturient egg rise is a phenomenon associated with pregnancy, lambing and lactation in sheep. Progesterone (P4) is the primary steroidal hormone that maintains pregnancy, an experiment was

conducted to investigate its effect on *Haemonchus contortus* populations. Eighteen female lambs with prior exposure to *H. contortus* infections were ovariectomized and assigned to 1 of 3 replacement regimens: 0, 25 or 250 mg of P4/day delivered IM. After 3 weeks of hormonal treatment, all lambs were inoculated with 100,000 infective larvae. After 8 weeks of steroidal treatment, a blastogenic assay was performed on blood lymphocyte populations, and the abomasum from each lamb was obtained for larval and adult worm recoveries. Lambs of the 25-mg P4 group had significantly ($P<0.05$) reduced blastogenic response to Concanavalin A and greater adult and larval populations, compared with controls. Lambs of the 250-mg P4 group had worm burdens and lymphocyte blastogenesis values intermediate between those of the other treatment groups. P4 induced a generalized suppression of the immune system with concomitant increase in worm population suggesting a role in periparturient egg rise. These results will be compared with those from short-term exposure to P4.

547 ELECTRON TRANSFER-FLAVOPROTEIN RHODOQUINONE OXIDOREDUCTASE FROM ANAEROBIC MITOCHONDRIA OF *ASCARIS SUUM*. Ma YC* and Komuniecki RW. Department of Biology, University of Toledo, Toledo, OH.

Muscle mitochondria of adult *Ascaris suum* synthesize branched-chain fatty acids as end products of anaerobic carbohydrate metabolism. The final reaction in this pathway is energy-linked and involves the transfer of reducing power from the electron-transport chain through a series of soluble flavoproteins to unsaturated 2-methyl branched-chain enoyl CoAs. Electron-transfer flavoprotein:rhodoquinone oxidoreductase(ETF:RO), which mediates this transfer, has been purified from isolated mitochondrial membranes. EPR spectroscopy of the purified ETF:RO revealed signals at g=2.076, 1.936 and 1.883, arising from an iron-sulfur center, as well as signals attributable to a flavin semiquinone. Potentiometric titration yielded an oxidation-reduction midpoint potential (E_m) for the iron-sulfur center of 25 mV at pH 7.4. The reduction of flavin occurred in two distinct steps, with a flavin semiquinone radical detected as an intermediate. The E_ms for the two steps in the complete reduction of flavin were 15 mV and -9 mV, respectively. Physiologically, ETF:RO accepts electrons from a low potential quinone, rhodoquinone, and functions in a direction opposite to that of the mammalian ETF:ubiquinone oxidoreductase. Incubations of *A. suum* submitochondrial particles (SMP) with NADH, 2-methylcrotonyl CoA, purified *A. suum* electron-transfer flavoprotein and 2-methyl branched-chain enoyl CoA reductase resulted in significant 2-methylbutyryl CoA(2-MB CoA) formation, which was inhibited by both rotenone and antisera to the purified ETf:RO. Quinone extraction of SMP with pentane resulted in almost the complete loss of 2-MBCoA formation. However, the reincorporation of rhodoquinone, but not ubiquinone, restored over 50% of the NADH-dependent 2-MBCoA formation. These results indicate that ETF:RO is a key component of the NADH-dependent enoyl CoA reduction and suggest that its reoxidation is rate-limiting under physiological condition.

548 *STRONGYLOIDES STERCORALIS* CONTROL OF DEVELOPMENTAL SWITCH POINTS BY CHEMOSENSORY NEURONS. Ashton FT, Bhopale VM, Fine AE, Cherry BR, and Schad GA*. Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Strongyloides stercoralis, an important nematode parasite of man and animals, has a complex life cycle. The direction of development taken at each of several switch points is presumably mediated by environmental signals. One switch point initiates development of an environmentally resistant, infective resting stage to a developing parasitic form. *Caenorhabditis elegans*, a related free-living nematode, has similar switch points. It can interrupt normal development by including an environmentally resistant resting stage, the dauer larva, in its life cycle. The decision to enter into and to exit from the dauer state is controlled by four chemosensory neurons, as demonstrated by neuronal ablation studies, as reported previously. The *S. stercoralis* infective, resting larva, which is

similar to the dauer larva of C. *elegans*, resumes feeding and development on receipt of signals (presumably chemical) from a host. We have found that the 12 labial and 4 cephalic sensilla are not open to the environment in this stage. Therefore, the 13 neurons we have found associated with the amphid are likely to include the relevant receptors.We have traced each of the amphidial neurons to its cell body, and have determined possible homologies with the amphidial neurons in C. *elegans* by comparing the position of the cell bodies in the two species. Using maps of the cell bodies, especially from the first stage larva, microlaser ablation studies can be conducted to determine which neurons are involved in the infective process.

549 ECTOPIC *MONILIFORMIS MONILIFORMIS* IN ITS USUAL DEFINITIVE HOST, *RATTUS NORVEGICUS*. Oetinger DF*. Department of Biology, Kentucky Wesleyan College, Owensboro, KY.

A living, 11.3 cm gravid female *Moniliformis moniliformis* was recovered from a tumorous mass within the greater omentum of a female outbred Sprague-Dawley rat five months post-infection. Paraffin sections of the omental mass were stained by the following procedures: Harris' Hematoxylin and Eosin, May-Grunwald, Periodic Acid-Schiff, Verhoeff's Elastic, and Weigert's Fibrin. Included within the omental mass was a small lobe of pancreas. The host's response had isolated the worm in a connective tissue tunnel in which there were notable abscess and inflammatory reactions. Eggs released by the worm elicited characteristic granulomatous reactions with stages similar to those which have been described for schistosome eggs. Since lymphocytes were abundant throughout the omental tissue, with large areas of perivascular infiltration, it is suspected that the eggs of *M. moniliformis* in this extra- intestinal site, have antigenic components capable of stimulating a cell-mediated delayed hypersensitivity reaction.

550 *NIPPOSTRONGYLUS BRASILIENSIS* INDUCED ENTERIC MASTOCYTOSIS IN SELF-CURING VS. NON-SELF-CURING MICE. Morawiecki PA*, Mayberry LF, and Bristol JR. Department of Biological Sciences, University of Texas at El Paso, El Paso, TX.

Recently, a Balb/C mouse strain which does not undergo typical self-cure was identified in the UTEP Animal Colony providing a natural system which can be used to demonstrate more precisely the involvement of mast cells in nematode expulsion. Therefore, three groups of mice were infected with 500 *N. brasiliensis* L3 larvae subcutaneously. Group 1 consisted of UTEP mice, group 2 was composed of self-curing C3H mice, and group 3 contained F_1 progeny of a C3H and UTEP mouse cross. Mice were sacrificed at 4 day intervals up to 28 days postinfection (PI) and the mast cells quantified per villus crypt unit (VCU) in tissue sections. Duodenal mast cell numbers per VCU were significantly increased on days 12-20, and 28 PI in Balb/C mice, and on day 12 PI in F_1 mice compared to uninfected controls. Jejunal mast cell numbers per VCU were significantly increased on days 12-28 PI in Balb/C mice, and on days 16 and 20 PI in C3H mice compared to uninfected controls. Ileal mast cell numbers per VCU were significantly increased on days 4, 12, and 16 PI in Balb/C mice and on days 16, and 20 PI in C3H mice compared to uninfected controls. Significant differences in mast cell numbers were observed among the three strains of uninfected mice.

551 SNAKE RENAL NEMATODIASIS. Stewart TB*, Veazey R, and Snider TG. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

A young Burmese python (*Python molurus bivitattus*) bought from a dealer died a month after purchase without eating a meal. Many species of snakes in mixed groups were stocked by the dealer. The snake presented to the Diagnostic Laboratory weighed 220 g, was in an adequate nutritional condition and showed mild postmortem autolysis. The ureters were packed with white granular material from the cranial pole of the kidneys to the cloaca. Sections of nematodes, 20-30 um in

diameter, were present in the collecting tubules of each kidney. Tubules were dilated and contained fibrin, sloughed epithelial cells and mucoid material. Multifocal infiltration of heterophils and lymphocytes were present throughout the interstitium and the pelvis and ureters were dilated with granular basophilic material. Several complete worms and many worm pieces were dissected from the formalin preserved kidneys. Only gravid females were found. The nematodes were identified as *Strongyloides* sp. Morphologically and morphometrically they appear similar to the parasitic females of *S. gulae*, parasites normally of the esophagus of many species of Louisiana snakes. Obstruction of the ureters by the nematodes in an unusual habitat probably resulted in pyelonephritis. Secondarily, enteritis and septicema were also involved in the disease process resulting in death.

552 NEW MORPHOLOGICAL CHARACTERS FOR IDENTIFYING INDIVIDUAL SPECIMENS OF *HAEMONCHUS SPP.* AND A KEY TO SPECIES OF RUMINANTS OF NORTH AMERICA. Lichtenfels JR*, Pilitt PA, and Hoberg EP. Biosystematic Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

The large stomach worms *Haemonchus contortus, H. placei* and *H. similis* are important pathogens of cattle and sheep. Newly described characteristics of surface cuticular ridges (synlophe) provide the first morphological means of identifying individual adult specimens of either sex. The diagnostic patterns of the synlophe can be observed at 400X on the anterior half of specimens in temporary mounts in glass slides. The synlophe can be studied in cleared preserved specimens or in living or freshly-thawed frozen specimens mounted in water. The synlophe of H. contortus has 30 ridges in the region of the posterior half of the esophagus, 4 fewer than *H. placei* and *H. similis*. The 4 extra ridges of *H. placei* and *H. similis* are consistently located bilaterally to the 3 ventralmost and the 3 dorsalmost ridges. The 4 extra ridges of *H. similis*extend to the end of the synlophe posterior to midbody, but in *H. placei* they extend only to the posterior end of the anterior quarter of the nematode. A key is included to the 3 species of *Haemonchus* parasitic in domestic sheep and cattle using characteristics of spicules, female reproductive system, female tail and the synlophe.

553 VALIDATION OF MICROTHRIX STRUCTURE IN *LACISTORHYNCHUS TENUIS* (CESTOIDEA: TRYPANORHYNCHA). Jacob BA* and Ruhnke TR. Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs CT.

The various tegumental structures (filiform, spiniform, palmate morphologies etc.) of tapeworms potentially offer a great deal of phylogenetic information. However, use of these characters is prohibited until the issue of homology among the various forms of these structures is resolved. One of the central questions is whether or not some or all of these structures should be classified as microtriches. The surface of the scolex of the trypanorhynch *Lacistorhynchus tenuis*, a species reported to exhibit at least three forms of surface projections, was examined with scanning and transmission electron microscopy in an attempt to at last resolve these issues. Scanning electron microscopy revealed four forms of surface projections on the scolex of *L. tenuis* including: filiform, spiniform, palmate and distally bifid structures. Transmission electron microscopy of these structure revealed that all four share a basic cytoarchitecture: (1) an electron-dense shaft mounted on but separated from an electron-lucid base by a multilaminated baseplate; (2) a proximally-oriented, circum-peripheral projection from the shaft/base plate surrounding the distal-most portion of the base; and (3) presence of plasmalemmal and glycocalyxial layers that overlie the entire tegumental structure. These fundamental characteristics are in agreement with the basic definition of a microthrix (sensu Rothman, 1963; Holy and Oaks, 1986). Given their homologous nature, the various microthrix morphologies are supported for use in phylogenetic analysis of cestodes.

554 CROSS-REACTIVITY BETWEEN *PLASMODIUM FALCIPARUM* AND AVIAN MALARIAL PARASITES IN THE ELISA FORMAT. Graczyk TK*, Cranfield MR, and Shiff CJ. School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD; and The Baltimore Zoo, Baltimore, MD.

An enzyme-linked Immunosorbent Assay (ELISA) with three *Plasmodium falciparum* antigens: 1) R32tet32, 2) P.F.R27, 3) Crude Red Blood Cell Extract (CRBCE) was tested for detection of anti-*P. relictum* and anti-*P. elongatum* circulating antibodies in the sera from experimentally infected Peking ducklings and naturally infected African black-footed penguins (*Spheniscus demersus*). Anti-chicken IgG was used for the duckling sera; anti-penguin IgG for the penguins (both coupled to alkaline-phosphatase). Anti-penguin IgG was generated in rabbits and purified by protein A low salt column. The ELISA clearly differentiated sera from infected and noninfected ducklings; birds infected with *P. elongatum* exhibited higher absorbance values than those infected with *P. relictum*. All penguins were positive for anti-*P. relictum* and/or anti-*P. elongatum* antibodies. The level of antibodies in the penguin sera was significantly correlated with the number of outdoor exposure years experienced by birds. The described assay can be used as a test for diagnosis of bird exposure to avian malarial parasites along with monitoring the level of antibodies in selected groups of birds.

555 ANTI-*PLASMODIUM* SPP. ANTIBODIES IN AFRICAN BLACK-FOOTED PENGUINS (*SPHENISCUS DEMERSUS*) DETECTED BY ELISA. Graczyk TK*, Cranfield MR, Skjoldager ML, and Shaw ML. Department of Immunology and Infectious Diseases, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD; and The Baltimore Zoo, Baltimore, MD.

An Enzyme-linked Immunosorbent Assay (ELISA) with three *Plasmodium falciparum* antigens: 1) R32tet32, 2) P.F. R27, 3) Crude Red Blood Cell Extract (CRBCE) was used for detection of anti-*P. relictum* and/or anti-*P. elongatum* circulating antibodies in the penquin sera. Blood for the trial was collected from 38 captive-reared various aged (from 1 to 26-yr-old), African black-footed penguins (*Spheniscus demersus*) from the Baltimore Zoo's open-air colony, deposited on filter paper, stored at 4° Celsius and eluted into buffer prior to the ELISA trials. The pooled sera from ten penguins known to be infected and parasitemic were used as a standard to determine the level of antibody titration units (ATU) in an individual bird sample. All sampled penguins were positive for anti-*P. relictum* and anti-*P. elongatum* antibodies. The group of 1- and 2-yr-old penguins had a significantly higher level of ATU (x=68.11 ATU) than the rest of the older birds (x=30.5 ATU). The decrease in ATU significantly correlated with the number of outdoor exposure years experienced by birds.

556 COMPARISON OF ANTIBODY TITERS TO *PLASMODIUM RELICTIM* AND *P. ELONGATUM* IN CHICKS AND MOTHERS OF AFRICAN BLACK-FOOTED PENGUINS *SPHENISCUS DEMERSUS*. Graczyk TK* and Cranfield MR. Department of Immunology and Infectious Diseases, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD; and The Baltimore Zoo, Baltimore, MD.

The ELISA method was used to determine the level of antibodies against *Plasmodium relictum* and/or *P. elongatum* in African black-footed penguins (*Spheniscus demersus*). Blood was collected from 14 chicks, deposited on filter paper, stored at 4°C and eluted prior to the ELISA trial. Chick mothers were bled at the time of oviposition and the blood samples were processed in the same manner. Three capture antigens of *P. falciparum* were used: 1) R32tet32, 2) P.F.R27, and 3) Crude Red Blood Cell Extract (CRBCE). The pooled sera from ten infected, parasitemic adult penguins were used as a standard to determine the level of antibody titration units (ATU) in individual birds. Chick and mother antibody titers were compared by a regression analysis. The level of maternal antibody (MA) varied widely among individual newly hatched chicks (NHC). The ATU in the NHC correlated strongly (r=0.866 for R32tet32, r=0.889 for P.F.R27, and r=0.948 for CRBCE) with those in mothers. The ATU were x=30.3% higher in the NHC than in their mothers.

557 MODIFIED MAURER'S CLEFTS IN MALARIA-INFECTED ERYTHOCYTES CULTURES UNDER NON-STANDARD CONDITIONS. Fujioka H* and Aikawa M. Institute of Pathology, Case Western Reserve University, Cleveland, OH.

Erythrocytes infected with *Plasmodium falciparum* and *P. berghei* exhibit cytoplasmic inclusions in the form of Maurer's clefts. We have examined by electron microscopy the morphological alterations engendered in these clefts by non-standard treatment of infected RBCs in culture. Parasitized blood cells were exposed to higher pH, higher PO_2, and lower temperature than usual. Under these conditions, the flattened, slit-like Maurer's clefts became markedly dilated. These dilated clefts were able to take up colloidal gold particles and tracer macromolecules such as Protein A, rhodamine-dextran, and lucifer yellow-dextran. In contrast, conventional Maurer's clefts in infected RBCs cultivated under standard conditions do not engage in such uptake. Our results indicate that the large membrane-lined inclusions in the cytoplasm of parasitized RBCs are the result of prefixation stress, and that they do not exist under physiological conditions.

558 DIFFERENTIAL LYSIS OF TWO DEVELOPMENTAL STAGES OF MALARIA SPOROZOITES BY THE ALTERNATIVE PATHWAY OF COMPLEMENT. Touray MG*, Seeley DC, and Miller LH. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD.

During sporogonic development of *Plasmodium gallinaceum* in the mosquito vector, 2 developmentally distinct sporozoite stages can be isolated. Sporozoites obtained from oocysts in abdomens of mosquitoes 10 days after an infective bloodmeal are poorly infectious to the vertebrate host; days later, sporozoites isolated from mosquito salivary glands are highly infectious. In a first step towards understanding the physiologic basis of this developmentally regulated infectivity to the vertebrate host, we determined the relative resistance of the 2 sporozoite stages to lysis by the complement system of the vertebrate host. Whereas 86% of oocyst sporozoites were lysed when incubated in fresh chicken serum *in vitro*, only 24% of salivary grand sporozoites were lysed under identical incubation conditions. The lytic activity of fresh chicken serum was abrogated by incubating for 45 minutes at 56°C or chelating with EDTA. Fresh chicken serum specifically depleted of Ca^{2+}, by chelating with EGTA in the presence of Mg^{2+}, retained its ability to lyse oocyst sporozoites. Because lysis by serum was heat sensitive and Mg^{2+}-dependent, but Ca^{2+} independent, we conclude that lysis was mediated by the alternative pathway of complement. One factor in the development from noninfectious oocyst sporozoite to infectious gland sporozoites is the ability to resist lysis by the alternative pathway of complement.

559 ANTIGENIC CHARACTERIZATION OF *PLASMODIUM YOELII* PARASITIZED ERYTHROCYTES GHOST. Terrientes ZI* and Chang AP. University of Panama, Faculty of Medicine, Center for Research and Diagnostic of Parasitic Diseases (CIDEP), Panama; and University of Hawaii, Department of Tropical Medicine and Medical Microbiology, Honolulu, HI.

Antigens exposed either at the surface of the parasite or in the infected host cell are more likely to play an impotant role in the development of immunity. Using a rodent malaria model, this study attempted to identify and characterize parasite-encoded proteins expressed using the techniques of SDS-polyacrilamide gel electrophoresis and imunoblotting, surface immunofluorescence and mebabolic labeling with ^3H-isoleucene. The surface antibodies to surface antigens was suggestive of the presence of novel surface antigens on parasitized erythocytes. *Plasmodium yoelii* infected red blood cel membrane antigens present in trophozoite ghosts. Some of these were expressed on the trophozoite surface (92.5, 79, 76,9,60, 43, 31, 30, 19, & 14.3 kDa) and some appeared to have co-migrating normal host membrane antigens (92.5, 80, & 69 kDa).

560 ADAPTATION OF A STRAIN OF *PLASMODIUM FALCIPARUM* FROM A MONTAGNARD IMMIGRANT TO IN VITRO CULTURE AND NEW WORLD MONKEYS. Collins WE*, Grady KK, Ciano J, Wick T, and Millet P. Division of Parasitic Diseases, Center for Infectious Disease, Centers for Disease Control, Atlanta, GA; and Georgia Institute of Technology, Atlanta, GA.

Gametocyte-producing strains of *Plasmodium falciparum* developing both in animals and *in vitro* are rare. Such parasites allow *in vitro* gametocyte production and infection of animals for immunologic and chemotherapeutic investigations. A strain of *P. falciparum*, Mont-1/Centers for Disease Control, was isolated from a Montagnard refugee who emigrated from the Vietnamese/Cambodian border and was relocated in North Carolina in November 1992. No treatment had been administered to the person before blood collection. The parasite adapted very quickly to culture conditions (RPMI supplemented with gentamycin, sodium bicarbonate, and 10% O+ human serum), and produced, after 18 to 22 days in culture, mature gametocytes infective for *Anopheles freeborni, An. stephensi, An. dirus,* and *An. gambiae.* Highest oocyst counts were obtained in *An. freeborni,* and sporozoites were isolated. *Aotus vociferans* and *A. nancymai* were inoculated with cultured asexual parasites; adaptation developed slowly. In addition, this strain was shown to cytoadhere to microvascular endothelial cells in an *in vitro* assay. Studies are continuing on characterization of parasite drug sensitivity in infected monkeys.

561 ARACHIDONIC ACID METABOLITE IN *PLASMODIUM FALCIPARUM.* Okoye VN, Williams HL, Johnson DJ, and Martin SK*. Department of Hematology, Walter Reed Army Institute of Research, Washington DC; Office of the Chief Medical Examiner, Washington DC; Department of Pharmacology, Duke University, Durham, NC; and United States Army Medical Research Unit-Kenya, Nairobi, Kenya.

The release of membrane arachidonic acid by phospholipase-A2 (PLA2) activity is the rate limiting step in the biosynthesis of ecosanoids. Prostaglandins, cytochrome P-450 metabolites, leukotrienes and HETEs are potent local host cellular and tissue function modulators. Previous studies have reported a 40-fold increase in serum PLA2 activity during a malaria infection. If malaria parasites initiate the production of ecosanoids, these biologically active lipid derivatives could then have a profound effect on host cellular and tissue function. Therefore, we incubated synchronized *Plasmodium falciparum* parasites with radiolabeled arachidonic acid for three hours and looked for more polar arachidonic acid metabolites in spent medium and pellet by high pressure liquid chromatography (HPLC). Unlike the ring stage, falciparum trophozoites released an arachidonate metabolite into the medium. This metabolite chromatographed as a single peak at (96.5min) relative to arachidonic acid (144.5 min). Release of this compound was neither inhibited by indomethacin (100 μM) nor nordihydroguaiaretic acid (10 μM). The role that this putative metabolite plays in parasite physiology and host pathophysiology is yet to be determined.

562 ULTRASTRUCTURAL OBSERVATIONS OF THE GAMETOCYTE DEVELOPMENT OF *PLASMODIUM FALCIPARUM.* Venugopal D* and Meszoely CA. Department of Biology, Northeastern University, Boston, MA.

In this ultrastructural study, we examined the changes in the cytoplasmic organelles and pellicular complex of the gametocytes of *Plasmodium (Laverania) falciparum* during development in order to find a set of characters with which to reliably differentiate the microgametocyte from the macrogametocyte. The gametocytes have a triple layered pellicular complex, cristate mitochondria, cytostome, numerous food vacuoles and a prominent system of subpellicular microtubules in the single and double form. The immature macrogametocyte has numerous osmiophilic bodies and pigment crystals that are tightly clustered in the cytoplasm. These structures are absent in the immature microgametocyte. The activation of the macrogametocyte is recognized when it becomes

spherical and by the alignment of the osmiophilic bodies along the pellicular complex. Some of the osmiophilic bodies are attached by small ducts to others or to the inner pellicular complex. The pigment crystals are then randomly dispersed in the cytoplasm. Activation of the microgametocyte is also recognized by the spherical shape of the parasite and the appearance of the kinetosomes. Axonemes develop from the kinetosomes with typical 9+2 configuration of ciliary or flagellar axonemes. A few pigment crystals are present in the cytoplasm. The exflagellating microgamete has a single axoneme, nucleus and a small amount of cytoplasm. The change in shape of the gametocytes is also accompanied by an increase in size. This is evident by disruptions in the inner and middle pellicular membrane. As a gametocyte matures, it induces changes in the host erythrocyte resulting in the breakup of the erythrocytic cytoplasm and the development of a translucent region around the parasite.

563 *PLASMODIUM VIVAX* HYPNOZOITES IN A HUMAN HEPATOMA CELL LINE. Karnasuta C *, Sattabongkot J, Chantakulkij S, Eikarat N, and Watt G. US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Dormant liver schizonts, or hypnozoites, have been implicated as the cause of relapses in *Plasmodium vivax* infections but there is little direct evidence of their existence. We attempted to gather additionalevidence of hypnozoites using human hepatoma cells (HepG2) infected with sporozoites dissected from laboratory reared *Anopheles dirus* mosquitoeswhich had fed on *P. vivax* infected human volunteers. HepG2 cultures were trypsinised at predetermined time points and the disaggregated cells were centrifuged onto poly *L*- lysine coated slides using Cytospin2, fixed and stained with Giemsa. There appeared to be 2 populations of schizonts; 1 which gradually enlarged, matured and ruptured and a secondwhich remained small (<5 μm in diameter). Only these small forms were seen beyond day 33 and remained detectable through day 45. Control experiments with non-relapsing malarias, drug-susceptibily studies, andattempts to determine the viability of these small schizonts are underway. Our findings suggest that the small liver schizonts present throughout the experiment are hypnozoites.

564 THROMBOMODULIN IS A MARKER FOR VESSEL WALL INVOLVEMENT IN FALCIPARUM MALARIA. Dietrich M *, von Riedesel J, Bierhaus A, Kern P, Hemmer CJ, and Nawroth PP. Department. of Medicine, Bernhard-Nocht-Institute for Tropical Medicine, Hamberg, Germany; and Heidelberg University Medical School, Heidelberg, Germany.

The vascular endothelium seems to be important in severe falciparum malaria. In vitro, patient serum induces transcription of several endothelial cell (EC) genes. To assess vessel wall involvement *in vivo*, we measured the plasma levels of Thrombomodulin (TM), a specific surface marker for endothelial cells. TM transcription was determined in cultured endothelial cells after incubation with patient or control serum. TM was measured by ELISA in 37 patients with falciparum malaria before and after antiparasitic therapy. TM transcription was assessed by Nuclear Run-on and Northern blot in cultured EC, after they had been incubated with patient or control serum. We also determined TM levels in culture supernatants from EC, that had been incubated with medium conditioned by falciparum-parasitized or non-parasitized erythrocytes. TM plasma levels were higher in falciparum malaria (median 45 ng/mL) than in healthy controls (< 25 ng/mL). The highest levels (> 250 ng/mL) were seen in the severest cases. TM levels correlated with parasitemia, TNFa serum levels, plasma levels of neutrophil elastase, and clinical severity. Incubation of EC with patient serum (as compared to control serum) downregulated TM transcription. This effect depended upon TNFa in the patient serum, as indicated by neutralization of TNFa by antibody. Thus, elevated TM plasma levels are not a direct effect of TNFa, since TNFa does not induce TM shedding from EC. When cultured EC were incubated with medium conditioned by falciparum-parasitized erythrocytes, significant TM amounts were released into the supernatant (compared with medium conditioned by control erythrocytes).

Elevated TM plasma levels *in vivo* seem to reflect the impact of parasitemia on the vascular endothelium and may indicate disease severity. Therefore TM may be of prognostic importance.

565 CHARACTERIZATION OF THE ALA-SYNTHASE GENE HOMOLOGUE OF *PLASMODIUM FALCIPARUM*. Wilson CM*. Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL.

The malarial parasite *Plasmodium falciparum* (Pf) has been shown to have an exquisite mechanism for detoxifying hemoglobin degradation products and has no evidence for classical heme scavenging. This would suggest a need for heme biosynthesis to meet the requirements of the heme containing molecules and pathways identified in Pf. In order to initiate studies on heme biosynthesis and its regulation in Pf we have cloned the ALA synthases (ALA-S) gene homologue of Pf. Initially a gene fragment was identified using PCR and degenerate oligonucleotides based on previously identified similarities among other ALA-S gene homologues. This fragment was used to select genomic clones which when sequenced revealed an open reading frame with significant similarity to other ALA-S gene homologues. The similarities with the gene homologues from the purple phototropic alpha-group eubacteria were particularly striking. Other interesting features of the predicted amino acid translation of the primary sequence are : 1) a 5' leader sequence with heme-binding/regulatory motifs involved in mitochondrial import regulation in other systems; 2) a 45 amino acid insertion with no apparent homology to other ALA-S homologues; 3) a 140 bp sequence with concensus intron splice-site motifs and located in an analogous position between exons 10 and 11 of the mouse and human ALA-S homologues. We are completing studies on the mRNA of this gene to further analyze the above observations and study its stage-specific expression.

566 ANALYSIS OF *mdr* GENES IN PROTOZOAN PARASITES TO FUNCTIONALLY COMPLEMENT THE YEAST *ste6* GENE. Volkman SK*, Chow LM, Harris DS, and Wirth DF. Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

The ATP-Binding Cassette (ABC) gene family contains gene thought to be involved in cell communication and drug resistance and encode proteins proposed to efflux or export molecule from cells. The protozoan parasites *Leishmania enrietti* and *Plasmodium falciparum* express genes which are homologous to this gene family. Another member of this family, the *ste6* gene of *Saccharomyces cerevisiae*, is known to encode a protein which exports a-factor mating phermone such that functional expression of this gene product can be directly quantitated in a mating analysis. As described previously, the expression of some members of the ABC gene family, the mouse *mdr3* gene and the human cystic fibrosis gene, can complement the *ste6* gene by restoring a mating phenotype in a *ste6* deleted strain. Since the function of the two protozoan genes, the *lemdr1* gene of *L. enriettii* and the *pfmdr1* gene of *P. falciparum*, is unknown, these genes were cloned into a yeast expression vector and the resulting plasmid was used to transform an *ste6*-minus yeast strain to determine if *lemdr1* or *pfmdr1* expression could complement the *ste6* deletion and function to export phermone. Initial results indicate that expression of the *lemdr1* and *pfmdr1* genes may complement the *ste6* gene in mating assays.

567 PF83/AMA-1, AN 83 KDA VACCINE CANDIDATE APICAL MEMBRANE ANTIGEN OF *PLASMODIUM FALCIPARUM*: SUBCELLULAR LOCALIZATION AND POSSIBLE FUNCTIONAL RELEVANCE. Narum DL* and Thomas AW. Department of Chronic and Infectious Diseases, Medical Biological Laboratories-TNO, Rijswijk, The Netherlands.

Understanding the biological functions of vaccine candidate molecules will facilitate analysis of protective responses. *In vitro* a protective (rhesus monkey trial) *Plasmodium knowlesi* 66 kDa merozoite apical membrane antigen (PK66), was inhibited by Fab fragments suggesting PK66 has a

receptor-like function. Therefore, the biological function of PF83/AMA-1, a highly conserved 83 kDa homologue in *P. falciparum* has been further studied using monoclonal antibodies against synthetic peptides representing the carboxyl and amino termini. PF83/AMA-1 is maximally expressed late in schizogony and post-synthetically processed to a 66 kDa molecule by cleavage of an amino terminal peptide at or around the time of schizont rupture and merozoite release. In the CVD-1 clone of *P. falciparum* the 83 kDa molecule is apically restricted, whereas the 66 kDa form may become circumferentially associated with the merozoite surface. After merozoite invasion, the 66 kDa molecule appears to co-localize within the parasitophorous vacuole with the rhoptry-associated-protein-1. After invasion, PF83/AMA-1 is rapidly degraded. It is possible that the unique amino terminal peptide may be involved in merozoite release while the 66 kDa molecule is subsequently involved in merozoite invasion.

568 POLYMERASE CHAIN REACTION AND A LIQUID-PHASE NON-ISOTOPIC HYBRIDIZATION FOR DETECTION OF *PLASMODIUM FALCIPARUM* INFECTION. Oliveira DA*, Holloway BP, Durigon EL, Lal AA. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA; Biotechnology Core Facility, Centers for Disease Control and Prevention, Atlanta GA; Division of Viral and Rickettsial Diseases, Centers for Diseases Control and Prevention, Atlanta GA; and Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

The use of rRNA probes has been considered to be better than DNA probes for the diagnosis of Plasmodium falciparum. When compared with DNA probes, previous studies utilizing 18S rRNA ^{32}P labeled probes showed higher sensitivity with a detection limit between 0.00046% and 0.000025% parasitemia. However, the use of radiolabeled probes is not suitable for field conditions because of its hazards and instability. In this study we present a strategy for *Plasmodium falciparum* detection that combines DNA amplification by polymerase chain reaction (PCR) with a nonradioactive hybridization in a solid-phase enzyme immunoassay. Genomic DNA from infected red blood cells was amplified by PCR utilizing biotinylated 5' primer and unmodified 3' primer prepared from a conserved small subunit rDNA sequence of *Plasmodium* species. The PCR amplification derived DNA fragment was allowed to react with a digoxigenin labeled probe complementary to a *P. falciparum* specific rDNA sequence followed by denaturing and annealing. Color was developed after capture of hybrids onto a streptavidin coated microtiter plate. The assay was specific for *P. falciparum* since the amplified PCR fragment did not react with digoxigenin labeled probes specific for *P. malariae*, *P. vivax* or *P. ovale*.

569 SEQUENCE OF TWO ALLELIC FORMS OF A MEROZOITE SURFACE ANTIGEN OF *PLASMODIUM FALCIPARUM* IN SRI LANKA. Ranasinghe C* and Ramasamy R. Division of Life Sciences, Institute of Fundamental Studies, Kandy, Sri Lanka.

It is clearly established that *Plasmodium falciparum* exhibits strain dependent antigenic polymorphisms. The sequences of many vaccine candidate antigens of *P. falciparum* are published. However, these sequences are derived from a few well established laboratory isolates. A determination of the sequences of antigens in Sri Lankan parasite isolates is required before the inclusion of antigens in a possible vaccine. There is at present little data on antigenic diversity of *P. falciparum* in Sri Lanka and the relationship of Sri Lanka isolates to those studied elsewhere. We have previously reported identifying a 45 kDa merozoite surface antigen that is a vaccine candidate. DNA from several isolates in Sri Lanka were amplified by the polymerase chain reaction (PCR) using primers specific for this antigen. The amplified gene was then DNA sequenced using a modified procedure developed in our laboratory. Two different sequences were obtained, showing that at least two allelic forms of the antigen are present in Sri Lanka. These are first sequences of functional genes determined in Sri Lanka.

570 TWO CYSTEINE-RICH PROTEINS OF *PLASMODIUM KNOWLESI* OOKINETES. Fried M * and Kaslow DC. Laboratory of Malaria Research, National Institute of Allergy & Infectious Diseases; National Institutes of Health, Bethesda, MD.

The late sexual stages (i.e., developing ookinetes) occur in the mosquito midgut. Because ookinetes-specific proteins may not be expressed while the parasites is in the vertebrate host they are not subjected to immune pressure and therefore may have limited antigenic diversity. Thus proteins on the surface of the sexual stages of malaria are suitable candidate for transmission-blocking vaccine development. The surface proteins of *Plasmodium vivax* ookinetes have not been characterized previously,in part because they are not readily available in large quantities. Due to their evolutionary relatedness, the primate malaria parasite *P. knowlesi* serves as an animal model for the human malaria parasite *P. vivax*. By producing mAb against developing *P. knowlesi* ookinetes we identified two major ookinetes proteins of molecular mass 24 and 20kDa, designated Pks24 and Pks20 respectively. Pks24 and Pks20 were localized to surface of developing ookinetes by immunofluorescence assay of live parasites. No reaction whatsoever was obtained with gametes or newly formed zygotes. Six hr old *P. knowlesi* zygotes were metabolically labelled with ^{35}S-cysteine for 1 hr. Pks24 and Pks20 were the dominantly labeled products. These data suggest that these two proteins are the homologoues of *P. gallinaceum* and *P. falciparum* sexual stages cysteine-rich proteins Pgs25/Pfs25 and Pgs28/Pfs28.

571 PURIFICATION REGIME FOR *PLASMODIUM FALCIPARUM*, PF83/AMA-1 AND THE APICAL MEMBRANE ANTIGEN-1 FAMILY. Thomas AW*, Welling GW, and Narum DL. Department of Chronic and Infectious Diseases, Medical Biological Laboratories-TNO, Rijswijk, The Netherlands; and Laboratory of Medical Microbiology, Rijksuniversiteit Groningen, The Netherlands.

A two step purification regime has been developed for a full-length baculovirus recombinant 83 kDa apical membrane blood stage vaccine candidate antigen of *Plasmodium falciparum*, PF83/AMA-1. The first step utilizes a novel approach to fast performance anion exchange chromatography (FPLC-IEC), in which, elution conditions are not only defined by charge, but by the hydrophilic/hydrophobic nature of proteins as well. FPLC-IEC purification of whole recombinant PF83/AMA-1 *Spodoptera frugiperda-9* cell extract requires two sodium chloride elution gradients (A and B), whereby a change in the non-ionic detergent polyoxyethylalkylether $C_{10}E_5$ concentration from 0.01% to 0.1% (w/v) (elutions A and B, respectively), results in a fraction of the B elution that is 9% recombinant PF83/AMA-1. A Q-sepharose CL 4B-28G2dc1 immuno-affinity column is used as the final purification step to generate a product that is approximately 60% pure when assessed by scanning laser densitometry of silver stained SDS-PAGE gels. Rat monoclonal antibody 28G2dc1 recognizes a highly conserved C-terminal region within the AMA-1 family and cross-reacts with all AMA-1 molecules tested so far, thus providing a method for the purification of all recombinant and native full-length AMA-1 molecules for immunological analysis.

572 IN VITRO EFFECTS OF LIPOXYGENASE INHIBITORS ON BLOOD-STAGE *PLASMODIUM FALCIPARUM*. Green MD*, Millet P, Grady KK, and Todd GD. Malaria Branch, Centers for Disease Control, Atlanta, GA.

Oxidation of erythrocytic membranes occurs as a result of plasmodial infection. This phenomenon is associated with the parasite's ability to grow and develop intraerythrocytically. Therefore, the host/parasite relationship consists of a delicate balance of oxidant and antioxidant systems that can be perturbed by many drugs including some antimalarial drugs. To target this area as a possible site for drug interactions, this relationship requires further understanding of the enzymes involved. Lipoxygenase (LO) is an enzyme that catalyzes the oxygenation of polyunsaturated fatty acids. Our

objective was to see if two well-known LO inhibitors, nordihydroguaiaretic acid (NDGA) and eicosatetraynoic acid (ETYA), could affect blood-stage plasmodium growth in an in-vitro system. Both compounds were tested on in- vitro cultures of blood-stage *P. falciparum*. At a concentration of 100 μM, each LO inhibitor prohibited the growth and development of the parasite within 48 hours. The inhibition was not associated with erythrocyte destruction. No significant change in parasite growth (parasitemia) was observed at concentrations of 1 or 10 μM for both NDGA and ETYA relative to the control. Further evaluations of these and other LO inhibitors will be discussed.

573 CLONING AND SEQUENCING OF A 93 KDA *PLASMODIUM CHABAUDI* ACIDIC PHOSPHOPROTEIN THAT INTERACTS WITH THE HOST ERYTHROCYTE MEMBRANE. Giraldo L*, Jennings GJ, Deleersnijder W, Lockyer JM, and Wiser MF. Department of Tropical Medicine, Tulane University Medical Center, New Orleans, LA; Department of Medicine, Tulane University Medical Center, New Orleans, LA; and Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Brussels, Belgium.

The malarial parasite alters the structure and function of the host erythrocyte membrane during the intraerythrocytic stage. Proteins synthesized by the parasite which interact with the erythrocyte membraneare potential mediators of these host alterations. A 93 kDa *Plasmodium chabaudi* phosphoprotein, referred to a Pc(em)93, that is associated with the cytoplasmic face of the erythrocyte membrane, has been previously characterized. Cloning and sequencing studies were carried out to learn more about the structure and possible function of this protein. A 2.1 kb insert DNA was cloned from a λ gt11 expression library and sequenced in its entirety. More than half of the cloned DNA fragment consisted of 17 blocks of tandem repeats of 75 nucleotides. The only open reading frame across the tandem repeats yielded a sequence rich and Asp and Glu residues consistent with the known amino acid composition of Pc(em)93. Genomic DNA was digested with various restriction enzymes and analyzed by Southern blotting using the 2.1 kb insert as probe. The probe hybridized with two restriction fragments inmost cases suggesting that the gene may exist in two forms. Attempts are currently underway to clone both fragments. These studies will provide information about the structure of Pc(em)93 and the molecular mechanisms by which the parasite alters the host.

574 THE DIHYDROOROTATE DEHYDROGENASE GENE HOMOLOGUE OF *PLASMODIUM FALCIPARUM*). LeBlanc SB* and Wilson CM. Department of Microbiology, Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham, AL.

Due to our interests in pyrimidine biosynthesis and mitochondrial targetting, we have cloned the gene homologue for dihydroorotate dehydrogenase (DHODase) of *Plasmodium falciparum* (pfDHOD). A fragment of the gene was initially identified by homology using the polymerase chain reaction (PCR) with degenerate oligonucleotides and *P. falciparum* genomic DNA. The design of the degenerate oligonucleotides was based on conserved sequences noted in other DHODase homologues and malarial codon bias. The remainder of the gene was cloned by screening a genomic λ library, inverse PCR, and size-selected DNA fragments. An open reading frame of 1704 base pairs was found to be significantly homologous to other DHODase genes. pfDHOD is 90% homologous to both the human and E. coli DHODase genes in the conserved regions identified previously. Differences found in the predicted amino acid sequence of pfDHOD include: 1) a single amino acid change in the consensus flaving binding site, 2) a 5' leader sequence which presumably acts as a mitochondrial targetting signal, and 3) a 42 amino acid insertion in the middle of the gene which does not appear to be an intron. We are now completing the characterization of pfDHOD by looking at mRNA, gene copy number, and chromosomal localization.

575 DIMORPHISM AND INTERGENIC RECOMBINATION WITHIN THE MICRONEME PROTEIN (MP-1) GENE FAMILY OF *PLASMODIUM KNOWLESI*. Prickett MD*, Smarz TR, and Adams JH. Department of Biological Science, University of Notre Dame, Notre Dame, Indiana.

The Microneme Protein-1 (MP-1) of *Plasmodium knowlesi* and *Plasmodium vivax* facilitates merozoite invasion of the erythrocyte by binding to Duffy blood group antigens. The erythrocyte binding domain of the MP-1 gene consists of six distinct regions. MP-1 nucleotide and deduced amino acid sequences from two isolates of *P. knowlesi* and one from *P. vivax* were compared for diversity. The MP-1 genes of *P. knowlesi* were dimorphic (alpha and beta types) based upon the central hydrophilic regions III and IV, where each type was highly conserved. However, these two distinct sequence types shared a common sequence motif found at different areas of regions III and IV. The other regions were conserved among all *P. knowlesi* genes with the exception of region II. Variability within regions I, V, and VI, both synonymous and non synonymous, were mostly individual events and not associated with any particular sequence type. The amino cystiene-rich region, region II, was characterized by two distinct sequence motifs and one hybrid, that were minimally 68% identical. All cystienes and aromatic amino acids were conserved in all sequences or within a sequence type of region II. There were two apparent recombination points within region II where switching occurred between types. Mutations within region II were strongly biased towards radical amino acid changes especially towards the carboxyl third, where there were three distinct types of sequence. Homologous regions of *P. vivax* were characterized by an increased number of synonymous changes and numerous radical amino acid changes.

576 PERSISTENCE OF IRRADIATED *PLASMODIUM BERGHEI* PARASITES IN THE HOST LIVER AND THEIR POSSIBLE ROLE IN THE INDUCTION OF PROTECTIVE IMMUNITY. Scheller LF* and Azad AF. Department of Microbiology & Immunology, University of Maryland School of Medicine, Baltimore, MD.

Immunization with irradiated sporozoites (irr-spzs) induces protective immunity against a challenge infection. Here we report the sequence of events that lead to the acquisition of protective immunity in irr-spz immunized animals. Intrahepatic portal inoculation was used to direct spzs into a defined area of the liver. Spzs were irradiated with varying doses of (γ)-rays and inoculated into animals. Spzs irradiated with 10,000 rads were found to retain their ability to invade hepatocytes although complete schizogony was not observed. In contrast, irr-spzs (20,000 rads) were unable to invade hepatocytes. The quantitative presence of irradiated parasites in the host liver, as a function of time, was monitored. Interestingly, spzs irradiated with 10,000 rads were not cleared immediately as reported by others. Although the number of irradiated parasites decreased by 2 fold within the first month post-inoculation, persistent parasites were detected as late as 6 months after primary inoculation. Upon challenge, focal infiltrates were observed in their liver, surrounding parasites resulting from the challenge. In addition to studying the antigenic profile of persisting parasites, immunohistochemical analysis was also performed to identify the nature of the cellular infiltrates. Plausible mechanisms leading to the protective immunity in irr-spz immunized animals will be discussed.

577 IMMUNOGENICITY OF MALARIA ANTIGEN DERIVED PEPTIDES. Wickramaratne C* and Ramasamy R. Division of Life Sciences, Institute of Fundamental Studies, Kandy, Sri Lanka.

There is a much interest at present in developing a synthetic vaccine against malaria. Different antigens, including sporozoite and merozoite surface antigens, have been identified as vaccine candidates. Three different peptides corresponding to known B-cell epitopes in a 45 kDa merozoite surface glycoprotein of the human malaria parasite *Plasmodium falciparum* were conjugated *via* 6-maleimido caproic acyl N-succinimide ester to bovine serum albumin. The conjugates were used to immunise groups of four Balb/c mice in saline with each of the following adjuvants: Freund's adjuvant, two muramyl dipeptide derivatives (murabutide and murametide) and aluminium

hydroxide. Antibody levels against peptides were measured by an enzyme linked immunoabsorbent assay. Freund's adjuvant produced the highest titre of antibodies (up to 10^{-5} after three injections). Antibody levels with alum absorbed antigen reached titres of 10^{-3} to 10^{-4} and antigen in saline alone yielded a titre of 10^{-3}. It was also observed that the location of the cysteine in the peptides used for coupling influences the specificities of antibodies elicited by the peptides. The results are important for designing peptide based immunogens for use as vaccines.

578 NITRIC OXIDE DEPENDENT PROTECTIVE IMMUNITY AGAINST PRE-ERYTHROCYTIC STAGE *PLASMODIUM BERGHEI* MALARIA. Seguin MC*, Green SJ, Goodbary M, Slayter M, and Klotz FW. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; Entremed Inc., Rockville, MD ; and Department of Veterinary Pathology, Armed Forces Inst. of Pathology, Washington, DC.

Immunization of mice or humans with irradiation attenuated malaria sporozoites confers protective immunity against subsequent challenge. Immunity is T cell mediated. Antigen specific T cells may generate cytokines such as γ interferon to induce responding cells to produce nitric oxide (NO). Nitric oxide is toxic to sporozoite infected liver cells *in vitro*. To determine whether nitric oxide is a mediator required to maintain protection in sporozoite immunized mice, we suppressed nitric oxide production in irradiated sporozoite immunized BALB/c mice by systemic administration of arginine analogs followed by viable sporozoite challenge. All of the 15 immune mice treated with N mono methyl L arginine (NMMLA) developed patent blood infection while 2 out of 15 of immune controls were not protected. There was no difference in patency periods for analog treated immune mice verses non-immune controls. NMMLA did not cause liver damage in treated mice. 5 out of 5 immunized BALB/c became suceptable to viable sporozoite challenge by treatment with N^G amino L arginine, while the N^G nitro L arginine analog had no suppressive effect on 5 immunized mice. N^G nitro L arginine is an inefficent inhitor of inducible nitric oxide synthetase. These data suggest that antigen specific T cells produce cytokines that induce hepatocytes or Kupffer cells to produce nitric oxide for the destruction of infected hepatocytes. Nitric oxide production is required to sustain protective immunity in BALB/c mice immunized with irradiated *Plasmodium berghei* sporozoites.

579 IMMUNOGENICITY OF RECOMBINANT *PLASMODIUM BERGHEI* MEROZOITE SURFACE PROTEIN-1 EXPRESSED IN *SALMONELLA*. Toebe CS*, Cardenas L, Jennings GJ, van Belkum A, van Doorn LJ, Clements JD, and Wiser MF. Department of Tropical Medicine, Tulane University Medical Center, New Orleans, LA; Department of Microbiology, Tulane University Medical Center, New Orleans, LA; and TNO Primate Center, Rijswijk, The Netherlands.

The use of *Salmonella* for the delivery of recombinant malarial antigens is potentially a safe and cost effective vaccine. In this study a portion of the merozoite surface protein-1 (MSP1) from*Plasmodium berghei* was expressed in *Salmonella* and examined for its ability to elicit an anti-malarial immune response in mice. A 500 bp insert corresponding to the highly conserved block 3 and the variable block 4 of MSP1 was cloned from a λ gt11 expression library. The block 4 sequence in *P. berghei* MSP1 consists in part of 7 tandem repeats composed of 10 amino acids. The insert was subcloned and expressed as a fusion protein with the B-subunit of heat labile toxin(LT-B) from *Escherichia coli*. Previous studies indicate that the immunogenicity of heterologous proteins fused to LT-B is increased. Mice inoculated orally with *Salmonella* expressing the MSP1/LT-B fusion protein expressed serum antibodies against MSP1 as determined by Western blotting. These results indicate that it is possible to induce a humoral immune response against recombinant malaria antigens via oral*Salmonella* inoculation. The *P. berghei* model will be used to determine which domains of MSP1 are capable of eliciting a protective response against challenge. This information will be applied to the development of an anti-*P. falciparum* vaccine.

580 EPITOPE MAPPING OF ANTIBODIES FROM PROTECTED VACCINATED VOLUNTEERS IMMUNIZED WITH *PLASMODIUM FALCIPARUM* CS SUBUNITS VACCINES. Boerger PR*, Theisen TW, Sylvester DR, Ballou R, Gordon DM, Cohen J, and Gross M. Division of Biopharmaceutical R&D, SmithKline Beecham Pharmaceuticals, King of Prussia, PA; SB Biologicals, Rixensart,Belgium; and Walter Reed Army Institute of Research, Washington, DC.

The clinical evaluation of vaccine candidates designed to induce protective immunity against the sporozoite stage of Plasmodium falciparum have concentrated primarily on the induction of antibodies directed towards the immunodominant B cell epitope, the repeating NANP (NVDP) tetrapeptide, of the circumsporozoite (CS) protein. Results from clinical studies utilizing various versions of vaccines containing the tetrapeptide repeat have been disappointing. Recently we have focused our efforts to include the regions flanking the repetitive domain in vaccine candidates. These candidates are: 1) RTS,S, which is composed of 19 repeats fused to the complete C-terminal flanking region of the CS protein and 2) RLF, which is devoid of repeats but contains the entire N and C-terminal flanking domains of CS protein. Sera samples from volunteers in these studies were analyzed by ELISA and Western Blot in an attempt to correlate protection against sporozoite challenge with reactivity against specific domains in the non-repetitive flanking regions. In this presentation we will describe the various peptides and recombinant proteins used to identify these potentially important epitopes within the CS protein flanking domains.

581 ABSENCE OF RELATIONSHIP BETWEEN IMMUNE RESPONSES TO Pf155/RESA EPITOPES OF *PLASMODIUM FALCIPARUM* AND HLA CLASS II ALLELES IN MADAGASCAR. Migot F *, Perichon B, Danze PM, Lepers JP, Chougnet C, Krishnamoorthy R, and Deloron P. INSERM Unité 13, Paris, France; INSERM Unité 120, Paris, France; Hospital de Lille, France; and Institut Pasteur, Antananarivo, Madagascar.

Fifty adults from the Highlands of Madagascar were longitudinally followed from 1988 to 1991. *Plasmodium falciparum* prevalence rates were 44% in 1988 and 6.5% in 1991, after a malaria outbreak in 1986-87. Cellular and Ab responses to the synthetic peptides $(EENV)_4$, $(EENVEHDA)_4$ and $(DDEHVEEPTVA)_3$ representing major Pf155/RESA epitopes were assessed at 3 to 9 instances in each individual, with an average of 5 determinations. Cellular responses were investigated by lymphocyte proliferation plus assays for IFN-γ and IL2 release. Anti-peptide Ab were determined by FAST-ELISA. The cumulative rates of cellular (range: 64-68%) and Ab responders (range: 70-74%) were similar for each peptide. HLA class II typing was performed by PCR-RFLP, technique allowing identification of 45 HLA-DRB 1 alleles (included into 10 DR specificities), 7 HLA-DQA 1, 14 HLA-DQB 1 and 18 HLA-DPB 1 alleles. For each peptide, the dominant specificities or alleles (frequency > 20%) were similar in responders and non-responders (both for cellular and Ab). These were HLA-DR 5 specificity and HLA-DQA 1*0601, 0101-2, HLA-DQB 1*0301 or HLA-DPB 1*0101 alleles. Other encountered alleles were observed at similar rates in the groups of responders and non-responders (Fisher's exact test, all $p \geq 0.07$). Despite repeated immunological measures which identify better the responder group, no HLA class II restriction of the cellular and Ab responses to Pf155/RESA epitopes was detected. If immune responses to Pf155/RESA epitopes are genetically regulated in humans, our study demonstrates that the studied HLA class II region appears not to be involved.

582 PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIUM VIVAX*-LIKE HUMAN MALARIA PARASITE. Qari SH*, Patterson P, Collins WE, Udhayakumar V, and Lal AA. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease, Atlanta, GA.

We have recently described a *Plasmodium vivax*-like human malaria parasite that appears morphologically similar to *P. vivax* but is genetically and antigenically different at the

circumsporozoite (CS) gene level. We have produced monoclonal antibodies against the CS protein repeat sequence APGANQEGGAA of this parasite. Two hybridoma lines, Pam-135 and Pam-172, were established from a single fusion using spleen cells from ICR mice that were hyperimmunized with a peptide (three copies of 11-mer repeat sequence shown above) conjugated to KLH. Culture supernatants from both of these clones showed peptide (APGANQEGGAA) specific reactivity using ELISA (titre of 1:4000). In an indirect immunofluorescence assay, these two MAbs recognized air-dried sporozoites of *P. simiovale*, which were used as surrogates for *P. vivax*-like malaria parasites since these two parasites have an identical CS protein sequence. Neither of these MAbs showed any reactivity to sporozoites from the four human malaria parasite species. Sporozoites from simian malaria parasites *P. cynomolgi*, *P. simium* and *P. brazilianum*, and a rodent malaria parasite *P. berghei*, also did not react with these MAbs. The minimal epitope recognized by these MAbs was located within the APGAN sequence of the repeats. Both the MAbs are of mouse IgG2b isotype. These MAbs will have many field applications including the characterization of sporozoites, and in epidemiologic and vector studies.

583 REPEAT REGION OF THE CIRCUMSPOROZOITE PROTEIN OF A *PLASMODIUM VIVAX*-LIKE HUMAN MALARIA PARASITE IS ANTIGENICALLY DISTINCT AND CONTAINS T-AND B-CELL EP. Udhayakumar V*, Patterson SH, Qari SH, and Lal AA. Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

The CS protein of a *Plasmodium vivax*-like malaria parasite has an 11-mer APGANQEGGAA repeat sequence, which is different from nanomer repeat sequences of P. vivax. Synthetic peptides containing a trimer of APGANQEGGAA (PL35) sequence in Freund's adjuvant were used to immunize inbred mice of different genetic background. A/J strain ($H\text{-}2^a$) showed highest antibody titer (IgG response) followed by C57BL/6 ($H\text{-}2^b$)and B10.BR ($H\text{-}2^k$). Balb/c and DBA/2 mice from the H-2d background failed to show any significant antibody response. Immune sera from A/J mice reacted with the sporozoites of P. simiovale, which were used as surrogates for *P. vivax*-like parasites in an indirect immunofluorescent assay, since they have an identical CS gene sequence. Anti-PL35 immune sera did not show any reactivity against peptides specific for CS repeats of *P. vivax*. The antigenic cross-reactivity between the CS repeats of *P. vivax* and *P. vivax*-like parasites was tested by competitive inhibition test using field sera. We found that anti-PL35 reactivity of sera was not cross inhibited by peptides specific for CS repeats of *P. vivax*. In conclusion these results show that CS repeat sequence of *P. vivax*-like parasite is antigenically distinct from *P. vivax* and has both B-and T-helper epitopes.

584 ANTIBODY RESPONSES TO *PLASMODIUM VIVAX* ANTIGENS IN HUMANS LIVING IN A MALARIA-ENDEMIC REGION OF HONDURAS. Alger J*, Ordonez L, Perez R, Montenegro-James S, and James MA. Department of Tropical Medicine, Tulane University Medical Center, New Orleans, LA; and Division of Vector-Borne Diseases, Ministry of Public Health, Honduras.

A seroepidemiological study involving 76 individuals was performed during August and September 1992 in Danli, Honduras, a *Plasmodium vivax*-endemic area of Health Region No.1. These persons were selected during an active detection of febrile, presumptive malarious cases.Fifty (66%) of the subjects were positive for *P. vivax* and were treated; 35 (46%) had no prior history of malaria; and 54% had antibody to the predominant repeat region of the circumsporozoite protein. The subjects were then monitored for a 5- week period as up to 4 serum samples were collected from each at 10-day intervals. Total antimalarial IgM and IgG levels were monitored by indirect immunofluorescence, the pattern of serologic reactivity determined by immunoblotting, and the kinetics of specific antibody responses to Pv/Pf70 measured by peptide-ELISA. Seropositivity rates for anti-Pv70 IgM and IgG during the follow-up period were 57% and 36%, respectively. Finally, the kinetics of the antibody responses to *P. vivax* antigens were compared among 4 age groups and correlated with the

parasitologic status at initial sampling. These data will be discussed as to the natural acquisition of immunity to *P. vivax* and the potential role of seroepidemiologic surveillance in the region.

585 TH1 AND TH2 CYTOKINE RESPONSES TO ASEXUAL BLOOD STAGE ANTIGENS IN HUMAN FALCIPARUM MALARIA. Al-Yaman F, Kazura JW*, King CL, Anders R, and Alpers M. Institiute of Medical Research, Maprik, Papua New Guinea; Case Western Reserve University, Cleveland, OH; and Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

The design of vaccines against asexual blood-stages of *Plasmodium falciparum* will be facilitated by an understanding of the nature of the CD4+ T helper (Th) cell memory engendered by naturally-occurring immunity. The objective of this study was to determine whether selected vaccine candidate molecules preferentially recall Th1- and/or Th2-like cytokine production by T cells from immune adult residents of a hyperendemic region of East Sepik Province, Papua New Guinea. Peripheral blood mononuclear cells were obtained from 5 asymptomatic 18-30 year old residents and stimulated with rRESA, rMSA-2 (FCQ27 strain), or PPD (positive Ag control in this BCG-vaccinated population). Cells from 2 subjects produced IFN-g in response to RESA (118 and 431 pg/105 cells/ml); RESA-stimulated IL-4 was not observed in these or the other subjects. MSA-2 stimulated IFN-g production in 2 persons (13 and 234 pg/ml) and IL-4 in two other individuals (10 and 17 pg/ml). PPD recalled IFN-g responses in 4 of the 5 subjects, whereas incubation of cells with the mitogens phorbol myristate acetate/ionomycin stimulated production of both cytokines in all persons. These data suggest that Th subset responses to asexual blood-stage Ags differ among immune adults and that such responses are exclusively Th1 or Th2 for a given individual. Correlation of antigen-specific Th1 or Th2 dominance with morbidity will provide insight into the role of specific cytokines in protective immunity.

586 ANTIBODY RESPONSE TO EPITOPES ON SPOROZOITE AND MEROZOITE SURFACE ANTIGENS RELATE TO MALARIA TRANSMISSION RATES. Ramasamy R* and Nagendran K. Division of Life Sciences, Institute of Fundamental Studies, Kandy, Sri Lanka; and Department of Parasitology, Faculty of Medicine, University of Jaffna, Jaffna, Sri Lanka.

Using sera collected from two different locations in Sri Lanka, antibody levels to repetitive epitopes on the circumsporozoite proteins of *Plasmodium falciparum, P. vivax,* and the *P. vivax* variant 247, and non-repetitive epitopes on two merozoite surface antigens of *P. falciparum* (the 185 kDa PMMSA and the 45 kDa GYMSSA) were determined by radioimmunoassay. Sera collected before, during and after the main monsoon - dependent transmission season were examined. The target antigens were either synthetic peptides or recombinant proteins. Levels of antibodies to the epitopes declined with decreasing transmission rates (measured by malaria incidence and entomological inoculation rates) with the fall for sporozoite antigens being particularly pronounced. Decreasing antibody levels were accompanied by a fall in the proportion of individuals having detectable antibodies. The changes in antibody levels and prevalence was more pronounced in the dry zone location that had the higher malaria transmission rates. Up to 59% of persons had in the dry zone site had antibodies to the variant *P. vivax* strain indicating, for the first time, that the variant was prevalent in Sri Lanka. Antibody levels in children aged 7 to 15 were lower than in adults suggesting that development of immunity may be a function of age. There was significant correlation between the antibody response to *P. vivax* and *P. falciparum* sporozoite antigens and also between the two epitopes of the two merozoite surface antigens. Our results show that antibody levels decline with a half-life of about three months after the end of transmission. The data therefore suggest that there may be a seasonal fall in immunity to malaria in endemic populations.

587 COMPARISON OF LEVELS OF INHIBITION IN ILSDA WITH PROTECTION BY PASSIVE TRANSFER OF MONOCLONAL ANTIBODIES IN MICE IN *PLASMODIUM YOELII.* de la Vega P*,

Mellouk S, Ak M, Bower JH, Charoenvit Y, and Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda, MD.

Passive transfer of monoclonal antibodies to the *Plasmodium yoelii* circumsporozoite (CS) protein can provide protection to mice during subsequent sporozoite challenge. Previous studies have shown, however, that the degree of this protection can vary. In order to relate levels of protection *in vivo* with inhibition *in vitro*, several Inhibition of Liver Stage Development Assays (ILSDA) were performed using sera from passive transfer mice and purified monoclonal antibodies at various concentrations. Three monoclonal antibodies against the repeat region of *P. yoelii* CS protein were used. Two of them (QGP-S1 and QGP-S2) were produced by immunization with a synthetic peptide from a sequence derived from the major repeat region of the CS protein $(QGPGAP)_4$, conjugated to keyhole limpet hemocyanin and the other antibody (NYS1) was produced by immunization with irradiated *P. yoelii* sporozoites. The monoclonal antibodies were IgG1, IgG2b and IgG3 respectively; unrelated monoclonal antibodies of the same subclasses were used as controls. The hierarchy of protection in mice is very clear: NYS1>QGP-S1>QGP-S2. Inhibition was observed in the ILSDA with all three monoclonal antibodies. NYSI and QGP-S1 both inhibited consistently and at low concentrations but one cannot differentiate the two based on ILSDA results alone. QGP-S2, on the other hand, inhibited only at high concentrations thereby correlating with *in vivo* results.

588 MONOCLONAL ANTIBODIES TO A *LACZ-PFMDR1* FUSION PROTEIN RECOGNIZED *PLASMODIUM FALCIPARUM* ANTIGENS. Ortiz AM and Serrano AE*. Department of Microbiology and Medical Zoology, University of Puerto Rico School of Medicine, San Juan, PR.

Drug resistance in *Plasmodium falciparum* (*P.f.*) is an increasing problem throughout the tropical world. In previous work we have demonstated that *P. falciparum* contains at least two genes related to the mammalian multidrug resistance (*mdr*) genes, *pfmdr1*, which encodes a polypeptide of ≈150kd, and *pfmdr2*. The purpose of this research work was to produce monoclonal antibodies (MAbs) to *pfmdr* gene product(s) as tools to help to elucidate the role of the polypeptide encoded by the *P.f.* homologues in parasite's drug resistance. A *lacZ-pfmdr1*-fragment fusion protein was constructed by cloning a sequenced *pfmdr1* gene fragment in pUR292 expression vector. MAbs were developed by immunizing BalbcJ mice with the *lacZ-pfmdr1* fusion protein. Mouse spleen cells were fused to SP/2 myeloma cells. Growing hybrids were screened for immunoglubulin secretion by ELISA. *P. falciparum* specificity was determined by ELISA and Western Blot to *P.f.* and uninfected red blodd cells (RBC) antigens. Six of the positive hybrids were cloned by limiting dilution. The antibodies secreted by the cloned cells were analyzed for immunoglubilin class and subclass by ELISA. Interesting, Western Blot analysis using *P.f.* and RBC antigens showed three patterns of MAbs recognition: 1) a polypeptide of approximately 72kd in *P.f.*, 2) a polypeptide of approximately 220kd in *P.f.*, and 3) a polypeptide of approximately 214kd in *P.f.* and two polypeptides in uninfected RBC. Analysis of the MAbs by immunofluorescence in permeabilized and fixed *P.f.* parasitized and uninfected RBC showed specific reactivity with *P.f.* intraerythrocytic stages. The third Mabs group reacts with the parasite and the RBC membrane.

589 PROPERTIES OF ANTI-MALARIAL IGG DURING THE IMMUNE RESPONSE TO *PLASMODIUM YOELII* INFECTION IN MICE. Price PW*, Evans CB, and Taylor DW. Department of Biology, Georgetown University, Washington, DC.

It is well established that antibodies (Ab) play a central role in the resolution of *Plasmodium yoelii* infections in mice. This study sought to determine if each of the IgG isotypes was equally effective in modulating malarial infections. To make the comparison, IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3 Ab were purified from hyperimmune plasma by protein-A chromatography. The isotypic preparations were adjusted to equivalent anti-malarial Ab titers, and passively transferred to BALB/CByJ mice immediately prior to infection with a lethal strain of *P. yoelii*. Results showed that IgG_{2a} and IgG_{2b}

were equally efficient in modulating *P. yoelii* parasitemias. The isotypic fractions were compared by immunoprecipitation, immunoblotting, and tested for the ability to enhance phagocytosis of ^{35}S-methionine-labelled parasites. Phagocytosis assays showed that the ability to mediate phagocytosis by the isotypes paralleled their efficacy *in vivo*. Immunoprecipitation and immunoblotting studies demonstrated concentration-dependent differences in the malarial antigens recognized by the different IgG isotypes, perhaps indicative of immunodominant-isotype-specific antigens. These results support the data showing that isotype as well as idiotype are important in controlling *P. yoelii* malaria.

590 ANALYSIS OF LYMPHOCYTE POPULATIONS DURING *PLASMODIUM YOELII* 17XNL INFECTION IN CBA MICE. Creswell KA* and Taylor DW. Department of Biology, Georgetown University, Washington, DC.

Changes in lymphocyte populations during the course of *Plasmodium yoelii*(17XNL) infaction were followed in CBA mice using flow cytometric methods. Primary (bone marrow and thymus) and secondary (spleen, lymph nodes, and blood) lymphoid organs were examined during infection and up to 70 days thereafter. MAb to B220, CD4, CD5, CD8, CD3, and TCR($\alpha\beta$, and $\gamma\delta$) were used with one and two color analysis. A significant decrease in the total number of mature B cells was seen inthe bone marrow. There were no changes noted in the composition of cells in the thymus. Similarly, no changes in cell populations in the lymph nodes were observed, although there was an increase in the total cell number. In the spleen there was a decrease in the percentage of cells that stained with T and B lymphocyte markers and an increase in"null" cells. However, the actual number of T and B cells increased due to the increase in the total number of nucleated cells in the spleen during infection. In the peripheral blood, there was an increase in the percent T cells, both $CD4^+$ and $CD8^+$, and a simultaneous decrease in the percentage of B cells. In addition the ratio of $\alpha\beta$ to $\gamma\delta$positive-T cells remained essentially unchanged during the course of infection. These data show that *P. yoelii* infection in CBA mice results in an increase in mature T and B cells and not immunosuppression.

591 ANALYSIS OF IMMUNE RESPONSES AGAINST *PLASMODIUM FALCIPARUM* MEROZOITE ANTIGENS IN A HOLOENDEMIC AREA IN SENEGAL. Dieye A *, Sarthou JL, and Heidrich HG. Immunology Unit, Institut Pasteur de Dakar, Senegal; and Max-Planck Institut für Biochemie, Martinsried, Germany.

In the aim to determine the possible role of HLA-antigens in malaria infection, sera from 50 HLA-typed donors from Dielmo (Senegal) were tested in immunoblotting (using crude merozoite antigen) and immunoprecipitation (using detergent-extracts from surface-iodinated merozoites as antigen).The donors were previously tested on lymphocyte proliferation *in vitro* and γ-interferon production and grouped into two classes : high responders and low responders. In immunoblotting and immunoprecipitation experiments, no specific differences were found in the antibody reactivity with native merozoite antigen in individuals with high (HR) or low(LR) *in vitro* proliferative T cell responses (LR). In other words, both groups of responders, high and low, showed antibodies in their sera against a wide range of different parasite antigens; although between individual donors striking differences were found. Individual donors had developed different levels of antibodies, or no antibodies at all, against individual natural antigens. These differences, however, could not be correlated with HR or LR. The band patterns obtained were compared with HLA-antigens of donors phenotypes. Results showed that there was no correlation found between the different merozoite antigens recognized by sera of the different donors or groups of donors (HR and LR) and the donors'HLA-phenotypes. The fact that donors with HLA-B51 all recognized $MSP1_{42}$ and donors with DR1 recognized $MSP1_{19}$, was not a convincing correlation .

592 CHARACTERIZATION OF *PLASMODIUM CHABAUDI ADAMI* SPECIFIC T-CELL LINES WHICH CONFER PROTECTION TO ATHYMIC MICE AGAINST CHALLENGE INFECTION. Kima PE, Srivastava IK*, and Long CA. Department of Microbiology & Immunology, Hahnemann University, Philadelphia, PA.

It has been established that cell-mediated immunity plays a significant role in the development of effective immune responses against sporozite, erythrocytic and exoerythrocytic stages of malaria parasites in addition to collaborating in antibody production. In this context, *Plasmodium chabaudi adami* (PCA) represents a very useful model to study plasmodial molecules capable of eliciting protective T-cell responses, since the resolution of acute erythrocytic infection with this parasite depends on the presence of CD4+ T- lymphocytes. One approach to identify protective PCA molecules is to generate antigen specific T-cell lines and clones and test them for their protective capabilities by adoptive transfer to syngeneic athymic mice. Using PCA molecules fractionated according to their isoelectric points, after substantial removal of host proteins particularly haemoglobin, we were able to develop several PCA- specific functional T-cell lines. Four of these lines were characterized in terms of their antigenic recognition patterns (using fractions collected after isoelectrofocusing or after continuous flow electrophoresis) or profiles of cytokines (IFN-γ, IL3, IL4, IL5 and IL6) secreted upon activation *in vitro* and finally their capability to protect syngeneic athymic mice in adoptive transfer experiments against otherwise lethal *P. chabaudi* infection. All the protective and non-protective lines secrete IFN-γ and none of the protective lines secrete a significant amount of IL4 implying that Th1 like cells can be both protective and non-protective. The two protective lines recognise PCA antigens in the molecular weight range of 28-35 kDa, 67-70 kDa and 100-105 kDa, suggesting that several molecules may be capable of activating protective T-cells.

593 ANTIGENS OF *PLASMODIUM FALCIPARUM* WHICH CROSS-REACT WITH ANTIBODIES INDUCED BY *P. YOELII* INFECTION. Kironde FA*, Ray P, Sahoo NC, and Singh B. Malaria Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India.

Antigens of malarial parasites are typically stage-, strain- and species specific. One way to address the strain- or species- related limitation of vaccine-induced immunity would be to identify epitopes that are common to antigens of different *Plasmodium* species (cross-specie reactive epitopes). Such epitopes might constitute biologically functional domains and could form the basis of chemo- or immuno-prohylxis against infection. However, include the cross-specie conserved epitopes in region II of circumsporozoite proteins and in the carboxyl terminal region of the *P. falciparum* merozoite surface antigen 2 (MSA2). In order to identify antigens containing potentially useful cross-specia reactive epitopes, we have investigated blood-stage antigens of the human-malaria parasite *P. falciparum* which cross-react with antibodies against the rodent parasite *P. yoelii*. Immunoprecipitation of [^{35}S]methionine-labeled antigens and immunoblot assays showed that at least 12 proteins (M_r range = 15 to 250 kDa) of *P. falciparum* blood-stages are hound at high affinity by antibodies in mouse anti-*P. yoelii* sera. Whereas digestion of *P. falciparum* antigens by trypsin abolished all observed binding of the anti-*P. yoelii* serum antibody to the antigen, periodate oxidation of the antigen did not alter the binding. These results suggest that the observed cross-specie reactive epitopes are protein. In immunofluorescent assays, antibodies in the anti-*P. yoelii* sera bind to various stages of *P. falciparum*. We found that in the presence of normal human macrophages, serum anti-*P. yoelii* antibodies inhibited *P. falciparum* growth *in vitro* in a dose-dependent fashion, showing that some of the cross-specie reactive epitopes may be targets of protective immune response. Thus, the cognate *P. falciparum* antigens are worthy of detailed investigation. Specific antibodies against these antigens will assist in their characterization and definition of their role in protective immunity.

594 PLASMA SOLUBLE CD14 LEVELS IN FALCIPARUM MALARIA. Pichyangkul S, Saengkrai P, Yongvanitchit K, Wongsrichanalai A, Viravan C*, Looareesuwan S, Kyle DE, and Pavanand K. U.S.

Army Medical Component, AFRIMS, Bangkok, Thailand; and Department of Clinical Tropical Medicine and Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

CD14, a 55-kD glycoprotein expressed on monocytes/macrophages and dendritic reticulum cells, is a receptor for complex of serum lipopolysaccharide (LPS) and LPS binding protein (LBP). Although soluble CD14 (sCD14) levels are elevated in patients with severe burn, the mechanism underlying the increased levels of sCD14 in this pathological condition is unclear. The present work extends those previous observations to a group of patients with falciparum malaria. Plasma samples from patients were collected during 1991-1992 and frozen at -20°C until assay. The plasma levels of sCD14 were measured by an enzyme-linked immunosorbent assay (ELISA). The mean sCD14 plasma levels were significantly higher in falciparum malaria patients than in healthy control ($P < 0.001$). Patients with severe malaria had mean sCD14 plasma levels (7.6 μg/ml) higher than those with non-severe malaria (5.7 μg/ml); however, the difference was not statistically significant. Eleveted plasma sCD14 levels were not found to be related to any of the clinical and laboratory parameters. During anti-malarial drug treatments, the plasma levels of sCD14 were dramatically reduced in 7 days and subsequently approached normal levels. We also studied the regulation of CD14 in monocyte cultures exposed to malaria pigment and cytokines, particularly those increased during falciparum infection (TNFα, IL-1β, IL6, IFNγ). Significant reduction of CD14 expression was observed in monocytes exposed to either malaria pigment (58%) or IFNγ (38%), whereas TNFα, IL-1β and IL6 did not show significant effect on CD14 expression. The reduction of CD14 expression observed in our studies apparently results from shedding of the antigen into culture supernatants as previously described. We detected increased levels of sCD14 in culture supernatants of monocytes exposed to malaria pigment or IFNγ, as compared to control cultures. These data suggest that elevated plasma sCD14 levels in falciparum malaria could reflect the activation of host monocytes/macrophages by malaria pigment and IFNγ.

595 LIFE STAGE VARIATION IN CUTICULAR HYDROCARBON PROFILES OF MEDICALLY IMPORTANT CULICIDAE. Pappas CD*, Christen JA, Rathe RR. Valdosta State University, Department of Biology, Valdosta, GA; and Peru State College, Division of Science & Technology, Peru, NE.

Unique cuticular hydrocarbon profiles identify individual adult specimens of three species of medically important Culicidae, *Aedes albopictus*, *A. triseriatus*, and *A. aegypti*. Individual pupae of these three species can also be identified by the cuticular hydrocarbon profile. Single fourth-instar larvae produce weak cuticular hydrocarbon profiles, but can be studied in pooled samples of five larvae. Chemical-ionization and electron-impact mass spectrometry have been used to identify the components of the cuticular hydrocarbon profiles of these species. Comparison of the pupal and adult cuticular hydrocarbon profiles suggests biochemical changes necessary between the larval, pupal, and adult life stages.

596 ENERGY-CONSERVATION, HYDROSTATIC BALANCE, AND SURVIVAL OF *AEDES AEGYPTI* MOSQUITO PUPAE. Romoser WS* and Lucas EA. Tropical & Geographical Disease Institute, Department of Biological Sciences, Ohio University, Athens, OH.

In response to a passing shadow or vibrations, *Aedes aegypti* mosquito pupae dive. A pupa alternates between being slightly less dense and slightly more dense than water. This buoyancy range is possible due to the presence of gas in the ventral air space, a cavity formed by the developing wings, legs, and mouthparts. The ventral air space, due to its bilaterally-symmetrical topography, assures that a pupa floats, dives, and ascends in an upright, balanced fashion. Pupae do not feed and must rely on energy reserves until they can obtain food as adults. Hence any energy-conserving adaptations have survival value. Earlier studies have shown (1) that the buoyancy of a pupa varies due to several

factors and diving behavior varies such that a pupa maximizes the time spent close to neutral buoyancy; and (2) that mechanical shock may disrupt the integrity of the ventral air space gas, and consequently hydrostatic balance, resulting in the pupa drowning. The objectives of our studies have been as follows: (1) to test the hypothesis that pupal behavior associated with changes in buoyancy is energy-conserving; and (2) to assess the effects of mechanical shock on pupal survival. By comparing the duration of survival as adults and caloric reserves in pupae with experimentally reduced buoyancy versus normally buoyant pupae, we provide evidence that pupal behavior and the ventral air space gas are adaptations which facilitate the conservation of energy. By exposing pupae to mechanical shocks of varying magnitude, we have found that once hydrostatic balance and/or buoyancy are compromised, pupae eventually drown. It is possible that mechanical shocks or vibrations might be of value in mosquito control.

597 REGULATION OF EXPRESSION OF TRYPSIN GENES IN *AEDES AEGYPTI*. Noriega FG*, Barillas-Mury CV, Wang XY, and Wells MA. Department of Biochemistry and Center for Insect Science, University of Arizona, Tucson, AZ; and Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA.

Aedes aegypti trypsin is synthesized *in vivo* and *in vitro* in two groups of forms: early trypsins, present during the first 6 h after feeding, and late trypsins, that appear about 6-8 h after feeding and are responsible of the peak of tryptic activity. The aim of this project is to use cDNA and antibody probes to understand the regulation of trypsin genes in *Aedes aegypti*. After emergence the midgut undergoes a process of "maturation" that includes the transcription of the early trypsin gene. The early trypsin mRNA level increases and remains untranslated "waiting" for a blood meal. When the mosquito ingests a blood meal, the early trypsin mRNA is immediately processedand translated. This mechanism will allow the mosquito to asses the quality of the meal by performing a small scale digestion with early trypsin before inducing large amounts of late trypsin. These digestion products are the initial signal for transcriptional activation of thelate trypsin gene. Soy trypsin inhibitor blocked transcription of the late trypsin gene by inhibition of early trypsin digestion. The result after administration of a pre-digested meal with cycloheximide indicates that some other factor(s), besides early trypsin, have to be translatedafter feeding to activate transcription of late trypsin.

598 REDUCED SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* TO PERMETHRIN ASSOCIATED WITH THE USE OF PERMETHRIN-IMPREGNATED BED NETS AND CURTAINS IN KENYA. Vulule JM*, Beach RF, Atieli FK, and Roberts JM.

Kenya Medical Research Institute, Kisumu, Kenya; and Division of Parasitic Diseases, NCID, Centers for Disease Control and Prevention, Atlanta, GA. The susceptibility of *Anopheles gambiae* s.l. to permethrin, measured by the WHO bioassay method, decreased following the installation of permethrin-impregnated (0.5 gm/m^2) bed nets or house curtains in four villages in western Kenya. During the first year of net and curtain use, the exposure time to 50% mortality (LT50) increased from 13 to 33 minutes. In villages where no intervention measures were used, LT50s of *An. gambiae* s.l. were unchanged. Two years after the nets and curtains were distributed, the LT50s were 28, 28, and 16 min. in bed net, curtain, and control villages, respectively. Ten percent of the *An. gambiae* s.l. in the study were identified to species; all were *An. gambiae* s.s. In selection experiments, the LT50 of a colony of An. gambiae was lengthened from 28 to 41 min. in two generations by exposing all females to permethrin-treated papers for 60 min. and rearing the offspring of the survivors. Permethrin-impregnated bed nets and curtains lower vectorial capacity. Reduced susceptibility to permethrin could counter this beneficial effect. Our findings may be of importance in countries where permethrin-treated bed nets or curtains are used for malaria control.

600 EVALUATION OF THE STABILITY OF SELECTED PYRETHROIDS IMPREGNATED ONTO BED-NET MATERIALS USING CHEMICAL AND BIOLOGICAL ASSAYS. Todd GD*, Mount DL, Van Cappellen VL, Sexton JD, and Steketee RW. Malaria Branch, Centers for Disease Control, Atlanta, GA.

The use of pyrethroid-impregnated bed-nets is receiving recognition as cost-effective disease control measures. Evolution of resistance to current insecticides is inevitable and requires ongoing evaluation of new ones. We are testing the stability of selected insecticides under a variety of storage and washing conditions, using both bioassays and chemical assays. Impregnated polyester bed-net samples were prepared using these generally recommended concentrations: permethrin at the levels of 295 and 522 mg/m^2, deltamethrin at 17 and 31 mg/m^2 and etofenprox at 310 and 445 mg/m^2. Samples are in storage for up to 1 year under different conditions. After 3 months the bioefficacy was not affected significantly, as determined by the WHO Cone bioassay with a susceptible strain of Anopheles gambiae (as determined by the WHO Tube Test). The pyrethroid content of each sample was then determined using gas-liquid chromatography. In washing experiments, samples impregnated with deltamethrin and etofenprox maintained a significant bioefficacy after three wash cycles, whereas the bioefficacy for permethrin impregnated samples was greatly reduced after one wash cycle. Cyfluthrin, β-cyfluthrin, and λ-cyhlothrin are also under evaluation.

601 THE LYTIC EFFECT OF A SYNTHETIC MAGAININ ON THE SPOROGONIC DEVELOPMENT OF *PLASMODIUM BERGHEI*. Rodríguez MC, Rodríguez MH*, Possani L, Villarreal C, Torres J, González L, and Zamudio F. Vector Biology Department, Centro de Investigación de Paludismo, Tapachula, Chiapas, México; and Molecular Biology Department, Instituto de Biotecnología, Cuernavaca, Morelos, México.

The search for compounds with parasiticidal activity form part of the global endeavor for the production of genetically engineered mosquitoes resistant to *Plasmodium* parasites. Previous investigators have shown that magainins have a lytic effect on *in vitro* culture of asexual stages of *Plasmodium falciparum*. Also, when synthetic magainins were introduced into the haemocoel of anophelines infected with *P. cynomolgi* and *P. knowlesi*, parasite development was disrupted but the parasiticidal dosage was near to that toxic to the mosquitoes. We investigated an alternative route to test the effect of a synthetic magainin, Shiva-3, on the development of *P. berghei* in *Anopheles albimanus*. To evaluate the effect of Shiva-3 on the parasite stages that occur in the mosquito gut, sexual parasite stages were first cultured *in vitro* and then fed to mosquitoes (via membrane feeders) at 2 hours intervals (0-6) and then at hourly intervals (6-10). Shiva-3 was added to the infected blood at the time of feeding at concentrations of 75 and 100 μM. Both concentrations were effective in reducing numbers of infected mosquitoes in all experiments. Complete inhibition was obtained if the 100 μM Shiva-3 was applied before 6 hours of parasite development. No adverse effects on mosquitoes were observed even with the administration of 750 μM of the magainin. These results indicate the feasibility of introducing genes of parasiticidal peptides into the mosquito genome as a possible alternative in the anti-malaria control strategy.

602 ULTRASTRUCTURE OF *NOSEMA ALGERAE* DEVELOPMENT IN *ANOPHELES ALBIMANUS*. Preciado MG*, Tsutsumi V, Rodriguez MH, Rodriguez MC, Villarreal C, and Martinez-Palomo A. Department of Experimental Pathology, Center for Research and Advanced Studies, IPN, Mexico, DF, Mexico; and Center for Malaria Reseach, Tapachula, Chiapas, Mexico.

Nosema algerae, a microsporidian parasite of anopheline mosquitoes is a potential biological control agent for malaria vectors. Dissected midgut samples of *Anopheles albimanus* spontaneously infected with Sporaonts, spores and sporoblasts of *Nosema* were found in the cytoplasm of midgut muscle and epithelial cells. Host cells usually exhibited different developmental stages of the parasite. Cytoplasmic components of most infected mosquito cells were severely damaged except near the cell

surface, where well preserved mitochondria and actin bundles lied beneath an apparently intact plasma membrane. Sporonts were ovoid (1.3 x 1.8 μm) and elongated (1.3 x 4.0 μm) with two closely attached nuclei, well developed rough endoplasmic reticulum cisternae, and numerous free ribosomes. Spores were ovoid (1.2 x 2.3 μm) and electrondense with cytoplasmic filamentous coils in the anterior end. Sporoblasts were very dense, with irregular profiles. Internal structures could not be identified, except for the presence of the polar filament coils at the periphery of the cell.

603 VIABILITY OF INFECTIVE LARVAE OF *HAEMONCHUS CONTORTUS, OSTERTAGIA OSTERTAGI,* AND *TRICHOSTRONGYLUS COLUBRIFORMIS* FOLLOWING EXSHEATHMENT. Conder GA* and Johnson SS. Upjohn Laboratories Division, The Upjohn Company, Kalamazoo, MI.

Various techniques were examined to determine optimum conditions for exsheathing infective larvae of 3 important ruminant parasites (*Haemonchus contortus, Ostertagia ostertagi,* and *Trichostrongylus colubriformis*). In repeated experiments, separate samples of $1x10^5$-$1x10^6$ infective larvae, 1-2 months old, of each parasite were incubated in each of 4 exsheathing media (distilled water, Earle's Balanced Salt Solution + carbon dioxide, nematode washing buffer + carbon dioxide, or sodium hypochlorite) for 1 or 18 hours. In each case, percentage of larvae exsheathed and infectivity for jirds was determined. Results of these studies indicate that no single exsheathing technique, of those studied, is optimum for every parasite.In addition, caution must be used in drawing conclusions from *in vitro* studies using exsheathed larvae, since techniques which routinely provide high percentages of exsheathment also appear to reduce viability.

604 IDENTIFICATION AND CHARACTERIZATION OF SYLVATIC FOCI OF *TRIATOMA INFESTANS* IN CENTRAL BOLIVIA. Bermudez H*, Balderrama F, and Torrico F. Programa de Control de Chagas-CCH/USAID, Cochabamba, Bolivia; and CUMETROP, Universidad Mayor de San Simon, Cochabamba, Bolivia.

Vector control efforts are crucial to the prevention of Chagas' disease in Bolivia. Sylvatic foci of the primary domestic vector, *Triatoma infestans,* could contribute to the reinfestation of rural homes. We searched for wild colonies of *T. infestans* and *Trypanosoma cruzi* infection of wild rodents (*Galea musteloides*) in two distinct ecologic zones: the high valleys of Cochabamba (Zone I) & the Mizque River region (Zone II). In Zone 1 (Jamach'uma), viable colonies with all evolutive instars of *T. infestans* & *T. infestans* were identified near rodent burrows. In all, 17/23 burrows (45%) were infested (53% with *T. infestans;* 19% with both). 73% of captured *T. infestans* were infected with *T. cruzi;* 24% of *T. infestans* were infected. Of 14 rodents evaluated, one (7%) was positive for *T. cruzi* by xenodiagnosis; all were *T. cruzi* seropositive by ELISA. Elsewhere in Zone I (Huerta K'ucha), we found only *T. infestans;* 10% of burrows were infested. *T. cruzi* was detected in 5% of captured *T. infestans;* no rodents were examined. In Zone II, only uninfected *T. infestans* were captured in 3 areas; one uninfected adult *T. infestans* was captured in a fourth area. This study confirms that sylvatic *T. infestans* are present and are probably transmitting *T. cruzi* among wild rodents in central Bolivia. This information is critical to designing effective longterm control measures.

605 NOCTURNAL ACTIVITY PATTERNS OF THE SAND FLY *LUTZOMYIA LONGIPALPIS* AT AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN COLOMBIA. Morrison AC*, Ferro C, Torres M, Pardo R, and Tesh RB. Department of Epidemiology and Public Health, Yale University School of Medicine; and Entomology Group, National Institute of Health, Santa Fe de Bogota, Colombia.

American visceral leishmaniasis (AVL) is a potentially fatal disease which is endemic in many regions of the New World tropics. *Lutzomyia longipalpis,* has been implicated as the major vector of AVLthroughout its distribution. Little information is available on the nocturnal activity patterns of

this medically important sand fly species. Nocturnal activity of *Lu. longipalpis* was studied in El Callejon, Colombia, a highly endemic focus of AVL. On 2-3 consecutive nights each month during the period from August 1991 to July 1992, hourly samples of adult sand flies were collected off cattle and from the walls of a pigpen. Climatic factors were montitored during each collection. Analysis of variance was used to evaluate differences in*Lu. longipalpis* relative abundance. In the pigpen, peak sand fly activity occurred between 2030-2230, then it diminished steadily until 0630; the ratio of females to males was 3:1 at 1730, ranged from 1:1.25 to 1:20 from 1830-0130, and then inverting again after 0130. In contrast, the sand flies remained active between 1830 and 0530 on cattle; males always outnumbered females from 5-35 fold. Adult *Lu. longipalpis* activity was significantly reduced at ambient temperatures <24°C or >30°C and at relative humidities >90%. Windspeed, rain and light intensity also appeared to be influential environmental factors affecting sand fly activity.

606 NATURAL POPULATIONS OF *AEDES ALBOPICTUS* FROM SOUTHERN THAILAND ARE PERSISTENTLY INFECTED WITH AN INHERITED GROUP III DENSOVIRUS. Kittayapong P*, Tesh RB, Braig HR, Gonzalez JP, and O'Neill SL. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; Department of Biology, Faculty of Medicine, Mahidol University, Bangkok, Thailand; and Institut Francais de Reserche Scientifique pour le Developpement en Coop, Paris, France.

A diagnostic PCR based assay for identifying Group III densoviruses was applied to recently colonized *Aedes albopictus* from southern Thailand. A previously undescribed densovirus was identified with this assay and phylogenetic analysis of the virus was performed using sequence data. On the basis of this analysis the virus appears distinct from previously identified densoviruses from mosquito cell lines. Instead it groups more closely to a densovirus recently described from Russia infecting *Ae. aegypti*. This newly discovered virus is avirulent to *Ae. albopictus* and appears to be maintained as a stable ingerited extrachromosomal element within the mosquito colony. As such, it appears to be an ideal candidate for development as a vector for expressing genes conferring refractoriness to disease transmission within mosquitos. Its extrachromosomal inheritance also makes it compatible with described genetic mechanisms (eg. cytoplasmic incompatibility) for rapidly spreading it into natural mosquito populations.

607 TRANOVARIAL TRANSMISSION OF ARBOVIRUS IN *AEDES ALBOPICTUS* MOSQUITOES CONCURRENTLY INGESTING MICROFILARIAE OF *DIROFILARIA IMMITIS*. Zytoon EM*, El-belbasi HI, and Matsumura T. Department of Medical Zoology, Kobe University, School of Medicine.

Vertical transmission of viruses; i.e., direct transfer from parent to progeny, can occur in arthropods by a variety or mechanisms, including transovarial transmission (TOT). Evidence from our previous studies indicates the probable dissemination of chikungunya (CHIK) through the legs and salivary glands of *Aedes albopictus* mosquitoes concurrently ingesting microfilaria (Mf) of *Dirofilaria immitis*. To determine the possible method of TOT of CHIK in mosquitoes concurrently ingesting Mf, two groups of *A. albopictus* mosquitoes were fed defribrinated sheep blood containing 5×10^7 PFU of an African strain of CHIK virus, with or without 20,000 Mf of *D. immitis*. After embryogenesis of the eggs was complete, half of the total number of eggs were immersed in water to induce hatching, and the other half were kept under the same insectary condition as before being assayed for virus titers. The virus was detected in the parent mosquitoes, eggs, pupae and F1 of the first ovarian cycle, and F2 of the second ovarian cycle. The control group that ingested virus alone did not have virus titer at any of the the stages examined. This TOT may make overwintering of arbovirus possible, and appears to be an efficient mechanism that could insure the survival of the viral agent during adverse environmental conditions in general.

608 DEVELOPMENT OF POLYMERASE CHAIN REACTION TO IDENTIFY *LEISHMANIA MAJOR* ISOLATES FROM THE SINAI PENINSULA. Francies WM*, Alwen A, Galloway DR, and Hedstrom R. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

Cutaneous leishmaniasis caused by Old World *Leishmania* spp. is endemic to North Africa and the Middle East and can occur on epidemic scale. Polymerase chain reaction (PCR) offers the advantage of being a rapid and extremely sensitive method for directly detecting these organisms in infected human and animal hosts. We report on the development of a PCR assay that can discriminate *L. major* from other *Leishmania* isolates from the Sinai Peninsula of Egypt. The primers for this assay were designed from the DNA sequence of the species- specific variable DNA region of minicircle or kDNA of *L. major*. This variable region extends just beyond both sides of the conserved DNA region common to all*Leishmania*. This region was sequenced by cloning a 350 base pair fragment from HaeIII digested minicircle DNA of *L. major* into the pUC18 vector. The insert DNA of the resulting plasmid was sequenced by the dideoxynucleotide method. Based upon the sequence analysis of these regions, DNA primers were identified which would specifically amplify kDNA minicircles of *L. major* and not of other Old or New World*Leishmania*. The results of PCR amplification of DNA from a variety of*Leishmania* isolates from different geographical areas demonstrate that these primers have *L. major* specificity and do not amplify DNA from other species.

609 GENOTYPIC AND PHENOTYPIC HETEROGENEITY OF *LEISHMANIA* ISOLATES FROM WESTERN CHINA. Lu HG*, Qu JQ, Zhong L, Hu XS, Guan LR, Chai JJ, and Chang KP. Department of Microbiology/Immunology, UHS/Chicago Medical School, North Chicago, IL; Institute of Parasitic Diseases, Chinese Academy of Medical Sciences, Shanghai, PR China; and Department of Parasitology, West China University of Medical Sciences, Chengdu, Sichuan, China; Institute of Endemic Diseases, Urumqi, Xinjiang, China. Leishmaniasis remains endemic in the north and northwest regions of China.

The endemic foci include geographically disparate places, e.g. mountain villages, oases of deserts, frontier oil towns and city suburbs of alluvial plains, scattered along the ancient "Silk Roads". Spreads of the diseases throughout these historic routes have long been suspected. Recent epidemic outbreaks in India, Pakistan, Afganistan and other neighboring Republics add to the urgency to study *Leishmania* speciation and evolution of leishmaniases in these foci. Preliminary studies were carried out mainly with samples from three foci: (1) Visceral leishmaniasis of low endemicity among the Uygur children of Kashigar, a islamic city and the last metropolis to Afganistan and Pakistan; (2) Simple cutaneous leishmaniasis among the Han children and great gerbils in Karamay, an oil town close to the border with Khazakstan; and (3) Visceral leishmaniasis of high endemicity among humans and dogs in Wen-Xian/Nanping, a densely populated hill village within the Great Wall. Primary isolations of *Leishmania* with the original NNN Medium (rabbit blood agar with Locke's) were often difficult. Most isolates gew better upon subculture with M199 plus 20% HIFBS as the overlay and could be passaged subsequently in this medium alone. When inoculated subcutaneously to BALB/c mice and hamsters, most cultured promastigotes produced no visible lesions, except one isolated from a great gerbil. RFLP analyses of chromosomal DNA for three multicopied genes divide the isolates at hand into four groups. One group of visceral isolates from mountainous areas displayed marked kDNA heterogeneity. Work is continuing to further characterize these isolates.

610 *TRYPANOSOMA (DUTTONELLA VIVAX)*: PURIFICATION, CHARACTERIZATION AND IMMUNOLOCALIZATION OF A LEUCINE AMINOPEPTIDASE. Ibitayo AL*, Olorunsogo OO, Wells CW, and Lonsdale-Eccles JD. Department of Biochemistry, University of Ibadan, Nigeria.

An aminopeptidase was purfied from a clone of *Trypanosoma vivax* (ILDat 2.1). The enzyme hydrolysed N-leucyl-amides and N-leucyl-peptides. An N-phenylalanyl-peptide was also hydrolysed but much more slowly than the equivalent leucyl compound.The enzyme is optically specific,

prefering peptides with the first two amino acids in the L-configuration. It would not cleave substrates with blocked N-terminal amino groups such as N-benzoyl-L-leucyl-L-tyrosinamide. The aminopeptidase has an isoelectric point of pH 7.5 and a pH optimum of 8.5. Using the substrate l-leucyl-L-glycylglycine, the enzyme had a V_{max} of 4.1 (±0.4) x 10^{-4} mmolmin^{-1}, a K_m of 34 ± 7mM. Amastatin and bestatin, known aminopeptidase inhibitors, inhibited peptidase activity. Bestatin, at a concentration of 333 μM and amastatin at a concentration of 250 μM gave maximal inhibition of 45% and 46% respectively. By immunoelectron microscopy rabbit antiserum produced against the purified ILDat 2.1 aminopeptidase, localized the enzyme in sections of homologous and heterologous *T. vivax* parasites to the luminal protion of large vesicles lying between the nucleus and the flagellar pcket, often in close proximity to the nucleus. The enzyme could not be detected enzymatically or immunologically in the plasma of ruminants infected with *T. vivax* even at peak parasitaemia.

611 COORDINATE REGULATION OF *TRYPANOSOMA BRUCEI* CYTOCHROME c REDUCTASE SUBUNITS DURING DIFFERENTIATION. Priest JW* and Hajduk SL. Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL.

Trypanosoma brucei is a flagellate protozoan parasite of the mammalian bloodstream. The organism cycles between a bloodstream form that satisfies its energy requirements by glycolysis and an insect form that has a fully functional mitochondrion and cytochrome-mediated electron transport. In earlier studies, we demonstrated that two nuclear encoded subunits of the cytochrome c reductase complex were coordinately regulated at the protein level. Using PCR, segments of the cytochrome c1 gene and the cytochrome c reductase band 4 gene have been amplified. Rnase T1 protection studies on bloodstream and procyclic RNA showed that the transcripts of these two genes are present in bloodstream form cells and that transcript levels increase approximately five-fold during transformation to the procyclic form. Northern blot analyses of bloodstream and procyclic RNA confirmed the RNase protection studies and revealed no differences in transcript size or polyadenylation. The presence of these mRNAs in bloodstream form trypanosomes that lack both spectrally detectable cytochromes and detectable subunit proteins suggests that the protein levels are regulated by a posttranscriptional mechanism. We are currently studying the mitochondrial targeting sequences of cytochrome c1 and cytochrome c reductase band 4 in an effort to determine the effect of mitochondrial import on regulation.

612 THE *IN VITRO* EFFECTS OF INHIBITORS ON THE UPTAKE OF HORSERADISH PEROXIDASE AND SUCROSE-^{14}C BY *TRYPANOSOMA CRUZI*. Ribeiro-Rodrigues R*, Bogitsh BJ, Carter CE. Department of Biology, Vanderbilt University, Nashville, TN.

Quantitative and ultrastructural studies were conducted to determine the *in vitro* effects of various inhibitors on the pinocytosis of horseradish peroxidase (HRP) and ^{14}C- sucrose by epimastigotes of *Trypanosoma cruzi* (strain Y). Ultrastructural studies using yeast mannose revealed no significant decrease in HRP reaction product in reservosomes. Pretreatment with the combination adenosine-homocysteine-erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), a selective inhibitor of fluid-phase pinocystosis, produced significant reductions in the number and size of reservosomes. Additionally, little or no HRP reaction product was observed following this pretreatment. Pretreatment with EHNA or iodoacetate inhibited the uptake of labeled sucrose by 31% and 74%, respectively. Pretreatment with yeast mannan, on the other hand, an inhibitor of receptor-mediated pinocytosis, resulted in a 12% inhibition. The effects of these inhibitors on the pinocytosis of HRP were also studied quantitatively using the o-dianisidine protocol. The results of these studies were used to clarify the observations following ultrastructural studies that suggested that epimastigotes transport most of the HRP by fluid-phase pinocytosis rather than receptor-mediated pinocytosis.

613 CHARACTERIZE LEISHMANIA ISOLATES FROM VISCERAL AND CUTANEOUS LEISHMANIASIS IN CHINA BY KDNA HYBRIDIZATION AND PCR AMPLIFICATION. Hu XS*, Ren HY, Luo P, Yang WT, Chen JP, Lu HG, Liu PN, Lin FQ, and Kan B. Laboratory of Parasitology West China University of Medical Sciences, Chendgu, P.R. of China; and General Hospital of Xinjiang Petroleum Bueau, Karamay; Sichuan Continuing Education College of Medical Sciences, P. R. of China.

In recent years, the frequency of visceral leiahmaniasis (VL) is increasing in several parts of China. In addition to VL, cutaneous leishmaniasis has begun to appear. We analyzed the kDNA sequence homology of *Leishmania donovani* (*L. d.*) isolates from the hill, plain and desert foci. By dot hybridization, the results showed that there are heterogeneous sequences between kDNA of *L. d.* isolates of plain foci an hill foci, which is in accordance with the differences of their epidemiological and clinical evidences. Recently, we used disgoxigenin -11- dUTP labeled kDNA of *L. infantum* from France [MHOM/FR/78/LEM 75] as a probe to hybridize to 15 isolate of different foci of China. The most interesting results obtained here that excellent hybridizating signals were shown with *L. d.* Sichuan human isolate, *L. d.* Xinjiang "901" isolate, and *L. d.* Xinjiang "911" isolate from the western part of china. As yet no publications area available about the kDNA sequence homology between *L. infantum* and those Chinese isolates. By Southern hybridization, the results revealed a 120 bp fragment hybridized only to kDNA of the same isolate, it may contained isolate - specific sequence. The clinical cases of cutaneous leishmaniasis in Karamay, Xianjiang, China were reported in 1984-93, however, no publications have been concerned the species/strain characterization of the pathogen. By using primers 13 A and 13 B the PCR amplified Leishmania kDNA from the cutaneous lesion of a confirmed patient was labeled with digoxigenin-11-dUTP as a probe to perform DD8 and *Leishmania* of big gerbil. The results revealed that strong hybridization occurred only with *L. tropica* and the positive controls, however, the other *Leishmania* species tested showed weak or extremely faint signals. Further studies are in progress.

614 SEXUAL REPRODUCTION IN THE PROTOZOAN PARASITE, *LEISHMANIA* (KINETOPLATIDA: TRYPANOSOMATIDAE). Kreutzer RD*, Yemma JJ, Grogl M, Tesh RB, and Martin TI. Department of Biology, Youngstown State University, Youngstown, OH; Division of Experimental Therapeutics, Walter Reed ARmy Institute of Research, Washington, DC; and Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven.

Parasitic protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) are of considerable public health importance. These parasites are generally thought to multiply by binary fision. However, our recent data from enzyme electrophoresis and quantitative microspectrophotometry indicate that natural hybridization occasionally occurs among *Leishmania* parasites and that nuclear fusion or sexual reproduction takes place in the intracellular amastigote form. As determined by microspectrophotometry on several different *Leishmania* species, the mean nuclear DNA content of all promatigotes and of some amastigoes was 0.098 +- 0.032 relative units; but other amastioges within the same macrophage had a mean nuclear DNA content of 0.219 +- 0.050. The latter population of 4N amastigotes are produced when the nuclei of a pair of 2N amastigotes fuse (possibly amphimixis or automixis). These 4N amastigotes later go through the meiotic cycle to reform the 2N condition. The demonstration of sexual reproduction in *Leishmania* may have significant impact on the future direction of research on this medically important parasite.

615 CHAGAS' DISEASE CONTROL IN BOLIVIA: A MODEL FOR COMMUNITY DEVELOPMENT AND VECTOR CONTROL. Balderrama F*, Bermudez H, Torrcio F, Bryan RT, Kuritsky J, Tonn RJ, and Arata A. Programa de Control de Chagas - CCH/USAID; Universidad Mayor de San Simon, Cochabamba, Bolivia; CDC, Atlanta, GA; Community & Child Health Project, USAID/Bolivia; Vector Biology & Control Project, Arlington, VA.

Three million Bolivians are at risk or already infected with *Trypanosoma cruzi*. Domestic insect vectors (*Triatoma infestans*) are the main route of infection and the prevalence of Chagas' disease is highest in rural areas where poverty, lack of education, and poor housing favor vector infestation. Because treatment options are limited and no vaccine exists, vector extermination and elimination of vector habitats are the most effective control measures. The Bolivian Control Program uses an integrated approach of community education, housing improvements, and limited insecticide application. A key objective is to ensure sustainability of control measures through community participation and social development. Education emphasizes the relationship of household conditions to vector infestation and the broader health benefits of better housing. A system of phased training enables program-trained community members (promoters) to educate families and supervise construction. Local construction materials are used; local culture and housing traditions are respected. Initially, most financial support for control efforts were Program-donated; participating communities now contribute 70% of overall costs and are playing increasingly greater roles in insecticide application, household maintenance, and vector surveillance. A recently implemented rotating fund should promote further community independence & program sustainability.

616 CHAGAS' DISEASE IN BOLIVIA: EFFECTS OF MATERNAL *TRYPANOSOMA CRUZI* INFECTION ON NONINFECTED NEWBORNS. Torrico F, Moore A, Balderrama F, Castro M, Dorado C, Arandia R, and Bryan RT. CUMETROP, Facultad de Medicina-UMSS and Programa de Control de Chagas-CCH, Cochabamba, Bolivia; and Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA

Chagas' disease is a public health priority in Bolivia where seroprevalence to *Trypanosoma cruzi* approaches 90% in some areas. Data collected by the National Chagas' Disease Control Program (CCH-Chagas 1991) from the disease-endemic areas near Cochabamba indicate that 20.3% of children <1 year old and 52.3% of women of childbearing age are *T. cruzi* seropositive. Although congenital transmission of *T. cruzi* is an important source of pediatric infection, no studies have addressed the effects of maternal infection on pregnancy or noninfected newborns. In this prospective study of 625 pregnant women and their 634 newborn children (9 twin deliveries), we found that 26.7% of women were infected. We analyzed the effects of maternal infection status on pregnancy outcome by comparing seronegative and seropositive mothers. Results indicate that maternal *T. cruzi* infection is associated with poorer pregnancy outcome. Uninfected newborns of seropositive mothers scored significantly lower in the variables assessed: APGAR scores, weight and length, head circumference, gestational age, and frequency of prematurity and low birth weight. These differences persisted even when mothers were stratified by socioeconomic status, suggesting that maternal *T. cruzi* infection may adversely affect newborns even in the absence of congenital infection.

617 BLOOD DONORS IN A VECTOR FREE ZONE OF ECUADOR ARE POTENTIALLY INFECTED WITH *TRYPANOSOMA CRUZI*. Escalante L*, Grijalva MJ, Rowland EC, Powell MR, and McCormick TS. Pathology Department, Instituto Nacional de Higiene y Medicina Tropical, Quito, Ecuador; and Department of Biological Sciences & Tropical & Geographic Disease Institute, Ohio University, Athens, OH.

Chagas' disease is a serious health problem for the population of South and Central America. Several studies have reported finding *Trypanosoma cruzi* infected blood in donated blood. This observation is evidence of the risk of transmission via blood transfusion. Serum samples were taken from the Red Cross blood bank in Quito, Ecuador and analyzed by Enzyme Linked Immunosorbent Assay, using crude epimastigote extract from *T. cruzi* Brazil strain. It was found that from 157 samples, 29 %, 45 % and 26 % presented O.D. values of <0.05 (group a), >0.05 <0.3 (group b) and >0.3 (group c), respectively, when blanked against a pool of negative controls. Western blot analysis of 7.5% SDS-PAGE separated *T. cruzi* epimastigote antigen proteins, using the same sera revealed the presence of a 205 kd doublet band which was present in 8%, 16% and 47% in groups a,b and c, respectively. These

preliminary results reveal a possible correlation between positive ELISA tests and reaction to the 205 Kd *T. cruzi* antigen doublet, although possible cross reactivity with *Leishmania* cannot be disregarded. These results indicate the necessity for long term screening of blood bank donors in order to reduce the risk of transfusion transmission of the disease in areas where the vector is not present.

618 PREVENTION OF TRANSFUSION-ACQUIRED LEISHMANIASIS: A COMPARISON OF THREE METHODS AVAILABLE TO BLOOD BANKS. Daugirda JL* and Grogl M. Department of Pathology, Walter Reed Army Medical Center, Washington, DC; and Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

On November 12, 1991, the DoD issued an advisory recommending that all individuals who traveled to the Persian Gulf after August 1, 1990 be deferred as donors of transfusable blood products. This action was taken because *Leishmania tropica* was isolated from the bone marrow of 7 of 8 patients with viscerotropic leishmaniasis. There have been six cases of transfusion-acquired leishmaniasis reported. In addition, we have found that blood units containing four infected monocytes per ml of blood with a mean of three amastigotes per monocyte, contain viable parasites for 15 days under blood banking conditions. On January 1, 1993 the outright ban was lifted although additional donor medical history questions were added to help identify those individuals who might have been infected with *Leishmania*. To assess the possibility of pre-treating blood to prevent transmission of leishmaniasis through blood transfusion, we studied three methods used routinely for other purposes in blood banks: chemotherapy, filtration and irradiation. Units of blood seeded with a known number of monocytes infected with *L. tropica* amastigotes were treated with gentian violet at a concentration of 125 mg/500 ml of blood; filtered with a leukocyte reduction filter BPF4; or irradiated with 2500 rads of Cesium-137 (^{137}Cs), and tested for parasite survival by culture, and staining with FDA. We report that gentian violet and white cell reduction filtration are effective tools to kill and/or remove *Leishmania* parasites from whole blood. Irradiation was inefective until large doses, 60,000 rads and greater, were administered. Prophylactic treatment of potentially infected units may provide safe, transfusable products.

619 CUTANEOUS LEISHMANIASIS IN U.S. RANGERS AND MARINES ASSOCIATED WITH JUNGLE WARFARE TRAINING IN FRENCH GUIANA DURING 1992-1993. Grogl M*, Gasser Jr. RA, Magill AJ, Johnson SC, and Oster CN. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.; Infectious Disease Section, Walter Reed Army Medical Center, Washington, DC; and Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

During a 12 month period in 1992-1993, 11 cases of cutaneous leishmaniasis were confirmed among U.S. military conducting jungle warefare training in French Guiana. The 11 cases came from two units: 7 cases from the 2nd RECON Battalion 2nd Marine Division, and 4 cases from the 1/75 Ranger Battalion, with attack rates of 58% (7 of 12) and 10% (4 of 40), respectively. However, all 4 cases from the 1/75 RGR BN belonged to 1 of the 4 platoons conducting jungle training (HQ Company) for an attack rate of 40%. This suggests the presence of concentrations of infected vectors in small, defined "microfoci" (Walton, 1968). All cases yielded positive *Leishmania* cultures, 5 were identified as *L. guyanensis* and 4 as *L. braziliensis* by cellulose acetate electrophoresis; two did not expand. The mean number of days in the endemic area was 22 days (from 14-28) for the 11 confirmed cases. The median incubation period calculated from day of arrival was 25 days (from 7-60). Six of the cases presented multiple lesions (2-6). The average ulcer size was 2.5 cm (from 0.5-6.0). Evaluation of different diagnostic techniques revealed that direct examination of tissues with a *Leishmania* specific monoclonal antibody was the most sensitive test. In contrast with published studies, serology (the indirect immunofluorescence test) was useful in suggesting infection (all patients had titers $\geq$ to 1:32). Non-duty days range from 51-138, the average time lost for each man being 92 days. The average cost

per patient was $17,541. This resulted in a total of approximately 1012 man-days of lost duty-time, at a cost of approximately $192,951 for the 11 cases.

620 INCIDENCE OF *TRYPANOSOMA CRUZI* INFECTION AMONG OPOSSUMS AND RACCOONS IN SOUTHEAST GEORGIA. Banks CW, Durden L, Krissinger MW, and Pung OJ*. Department of Biology, Georgia Southern University, Statesboro, GA; Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, GA.

Chagas' disease is rarely diagnosed in people living in the United States, even though various investigators have reported finding *Trypanosoma cruzi*-infected animals and triatomine bugs in different parts of the country. Vector transmission of *T. cruzi* to humans in this country is considered unlikely, due to the behavior of indigenous triatomines. However, hunting, trapping and consumption of opossums and raccoons is not uncommon in many locales and human contact with infected animal tissues could result in direct transmission of the parasite. This study was initiated to determine the prevalence of *T. cruzi* infection among wild opossums and raccoons in southeast Georgia. A total of 21 opossums and 5 raccoons were live-trapped, primarily in Bulloch County, Georgia, from September, 1992 to May, 1993. Animals were anesthetized and bled and a wet mount of blood from each animal was examined. In addition, 1 ml of blood was cultured in liver infusion tryptose (LIT) medium at 27°C in 25 cm^3 T-flasks and monitored for trypanosomes every 2 wk for 10 wk. Trypanosomes were not found in wet mounts of blood but we did observe epimastigotes typical of *T. cruzi* in LIT cultures of blood from 3 opossums (14.3% of total) and in 2 raccoons (40% of total). These results suggest that hunters and trappers in southeast Georgia are quite likely to come into contact with infected animals. To examine the potential risks associated with contact with these organisms, future studies are planned which will assess the pathogenicity of southeast Georgia trypanosome isolates in inbred laboratory mice. Other parasite infestations of animals trapped will also be presented.

621 EVALUATION OF DOT ELISA EMPLOYING NATIVE gp63 FROM *LEISHMANIA MAJOR* FOR LEISHMANIASIS DIAGNOSIS. Mohareb EW*, Youssef FG, and Galloway DR. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

We report on the use of *Leishmania major* surface glycoprotein, gp63, derived from *L. major* to detect antibodies in the sera of humans and animals infected with different species of *Leishmania*. *L. major* promastigotes (1.2×10^{11}) were lysed and membrane antigens were extracted using octylglucoside. The extract was purified by binding successively to Con A followed by a Mono Q column to harvestelectrophoretically pure gp63. This protein served as an antigen in a Dot ELISA procedure. Sera were obtained from the following sources: rabbits immunized with *L. major*, *L. tropica*, and *L. donovani*, cutaneous leishmaniasis patients infected with *L. major*, BALB/c mice infected with*L. major* showing skin and spleen pathology, BALB/c mice inoculated with a viscerotropic *L. tropica* strain. All rabbit antisera produced a strong reaction demonstrating cross-reactivity between the different species' gp63. Antisera from BALB/c mice and humans infected with *L. major* all reacted with gp63 but at different intensities. Antisera from mice inoculated with the viscerotropic strain also gave a strong reaction with the antigen. These results suggest that native gp63from *L. major* may be a sensitive diagnostic marker for simple cutaneous as well as visceralising forms of leishmaniasis.

622 IDENTIFICATION OF A MISLEADING TRYPANOSOMATID PARASITE FROM *GERBILLUS PYRAMIDUM* AND *G. ANDERSONI* IN A *LEISHMANIA MAJOR* ENDEMIC AREA IN NORTH SINAI. Mikhail EM*, Mansour NS, Mohareb EW, Francies WM, Galloway DR, Fryauff DJ, and Modi GB. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt.

During an epidemiologic investigation of cutaneous leishmaniasis at a focus in North Sinai, Egypt, between June 1989 and December 1991, a total of 1294 desert rodents were trapped and examined to identify reservoir hosts of *Leishmania major*. Mixed forms of epimastigotes and promastigotes were isolated in Tanabe's medium from 4 *Gerbillus pyramidum* and 1 *G. andersoni*. The two forms were later grown and separated as distinct cultures in Schneiders' medium. The isoenzyme profile of the gerbil promastigotes was identical to *L. tropica* but differed from those of *L. major* and the gerbil epimastigotes. However, the protein pattern by SDS-PAGE and the *Hae*III fingerprinting of PCR amplified minicircles of both forms were distinct from each other and from those of *L. tropica* and *L. major*. The gerbils' promastigotes were morphologically similar to but 30% broader with smaller nucleus than those of *L. tropica*. Following several subcultures epimastigotes were found to transform to promastigotes. These observations indicate that the two forms belong to *Trypanosoma* spp. Further studies are being done to identify these trypanosomas in which the promastigote-stages gave isoenzyme patterns identical to *L. tropica* and can be misidentified microscopically as *Leishmania* promastigotes.

623 COMBINATION CHEMOTHERAPY OF DRUG RESISTANT *TRYPANOSOMA BRUCEI RHODESIENSE* INFECTIONS IN MICE USING DL-α-DIFLUOROMETHYLORNITHINE AND SURAMIN. Bacchi CJ*, Yarlett N, McCann PP, Sjoerdsma A, Saric M, and Clarkson, Jr. AB. Haskins Laboratories and Biology Dept., Pace University, New York, NY; Marion Merrell Dow Research Institute, Cincinnati, OH; and Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY.

Chemotherapy for East African sleeping sickness is unreliable because of resistance to arsenicals and other agents and new drugs areneeded. DL-α-difluoromethylornithine (DFMO, eflornithine, Ornidyl®) was recently approved by the U.S. FDA for use against African disease. Animal studies were done to determine whether combinations of DFMO + suramin would be synergistic against drug-refractory clinical isolates of *Trypanosoma brucei rhodesiense*. Combinations of DFMO with suramin were effective against acute infections resistant to these drugs when used singly. An infection with a melarsen oxide refractory isolate was cured by a combination of low dose DFMO (0.5% in the drinking water for 3 days) plus low dose suramin (1 mg/kg, i.p.). Another strain, moderately resistant to arsenical drugs, was cured with 4% DFMO + 5 mg/kg melarsen oxide. A combination of DFMO (2% for 14 days) and suramin (20 mg/kg, one dose) was 100% curative in a CNS model, although the same dosage of these drugs used singly was ineffective. The synergism of DFMO and suramin againstan acute infection was improved when suramin was given at the middle or the end of the DFMO dose. No adverse effects were seen when high doses of DFMO were administered to uninfected mice combined with high doses of suramin. These results suggest that combinations of DFMO and suramin should be examined clinically in arsenical refractory cases of East African sleeping sickness.

624 CHANGING PATTERN OF AMERICAN VISCERAL LEISHMANIASIS: FURTHER OBSERVATIONS FROM THE URBAN OUTBREAK IN NATAL, BRAZIL. Jeronimo SB*, Oliveira RO, Mackay S, Costa RM, Sweet J, Nascimento ET, Luz KG, Fernandes MZ, Jernigan JA, and Pearson RD. Department of Biochemistry, Universidade Federal do Rio Grande do Norte; Fundaão Nacional de Saúde; Hospital Gizelda Trigueiro, Natal, RN, Brazil; and Department of Medicine, University of Virginia, Charlottesville, VA.

The epidemiologic pattern of visceral leishmaniasis is changing in northeastern Brazil. The disease was typically seen in rural, endemic areas, but it is now occurring as an epidemic in the city of Natal, where 316 cases have been reported since 1989. The majority of cases have occurred in the northern suburbs of Natal where thousands of people have settled in dune areas. These districts were sites of tremendous population growth during the preceding decade as Natal spread outward from its center. Cases have been reported from all age groups, but 49% were in children less than 5 years. The

epidemic in Natal has been accompanied by similar increase in the number of cases of visceral leishmaniasis in other areas of the state of Rio Grande do Norte. Isolates (n=5) were identified as *Leishmania chagasi* by isoenzyme pattern. *Lutzomyia longipalpis* was identified in the houses of patients. The rate of seropositivity of dogs determined by immunofluorescence ranged from 0.8% to 9.6%. In order to determine the extent of infection in the community two hundred and ten household contacts and neighbors of patients from the Alvorada district were examined for evidence of visceral leishmaniasis. Six additional cases were diagnosed, and 38% of housemates and neighbors were Montenegro skin test positive indicating that they had had subclinical infection. Significantly more housemates than neighbors had positive skin tests ($p<0.001$, Fisher exact test). In summary, visceral leishmaniasis has become an important urban health problem in Natal where thousands of residents in the suburban areas are now at potential risk of *Leishmania chagasi* infection.

625 CHARACTERIZATION OF PARASITES CAUSING CUTANEOUS AND VISCERAL LEISHMANIASES IN PAKISTAN. Masoom Yasinzai M* and Chang KP. Institute of Biochemistry, University of Balochistan, Quetta, Pakistan; and Department of Microbiology/Immunology, UHS/Chicago Medical School, North Chicago, IL.

Both cutaneous and visceral leishmaniases are endemic to Pakistan. The cutaneous disease is predominant in the southwest province of Balochistan, which extends from the coast of Arabian sea north to Afganistan and east to Iran. The visceral disease exists largely in the northern region bordering China. The reservoir animals are presumably rodents and canines for cutaneous and visceral leishmaniasis, respectively. Outbreaks of the cutaneous disease have been reported in recent years with increasing frequency, especially after the migrations of populations into the endemic regions. We have begun to grow the parasite isolates *in vitro* for laboratory studies relevant to their chemotherapy, pathogenicity and epidemiology. One visceral and two cutaneous isolates were provided as *Leishmania major, L. tropica* and *L. infantum*. All three can be passaged in NNN medium. The two cutaneous isolates, but not the visceral one, can be adapted to grow in M199 plus 20% HIFBS. Peak cell density varies considerably at stationary phase among the three isolates. One of the cutaneous isolates produces nodular lesions in BALB/c mice. The visceral isolate also appears to give cutaneous lesions in hamsters. Work is under way to characterize these isolates by studying their genomic and kinetoplast-DNAs. The sensitivity of these isolates to neomycin congeners *in vitro* is under study to complement the field trials of paromomycin by topical application. Additional isolates are being collected for these and other laboratory studies.

626 HIGH SEROPREVALENCE OF CHAGAS ANTIBODY POSITIVE SPECIMENS FOUND IN THE U.S. SHOULD A DIAGNOSTIC TEST FOR *TRYPANOSOMA CRUZI* BE IMPLEMENTED ?. Pan AA*, Schur JD, Brashear RJ, Winkler MA, Cantrell L, Rivera D, Shih J, and Holzer T. Diagnostic Biology Research, Abbott Laboratories, North Chicago, IL; and Quality Scientific Support, Abbott Laboratories, North Chicago, IL.

In the U.S. there are six required procedures that are performed on donated blood to insure its safety for transfusion; these include Syphilis, HBsAg, Hepatitis B core, HCV, HIV-1 and 2, and HTLV-I. At present there is no recognized standard for establishing the presence of antibodies to *Trypanosoma cruzi* in the U.S. However, it is well known that transmission of Chagas' disease by blood transfusion in Latin America is second in frequency compared to infection by the insect vector. Several countries require testing for antibodies to this parasite in all blood donations. There is more testing done in Latin America for Chagas' disease than for Hepatitis or HIV. In the U.S. several recent seroprevalence studies have indicated the presence of antibodies to *T. cruzi* : (a) Immigrants from Central America to the Washington DC area were found to have a 4.9% seroprevalence rate. (b) A study of 1027 consecutive blood donors in Los Angeles County found 10 initial reactives and one confirmed case. (c) A study among 7835 Hispanic-surname blood donors in the Southwestern and Western areas of the U.S. tested with the Chagas Antibody EIA indicated a 0.16% prevalence rate (1 out of 603). (d) A

further evaluation of the Chagas Antibody EIA at the Sacramento Medical Foundation indicated a rate of 0.1% (1/1072) in the general blood donor population. It has been estimated that the overall national rate is approximately 1 per 5000. The use of the Chagas Antibody EIA to detect the presence of antibody to this parasite will aid in the diagnosis of this blood borne pathogen.

627 *PNEUMOCYSTIS* PREPARATIONS OF HIGH PURITY AND VIABILITY. Kaneshiro ES*, Ellis JE, Wyder MA, Zhou LH, Langreth SG, and Voelker DR. Department of Biological Sciences, University of Cincinnati, Cincinnati, OH; Department of Microbiology, Uniformed Services University of Health Sciences, Bethesda, MD; and Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

Continuous culture methods are currently lacking for *Pneumocystis*. Biochemical analyses of organisms isolated from infected hosts can best be interpreted if the purified preparation has been characterized with respect to the degree of possible contaminants. Mixed life cycle stages of *P. carinii* were isolated from immunosuppressed, viral antibody-negative rats, which had been infected by intratracheal intubation of organisms, tested negative for common bacteria and fungi. Protocols were developed to optimize separation of cells from host material, recovery efficiency, preparation purity, and organism viability. A HEPES-buffered salt solution containing the mucolytic agent glutathione (0.5%) was used throughout a protocol in which excised infected lungs were minced, homogenized in a Stomacher lab blender, sieved (50 - 60 mesh), then subjected to two rounds of high (925 g) and low (60 g) spins for 10 min. The final supernate was filtered through two tandemly arranged 25-mm diameter polycarbonate filters (8 then 5 mm pore diameters). The purity of the preparations was characterized by several criteria. (1) Light microscopy with phase- and differential interference-contrast optics indicated the absence of intact host cells. (2) Electron microscopy indicated <5% of host debris. (3) The lung surfactant marker surfactant protein-A, quantified by an enzyme-linked immunosorbant assay, was <0.3% total protein; <2.6 mg SP-A/109 organisms. (4) Phosphatidylglycerol, concentrated in secreted lung lamellar bodies, was 1.4 wt. % of total phospholipids of final *P. carinii* preparations. (5) Cholesterol decreased from 5.7 to 0.5 mg/109 parasites (initial value - lung plus organisms; final value - taken as the concentration in *P. carinii*). (6) Exogenous stigmasterol, added as an extracellular marker, was undetectable in the final preparation. Cholesterol/stigmasterol increased during the purification procedure. (7) Total non-*P. carinii* protein, estimated by the total protein of control pellets (from uninfected lungs) was <6%. Viability of the organisms was 80 - 95%, as determined by the Calcein-AM-propidium iodide assay. Electron micrographs showed cytoplasm-filled *P. carinii* cells and normal organelle morphologies. These preparations were morphologically superior to those subjected to a hypotonic shock, a treatment used in some protocols to lyse host cells. *Pneumocystis* cells were not adhered to each other or to host material, indicating the effect of the mucolytic agent. Reliable biochemical analyses and short-term metabolic studies can be done using these preparations. For example, <2% of *P. carinii* total lipids were found to be sterols absent in control rat lungs.

628 A NOVEL *IN SITU* MODEL TO STUDY *PNEUMOCYSTIS CARINII* ADHESION TO LUNG ALVEOLAR EPITHELIAL CELLS. Pavia-Ruz N*, Ortega-Barria E, Alroy J, and Pereira ME. Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospitals, Boston, MA; and Department of Pathology, Tufts University School of Medicine and Veterinary Medicine, Boston, MA.

Pneumocystis carinii (P.c.), is a major cause of death in AIDS patients in the United States. The mechanisms of parasite adherence remains unknown, lagerly because of difficulties in isolating type I pneumocytes and maintining them *in vitro*. As a first step to understand P.c. adherence to its natural substrate, we developed an *in situ* method to directly study parasite binding to lung alveolar cells. We used formaldheyde fixed sections of normal rat lung as substrate for adhesion. $2.5x10^5$ FITC-labeled P.c. were overlaid over each lung sections and incubated 2 h at room temp. The slides were washed,

fixed in 4% formalin and analysed by fluorescence microscopy. To determine whether P.c. adheres to alveolar epithelial cells by a lectin-type interaction, we tested the effect of several carbohydrates and compared the ability of glycoproteins to inhibit P.c. adhesion *in situ*. Adherence was saturable, time and dose dependent, and selectively blocked by glycoconjugates, in particular bovine submaxillary mucin, fetuin, and asialofetuin, suggesting that it may be mediated by a lectin type of interaction.In order to determine the type of lung alveolar epithelial cells P.c. adhered to, we stained the sections with *Erythrina cristagalli* and *Maclura pomifera* lectins and incubated with the parasite. As in its binding to the lung *in vivo*, P.c. adhered preferentially to type I pneumocytes. Inmune serum and purified anti-P.c. antibodies from infected rats, also inhibited parasite attachment as compare with normal serum. The results suggest the usefullness of the in an *in situ* model for studying the mechanisms of *Pneumocystis carinii* adherence to alveolar cells.

629 ALBENDAZOLE INHIBITS *PNEUMOCYSTIS CARINII* IN MOUSE MODELS. Bartlett MS*, Edlind TD, Shaw MM, Smith JW. Department of Pathology, Indiana University School of Medicine, Indianapolis, IN; and Department of Microbiology, Medical College of Pennsylvania, Philadelphia, PA.

Albendazole, an anti-hemminthic benzimidazole which inhibits microtubule polymerization, has been shown to be effective against *Pneumocystis carinii* in HEL tissue culture and is now evaluated in two BALB/c mouse models which were transtracheally inoculated with mouse *P. carinii*. Both models demonstrated albendazole's effectiveness in eliminating heavy infections of *P. carinii*. Mice were immunosuppressed with either dexamethasone at 1.2 mg/kg/day or monoclonal antibody L3T4+ from clone Gk1.5 at 0.2 mg twice a week, inoculated, allowed to develop infection for 3 weeks then treated with albendazole at 300 or 600 mg/kg/day for 3 weeks. Severity of infection was scored by microscopic examination of Giemsa-stained impression smears and by ELISA. In dexamethasone-immunosuppressed mice, albendazole decreased infection so that scores of *P. carinii* per 1000X field (on a roughly logarithmic scale) were 2.4 ± 0.5 for 600 and 3.0 ± 0.5 for 300 mg doses compared to 4.4 ± 0.2 for untreated (over 90% decrease). ELISA O.D. values were 0.203 ± 0.015 (600) and 0.220 ± 0.033 (300) versus 0.458 ± 0.044 for untreated control. In L3T4+-immunosuppressed mice scores were 0.8 ± 0.3 (600) compared to 4.4 ± 0.2 for untreated controls (over 99% decrease). In both models, trimethoprim/sulfamethoxazole at 50/250 mg/kg/day treatment groups had scores of 0.1 ± 0.1. Albendazole was more effective in the L3T4+-immunosuppressed mice. Albendazole might be useful for treating *P. carinii* infections in humans.

630 ENTEROPATHOGEN PARASITES IN STOOLS OF HIV-POSITIVE PATIENTS WITH DIARRHEA. Houze-Savage S*, Van Gool T, Lemann F, Bouchaud O, Verdon R, Leport C, Ruggeri C, and LeBras J. Service de Parasitologie, Hospital Bichat-Claude Bernard, Paris, France; Service de Maladies Infectieuses et Tropicales, Hospital Bichat-Claude Bernard, Paris, France; and Medical Microbiology, Academic Medicla Center, Amsterdam, The Netherlands.

Chronic diarrhea is a common feature observed in patients with AIDS. Numerous microorganisms may be responsible for diarrhea, but in 30% of cases, no parasites may be found. *Enterocytozoon bieneusil*, a new species of *Microspora*, was recently identified as an etiologic agent of diarrhea. During one year, 350 stool samples from 280 HIV-seropositive patients with diarrheal episodes were examined for parasites. Microsporidian spores in the stools were diagnosed by Chromotrope 2R and Uvitex 2B techniques. Modified acid-fast straining was used to identify cryptosporidian oocysts. The other parasites were searched for by direct microscopic examination after a concentration technique. 31 cases of microsporidiosis with *E. bieneusil* (11%), 29 cryptosporidiosis (10%), 16 giardiasis (6%), and 4 isosporidiosis (1.5%) were diagnosed. In two cases, both cryptosporidiosis and microsporidiosis were present. *Giardia intestinalis* cysts and *E. bieneusil* spores were associated in two other cases. In one patient, two microsporidian spores, *Enterocytozoon* and *Encephalitozoon*, as well as cryptosporidian oocysts, were found in the same stool. The differential diagnosis was obtained with Uvitex 2B

fluorescent staining. Our results suggests that, due to the high frequency of microsporidiosis in HIV positive patients, microsporidian spores and other parasites should be looked for in the stool.

631 WILL POOLING THREE SPECIMENS INCREASE THE DIAGNOSTIC YIELD COMPARED TO ROUTINE EXAMINATION OF THREE SAF PRESERVED STOOLS?. MacPherson DW and Stephenson BJ*. Regional Parasitology Lab, St. Joseph's Hospital, Hamilton, Ontario; Infectious Diseases and Tropical Medicine Clinic, Chedoke-McMaster Hospitals; and Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Canada.

A three part study measuring diagnostic agreement was undertaken to determine if a single examination on three individual specimens compared to pooled samples from these three samples would increase diagnostic yield. 1) Agreement between three individual single samples was compared to a pooled sample; 2) Of the pooled disagreements, samples with sufficient quantity were re-analyzed twice to assess the increase n diagnostic yield; and 3) Three individual specimens were assessed for progression in diagnostic yield. With a positive trate 42.3%, interim analysis of 482 pairs (standard vs. pooled) yielded a sensitivity of 80% and a specificity of 98% for the pooled specimens. Evaluation of the disagreement was highly significant (Z_c=4.87; $p<0.001$). Of the 40 pooled disagreements, 23 with suficient quantity were re-analyzed twice,increasing the sensitivity to 97%; specificity remained at 98%; and disagreement was no longer significant (Z_c=-0.29; $p>0.005$). In three individual specimens progression of diagnostic yield was 84% for one, 10% for the second, and an additional 6% for the third. 1) Pooling three SAF specimens has a significantly lower diagnostic yield than three examination independently; The first examination of SAF will yield all positive findings in 84% of three specimens, the second will increase the yield by 10% and the third will increase the yield by 6%; and 3) Three separate exminiation of one pooled specimen equals three separate examination of three individual SAF specimens; and 4) There is no diagnostic advantage to pooling SAF samples.

632 STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF LIGAND BINDING TO URACIL PHOSPHORYBOSYLTRANSFERASE FROM *TOXOPLASMA GONDII*. Tankersley KO* and Iltzsch MH. Department of Biological Sciences, University of Cincinnati, Cincinnati, OH.

The eucaryotic organism *Toxoplasma gondii* is an obligate and intracellular parasitic-protozoan. Although the parasite remains benign in the general population, it does pose serious health problems for immunosuppressed individuals; i.e., those people with acquired immuno-deficiency syndrome (AIDS). As many as 40% of the patients with AIDS in the United States will be affected by this parasite. As a result, toxoplasmic encephalitis is currently the most common opportunistic infection of the central nervous system. Current durg therapies for toxoplasmosis only affect viable tachyzoites and are unable to affect dormant pseudocycts. Therefore, current treatments only suppress the infection rather than cure it. One possible target for the development of new chemotherapeutic agents are the pathways responsible for nucleotide biosynthesis; specifically, the pyrimidine salvage pathway in *Toxoplasma gondii*. The key enzyme in pyrimidine salvage is uracil phosphoribosyltransferase (UPRTase). UPRTase is a favorable target because it is not found in the mammalian host. Using apparent K_i values, a structure-activity relationship study was formulated from structurally similar uracil analogs. The low apparent K_i's of three analogs, 1-deazauracil, trithiocyanuric acid and 4-mercaptopyridine, represented unique analogs that may either be inhibitors or substrate analogs of UPRTase. Although none of the eighty-five compounds tested bound tighter to UPRTase than the highly-specific substrate; uracil, these three compounds did have a lower K_i than two known substrates; 5-fluorouracil and emimycin. Thus providing potentially new anti-toxoplasmic agents.

633 DIFFERENTIAL MODES OF ACTION OF DICLAZURIL AGAINST THE RELATED PROTOZOANS *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM IN VITRO*. Lindsay DS*, Toivio-Kinnucan MA, and Blagburn BL. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

Toxoplasma gondii and *Neospora caninum* are important causes of abortion and neonatal disease in domestic mammals and *T. gondii* is an important opportunistic pathogen causing encephalitis in AIDS patients. Both reproduce by endodyogeny *in vitro*. Diclazuril, a benzeneacteonitrile anticoccidial, has excellent activity against both of these protozoans at levels of 0.005 mcg/ml. Because our phase contrast microscopy examinations of infected tissue culture flasks indicated that the structural mode of action of diclazuril was different for the 2 parasites, we used transmission electron microscopy to further investigate the mode of action of diclazuril. Diclazuril apparently inhibited cytoplasmic division of the RH isolate of *T. gondii* because multinucleate meronts were observed at 2 days PI. Tachyzoites were observed attempting to bud from these stages. Many of these stages developed cytoplasmic vacuoles. The parasitophorous vacuole enclosing these multinucleate stages became enlarged and often had increased amounts of intravacuolar tubules. Most *T. gondii* stages were degenerate by 5 days PI. No stages with more than 2 nuclei were observed in cultures containing *N. caninum* at 2 or 5 days PI. Enlarged lipid-bodies were the main diclazuril induced changes observed in *N. caninum* tachyzoites.

634 EXPERIMENTALLY INDUCED OCULAR TOXOPLASMOSIS IN NURSING PIGS. Pinckney RD*, Lindsay DS, McLaughlin SA, Boosinger TR, and Blagburn BL. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL; and Department of Small Animal Surgery and Medicine, College of Veterinary Medicine, Auburn University, AL.

Toxoplasma gondii is a ubiquitous protozoan parasite of man and animals. Our study examined the safety of a temperature-sensitive mutant (TS-4) and its parent RH isolate of *Toxoplasma gondii* in 3 day-old nursing pigs. Hanks' balanced salt solution (HBSS), or 3 x 10^5 tachyzoites of either the TS-4 or RH isolates were used for experimental intravenous inoculations. Clinical signs were not seen in pigs given HBSS or TS-4; results of ophthalmic examinations of these pigs were not remarkable. Pigs inoculated with the RH isolate developed severe clinical signs consisting of diarrhea, dyspnea, rales, and lethargy 5 to 14 days postinoculation. One pig died 7 days postinoculation. Severe ophthalmic signs consisting of buphthalmos, lagophthalmos, and periorbital edema were seen in one RH inoculated pig 9 days postinoculation. Ophthalmic examination revealed exposure keratitis, conjunctivitis, corneal ulceration, hypopyon, and secondary glaucoma. Ophthalmic examinations of the other RH inoculated pigs demonstrated progressive lesions of retinitis and retinochoroiditis. Results indicate that the TS-4 isolate is safe in nursing pigs and in contrast to the parent RH isolate, does not induce ocular lesions.

635 TRANSMISSION OF TOXOPLASMA IN PANAMA. Frenkel, JK*, Quintero R, Hassanein KM, Hassanein RS, Brown EF, and Thulliez, PH. Department Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS; Gorgas Memorial Laboratory, Panama City, Rep. of Panama; Instit. de Seguro Social, Panama City, Rep. of Panama; Department Biometry, University of Kansas Medical Center, Kansas City, KS; and Lab. de Serologie Neo-natale, Inst. de Puericulture, Paris, France.

A 5-year prospective cohort study of children, cats, rodents and birds was conducted in Panama City to define transmission and preventive mechanisms. 500 Children between 1 and 6 years were studied by questionnaires, medically, and serologically 4 times yearly from 1987-1992. Cats, soil, rodents and birds in the children's environment were examined serologically or by isolation, at intervals. Seventy-two children seroconverted over a 5 year period. 46% of 241 cats, 0.5% of 383 cat feces, 1.1% of 974 soil specimens, 0.35% of 571 house mice, 23.0% of 221 Norway rats and 12.1% of 223 ground-feeding birds were positive for Toxoplasma. Questionnaire variables were selected based on

theoretical and practical knowledge of biologic possibilities. 57 Initial variables were subjected to stepwise discriminate analysis for their effect on seroconversion. The following 5 variables were found to be significantly related to seroconversion and they classified 88% of children as to seroconversion or remaining negative: Many flies, living on ground floor, eating beef, living in apartment building, 8+ cats. When many flies were replaced by not having screens, 81% of seroconversions were predicted. The 5 significant variables were then submitted to stepwise logistic regression to build a model with practical applications. Just 3 variables, many flies, consuming beef and 8+ cats predicted 86%, and many flies, living on ground floor rather than higher up and 8+ cats predicted almost 88% of seroconversions. In these studies fly transmission of oocysts was a new factor identified, whereas soil contamination, in the house and park was widespread, but oocysts in the soil very rare. Beef transmission of tissue cysts was rare and mentioned for only 8 children, 5 seroconverted and 3 seronegative.

636 BOVINE *NEOSPORA* ISOLATES: CULTIVATION, CHARACTERIZATION AND EXPERIMENTAL INFECTIVITY. Conrad PA*, Barr BC, Sverlow K, Rowe J, Tarantal A, Marsh A, BonDurant R, Ho M, and Hendrickx AG. School of Veterinary Medicine, University of California, Davis, CA; and California Regional Primate Research Center, Davis, CA.

Neospora is a newly recognized *Toxoplasma*-like protozoal parasite of domestic animals which we have identified as a major cause of abortion in California dairy cattle. Recently, we established the first continuous cultures of bovine Neospora isolated from the brains of aborted fetuses and congenitally infected calves. In addition, we showed that cultivated parasites inoculated into pregnant heifers could be transmitted transplacentally and cause fetal death with lesions like those seen in naturally infected fetuses. To investigate the zoonotic potential of *Neospora*, primate fetuses were inoculated *in utero* with culture-derived tachyzoites at 65 days gestation and removed at 13 and 22 days post-infection when ultrasound examination showed decreased, flocculent amniotic fluid and decidual laking. Both fetuses had inflammatory lesions, similar to those seen in fetal toxoplasmosis. *Neospora* tachyzoites were seen by immunohistochemistry and re-isolated from multiple tissues from the infected fetuses, but not the control. Current studies to determine the effect of transplacental *Neospora* infection on primate fetuses will be discussed.

637 IMMUNOGENICITY OF *TRICHOMONAS VAGINALIS* HEAT SHOCK PROTEINS IN HUMAN INFECTIONS. Davis SR*, Finley RW, and Lushbaugh WB. Parasitology Division, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson MS; and Infectious Diseases Division, Department of Medicine, University of Mississippi Medical Center, Jackson MS.

The immunogenicity of *Trichomonas vaginalis* heat shock proteins (hsp) was investigated by immunoprecipitation of heat shocked *T. vaginalis* proteins with sera from infected humans. Two virulent isolates of *T. vaginalis* and one drug resistant isolate were compared. Sera from culture positive males and females were reacted with *T. vaginalis* proteins radiolabeled with Trans ^{35}S Label (ICN) under control and heat shock conditions. Immune complexes were precipitated with Staph A, and the immunprecipitate was subjected to sodium-dodecyl-sulfate-polyacrylamide gel electrophoresis and autoradiography. Several immunogens were specific for heat-shocked cells, but all serum samples recognized proteins in both control and heat-shocked cells of the three isolates. The predominant heat-shock immunogens in all experiments were hsp of approximately 65, 49, and 32 kilodaltons. Serum recognition patterns differed among strains. Variations existed among isolates in the intensity of the bands and in the number of immunogens present. The two virulent isolates yielded similar immunogenic responses whereas the response to the drug resistant isolate was diminished. Variation in the banding pattern was dependent on the isolate of parasite rather than the source of serum. All serum samples recognized similar proteins in individual isolates of the parasite.

638 USE OF VERO-CELL CULTURES TO ASSESS CYTOPATHOGENICITY AND VIRULENCE OF *NAEGLERIA* SPECIES. John DT* and John RA. Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK; and Symex Corp., Tulsa, OK.

Naegleria is a genus of free-living ameboflagellates to which belong 2 pathogenic and 4 nonpathogenic species. *N. fowleri* is responsible for a rapidly fatal human disease known as primary amebic meningoencephalitis. *N. australiensis*, an environmental isolate, is pathogenic for mice but so far has not been associated with human infection. The cytopathogenicity of *N. fowleri* and *N. gruberi* for African green-monkey kidney (Vero) cells increases with serial cell culture passage. Are the more cytopathic *N. fowleri* and *N. gruberi* also more virulent or pathogenic for mice? The purpose of this study was to answer this question by determining whether continuous passage in Vero-cell cultures would enhance the virulence of *N. fowleri* for mice and produce pathogenic *N. gruberi*. Additionally, because only *N. fowleri* and *N. gruberi* had been shown to produce a cytopathic effect (CPE) in Vero cultures, we evaluated the cytopathic potential of the 6 species of *Naegleria* for Vero cells. All species of *Naegleria* produced CPE in Vero cultures and CPE was dependent on incubation temperature and ameba:target cell ratio. Continuous growth in Vero-cell cultures enhanced the virulence of a weakly virulent strain of *N. fowleri*. We propose that maintenance of *N. fowleri* in Vero-cell cultures will serve as an alternative to serial passage in mice for restoring the virulence of weakly virulent strains of *N. fowleri*. The nonpathogenicity of *N. gruberi* remained unaffected by maintenance in Vero cultures.

639 EPIDEMIOLOGICAL CHARACTERISTICS OF *ACANTHAMOEBA* KERATITIS IN SUB-SAHARAN AFRICA. Resnikoff S*, Le Flohic AM, Traore L, Huguet P, and Peyramaure F. Institute of African Tropical Ophthalmology, OCCGE, Bamako, Mali; and Department of Parasitology, Faculte de Medecine de Brest, France.

Acanthamoba Keratitis (AK) is a recently identified cause of blindness in sub-Saharan Africa. With the aim of defining its clinical and epidemiological characteristics we carried out a case-control study. 116 patients were included : 16 AK and 100 controls. In sub-saharan Africa AK appeared in the form of blinding corneal ulceration without any pathognomonic characteristics other than the rarety of neovascularisation (OR=0.2 p=0.05). A mycosis was associated in both the cases (37.5%) and the controls (22%). The contamination seems to be linked to the utilization of surface water and traditionally-made eye-drops (roots and leaves decoctions).

640 CHARACTERIZATION OF IMMUNODOMINANT PEPTIDES OF *THEILERIA SERGENTI* MEROZOITE ANTIGEN. Baek BK*, Rhim BM, Kim BS, Park KH, Rhim TE, Hansen RD, Vodkin MH, McLaughlin GL, and Kakoma I. Chonbuk National University, Chonju, Korea; University of Illinois, Urbana, IL; and Purdue University, West Lafeyette, IN.

Western immunoblot analysis utilizing highly specific polyclonal bovine anti-*Theileria sergenti* (Korean isolate). The major peptides as demonstrated by SDS-PAGE were 116, 105, 80, 77, 66, 60, 56, 53, 49, 47, 38, 35, 34, 29, and 18 KD. Specific immunodominant bands revealed by hyperimmune serum were 105, 35, 34, and 29 KD. Two weeks post challenge, vaccinated animals showed a significant increase in intensity of the various bands. Four of the major bands, 18, 29, 35, and 47 KD were excised after electrophoresis and transfer to PVDF membrane. The individual bands were sequenced. Hydropathy plots and Chou-Fausam prediction sites for antigen determinants were established. A high degree of heterogeneity in location and density was observed. Candidate peptides were selected, charged and utilized in immunizing rabbits and cattle. All rabbits developed specific antibody to *T. sergenti* as measured by the enzyme immunoassay and recognized similar immunodominant peptides as demonstrated by polyclonal hyperimmune antisera. These data combined with merozoite

neutralization studies confirm the suitability of synthetic peptides as potential candidates for vaccines against bovine theileriosis in Korea. A correlation (90-100%) between immunogenicity, antigenicity and Chou-Fausam predictions was demonstrated.

641 *TETRATRICHOMONAS GALLINARUM* ASSOCIATED ENCEPHALITIS IN A MOCKINGBIRD (*MIMUS POLYGLOTTOS*). Patton S* and Patton CS. Department of Environmental Practice, University of Tennessee College of Veterinary Medicine, Knoxville, TN; and Department of Pathobiology, University of Tennessee College of Veterinary Medicine, Knoxville, TN.

A juvenile male Mockingbird died shortly after presentation with blindness and seizures. Numerous, poorly staining (H & E), rounded to fusiform, uninuclear protozoa were seen histologically associated with a necrotizing encephalitis of one cerebral hemisphere and a severe mononuclear/heterophilic meningitis. Meningitis extended to the optic nerves and caudal to the left eye. The adventitia of an ultimobranchial body contained similar organisms. Systemic spread of the infection was attributed to lamina proprial invasion at a site of small intestinal, chronic pyogranulomatous diverticulitis. Ultrastructurally, four anterior flagella, an undulating membrane and a costa, characteristic of trichomonads, were seen. A Bodian silver stain demonstrated flagella and an undulating membrane. These organelles were not demonstrable by PAS and Gram, Giemsa, iron hematoxylin, Grocott's methenamine silver or modified Steiner silver stains. Enteric trichomoniasis of birds has been attributed to *Trichomonas gallinae* and *Tetratrichomonas gallinarum*. Pigeons (and other birds) develop adherent caseous mucosal masses from the mouth to the proventriculus with *T. gallinae* infection; systemically, necrotizing hepatitis and nephritis may occur. *T. gallinarum* inhabits the ceca and adjacent small intestine; it is a cause of necrotic typhlitis and hepatitis in turkeys. Although *T. gallinarum* has not previously been reported from mockingbirds or from the brain of any bird, the four anterior flagella, costa, undulating membrane and a free recurrent flagellum identify this organism as *T. gallinarum* rather than *T. gallinae* whose recurrent flagellum terminates at the undulating membrane.

642 *CARYOSPORA BIGENETICA* DEVELOPMENT AT LOW TEMPERATURE. Sundermann CA*. Department of Zoology & Wildlife Science, Auburn University, AL.

The coccidian *Caryospora bigenetica* uses certain snakes as primary hosts and mammals as secondary hosts. The development of *C. bigenetica in vitro* at 37°C in human fetal lung cells (HFL) parallels development in the secondary host. Development in the primary host is incompletely known but is different than development in mammals. Thus attempts were made to culture the parasite at lower (29-30°C) temperatures in viper spleen cells (VSW). Purified sporozoites of snake origin were inoculated onto VSW and viewed by light microscopy. Multiplication occurred and at least 2 types of zoites (Type I & II) were present by 2 days post- inoculation (DPI), however meronts were not observed. By 4 DPI, Types I & II zoites, sporozoite-shaped meronts, spherical meronts with 2-4 nuclei, and large, dark staining zoites were present. Beginning 5-6 DPI, the classical coccidian merozoites were produced. Asexual multiplication continued, but gamonts and oocysts were not produced 9-11 DPI as in stages grown at 37°C and occurred much later. Development at 29°C was not merely a slow version of development at 37°C. Oocysts were resistant to full strength sodium hypochlorite and produced clinical signs in rats. Merozoites were removed from VSW 8-9 DPI and inoculated onto HFL at 37°C. They did not immediately revert to the mammalian cycle and produced thin-walled oocysts but they did enter host cells.

643 PARTIAL SEQUENCING AND ISOLATION OF DNA POLYMERASE δ FROM *CRYPTOSPORIDIUM PARVUM*. Mead JR*, Lloyd RM, You XD, Arrowood MJ, Slemenda SB, Pieniazek NJ, and Schinazi RF. Emory University, Atlanta, GA; VA Medical Center, Decatur, GA; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

Targeted, rational approaches to the development of anti-cryptosporidial agents have been impeded by models of limited capacity to characterize the parasite's biologic and metabolic pathways. The difficulty in obtaining significant quantities of parasite proteins warrants the large-scale production of recombinant proteins. Because DNA polymerase plays a critical role in cell replication and because we have available drugs that interfere with this enzyme, we consider this enzyme to be an excellent target for chemotherapy. We have recently designed primers based on conserved regions of the DNA polymerase δ gene from other organisms. These primers were used in a polymerase chain reaction (PCR) applied to genomic DNA of *Cryptosporidium parvum*. Partial sequencing of the PCR-generated 1.3 Kb and 0.86 Kb DNA fragments revealed distinct similarities with DNA polymerase δ from *Plasmodium*. The fragments were radiolabeled and used as probes to screen a *C. parvum* cDNA library yielding several positive clones. Cloning and expression of the DNA polymerase gene should make possible the evaluation of drugs active against this enzyme in a cell-free assay.

644 *CRYPTOSPORIDIUM PARVUM* SURFACE GLYCOPROTEINS ARE THE TARGET OF PROTECTIVE ANTIBODY. Doyle PS*, Lewis S, Barnes DA, and Petersen C. University of California, San Francisco, CA; San Francisco General Hospital, San Francisco, CA; and ImmuCell Corp., Portland, ME.

Cryptosporidium parvum causes severe gastrointestinal disease in immunocompromised patients for which there is no treatment. However, oral immunotherapy has improved symptomatology or eradicated disease in AIDs patients. Monoclonal and polyclonal antibodies neutralize *Cryptosporidium* infection *in vitro* and prevent infection in experimental animals. In order to identify immunogenic proteins which might be targets of protective antibody, we have raised antibody reagents (e.g. monoclonal antibodies against sporozoites and intracellular stages, polyclonal mouse sera, and anti-recombinant mouse ascites). We have also radioiodinated sporozoite membranes and immunoprecipitated labeled surface antigens. In addition, we have screened two *Cryptosporidium* genomic expression libraries with the antibodies described above. A highly glycosylated Triton X-100 soluble protein (GP900) was identified in sporozoites and merozoites. GP900 is a surface antigen localized to the anterior end of sporozoites, and presumably present also in apical complex organelles. Monoclonal antibodies to GP900 cross-react with another sporozoite surface antigen of Mr 250 kD (GP250). These surface molecules are the major targets of protective hyperimmune bovine colostral and of several monoclonal antibodies. Clones partially encoding GP900 were identified from our *C. parvum* genomic expression libraries and sequenced. The predicted aminoacid sequence indicates the presence of an unusual aminoacid repeat. Antibody to the recombinant GP900 protein significantly inhibited *Cryptosporidium* infectivity *in vitro* as compared to non-treated and ascites controls.

645 CLINICAL EFFICACY OF AMINOSIDINE SULPHATE IN THE TREATMENT OF AIDS-RELATED CRYPTOSPORIDIOSIS. Scaglia M, Atzori C*, Marchetti G, Olliaro P, Malfitano A, and Maserati R. Inst. Infectious Diseases, University-IRCCS S. Matteo, Pavia; Department Infectious Diseases, Hospital, Busto Arsizio (VA); and Farmitalia-C.Erba, Milano, Italy.

The objective of this study was to assess the clinical efficacy of aminosidine sulphate (AMs) in the treatment of diarrhoeal cryptosporidial enteritis in AIDS patients (pts). 35 adult HIV-pos subjects (26 males, 9 females; mean age: 35.7 yrs, range: 24-48 yrs) with a mean CD4 value of 40.2 cells/mm^3 (range: 4-200), being affected by enteric cryptosporidiosis, were treated by oral administration of AMs (Gabbroral®, Farmitalia-C.Erba) with the following treatment schedule: 2 g/day for 4 weeks, then 1 g/day for 4 further weeks. All pts received the drug for at least 3 weeks (mean: 6.9 weeks; range: 3-8). 15 pts completed the therapeutic protocol. AMs reduced the daily bowel movements in 28 treated pts, abdominal pain in 15, increased stool consistency in 26 and body weight in 15. Weekly scoring of *Cryptosporidium* oocyst number in concentrated and acid-fast stained stool samples demonstrated a

reduction of coccidian burden in 21 (60%) of the pts and disappearance of oocysts in 7 (20%), whereas no changes were observed in 6 (17.14%) and 1 pt (2.86%) deteriorated. In 3 of the 7 pts who cleared *C. parvum* oocysts from the stools, small-bowel biopsy confirmed the absence of parasites. In our trial AMs appeared to be an useful drug that often improves symptoms and, sometimes, induces recovery from cryptosporidiosis in HIV subjects. Further controlled, double-blind therapeutic trials are needed to confirm the efficacy of AMs in cryptosporidial opportunistic enteritis and to determine the optimal dosage and length of the treatment.

646 TESTING ANTI-CRYPTOSPORIDIUM AGENTS IN A CHRONICALLY INFECTED IMMUNODEFICIENT MOUSE MODEL. Leitch GJ* and He Q. Department of Physiology, Morehouse School of Medicine, Atlanta, GA.

Rodent models of crytosporidiosis have been used to assess the efficacy of potential chemotherapeutic agents. In this study, adult athymic nude mice were orally inoculated with *Cryptosporidium parvum* oocysts of calf origin. The resulting *Cryptosporidium* infections lasted until the animals died 6-15 months later. During this period of chronic infection, agents were tested that had been shown by other to ameliorate cryptosporidiosis in dexamethasone (DEX) immunosuppressed rat and mouse models. Dehydroepiandrosterone was administered subcutaneously twice a day (120mg/Kg day) for 8 days or continuously in a subcutaneously implanted timed release capsule (160mg/Kg day) for 21 days without effect on oocyst excretion. When lasalocid was administered orally at a dose approaching the LD_{50} (120mg/Kg day) twice a day for 3 days, there was a small but statistically significant transient reduction in oocyst excretion. Similarly, when this dose of lasalocid was administered for 3 days at the onset of the infection, there was a statistically significant delay in the onset of oocyst excretion following inoculation with 10^5 oocysts. Paromomycin (120mg/Kg day) administered orally twice a day for 8 days also was without effect on oocyst excretion in chronic cryptosporidiosis. These data suggest that chemotherapeutic agent testing performed in DE immunosuppressed rodent models at the onset of a cryptosporidium infection may yield unrealistically promising results while the chronically infected immunodeficient mouse model may more closely mimic the infected AIDS patient in its response to antiparasitic agents.

647 FURTHER EFFICACY EVALUATION OF DICATIONIC MOLECULES AGAINST *CRYPTOSPORIDIUM PARVUM* IN HsD/ICR SWISS MICE. Blagburn BL*, Lindsay DS, Parsons LC, and Rippey NS. Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

Dicationic molecules (pentamidine and its analogues) are broad spectrum antimicrobial agents with demonstrated activity against numerous species of parasitic protozoa and *Pneumocystis carinii*. In previous studies we have demonstrated that the dicationic class of molecules possesses antiparasitic activity against *Cryptosporidium parvum* and *Toxoplasma gondii*, important AIDS-associated opportunistic agents. As part of a continuing series of studies we herein demonstrate activity of additional dicationic molecules against *C. parvum*. All dicationic compounds were solubilized in distilled water or 1% DMSO in distilled water, and evaluated against *C. parvum* utilizing our established suckling murine assay. Compounds and dosages at which they were evaluated were as follows: trans-1.2-Bis (5-amidino-2-benzimidazoyl) ethene (BBE-5 mg/kg]; 2,4-Bis-(4-amidino-phenyl) pyrimidine-trihydrochloride (DB100-25, 5, 0.1 mg/kg]; Bis-(5-amidino-2-benzimidazoyl) ethane (SL 031- 4mg/kg]. All dicationic molecules tested significantly reduced ($p<0.05$) the numbers of oocysts recovered from treated mice compared to diluent-treated control mice. Percent efficacies were as follows: BBE-64%; DB100 (25 mg/kg]-50%; DB100 (5 mg/kg]-68%; DB100 (0.1 mg/kg]-30%; SL 031-62%. A polyether compound maduramicin was shown previously to be highly effective against *C. parvum* in this same murine test system. To validate the assay, maduramycin was tested as a positive control concurrent with the test molecules. Maduramicin at 3 mg/kg significantly reduced oocyst excretion in treated mice by 97% compared to 1% ethanol-treated control mice.

648 *CRYPTOSPORIDIUM* INFECTION INDUCES A DOSE AND TIME DEPENDENT DECREASE IN RESISTANCE ACROSS CACO-2 CELL MONOLAYERS. Griffiths JK*, Moore R, Keusch GT, and Tzipori S. Department of Comparative Medicine, Tufts University Schools of Medicine and Veterinary Medicine; Division of Infectious Diseases, St. Elizabeth's Hospital; Division of Geographic Medine and Infectious Diseases, Tufts-New England Medical Center, Boston, MA; and Department of Pathology, Tufts University Schools of Medicine and Veterinary Medicine, N. Grafton, MA.

Cryptosporidium-associated mucosal injury and diarrhea is poorly understood. As part of our studies on the cellular pathobiology of cryptosoridiosis, we devised a model system with CACO-2 cells grown atop Transwell permeable filters (F). F are seeded with 10^5 cells and develop transmonolayer electrical resistances of ~200 ohm*cm^2 by day 7, a direct electrophysiological measure of passive ion permeability. 10^4-10^6 oocysts (OO) when used to infect cells on day 7 induce significant [$p<0.01$-0.001] decreases in resistance (R) after 24-72 hours, with final R values near those of bare F (~50 ohm*cm^2). The onset of R decline is inversely related to the number of OO. Inactivated oocysts or sporozoites do not induce a fall in R compared to controls. The decline in R does not correlate to cell death as the mean number of cells/F (released with trypsin-EDTA) was unaltered during infection (6.2±0.9 vs. 6.7±1.4 x 10^5 cells/F, uninfected vs. infected). These results suggest that the dose-dependent fall in R, and rise in passive permeability, is due to a direct pathophysiological effect of infection, and not due to a nonspecific toxic effect, and that this model may assist in elucidating the pathophysiology of mucosal injury and diarrhea during *Cryptosporidium* infection.

649 RISK FACTORS FOR CRYPTOSPORIDIOSIS AMONG PERSONS WITH AIDS IN LOS ANGELES COUNTY. Sorvillo FJ, Lieb LE, Ash LR, and Kerndt P*. HIV Epidemiology Program, Department of Health Services, Los Angeles County, CA; and Department of Epidemiology, School of Public Health, University of California at Los Angeles, CA.

The protozoan *Cryptosporidium* is an important cause of severe and untreatable morbidity among persons with AIDS. Yet risk factors and sources of infection in this high risk group are unknown. To attempt to determine potential risk factors and sources of *Cryptosporidium* among persons with AIDS we analyzed the occurrence and determinants of cryptosporidiosis among AIDS patients reported to the Los Angeles County AIDS surveillance registry from 1983-1991. A total of 570 (3.7%) cryptosporidiosis cases among 15688 persons with AIDS were reported. Prevalence was highest among children <5 years of age (6.1%), gay and bisexual men (3.9%) and adult females (3.8%) (odds ratios 3.1, 1.8 and 1.7 respectively, relative to non-gay, adult males). The prevalence of cryptosporidiosis declined with increasing age among gay males ($P<.001$) but not among females and non-gay males. A temporal trend of declining prevalence was observed in reported cryptosporidiosis among gay and bisexual men from 1983-1986 ($P<.001$) which corresponds to a time of modified sexual behavior in this group. No such decrease was noted among females or males in other risk groups. These data suggest the existence of mechanisms including sexual transmission of cryptosporidiosis among gay and bisexual men with AIDS and a possible role of child contact in the transmission of *Cryptosporidium* among women and children with AIDS in Los Angeles County. Such information may be valuable in recommending strategies for prevention.

650 *CRYPTOSPORIDIUM* INFECTIONS IN A SUBURBAN COMMUNITY IN MARACAIBO, VENEZUELA. Chacin-Bonilla L*, de Young MM, Cano G, Guanipa N, Estevez J, and Bonilla E. Instituto de Investigacions Clinicas, Universidad del Zulia, Maracaibo, Venezuela.

A point prevalence survey for *Cryptosporidium* was conducted in 212 subjects two months to 70 years of age in a suburban area with a low socioeconomic status in Maracaibo City, Venezuela. Single stool specimens were collected. Modified Ziehl-Neesen carbol-fuschin staining of 10% formalin-

preserved stool was used to identify *Cryptosporidium* oocysts. Direct wet mounts, iron-hematoxylin-stain smears and formalin-ether concentrates were examined to determine the presence of other intestinal parasites. *Cryptosporidium* infections were identified in 21 subjects (9.9%), with a high percentage of asymptomatic carriers (15 of 21, 71.4%). Six children (28.5%) has gastrointestinal symptoms and four of them were infants. *Cryptosporidium* was the single detectable potential pathogenic parasite in only five (23.8%) of 21 patients. The infection rate with one or more parasites was high (82.0%) and multiple infections, including pathogenic helminths and protozoa, were observed in the majority of patients who passed oocysts. Our findings suggest that although *Cryptosporidium* is an important pathogen, the proportion of asymptomatic carriers may be high in areas of low socioeconomic status in developing countries.

651 IMPROVED METHODS FOR ASSESSING CRYPTOSPORIDIAL PARASITEMIA USING FLOW CYTOMETRY. Arrowood MJ*, Hurd MR, Brandt FB, and Mead JR. Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta, GA; Emory University, Atlanta, GA; and VA Medical Center, Decatur, GA.

A flow cytometric method for the quantitation of *Cryptosporidium parvum* oocysts in stool specimens was developed to replace conventional immunofluorescent microscopic assays (IFA). Fecal pellets were collected from control (uninfected) severe combined immune deficient (SCID) mice, suspended in 2.5% $K_2Cr_2O_7$ at a ratio of 400 µl per pellet and homogenized by vortexing. Purified oocysts were added to the samples (10^5, 10^4, 10^3 and 10^2 per ml). Aliquots (200 µl) of the vortexed samples were centrifuged over micro-scale discontinuous sucrose gradients essentially as previously described. The oocyst-containing fractions were collected, washed, and incubated with an oocyst-specific monoclonal antibody (labeled with fluorescein isothiocyanate) for 30 min at 37C. Samples were adjusted to 600 µl with PBS and assayed using logical gating of forward/side scatter and fluorescence signal on a Becton Dickinson FACScan flow cytometer. Seeded samples showed a linear correlation in the number of oocysts recovered from the gradients. Analyses of stool samples from chronically infected SCID mice revealed that the flow cytometric method was approximately 10 times more sensitive than IFA.

652 CHRONIC DYSENTERY, THE OLD NAME OF AMEBIC DYSENTERY. Acuna-Soto R*. Division of Infectious Diseases, Beth Israel Hospital, Harvard Medical School, Boston, MA; Department of Tropical Public Health, Harvard School of Public Health, Boston, MA.

This work is based in the medical literature before 1875,the year of the description of *Entamoeba histolytica* by Fedor Alexandrovich Lesh. Reports of diseases compatible with amebic dysentery before that year have been dismissed with the argument that dysentery can be caused by numerous conditions. This report has two aims: i) To establish that the disease we know today as amebic dysentery was known by centuries as chronic dysentery. The disease was accurately defined, it was described by Hippocrates and many Roman and medieval phisicians. ii) To acknowledge the first description of the trophozoites of *Entamoeba histolytica* to William Baly, who described trophozoites in 1849. His observation has been forgotten for more than one hundred years since it was published before the descriptions of Lesh, Lamb or Lewis. Chronic dysentery was defined as the occurence of attaks of variable severity of a disorder characterized by passing numerous stools with abundant mucus mixed with blood and intense tenesmus for many weeks months or years. Descriptions of chronic dysentery emphasized its cruelty and long duration. Patients with chronic dysentery were helplessly consumed and died in a state of cachexia. Some of the arguments in favor of the relationship of chronic dysentery with amebic dysentery are: 1). There were many reports in which chronic dysentery was diagnosed before or simultaneosly to the occurence of liver abscess. Cases resembling fulminating colitis and ameboma were also associated with chronic dysentery. 2). The use of large doses of ipecacuanha, plant from which emetine is extracted, produced good results in many cases. 3). The geographical distribution corresponds to that of amebic dysentery. 4). Colonic ulceration

in chronic dysentery was well known. Finally, it is important to remember that the patient J. Markow in which Lesh described trophozoites of *Entamoeba histolytica* in 1875 was diagnosed as having chronic dysentery. He suffered periodic attaks of dysentery for two years before he died in a state of "severe anemia and generalized exhaustion".

653 STUDY OF THE SERUM IgM ANTIBODY RESPONSE TO THE GALACTOSE-INHIBITABLE ADHERENCE PROTEIN OF *ENTAMOEBA HISTOLYTICA*. Abd-Alla MD, Jackson TF, El-Hawey AM, and Ravdin JI*. Case Western Reserve University and the Cleveland VA, Cleveland Ohio; El-Hussain University Hospital, Cairo, Egypt; and Medical Research Council (Natal), South Africa.

Serum IgG antibodies to the galactose-inhibitable adherence protein (GIAP) aren't usually found until 7 days of illness; serum GIAP antigen can be detected acutely. We found serum anti-GIAP IgM antibodies in 55% of 100 patients with amebic colitis, 78% of 12 with amebic liver abscess, 3% of regional controls, 13% with asymptomatic intestinal infection with culture-proven nonpathogenic zymodemes, and none of 27 with other parasitic infections ($p<0.01$ for ALA and colitis compared to other groups). In the 69% of amebic colitis patients who lacked serum IgG ab. to the GIAP, 41% had serum anti-GIAP IgM abs. All IgG (-) IgM (+) sera contained serum GIAP antigen as detected by ELISA with anti-GIAP monoclonal abs. Of patients with a clinical diagnosis of amebic colitis, 23% were negative for serum anti-GIAP IgG and IgM and did not have serum GIAP antigenemia. Therefore, despite a (+) stool microscopy, their symptoms were likely not due to pathogenic *Entamoeba histolytica* infection. In conclusion, ELISA for serum anti-GIAP IgM ab is more useful than study of IgG ab in the setting of acute amebic colitis (< 7 days), but is no more sensitive than assay of serum GIAP antigen.

654 TRANSFORMING GROWTH FACTOR-β_1 PRIME MACROPHAGES FOR AN ENHANCED NITRIC OXIDE-DEPENDENT CYTOTOXICITY AGAINST *ENTAMOEBA HISTOLYTICA*. Lin JY*, Seguin R, Keller K, and Chadee K. Institute of Parasitology, McGill University, Ste Anne de Bellevue, PQ. Canada.

Nitric oxide (NO) produced by activated macrophages is the major cytotoxic molecule for cytotoxicity against *E. histolytica* trophozoites. Transforming growth factor-β_1 (TGF-β_1) is a potent negative regulator of several macrophage functions including NO production. In this study we investigated the effect of TGF-β_1 on macrophage amoebicidal activity and the underlying mechanisms involved. TGF-β_1 by itself was incapable of inducing mouse bone marrow-derived macrophage (BMM) amoebicidal activity and nitric oxide (NO) production (as measured by nitrite). In constrast, TGF-β_1 pretreatment (4 hr, 2ng/ml) primed BMM for an enhanced amoebicidal activity by 15% and 23% in response to IFN-γ + TNF-α or IFN-γ + LPS, concomitant with increased NO production by 85% and 27%, respectively. The priming effect of TGF-β_1 on NO production was dependent on the concentration of LPS (>10ng/ml), but independent of TNF-α triggering dose (100-1000 U/ml) in the presence of IFN-γ (100 U/ml). TGF-β_1 pretreatment increased the kinetics (4-48hr) of NO production in response to IFN-γ + TNF-α/LPS stimulation. By Northern blot analysis, the increased NO production of TGF-β_1 pretreated BMM was preceded by markedly enhanced expression of mRNA for the inducible form of macrophage NO synthase (mac-NOS). These results demonstrate that TGF-β_1 can prime BMM for increased mac-NOS mRNA expression for NO-dependent cytotoxicity against *E. histolytica* in response to IFN-γ + TNF-α/LPS.

655 CLONING, STRUCTURE AND EXPRESSION OF A MYOSIN LIGHT CHAIN KINASE GENE OF *ENTAMOEBA HISTOLYTICA*. Que X* and Reed SL. Department of Pathology, UCSD Medical Center, San Diego, CA.

The motility of *Entamoeba histolytica* trophozoites is important for invasion of host tissue. Phosphorylation of myosin light chains by myosin light chain kinase (MLCK) is required for cross-linking F-actin to generate the contractile component of amebic movement. To explore the role of the MLCK and associated signal-transducing pathways in the regulation of invasion, we amplified a gene fragment of a myosin light chain kinase by PCR, and isolated the full-length gene from an *E. histolytica* genomic library. This gene, designated *Ehmlck*, contained a 358 bp intron in the catalytic domain. The only other intron described in *E. histolytica* is in a protein kinase $p34^{cdc2}$ homologue. The deduced amino acid sequence of the catalytic domain (321 aa) matched most closely with the *Dictyostelium* MLCK (48% identity) and showed high overall homology to the calcium/calmodulin-dependent protein kinase family (37% - 43% identity). The amino terminal portion of the sequence (139 aa) was highly positively charged and contained a nuclear localization signal sequence. The catalytic domain of *Ehmlck* from *E. histolytica* cDNA was amplified and fused in frame into the pGEX-KG expression vector. The fusion protein, GST-EhMLCK, was expressed in *E. coli*, and purified for further characterization. This is the first myosin light chain kinase homologue isolated from *E. histolytica* and should allow further analysis of the regulation of amebic motility and its role in invasion.

656 IN VITRO ACTIVITY OF ATOVAQUONE AGAINST *ENTAMOEBA HISTOLYTICA* AND *E. DISPAR*. Ashley LS*, Ragland BD, Rogers MD, Petri WA. University of Virginia, Charlottesville, VA; and Burroughs Wellcome, Research Triangle Park, NC.

Current therapy of amebiasis is complicated by the need to use different drugs for the different sites of infection (bowel lumen, intestinal submucosa and liver) and by drug toxicities. Atovaquone (1,4-hydroxy-naphthoquinone) is known to have activity against *Plasmodium falciparum, Toxoplasma gondii* and *Pneumocystis carinii*. We evaluated its activity against *Entamoeba histolytica* and *E. dispar* (formerly called nonpathogenic *E. histolytica*) trophozoites *in vitro*. Atovaquone was added at time 0 in DMSO (final concentration 1.0% in drug-treated and control cultures) to trophozoites cultured in medium TYI-S-33. Parasite viability was assessed microscopically at 24 to 96 hours. At a mean concentration of 50 μg/ml atovaquone we observed 60% killing of *E. histolytica* at 48 h, 90% kill at 72 h and 100% kill at 96 h. At a concentration of 100 μg/ml atovaquone killed 97% of *E. histolytica* trophozoites at 24 h. Atovaquone (50 μg/ml) killed 73% of *E. dispar* at 72 h. Conclusion: In these *in vitro* studies atovaquone achieved significant killing of *E. histolytica* and *E. dispar* at concentrations achievable *in vivo*. Further studies are needed to determine the role of this drug in treating amebiasis.

657 MONOCLONAL ANTIBODY-BASED ELISAS TO DETECT *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* INFECTION IN STOOL. Haque R, Kress KD, Lyerly D, Wilkins T, and Petri, Jr. WA*. Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh; TechLab Inc., Blacksburg, VA; and University of Virginia, Charlottesville, VA.

Two morphologically identical but genetically distinct species of *Entamoeba histolytica* exist, only one of which causes amebic liver abscess and colitis. Current diagnosis of *E. histolytica* infection requires the direct microscopic identification of the parasite, a technique that is insensitive and which cannot distinguish the pathogenic (*E. histolytica*) from the noninvasive species (now renamed *E. dispar*). Previously we reported the use of *E. histolytica*-specific monoclonal antibodies (mAbs) in a stool antigen detection test, as described previously. We have now used mAbs directed against cross-reactive and *E. histolytica*-specific epitopes of the amebic galactose adhesin in ELISAs to distinguish *E. histolytica* from *E. dispar* infection in stool. These assays were applied to single stool specimens from 141 patients in Bangladesh, including 33 stools with culture and zymodeme-confirmed *E. histolytica* infection, 38 with culture and zymodeme-confirmed *E. dispar* infection, and 70 specimens that were negative by microscopy for *Entamoeba*. The assay using *E. histolytica* -specific mAbs was positive in

32/33 *E. histolytica* (+)stool specimens, 3/38 *E. dispar* (+) stools, and in 3/70 patients with other or no intestinal parasites, for a specificity and sensitivity for the detection of pathogenic *E. histolytica* infection of 94% and 97% respectively. The assay using cross-reactive mAbs was positive in 20/21 *E. histolytica* (+) stools, 13/16 *E. dispar* (+) stools and 1/30 stools negative by microscopy for *Entamoeba*, for a specificity and sensitivity of 97% and 89% respectively. As the ELISA tests can be completed in several hours, they offer promise as rapid and sensitive means to detect infection by *E. histolytica* and *E. dispar*.

658 AMEBICIDAL AND TRICHOMONICIDAL ACTIVITY OF NALIDIXIC ACID. Anaya-Velazquez F*, Lopez-Godinez J, and Moreno-Rodriguez M. I.I.B.E., Fac. de Quimica, University de Guanajuato, Guanajuato, Gto. Mexico.

Amebiasis caused by *Entamoeba histolytica* and urogenital trichomoniasis produced by *Trichomonas vaginalis* are commonly treated with metronidazole (MET). Although resistant amebas to Met have not been reported, natural resistant can be found in *Trichomonas* obtained form patients. In order to search for alternative drugs we have previously showed that nalidixic acid (HNal) has amebostatic activity *in vitro* (88 μg/ml). In this work we investigated by three methods in test tube if HNal has *E. histolytica* is killed by 385 μg/ml of HNal. Axenic amebas required less drug than monoxenic amebas (*C. symbiosum*-associated). When amebostatical concentrations of HNal were added to amebas in phase log of growth amebas were almost not sensible to drug. However, amebicidal concentrations were effective at all times of amebal growth curve. On the other hand, five axenic strains of *Trichomonas* showed high sensitivity to 100 μg/ml of HNal but they differed in its sensitivity (76-95%). We did not find a correlation between resistance to metronidzole and synergism between metronidazole and HNal against amebas. Finally, besides being inhibited in their growth by HNal, ultrastructurally both parasites showed severe alterations in cytoplasm, vacuoles, nucleus, and cell shape after being treated with lethal concentration of HNal (400 μg/ml for amebas and 200 μg/ml for *Trichomonas*). Our results suggest HNal is active *in vitro* against both *E. histolytica* and *T. vaginalis*. Therefore, this drug could be potentially used for the treatment of patients harboring these parasites.

659 ISOLATION FROM *GIARDIA* OF A GENE ENCODING FIBRILLARIN, A PROTEIN REQUIRED FOR PRE-RIBOSOMAL RNA PROCESSING. Narcisi EM*, Glover CV, and Fechheimer M. Department of Zoology, University of Georgia, Athens, GA; and Department of Biochemistry, University of Georgia, Athens, GA.

Giardia lamblia is a flagellated protozoan parasite shown by 16sRNA sequence comparisons to be one of the most primitive of the eukaryotes. We have isolated a gene encoding *Giardia* fibrillarin, a protein involved in pre-rRNA processing, methylation, and ribosome assembly. The fibrillarin gene was isolated from a *Giardia* genomic library in phage λ EMBL3 kindly supplied by Dr. D. Peattie. The sequence of 1200 base pairs contains an open reading frame of 981 base pairs (327 amino acids). The deduced protein sequence of 35.3 kDa is similar to other known fibrillarin sequences, containing a glycine rich amino terminal region, and a highly conserved putative RNA binding domain. Antibodies to yeast fibrillarin (kindly supplied by Dr. J. Aris) cross-react with a 36 kDa polypeptide from *Giardia* on Western blots, and stain both nuclei of *Giardia* by immunofluorescence microscopy.

660 PRODUCTION AND SECRETION OF PROTEASES BY *GIARDIA LAMBLIA* TROPHOZOITES. Alvarado L*, Cedillo-Rivera R*, Munoz O, Ortega-Pierres MG. U.I.M.E.I.P., Hospital de Pediatria, CMN SXXI, IMSS., Mexico, D.F., Mexico; Department of Genetics and Molecular Biology, CINVESTAV-IPN, Mexico, D.F., Mexico.

Protease activity in some microorganisms has been related to their virulence and invading capacity . In *Giardia lamblia* there are few reports regarding the study of protease activity. To further study

proteases of this parasite and their role in host-parasite relationship, protease activity was analyzed in isolates and clones of *G. lamblia* as well as in the interaction of the parasite with MDCK cells. Protease activity was determined in extracts of six isolates from asymptomatic carriers, two from symptomatic patients and strains WB and P-1. Secretion of proteases was analyzed in concentrated supernatant of clones cultured alone or after their interaction with MDCK cells. Electrophoretic analyses of trophozoite lysates in SDS-PAGE co-polymerized with 0.2% gelatin revealed 10 bands of protease activity with M.W. from 18 to185 KDa. An increased number of bands was observed when trophozoites were maintained in continuous cultures and the pattern of activity was similar in all isolates. Analysis of concentrated supernatants from trophozoites cultured alone showed only four bands with low protease activity and M.W. between 21 and 85 KDa. Interestingly, an increased protease activity of such molecules and other 3 was observed in supernatants of co-cultures of trophozoites with MDCK cells. These results suggest that clones and isolates of *G. lamblia* express similar proteases. Some of these are *in vitro* secreted and increase upon interaction of trophozoites with MDCK cells.

661 RISK FACTORS FOR DEVELOPMENT OF FIRST SYMPTOMATIC *GIARDIA* INFECTIONS AMONG INFANTS OF A BIRTH COHORT IN RURAL EGYPT. Mahmud MA*, Chapell C, Hossain M, Habib M, and DuPont HL.

Giardia infection is associated with diarrheal diseases in both developed and developing countries among infants and young children. A study was conducted to demonstrate the predisposing factors for developing first symptomatic infection among infants in rural Egypt. The study cohort was followed from birth to first year of life. Univariate and multivariate analyses of data revealed that infants under six months age were at special risk (RR=12.3; CI=5.5-27.5; $p<0.001$) for developing first symptomatic infection compared to infants above six months. Other factors that were associated with increased risk include household without latrine (RR=2.63; CI=1.4-4.9; $p<0.05$), floor of sleeping rooms constructed of mud (RR=1.79; CI=1.03-3; $p<0.05$), infants, family owned one or more buffalos (RR=1.99; CI=0.86-4) and family having more than ten chickens (RR=2.5; CI=1.13-5.56; $p<0.05$). In contrast, mother's education above primary level (RR=0.28; CI=0.09-0.85; $p<0.05$), drinking water stored in metallic containers (RR=0.33; CI=0.11-0.98; $p<0.05$), infants' family owned a color TV set (RR=0.18; CI=0.03-1.3) and male sex (RR=0.52; CI=0.3-0.89; $p<0.05$) had decreased risks for infections. The authors concluded that infants at an earlier age (within six months) were more prone to develop first symptomatic infection. It was also concluded that besides immunity, poverty, low education, gender discrimination and certain environmental conditions potentiate the risk for developing first symptomatic infection.

662 DETECTION OF *GIARDIA* CYSTS IN FOODS USING DIRECT IMMUNOFLUORESCENCE. Dixon BR*. Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada.

Several foodborne outbreaks of giardiasis have been reported in North America within the last fourteen years. In the majority of cases, the food was apparently contaminated through the unhygienic practices of food handlers who were either infected and shedding cysts themselves, or who had been in close contact with an infected individual, such as an infant in diapers. While a variety of food items have been implicated in such outbreaks, *Giardia* cysts are rarely detected in the suspect food. Commercial kits are available for the detection of *Giardia* cysts in environmental samples and clinical specimens by means of direct immunofluorescence. However, the use of immunofluorescence in the detection of cysts in foods has not been previously reported. An evaluation was performed on the effectiveness of this technique on cyst-spiked foods. Preliminary results indicate that direct immunofluorescence is considerably more sensitive than conventional methods, and may be useful in positively identifying the source of *Giardia* infection in any future foodborne outbreaks.

663 DETECTION AND DIFFERENTIATION OF *GIARDIA LAMBLIA* AND *GIARDIA MURIS* CYSTS IN SURFACE WATER BY IMMUNOFLUORESCENCE FLOW CYTOMETRY. Stibbs HH*. Department of Cell and Molecular Biology, Tulane University, New Orleans, LA.

Cysts of *Giardia lamblia* and of other *Giardia* species and strains are common contaminants of surface water throughout North America. These cysts at times survive municipal drinking water treatment, including chlorination, and infect consumers of the treated water. Many cities now test incoming untreated surface water as well as their treated water for the presence of *Giardia* cysts. The current EPA-endorsed method for cyst detection involves immunofluorescence microscopy with sediment obtained from wound filters; this method is very time-consuming, and does not permit the differentiation of cysts of *Giardia lamblia* strains from those of *G. muris*. We now report that immunofluorescence-based flow cytometry, coupled with the use of species-specific, FITC-labeled monoclonal anti-cyst antibodies developed in our laboratory, can reliably detect and quantitate cysts of these species in sediment obtained from surface water, and can differentiate *G. lamblia* (including all *duodenalis* types) from *G. muris*. The single-step antibody treatment of the parasite concentrates obtained from the surface water sediment takes place in a centrifuge tube and requires only about 45 minutes. Subsequent analysis by flow cytometry requires less than 10 minutes of analysis per sample. Results are totally quantitative and are stored on microcomputer diskettes.

664 *BLASTOCYSTIS HOMINIS* AND *CRYPTOSPORIDIUM* IN PATIENTS WITH DIARRHOEA IN SLOVENIA. Logar J*, Andlovic A, Poljsak-Prijatelj M, and Golog V. Institute of Microbiology, Medical Faculty, University of Ljubljana, Zalska, Slovenia.

Blastocystis hominis, previously considered a harmless yeast, is now classified as a protozoon. The association of *B. hominis* with human diarrhoea has been controversial. *Cryptosporidium* is a well-known protozoan parasite causing diarrhoea in animals. Also, it may produce self-limited diarrhoea in immunocompetent humans, and even fatal forms of diarrhoea in immunologically deficient patients.The purpose of this study was to evaluate the occurence of both parasites as potential diarrhoeal agents in the faeces from 1,066 patients examined between Sept. 1, 1992 and the end of March 1993. None of them was infected with HIV. Patients of both sexes were divided into two groups as follows: a group of 645 younger subjects, ranging in age from one month to 14 years, and a group of 421 subjects 14 to 90 years of age. For the detection of *B. hominis* and *Cryptosporidium* the Gomori's trichrome modification procedure and safranin-methylene-blue stain, respectively, were used. The faeces was also examined for other enteropathogenic parasites, bacteria and rotavirus. No significant differences were found between males and females in any age group. There were no significant differences between the patients from different geographic areas either. Of the 1,066 diarrhoeal faeces, 39 (3.7 %) were positive for *Blastocystis hominis*. The median age of *B. hominis*-positive patients was 5.5 years in the younger group, and 62 years in the older group. *B. hominis* alone was more frequently identified in the older patients (9 of the 12 patients), as compared to the younger ones (2 of the 27 patients) ($p<0.005$). Of the 1,066 stool samples examined, 217 (20.4 %) were positive for *Cryptosporidium* . The median age of *Cryptosporidium* -positive patients in the younger group was 3 years, and in the older group 55 years. Also, *Cryptosporidium* oocysts alone were more frequently detected in the older group (70 of the 98 patients) than in the younger patients (69 of the 119 patients) ($p<0.05$). The presence of these parasites in the faeces samples from patients with diarrhoea in which any other enteropathogen has been ruled out suggests an etiology that should get more attention in Slovenia in future.

665 *BLASTOCYSTIS HOMINIS*: A CAUSE OF DIARRHEA IN PRE-SCHOOL CHILDREN. Nimri LF*. Department of Biological Sciences, Jordan University of Science and Technology, Irbed, Jordan.

Blastocystis hominis is now gaining acceptance as an agent of human intestinal disease. A case-control study was conducted to study the cause of gastroenteritis in children (<) 6 years old. A total of 500 stool specimens were examined by wet mount preparations, formaline ether concentration, Sheather's sugar flotation technique, and permenant stains when necessary. *Blastocystis hominis* was found in 63/250/(25%) of the stool specimens examined of the cases, was noticed that the appearance of severe symptoms was associated with the number of the parasites in the diarrhiec specimens ((>5) parasites per 400x field). The most common symptoms were abdominal pain, recurrent diarrhea, cramps, anorexia, and fatigue. Contaminated water is suspected to be the major source of infection, since several cases were associated with *Giardia*. These findings support the concept of considering *Blastocystis hominis* as a potential pathogen in children with gastroenteritis.

666 TAXONOMIC UNCERTAINTY OF *BLASTOCYSTIS* (PROTOZOA:SARCODINA). Hollebeke NL* and Mayberry LF. Department of Biological Sciences, University of Texas at El Paso.

The taxonomic status of *Blastocystis* has long been controversial. Recently, however, it was suggested that *Blastocystis* be assigned its own subphylum (Blastocysta) based on a newly proposed life cycle. The concept of "expressional forms" is preferred to "life cycle stages", since arguments can be made that these forms are produced by regulatory genes acting upon a small suite of paralogous genes. The primitive "expressional forms"was considered to be the amoeboid state, rather than the vacuolated form described by Jiang and He, and the other forms derived states. The taxonomy of the parasites allocated to the genus *Blastocystis* is based more on a shared absence of character states rather than presence. A clandistic analysis of described *Blastocystis* species utilizing various characters suggests that these species are monophyletic with respect to the sarcodina outgroup taxa, but the results were ambiguous with respect to the relationship of the genus to these outgroups. The justification for assigning *Blastocystis* a separate subphylum breaches the current accepted criteria for macrotaxonomy. Preliminary indications argue that the genus *Blastocystis* be placed *incerta sedis* with the Protozoa until more comprehensive studies can be conducted.

667 LYME DISEASE IN THE RHESUS MONKEY, I: A MODEL FOR THE INFECTION IN HUMANS. Philipp M*, Aydintug MK, Bohm, Jr. RP, Cogswell FB, Dennis VA, Gu Y, Lanners HN, Lowrie, Jr. RC, Roberts ED, Conway MD, Gubler DJ, Johnson BJ, and Piesman J. Departments of Parasitology, Veterinary Sciences and Pathology, Tulane Primate Center, Covington, LA; Centers for Disease Control and Prevention, Fort Collins, CO; and Louisiana State University Eye Center, New Orleans, LA.

The study population consisted of 12 male, 2-year-old Chinese *Macaca mulatta*. Three animals were given *Borrelia burgdorferi* (strain JD1) by needle inoculations, 6 were exposed to the bite of infected *Ixodes dammini* nymphs, and 3 animals were uninfected controls. Clinical, bacteriological, immunological and pathological signs of infection were searched for over a 13-week period after inoculation. *B. burgdorferi* could be recovered from all animals given the spirochete. Spirochetes were detectable until week 6 post-inoculation (PI) in blood, until week 8 PI in skin biopsies taken near the inoculation site, and at 10 weeks PI in the conjunctiva of one animal with conjunctivitis. Erythema migrans (EM) appeared in one of the 3 needle-inoculated animals and in 5 of 6 infected by tick bite, but in no control monkeys. Deep dermal perivascular lymphocytic infiltrations (characteristic of human EM) were observed in all tick-infected animals and in the one needle-inoculated monkey showing gross EM, but not in the other 2 needle-inoculated animals or in controls. In addition, lethargy, splenomegaly, bradycardia and marked CSF pleocytosis were noted. The appearance rate of IgM and IgG antibodies to *B. burgdorferi* as well as the spectrum of antigens recognized were remarkably similar to those observed in infected humans. Serum antibodies from infected animals were able to kill *B. burgdorferi in vitro* in the presence of rhesus monkey complement. PBMC blastogenic responses were observed early in infection followed, as it often happens in humans, by a period of *B. burgdorferi* antigen-specific immune unresponsiveness. The

rhesus monkey appears to be a useful model for investigating the immunology and pathogenesis of Lyme disease and for the development of diagnostic, chemotherapeutic, and immunoprophylactic protocols.

668 LYME DISEASE IN THE RHESUS MONKEY, II: LONGITUDINAL ASSESSMENT OF THE IgM AND IgG ANTIBODY RESPONSES TO *BORRELIA BURGDORFERI*. Aydintug MK and Philipp M*. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

We investigated the IgM and IgG antibody responses of rhesus macaques (*Macaca mulatta*) against *Borrelia burgdorferi* (strain JD1) by Western blot. Animals were studied for 54 weeks post-infection (PI), and were infected either by tick bite (3 animals) or needle inoculation (3 animals). The IgM response peaked between weeks 2 and 5 PI. Major bands were visualized at 18.0, 25.0, 38.5, 39.5 (P39), 42.0 (flagellin), and 92.0 (P93) kDa regions. After the first 4-5 weeks PI all animals produced IgG antibodies to antigens of 18.0, 25.0, 28.5, 30.5, 39.5 (P39), 42.0 (flagellin), 48.8, 49.5, 63.0, and 92.0 (P93) kDa. These results illustrate the similarity between the antigen recognition pattern of humans and rhesus monkeys. As found before in other model systems, antibodies to OspA and OspB antigens were detectable in needle-inoculated monkeys, but not in tick-inoculated animals. The anti-OspA/B IgG responses were evident by week 2 PI, and disappeared by week 10 PI. Responses to a few other antigens were also transient, and new antigenic specificities were recognized after week 20 PI. This latter observation, previously made in rodents, indicates that antigenic mutation (or variation), phenomena hitherto observed only *in vitro*, may also occur *in vivo*. Several antigens were uniformly recognized throughout the 54-week period investigated.

669 LYME DISEASE IN THE RHESUS MONKEY, III: LONGITUDINAL ASSESSMENT OF ANTIBODY-DEPENDENT COMPLEMENT-MEDIATED KILLING OF *BORRELIA BURGDORFERI*. Gu Y*, Aydintug K, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

The role of rhesus monkey (*Macaca mulatta*) antibody and complement in the *in vitro* killing of *Borrelia burgdorferi* was investigated as function of time and mode of infection. Three needle-inoculated, two tick-inoculated and one control animal were investigated throughout a 36-week period post-infection (PI). Complement alone killed only 10% of the bacteria after 6 hours of incubation. Addition of serum antibodies from both tick-inoculated and needle-inoculated animals enhanced this % killing significantly. Even though the heat-inactivation of the complement eliminated the antibody-dependent killing in most animals, some animals developed complement-independent borreliacidal antibodies in low concentrations and only at some time points PI. Treatment of early serum samples with anti-human IgM, and late serum samples with anti-human IgG antibodies abrogated the killing. Antibody-dependent complement-mediated killing was also significantly inhibited ($P<0.05$) by recombinant OspA and B antigens when serum samples from needle-inoculated, but not tick-inoculated, animals were preincubated with these antigens. In addition, in the presence of rhesus monkey complement, mouse monoclonal antibodies against OspA and B antigens were also able to kill *B. burgdorferi*. Killing antibody titers varied concomitantly with anti-OspA/B antibody levels as a function of time PI. These results indicate that experimentally infected rhesus macaques produce antibodies capable of mediating killing of *B. burgdorferi* in the presence of complement, and that these antibodies target, at least in part, OspA and B molecules. However, since antibody-dependent complement-mediated killing of the spirochete persisted in the absence, or in the presence of antigen-bound, anti-OspA/B antibodies, other surface-antigens must also be targeted by this important host-protective immune effector mechanism.

670 LYME DISEASE IN THE RHESUS MONKEY, IV: TISSUE LOCALIZATION OF SPIROCHETES BY PCR. Cogswell FB*, Roberts ED, Lowrie, Jr. RC, Lanners HN, and Philipp M. Departments of Parasitology and Pathology, Tulane Regional Primate Research Center, Covington, LA.

We have shown that the rhesus monkey is a suitable model for the investigation of the pathogenesis of Lyme disease. We sought to address the question of the location of spirochetes in the host relative to the course of infection and disease. To that end, a group of 5 rhesus monkeys were infected by tick bite with the JD-1 strain of *Borrelia burgdorferi*. Samples taken for culture and examination by PCR included skin, blood, cerebral spinal fluid (CSF), urine, spleen, lymph nodes, lung, kidney, bladder, liver, joints, and ocular tissues. All tissues and negative culture supernatants were examined for the presence of *B. burgdorferi* DNA with the PCR using primers specific for both chromosomal and plasmid-derived DNA fragments. During the first 8 weeks post infection (PI) we were able to find the spirochete in skin, blood, and conjunctival tissues, but not in urine or CSF. We are currently examining tissues taken at 6 months PI for evidence of the spirochete. Knowledge of the distribution of spirochetes in the monkey model would provide singular insight into the course of infection and dissemination of bacteria in the human host.

671 LYME DISEASE IN THE RHESUS MONKEY, V: LONGITUDINAL ASSESSMENT OF LYMPHOPROLIFERATIVE RESPONSES TO *BORRELIA BURGDORFERI* ANTIGENS. Dennis VA, Lasater BL*, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA.

Little is known about the role of cell-mediated immune responses in the pathogenesis of, and protective immunity against, Lyme borreliosis. Human peripheral blood mononuclear cells (PBMCs) proliferate in response to *B. burgdorferi* (Bb) antigens *in vitro*, but these cells can often be unresponsive at certain points during infections in humans. To gain insight into the dynamics of cell-mediated immunity in Lyme disease we investigated PBMC proliferative responses to Bb antigens *in vitro* in the rhesus monkey model over a period of 90-weeks post-infection (WPI). Three needle- inoculated animals (NIA), 6 tick- inoculated animals (TIA) and three controls were used. Inoculations were with the Bb strain JD1. Significant responses (SI >3.85) were observed in 2 of the 3 NIA and in 5 of the 6 TIA, but these fluctuated over the 90-wk period. An early response was detected between 2-8 WPI; this response was marked in the NIA ($22 \leq SI \leq 44$) and less pronounced in the TIA ($4 \leq SI \leq 17$). A transient period (10-22 WPI) of low or no responsiveness was followed by an enhanced and sustained response appearing between 24-52 (NIA) and 24-38 (TIA) WPI. A second interval of unresponsiveness was observed in NIA between 54 and 84 WPI, and in all but one of the TIA between 42 and 90 WPI. Throughout the study period PBMCs continually responded to Con A. The apparent Bb antigen-specific unresponsiveness was not a consequence of T cell receptor dysfunction, since cells from all animals responded in a one-way allogeneic MLR assay. At selected times, but not always, PBMCs of unresponsive animals proliferated in response to *Candida albicans* antigens, thus indicating the specificity of the unresponsiveness. After the 10th WPI the IgG antibody levels to Bb antigens, as well as the spectrum of antigens recognized remained largely unchanged. However, several new antibody specificities appeared at different times PI, probably due to the emergence of mutant or variant spirochetal populations. The fluctuating lymphoproliferative responses could also be due to this phenomenon and/or to infection-induced immunoregulatory mechanisms as yet undefined. These mechanisms sound a note of caution for the interpretation of the immunological status of Lyme disease patients on the basis of single time point measurements of their PBMC blastogenic responses to Bb.

672 POTENTIAL DIAGNOSTIC ANTIGENS FOR LYME DISEASE RECOGNIZED BY HUMANS AND RHESUS MONKEYS. Povinelli L*, Burkot T, Johnson B, and Philipp M. Department of Parasitology, Tulane Regional Primate Research Center, Covington, LA; and Centers for Disease Control and Prevention, Fort Collins, CO.

Diagnosis of Lyme disease, a disorder caused by the spirochete *Borrelia burgdorferi*, is complicated by the fact that the spirochete cannot be recovered reliably from cultures of patients' body fluids or tissues (other than erythematous skin) and because the available diagnostic tests are insufficiently sensitive and specific, and often imprecise. This situation could be improved if the *B. burgdorferi* antigens recognized by human antibodies were defined accurately as a function of time after infection. Requisites to achieve such a temporal definition are 1) certainty that the infection has occurred, 2) precise knowledge of the time of infection and the *B. burgdorferi* strain causing it, 3) availability of serum samples collected frequently and longitudinally. Only a few samples satisfying these criteria are presently available. A plausible alternative could be an animal model which, on the one hand, was sufficiently close to the human taxonomically to permit the detection of antibody using the same reagent used for humans and, on the other, would mirror the clinical and immunological manifestations of Lyme disease. Recently, we demonstrated that the rhesus monkey infected with *B. burgdorferi* is indeed such a model. Here we present an initial Western blot longitudinal study in which the antigens recognized by serum antibodies from 9 Lyme disease patients that satisfy some of the above criteria and the antigens recognized by serum antibodies from 6 rhesus monkeys are compared over a time period of 12 weeks post-infection. Two antigens, Mr 27 ± 1 and 47 ± kDa, were uniformly recognized by all human and non-human primate serum samples.

673 PREDICTING *IXODES SCAPULARIS* ABUNDANCE ON WHITE-TAILED DEER USING GIS. Glass GE*, Amerasinghe FP, Morgan JM, Scott TW. Department of Immunology & Infectious Diseases, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD; Department of Entomology and Center for Agricultural Biotechnology, University of Maryland, College Park, MD; and Department of Geography and Environmental Planning, Towson State University, Towson, MD.

We collected 1410 *Ixodes scapularis* from 139 white-tailed deer in Kent County, MD during the 1990 hunting season. A geographic information system (GIS) was used to extract 41 environmental variables in the areas surrounding the collection sites of the deer. Stepwise linear regression was used to evaluate the association between the abundance of ticks on deer and the environmental data. A significant statistical association was observed between the abundance of adult *I. scapularis* and seven environmental variables (R = 0.69). Tick abundance was negatively correlated with urban land use patterns, wetlands, the amount of privately owned land, soils that tended to be saturated with water, and one drainage system. Tick abundance was positively correlated with well drained, sandy soils having low water tables. These results indicate that geographically referenced environmental data may be useful in anticipating the risk of exposure to vectors over large areas.

674 DETACHMENT PERIODICITY OF JUVENILE *IXODES PACIFICUS* TICKS FROM COMMON VERTEBRATE HOSTS. Vredevoe LK*, Richter PJ, and Kimsey RB. Department of Entomology, University of California, Davis, CA; and Department of Comparative Pathology, University of California, Davis, CA.

We sought to determine the temporal detachment pattern of juvenile Western Black-Legged ticks, *Ixodes pacificus*, by recording the time that ticks detached from common laboratory and field-derived hosts. Approximately half of the engorged sub-adult ticks which naturally infested rodents detached during the afternoon, while around one-quarter detached during all other periods. Ticks did not usually drop-off during the night. In a similar experiment using BALB/C white mice, Deer mice (*Peromyscus leucopus*), Western Fence swifts (*Sceloporus occidentalis*), and Wood rats (*Neotoma fuscipes*) infested with laboratory reared larvae, we also found peak detachment time to be in the afternoon. Because ticks leave diurnally active and nocturnally active hosts at the same time of day, tick detachment must be independent of temporal patterns of host activity. We, therefore, suggest the existence of a detachment mechanism in *I. pacificus* which is independent of host-associated factors and resembles the post-attachment mechanisms proposed by Graf *et al.* in *I. ricinis*, and by Mather *et*

al. in *I. dammini.* Diurnal detachment will result in the distribution of engorged ticks in the nests of nocturnal hosts and on the surface of the ground from diurnal hosts. Thus, we propose that the spatial distribution of the Western Black-Legged tick is mediated by host activity.

675 DIFFERENTIATION OF *BORRELIA* SPECIES AND STRAINS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS. Nicholson WL* and Glass GE. Department of Immunology & Infectious Diseases, Johns Hopkins School of Hygiene & Public Health, Baltimore, MD.

The use of random amplified polymorphic DNA (RAPD) markers for the differentiation of *Borrelia* strains and species was investigated. DNA from *Borrelia anserina, B. burgdorferi,,B. coriaceae, B. hermsii, B. parkeri, B. turicatae,* and a novel *Borrelia* isolate (FCB-1) were extracted using a Chelex-100 lysis method. Single decamer primers of arbitrary sequence were used to generate polymerase chain reaction (PCR) products which were analyzed by agarose gel electrophoresis. The patterns of banding were examined for their ability to discriminate among the species and strains. Several primers were useful in differentiation at the species level, while others were useful at the strain level. The FCB-1 isolate geve unique banding with the primers tested indicaitng, that the isolate may represent a new taxon. Further work is suggested for using RAPD markers in the development of DNA probes, and for the use of markers generated by various combination of the primers.

676 PHENOTYPIC AND GENOTYPIC VARIATION IN *BORRELIA BURGDOFERI* CULTURED FROM CHRONICALLY INFECTED *PEROMYSCUS LEUCOPUS* CAPTURED LONGITUDINALLY AT A LYME DISEASE ENZOOTIC SITE IN MARYLAND. Hofmeister E* and Childs J. The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD; and Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA.

Thirty isolates of *Borrelia burgdorferi* cultured from 15 naturally infected *Peromyscus leucopus* were examined for evidence of variation in protein expression and plasmid profile. Early and late isolates of *B. burgdorferi* were obtained an average of 6.4 months apart (range = 1.5 to 14 months) from ear biopsy samples removed from *P. leucopus* captured longitudinally at a Lyme disease enzootic area of Maryland. All cultured spirochetes were identified as *B. burgdorferi* by IFA using monoclonal antibody H5332 and by specific amplification of ospA by polymerase chain reaction. Variation in protein expression by SDS-PAGE was observed between early and late isolates in 5/15 (33%) of mice. Plasmid profile analysis detected variation both among mice and between early and late isolates of *B. burgdorferi* in 11/15 (73%) of mice. Variation in protein expression and plasmid profile was confirmed by examination of additional isolates of the organism from several mice in the sample group. Serological response to chronic infection with *B. burgdorferi* was examined by Western immunoblotting. We conclude that phenotypic and genotypic variation may contribute to maintenance of chronic infection with *B. burgdorferi* in naturally infected *P. leucopus.*

677 INCIDENCE OF SEVERE ANEMIA DURING THE FIRST YEAR OF LIFE IN INFANTS IN TANZANIA. Redding-Lallinger R*, Ting DY, Mmari MP, Kalokola F, Wilkinson WE, Lillinger G, and Durack DT. Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania; and Duke University Medical Center, Durham, NC.

In a longitudinal study to determine the incidence, risk factors and natural history of anemia in very young children in urban Tanzania, 426 healthy infants were enrolled at birth and followed monthly thereafter. We present here data on 312 infants who had reached one year of age and for whom complete data was available. During the first year, 15% of the infants developed severe anemia, defined as Hb < 6.0 mg/dL. The mean age + S.D. for the development of severe anemia was 9.5 + 2.1 months, with 88% of infants falling between 9 and 12 months of age when they became severely

anemic. Patent malaria parasitemia at the time of diagnosis of severe anemia was present in 50% of these infants. On average, malaria parasitemia was present on 21% of all clinic visits of the infants who developed severe anemia, and in 5% of all visits of those who did not develop severe anemia. Fifty percent of the severely anemic group had never had malaria parasitemia during the first year of life, compared with 83% of the rest of the cohort. Seventy-seven percent of severely anemic infants had more than one diagnosis as the etiology for anemia. Iron deficiency was diagnosed in 71% of severely anemic infants, with additional hematologic diagnoses for the cause for anemia found in 56%. In addition, almost all infants experienced one or more infections other than malaria during the first year of life. The mean number of infections for severely anemic infants was 11.6 + 4.5 per infant, compared with 10.1 + 4.3 infections per child for the remainder of the cohort. Mortality during the first year of life was 4.2% for severely anemic infants, and 6.3% for infants who were not severely anemic. We conclude that severe anemia is common in very young children in Dar es Salaam, and it is usually associated with more than one etiologic factor. In this cohort, severe anemia was not associated with excess mortality, although these infants were treated after diagnosis in this study.

678 SAFETY AND PHARMACOKINETICS OF A HUMAN HYPERIMMUNE BOTULINUM ANTITOXIN IN VOLUNTEERS. Hack DC*, Sjogren MH, and Crabbs C. Medical Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD; and Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, MD.

Between 1978 and 1981, 2,753 units of immune plasma, plasmapheresed from volunteers hyperimmunized with pentavalent botulinum toxoid, were pooled into 4 lots (1A,1B,2A and 2B). Human botulinum immune globulin was produced from this immune plasma by a patented procedure designed to concentrate the IgG and remove any adventitious agents. Lot 2A was tested in 8 volunteers, 4 by the intramuscular (i.m.) route and 4 by the intravenous (i.v.) route at a dose of approximately 15 mg/kg (10 ml). Sequential blood samples were drawn and assayed for neutralizing antibody to botulinum toxin, for safety data (hepatitis A,B,C; HIV; rheumatoid factor; CBC; liver enzymes), and immunoglobulin and complement levels over 6 months. Neither route of administration produced significant side effects other than local soreness from injection. Human botulinum IgG administered i.v., supplied high levels of antitoxin antibody immediately without any significant differences in the duration of measurable antibody.This is in contrast to IgG given i.m., with the concurrent delayed level of antibody. Compared with the data obtained from a previous clinical study of a despeciated equine antitoxin, after the first week, the human preparation yielded significantly higher neutralization titers than did the equine product, although the equine product had 50-100 times the neutralization activity of the human-derived product. This higher level persisted; the neutralizing levels of the human antitoxin measured at 60 days were similar to the levels of the equine product at 21 days. Lots 1B and 2B were given to 4 volunteers i.v. with similar results. Lot 1A is scheduled for testing in the near future.

679 VARIATION IN BCG EFFICACY BY GEOGRAPHIC LATITUDE: SOME PLAUSIBLE HYPOTHESES. Wilson ME* and Fineberg HV. Division of Infectious Disease, Department of Medicine, Mt Auburn Hospital, Cambridge MA; and Harvard School of Public Health, Harvard Medical School, Boston MA.

A recent meta-analysis of BCG efficacy for the prevention of tuberculosis found about a 50% protective effect across many populations and study designs, though individual studies varied widely in their results. In a random effects regressive analysis of prospective studies, geographic latitude alone could explain 40% of the between-study variance. Study sites at greater distance from the equator were associated with higher efficacy of BCG. A number of factors that vary with latitude could potentially influence the effectiveness of BCG through impact on the susceptibility of human hosts, pathogenicity of the organism, or host-agent interactions. These include socioeconomic status of the

population, genetic composition of the population, climate, exposure to sunlight, diet and nutrition, presence of non-tuberculous mycobacteria in the environment, completeness of surveillance and follow-up in BCG studies, virulence of locally prevalent *M. tuberculosis* strains, and storage and viability of BCG. This paper describes the biologic plausibility, epidemiologic evidence and other scientific data bearing on the influence of these factors on BCG efficacy. Better understanding of the determinants of BCG efficacy can help health officials make more informed judgments on use of the vaccine.

680 MORE THAN MICROBES: THE SOCIAL AND CULTURAL BASES OF INFECTIOUS DISEASE. Etkin NL*. Department of Anthropology, University of Hawaii, Honolulu, HI.

More than they are medically and biologically remarkable, infectious diseases are fundamentally a factor of human behavior: in ways that the most exacting laboratory studies can never reveal, infectious diseases represent the confluence of a host of social, cultural, and political factors that define disease risk and recognition, as well as access to prevention and therapy. This has been recognized in principle since the earliest public health initiatives, and is embodied in today's emphasis on "community-based" disease control. But, in fact, the behavioral dimension of human health is poorly understood by the medical sciences, which lack the conceptual perspective and training to study human society and culture as it affects disease control. I propose that collaboration among biological and social scientists will prepare us better solve tropical disease problems for contemporary peoples. More specifically, I suggest that medical anthropology can offer a tested methodology to operationalize for a particular study setting the abstractions that litter the tropical disease and development literature -- "involve the community," "learn local disease models," "identify high risk populations." Given the great variety of cultures, one cannot, of course, anticipate all potential scenarios and contingencies. But, I can provide a general outline for disease-related in-field ethnography, as well as offer a small number of very specific examples in order to illustrate the process through which one comprehends the most salient social and cultural variables for studying tropical diseases outside the laboratory.

681 CASE-CONTROL STUDY OF ENDEMIC DIARRHEAL DISEASE IN EGYPTIAN CHILDREN. Mortagy AK*, Mourad AS, Bourgeois AL, Kilpatrick ME, Kleinosky M, and Murphy JR. US NAMRU-3 & Faculty of Medicine, Ain Shams University, Egypt; Faculty of Medicine, Alexandria University, Egypt; Naval Medical Research Institute, Bethesda, MD; Naval Hospital, Orlando, FL; and Center for Infectious Diseases, University of Texas, Houston, TX.

From 1 May 1992 to 30 April 1993, 343 children <5 years old presented with diarrhea to a rural clinic in Abees, Egypt. These were matched for age and sex with a similar number of children, without diarrhea, who presented to the same clinic. Seventy-five percent of the children were less than 12 months of age; 4% of the cases presented with persistent diarrhea (more than two weeks diarrhea) and 8% presented with bloody diarrhea. *Shigella* species was the most frequently isolated bacterial pathogen (5% of the children), followed by enterotoxigenic *E. coli* (4%), *Campylobacter* (3%) and *Salmonella*(1%). The difference in detection of enteric pathogens between cases and controls was significant ($p<.05$) for *Shigella* and ST positive *E. coli*. LT positive *E. coli* was isolated more often from controls than cases. *Campylobacter* was significantly ($p<0.05$)associated with diarrhea only in children less than 12 months of age. Although mortality from diarrhea has declined in Egypt in the last decade, this study demonstrates that morbidity is still a major problem in Egypt.

682 MODULATION OF CELL PROLIFERATION IN BACTERIAL MENINGITIS BY SOLUBLE IL-2 RECEPTORS. El Ghorab NM*, Mansour MM, Girgis NI, Salah LA, and Abu-Elyazeed RR. U.S. Naval Medical Research Unit Number Three, Cairo, Egypt.

It has been demonstrated that soluble IL-2 receptor (sIL-2R) has an immunoregulatory function during cell growth and differentiation. This study was designed to investigate the role played by this receptor in modulating the immune response of patients with bacterial meningitis.The lymphoproliferative responses, peripheral blood subpopulations and levels of IL-2 and sIL-2R in cell-free culture supernatant, CSF and plasma of 20 patients were measured before and after antibiotic and steroid treatment. A significant improvement in the lymphoproliferative responses to PHA was observed after treatment. This improvement was accompanied by a significant increase in the IL-2 levels in the cell-free culture supernatants and significant decrease in plasma sIL-2R as well as the absolute counts of the B cells. A significant positive correlation between plasma sIL-2R and the B-cell counts was observed (r=0.70). Before treatment there was no differencein the IL-2 levels of CSF and plasma but sIL-2R levels were significantly higher in plasma suggesting systemic activation. These results demonstrate that activation of B-cells by bacterial antigens can induce the release of high levels of sIL-2R. This, in turn, blocks IL-2 secretion by T cells which leads to impaired mitogenic response.

683 A COMPARISON OF REDUCED DOSAGE CIPROFLOXACIN WITH THE STANDARD DOSAGE AS EMPIRIC THERAPY FOR TRAVELERS' DIARRHEA. Ferguson DD, Cimino AL, Panza N, Basnyat B, and Bia FJ*. Department of Internal Medicine, Yale University School of Medicine; Department of Laboratory Medicine, Yale University School of Medicine; and Nepal International Clinic, Kathmandu, Nepal.

Infectious diarrhea affects as many as 20% to 70% of travelers. Ciprofloxacin (Cipro) has a broad antimicrobial spectrum, relatively few side effects, and has been shown effectively to reduce the duration and severity of diarrhea. However, its expense is a significant drawback. To determine if low dose Cipro is as effective as standard therapy, 52 patients who presented at the Nepal international Clinic in Kathmandu complaining of diarrhea were treated randomly and in placebo-controlled, double-blind fashion with standard dose Cipro (SD - 500 mg twice daily for 5 days) or low dose Cipro (LD - 500 mg daily for 3 days). Nineteen patients treated with SD and 17 treated with LD for whom follow-up was available were included in analysis. The groups did not differ significantly with respect to duration of travel abroad, duration of diarrhea, stool frequency, symptoms (fever, abdominal pain, vomiting, weight loss) or the presence of fecal white or red cells prior to treatment. *Aeromonas hydrophila* was the most common pathogen isolated (3 cases). Neither *Salmonella, Shigella* nor *Campylobacter* species were isolated. Pretreatment pathogens were eradicated after therapy in both groups. Stool specimens were examined microscopically and with an antigen detection system for protozoa. *Giardia lamblia* and *Cryptosporidium* were each detected in one patient which excluded them from analysis. *Endolimax nana* was detected in one patient, and *Blastocystis hominis* was detected in five patients. These patients were included in the analysis. Comparison of LD with SD after treatment showed no significant difference in the daily frequency of diarrhea, total episodes of diarrhea (4.2 ± 0.9 vs 4.3±0.9, $p>0.25$), number of cures (17/17 vs 19/19), number of failures (0.17 vs 0.19), number of relapses (3/17 vs 3/19), average duration of diarrhea (1.6 ± 0.3 vs 1.98 ± 0.3 days, $p>0.25$), or the number of types of side effects. The subgroups of patients treated with LD for severe diarrhea or dysentery tended to have a longer duration of diarrhea compared with patients treated with SD. In contrast, patients taking LD for mild-moderate diarrhea and without dysentery tended to have a shorter duration of diarrhea compared with patients taking SD. These differences were not significant. These results suggest that low dose Cipro is as effective as a standard dose for mild-moderate, nondysenteric diarrhea and may be effective for severe and dysenteric diarrhea. A lower dosage has the benefits of reduced cost and, potentially, a reduction in the incidence and severity of side effects.

684 CANINE HOOKWORM INFECTIONS: A LEADING CAUSE OF HUMAN EOSINOPHILIC ENTERITIS IN AUSTRALIA. Loukas A*, Croese J, Opdebeeck J, and Prociv P. Department of

Parasitology, The University of Queensland, Queensland, Australia; and Townsville General Hospital, Townsville, Queensland, Australia.

Human eosinophilic enteritis (EE), supposedly a rare disease of unknown etiology, occurs frequently in north-eastern Australia. When adults of the common dog hookworm, *Ancylostoma caninum*, were found *in situ* in several cases, we used ELISA and Western blot to seek antibody responses to the secretions (ES antigen) of this parasite. The study group comprised 25 patients with EE or unexplained abdominal pain and blood eosinophilia (PE); the control groups were: 3 patients harbouring *A. caninum*; 42 with diagnosed gastrointestinal disorders causing abdominal pain; 8 with human hookworm infection; 27 with other parasitic infections; 100 blood donors from Tasmania, where *A. caninum* does not occur. By ELISA, 88% of EE/PE patients were positive for IgG and IgE antibodies. All 8 with human hookworm (confirmed as *A. duodenale* in 3) were positive, while most with other infections, as well as sick and healthy control subjects, were negative. In Western blots, 92% of EE/PE patients had IgG and IgE antibodies to a 68 kDa component (Ac68) of *A. caninum* ES antigen, as did all those infected with human hookworms. Very few sera from other groups reacted positively. This indicates that *A. caninum* can develop in the human gut, and is a major cause of EE and PE in northeastern Australia. Our ELISA and Western blot should be helpful in diagnosis. The disease probably results from allergy to hookworm secretions, and is likely to occur wherever people are exposed to infective larvae of *A. caninum* and, perhaps, related species.

685 CANINE HOOKWORMS: A CAUSE OF HUMAN INTESTINAL DISEASE. Croese J, Prociv P*, Loukas A, Opdebeeck J, and Fairley S. Department of Parasitology, The University of Queensland, Brisbane, Queensland, Australia; and Townsville General Hospital, Queensland, Australia.

While serology indicates that *Ancylostoma caninum*, a common dog hookworm, is the leading cause of human eosinophilic enteritis in Australia, the parasite itself has only rarely been found in the human gut. We here summarize findings in 9 patients who were found to harbour a zoonotic hookworm, from 2 cities with a combined population less than 2 million in the state of Queensland. Six were diagnosed in Townsville, and 3 in Brisbane. Three were diagnosed over 6 years and 6 presented in the last 2 years of the study. Three had laparotomy for acute abdominal pain, and 6 were colonoscoped, 5 with abdominal pain and one without symptoms. Six had blood eosinophilia and 5/8 tested had elevated IgE levels; 6/8 had eosinophilic inflammation of the gut. A solitary hookworm was found in each case. They were immature adults of either sex, 7.0-11.4 mm long: 6 were A. caninum, but buccal capsule damage in the remaining 3 precluded specific identification. However, no patient had been to areas endemic for human hookworms. Of 8 patient sera tested by ELISA and Western blot, 7 showed IgG and/or IgE antibody responses to *A. caninum* secretions. Given the parasite's broad geographic distribution, anatomical inaccessibility and provocation of nonspecific symptoms, human enteric infection is likely to be far more common and widespread than indicated by our findings. In some cases a localised allergic reaction induces eosinophilic enteritis, but infection may also be asymptomatic.

SYMPOSIUM ABSTRACTS

S1 ALTERNATIVE PATHWAYS OF IFN-γ PRODUCTION AND PROTECTIVE IMMUNITY INDUCED BY AN ATTENUATED *TOXOPLASMA GONDII* VACCINE. Gazzinelli RT*, Denkers E, Wysocka M, Trinchieri G, and Sher A. Immunology and Cell Biology Section, Laboratory of Prasitic Diseases, NIAID - NIH, Bethesda, MD; and Wistar Institute, Philadelphia, PA.

Mice vaccinated with a temperature sensitive mutant (TS-4) of *Toxoplasma gondii* develop complete resitance to lethal challenge with a highly virulent toxplasma strain (RH). This immunity is known to be dependent on IFN-γ synthesis. CD4+ cells from vaccinated mice produced high levels of Th1 cytokines (IL-2 and IFN-γ) but not Th2 cytokines (IL-4, IL-5 and IL-10), whereas CD8+ cells produced

high levels of IFN-γ when exposed in culture to exogenous IL-2 and parasite antigen. *In vivo* treatment with culture to exogenous IL-2 and parasite antigen. *In vivo* treatment with anti-CD4 plus anit-CD8 or anti-IFN-γ antibodies during challenge infection completely abrogated resistance to *T. gondii*. While anti-CD8 treatment partially decreased vaccine induced resitance, anti-CD4 antibodies when given during vaccination, as opposed to after challenge, were capabel of blocking protective immunity. To further investigate the role of CD8+ lymphocytes in resitance to this coccidium we used β2-microglobulin knockout mice which fail to express class I MHC molecules and CD8+ lymphocytes. Interestingly we observed the emergence of a major population of protective cells (NK1.1+/CD3-/αβ-) as a major source of IFN-γ and protective immunity. Since, the newly identified cytokine IL-12 has been shown to have a major role in CD4+ T cell differrntiation towards a Th1 phenotype, as well as CD8+ lymphocyte maturation and indicution of IFN-γ synthesis by NK cells, we are investigating tghe role of the cytokine in both gneration and maintenance of protective immunity induced by TS-4. Our results show that either live tachyzoites or soluble parasite antigens trigger macrophages to produce high levels of both chains (p40 and p35) of the IL-12 heterodimer. We are currently examining the *in vivo* role of IL-12 in establishing cell mediated protective immunity induced by this attenuated tachyzoite vaccine.

S2 INDUCTION OF IMMUNITY TO *TRICHURIS SUIS* IN SWINE USING EXCRETORY/SECRETORY PRODUCTS FROM ADULT WORMS. Hill DE* and Urban JF. Biosystematic Parasitology Laboratory, USDA, Agricultural Research Service, Beltsville, MD; and Helminthic Diseases Laboratory, USDA, Agricultural Research Service, Beltsville, MD.

Trichuris suis is a nematode infection of swine which causes anemia, anorexia, mucohemorrhagic diarrhea, and death in heavy infections. Acquired resistance to Trichuriasis is inferred from the fact that infective eggs are ubiquitous and long lived, but infections are mainly observed in growing pigs. There are few reports on immunity to *T. suis* in swine. The purpose of this study was to determine the effects of immunization of pigs with *T. suis* adult excretory/secretory products (ESP) on the development of resistance to *T. suis* infection. Procedures were developed for the *in vitro* cultivation of adult *T. suis* that result in the production of culture derived ESP. Pigs were immunized with ESP in alum or Freunds adjuvants on day 0 and day 7 of the experiment. Pigs were challenged with 2000 eggs/kg body weight orally on day 21, and then placed on a dirt lot contaminated with *T. suis* eggs for 52 days. Control, immunized pigs had 2205 plus or minus 465 *T. suis* adults recovered at necropsy, while immunized pigs had adult recoveries reduced by 31% in the Freunds group, 86% and 94% in the alum groups. Immunized pigs had increased serum lgG, IgA and IgM antibodies to ESP. In vitro peripheral blood lymphocyte responses to ESP were also elevated. Larval stages may be targeted by immune effectors induced by the immunization with ESP. In addition, immunization with ESP completely eliminated the pathology (weight loss, diarrhea, dehydration) normally associated with trichuriasis.

S3 CASEIN KINASE II IS CONSTITUTIVELY EXPRESSED IN BOVINE LYMPHOCYTES TRANSFORMED BY INTRACELLULAR PROTOZOAN PARASITE *THEILERIA PARVA*. ole-MoiYoi OK, Brown WC, Iams KP, Nayar A, and Maclin MD. International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya; Department of Microbiology and Parasitology, Texas A&M university, College Station, TX; Department of of Physiology, University of Alberta, Calgary, Alberta, Canada; Department of Chemistry, Indiana University, Bloomington, IN; and Agracetus, Middleton, WI.

Theileria parva is an obligate, intracellular, parasitic protozoan that causes East Coast fever, an acute leukemia-like disease of cattle. *T. parva* and the related parasite of cattle and domestic buffalo, *T. annulata*, are unique among protozoa in that their intralymphocytic schizonts induce transformation of bovid lymphocytes. Comparison of *in vitro* protein kinase activities in 100,000xg particulate fractions prepared from control IL 2-dependent bovine T lymphoblast clones with those from the cells

after infection with *T. parva*, revealed a 4.7- to 12-fold increase in phophorylation of endogenous protein substrates, some of which were *Theileria* infection-specific. The protein kinase which phosphorylated these substrates were serine/threonine-specific with substrate, messenger and effector specificities of casein kinase (CK) II. Northern blot analysis revealed a 3.9- to 6.0-fold increase in CKIIα transcripts in mRNA from *T. parva*-infected lymphocytes. Furthermore, a marked increase of CKII antigen was observed on Western blots of materials prepared from the infected cell lines. The anti-bovine CKII polyclonal IgG used in these studies immunoprecipitated a protein kinase that phosphorylated casein in a reaction that was inhibited by low (nM) quantities of herarin. Our data show marked increases of bovine CKII at the transcriptional, translational, and functional levels in *T. parva*-infected lymphocytes relative to quiescent cells or IL 2-dependent parental lymphocytes. Bovine CKII thus appears to be constitutively activated in these cells and we propose that this kinase may be an important element in a signal-transducting pathway activated by *Theileria* in bovid lymphocytes and also perhaps in some leukemic cells. The role of the transformed lymphocytes in disease pathology and immunity will be discussed.

S4 THE ROLE OF NATURAL KILLER CELLS, IL-12 AND IFN-γ IN RESISTANCE TO *LEISHMANIA MAJOR*. Scott P*. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.

The outcome of *Leishmania major* infection in mice is dependent on the CD4+ T cell subset that predominates. Resistant C3H/HeN mice develop a Th1 type of response, characterized by the production of high levels of IFN-γ, while BALB/c mice exhibit a Th2 response and are unable to control the infection. The signals that drive the differentiation of Th1 cells following leishmanial infection include the presence of IFN-γ, since a single injection of anti-IFN-γmonoclonal antibody prior to infection abrogates Th1 development. We have recently found that NK cells are one source of this IFN-γ following infection of C3H/HeN mice with *L. major*, while BALB/c mice are unable to exhibit this response. Since IL-12 stimulates the production of IFN-γ by both NK and T cells, we assessed the ability of IL-12 to induce a Th1 response to a soluble leishmanial antigen (SLA) preparation in the normally susceptible BALB/c mouse. We found that subcutaneous administration of SLA with IL-12 induced an NK cell response in the draining lymph node, and primed CD4+ T cells to differentiate into Th1 type cells within 10 days. Furthermore, when BALB/c mice immunized with SLA and IL-12 were subsequently challenged with *L. major* parasites, they were found to be completely resistant to the lethal effects of this infection. These data indicate that IL-12 may function as an adjuvant for vaccines requiring the induction of cell-mediated immunity.

S5 IMMUNIZATION OF MICE WITH IRRADIATED *PLASMODIUM YOELII* SPOROZOITES. Sedegah M*, Weiss WR, Charoenvit Y, Hoffman SL. Malaria Program, Naval Medical Research Institute, Bethesda MD.

Immunization of mice with radiation attenuated *Plasmodium yoelii* sporozoites (irrspz) protects against challenge with sporozoites (spz), but not with infected erythrocytes; the protective immune responses are directed against spz in circulation or infected hepatocytes. This notion is consistent with the fact that irrspz invade, but only partially develop within hepatocytes. We now summarize our data regarding the kinetics and duration of immune responses after single or multiple immunizations of *P. yoelii* irrspz, the effects of chronic erythrocytic stage infection on irrspz-induced immunity, and the identification of the mechanisms and targets of protective immunity induced by irrspz. There is a direct correlation between number of immunizations and protection. Humoral and cellular immune responses to spz are diminished by chronic blood stage infection. Finally, existing data indicates that although the irrspz vaccine induces antibodies that can prevent effective spz invasion of hepatoyctes during the 5-10 min required for extracellular spz to reach the liver, T cells that recognize parasite peptides on the surface of infected hepatocytes are primarily responsible for the

sterile immunity. Different subsets of T cells are apparently more or less important depending on the strain of mouse and the parasite. At least 2 targets of protective CD8+ CTL have been identified, the CSP and Sporozoite Surface Protein 2, and work is in progress to identify additional targets. Virtually all successes and failures in human pre-erythrocytic malaria vaccine development have been predicted by work in the *P. yoelii* rodent model system, which we believe is a faithful predictor of immunity to *P. falciparum* in humans.

S6 VACCINATION WITH RADIATION-ATTENUATED CERCARIAE OF *SCHISTOSOMA MANSONI*: ROLE OF CYTOKINES IN THE PROTECTIVE RESPONSE. Wilson RA*. Department of Biology, University of York, York, UK.

Percutaneous exposure of C57Bl/6 strain mice to optimally irradiated cercariae of *Schistosoma mansoni* induces 60-70% protection against a challenge with normal parasites. The radiation-attenuated cercariae differ from normal larvae in their pattern of migration. A proportion lodge in the skin-draining lymph nodes where they stimulate the proliferation of CD4+ T cells with Th1 characteristics, particularly the ability to secrete interferon γ (IFNγ) when cocultured with antigen *in vitro*. Other attenuated larvae travel to the lungs where they induce a leukocytic infiltrate, the lymphocytes of which persist at least to 10 weeks. These cells, predominantly of the CD4+ T helper subset, are reluctant to proliferate *in vitro* but secrete abundant IL-3 and IFNγ upon antigenic stimulation. On the basis of function and phenotype, the T cells appear to be a short-term effector/memory population which may arm the lungs against the arrival of challenge larvae. When such a larva reaches the primed lung, it provokes an intense focal accumulation of T cells and macrophages. This cellular aggregate appears to act by blocking schistosomulum migration rather than by a direct cytotoxic mechanism. In vivo neutralization of IFNγ after challenge of vaccinated mice results in a 90% abrogation of immunity. Pulmonary inflammation is exacerbated by this treatment but the foci which develop are looser in composition and contain numerous multinucleate giant cells. Neutralization of TNF and inhibition of nitrite production have no comparable effect on immunity. A plausible role for IFNg in the protective mechanism may be to upregulate the expression of adhesion molecules on the cells which comprise the inflammatory focus, thereby contributing to its density, integrity and blocking capacity.

S7 WRITING SCHOLARLY SCIENTIFIC BOOKS: DON'T QUIT YOUR DAY JOB. Brooks DR*. University of Toronto, Toronto, Canada.

Writing for love and joy is discouraged within the scholarly scientific arena, and the market for specialized scientific books is too small for direct financial reward. Such volumes thus are written for a severely limited and bitterly contested professional resource, recognition, and prestige within the scientific community above that gained through research articles. Thus, the only rationale for entering this arena is a strong conviction and compulsion. This means you must have something novel to say, have the courage to say it, and be able to say it effectively. Your book may present either a coherent account of an area of research derived by synthesizing many studies, or a novel conceptual framework that could not be explored fully in research articles. Success in this arena requires outstanding scholarship, personal discipline and emotional strength, qualities editors tend to associate with long careers and prestigious institutions. If you or a co-author have excellent literary skills, you will be ahead of the game, because readable specialized volumes are rare. Such ventures become less risky as your career progresses, because administrators can count, but cannot read; thus young scientists generally are advised to publish lots of research articles rather than a single book. Unlike research articles, books are reviewed publicly after publication, and it is easier to survive reviews if you are already tenured.

S8 A TASTE OF WRITING TEXTBOOKS. Roberts LS*. Department of Biological Sciences, Florida International University, Miami, FL.

Writing textbooks differs in some respects from other kinds of writing. It is a hybrid of scholarship, teaching and the craft of creativity of writing. An introductory text, such as *Biology of Animals* or *Integrated Principles of Zoology* demands the scholarly expertise to integrate material from an enormous range of subjects. You will have no first-hand experience in some or most of the subject areas, yet you must present them accurately and in an inviting, interesting style. An upper division book, such as *Foundations of Parasitology*, requires a somewhat different type of scholarship. Requiring a deeper treatment in a single discipline, it is written from the primary literature, just as a review in a scholarly journal. Readers who are specialists in the field must judge that the results satisfies their standards, but students who use the book should find it interesting - or you will not sell many copies. Writing a text in an interesting style encompasses not only the teaching aspects of writing textbooks, but also the craft of writing itself. You must, of course, be able to spell, punctuate, and construct sentences and paragraphs, while summoning the creativity necessary to tell your story. Getting your words down on paper (or on your computer screen) is only a beginning. You must also obtain illustrations, write legends and place the illustrations, read and correct galley page proofs, and consult with your editors on a myriad of other details. If your text is successful, as you hope it will be, you are faced with revising it and going through the whole thing every four years, edition after edition.

S9 THE TRADE BOOK MARKET: DOORSTEP TO THE WORLD'S MIND. Janovy J. School of Biological Sciences, University of Nebraska, Lincoln, NE.

Trade books are those sold on the open market to the general public through bookstores, book clubs, etc. Such books differ from "scholarly" books in that trade books rarely satisfy content reviewers unless the subject matter might inspire a lawsuit. However, trade books must satisfy literary and commercial criteria to which scholarly books are not always subject. Through the trade market, therefore, a writer can reach an audience that would never think of picking up a scholarly book. Potentially this audience is very large and includes people from government, business, the arts, not only in the United States, but around the world. The publication of a trade book gets you on the doorstep of this audience. The words you've put into that book get your foot in the door. The ideas in that book get you into the audience's mind. I contend that parasitologists, because of the breadth forced upon them by their subject matter, have enormous potential for influencing the general public's attitudes about nature. The scientific community needs desperately for young biologists to see themselves, eventually, in the role of influential writers for the general public.

S10 THE PUBLIC-PUBLISHING PARASITOLOGIST. Desowitz RS*. Department of Tropical Medicine and Medical Microbiology, University of Hawaii, Honolulu, HI.

The best-received, most critically acclaimed public/popular books on science are written by professional scientists who write rather than professional writers who are not scientists. This is reflected in the yearly shortlist selection of books for the Rhone-Poulenc Prize, the premier award given to books that advance the public's understanding of science. Worms and other parasitic animacules, so beloved by members of this Society, have, in the past, rarely captured the public's reading interest. The fault lays as much with the parasitologist as the parasite-a DeKruif has yet to emerge from our ranks. Nevertheless, there is a growing public curiosity about the ways of parasites and gow they have affected the health and social structure of societies, how the interact with ecological perturbations, and their relationships to AIDS. It is our professional responsibility, as much as the labors at the bench, to meet the demands of that public interest. But in doing so, an honest account must be given - the "warts and all" of reality. We have fascinating and important

stories to tell. Paradoxically, the telling benefits our skills as scientists as much as advancing the knowledge to the public audience.

S15 BIOLOGY OF THE MALARIA VECTOR *ANOPHELES GAMBIAE* IN BAMAKO AND BETHESDA. Vernick KD*, Lanzaro GL, Toure YT, Traore S, and Miller LH. Laboratory of Malaria Research, NIAID, National Institutes of Health, Bethesda, MD; and National School of Medicine and Pharmacy, Bamako, Mali, West Africa.

Anopheles gambiae is the primary vector of human malaria in Africa. It is an extremely efficient vector for reasons of both physiology and behavior. We are using molecular genetic tools to understand gene flow and population structure of *An. gambiae* populations in West Africa. These studies are a prelude to efforts to control malaria transmission by genetic manipulation of vector populations in order to render mosquitoes refractory to infection with malaria. We are also studying the molecular basis of refractory traits which could be candidates for field introduction. In one such system, malaria ookinetes are lysed within hours of invading midgut epithelial cells of the refractory mosquitoes. Development proceeds normally in midguts of susceptible mosquitoes selected from the same parent colony.

S50 CRYPTOSPORIDIOSIS: NEW TARGETS FOR CHEMOTHERAPY. Nelson RG*. Medicine and Pharmaceutical Chemistry, San Francisco General Hospital, San Francisco, CA .

The coccidian parasite *Cryptosporidium parvum* infects the microvillus border of intestinal epithelium and causes a debilitating, life-threatening diarrheal disease in AIDS and other immunocompromised patients. No prophylactic or therapeutic regimens have been identified for cryptosporidiosis and effective drugs are urgently required. To date, little is known about specific or unique aspects of *C. parvum* metabolism, biochemistry or cell biology that could serve to focus drug discovery efforts. To circumvent this lack of knowledge, and to expedite discovery and testing of potential anti-cryptosporidial drugs, we have begun to isolate and characterize *C. parvum* genes whose protein products are expected to have indispensable roles in the parasite's life cycle; several of these proteins are important targets of clinically effective antimicrobial and/or antineoplastic chemotherapy in other organisms. We have focused on proteins whose functions are essential for DNA synthesis and replication, chromosome segregation and cell division. These include enzymes of folate homeostasis and de novo folate and thymidylate biosynthesis as well as the proteins composing cytoskeletal microtubules, microfilaments and their associated motor proteins. To date, we have developed an *in vitro* culture system that supports parasite invasion, schizogony and gamogony and have cloned and sequenced *C. parvum* genes encoding several proteins thought to be essential for completion of its intracellular life cycle including: thymidylate synthase-dihydrofolate reductase; the microtubule proteins α-, β- and γ-tubulin; the microfilament protein actin and a myosin II motor protein.

S51 *PNEUMOCYSTIC CARINII* PNEUMONIA: RECENT ADVANCES IN EXPERIMENTAL AND CLINICAL CHEMOTHERAPY. Gutteridge WE*. Wellcome Research Laboratories, Langley Court, Beckenham, Kent, UK.

Cotrimoxazole remains the drug of choice for the chemoprophylaxis and treatment of this disease. However, there are continuing concerns about the high level of adverse events associated with its use. In addition, by analogy with other antimicrobial drugs, including those used in combination, there is always the possibility that its use will ultimately be compromised by acquired drug resistance. An overview will therefore be given of progress in experimental and clinical chemotherapy, by both the rational and empirical routes, towards the discovery and development of novel drugs for this disease. Included here will be: the development of an automated *in vitro* drug screening system; the

use of SCID mice for *in vivo* testing; molecular and structural work on dihydrofolate reductase and some of the enzymes of de novo folate biosynthesis; new experimental leads such as 8-aminoquinolines, biguanides and 1,2,4-trioxanes; and clinical updates on drugs such as dihydrofolate reductase inhibitors with and without sulphonamides/sulphones, nebulised pentamidine, eflornithine and clindamycin/primaquine. In addition, a progress report on the development of atovaquone (566C80, now marketed as Mepron®), discovered originally as and still also in development for malaria, will be presented.

S52 TOXOPLASMOSIS: RECENT ADVANCES IN CHEMOTHERAPY. Derouin F*. Lab. Parasitol-Mycologie, Hopital Saint Louis, Paris, France.

Toxoplasma infection is highly prevalent throughout the world and is the cause of serious disease in congenitally infected children and in immunocompromised patients. In those infected by HIV, toxoplasmic encephalitis (TE) has been recognized as a major opportunistic infection. The combination of pyrimethamine and sulfadiazine is the standard therapy for TE; recent clinical trials have also suggested that the combination of trimethoprim and sulfamethoxazole (cotrimoxazole) could be efficient for prophylaxis of both TE and PCP. However, these folate inhibitors frequently cause a high incidence of side effects, especially in AIDS patients. Thus, there is a critical need for new drugs or drug combinations that are effective and safe for long term prophylaxis. A new *in vitro* model has been developed in order to identify drugs that are effective against intracystic parasites: atovaquone (hydroxynaphthoquinone), azithromycin (macrolide) and arprinocid-N-oxide are the only drugs that were found to have some activity on Toxoplasma cysts, but only the two former have been evaluated *in vivo*. In a model of acute infection, a significant protection is obtained when atovaquone or macrolides such as azithromycin and clarithromycin are administered alone; in chronically infected mice, long term administration of these drugs results in a significant reduction of brain cysts. A significant synergistic activity is observed when atovaquone is combined with pyrimethamine or sulfadiazine. Similarly, the combinations of azithromycin or clarithromycin with sulfadiazine, pyrimethamine or minocycline are highly synergistic *in vivo* but not *in vitro*; in a murine model of acute toxoplasmosis in which infection is sequentially followed in blood, lungs and brain tissues, this synergy is clearly evidenced by a dramatic reduction of parasite burden in mice treated with these combinations. These results suggest that these drugs or drug combinations could represent a complete alternative to the use of folate inhibitors in the treatment and prophylaxis of toxoplasmosis in humans, particularly those intolerant to sulfadiazine and/or pyrimethamine. This also offers a rationale to explore the possibilities of preventing multiple opportunistic infections, as these drugs have a wide anti-parasitic spectrum.

S53 THE MYCOBACTERIA: RAPID *IN VITRO* DRUG SCREENING OF SYNTHETIC AND NATURAL PRODUCTS. Franzblau S*. GWL Hansen's Disease Center, Baton Rouge, LA.

Worldwide, the mycobacteria have been responsible for more morbidity and mortality than any other group of bacteria. The difficulty in treating the mycobacterioses is further exacerbated in HIV-infected individuals. No new drugs have been added to the anti-tuberculosis and anti-leprosy armamentaria for over 20 years, due in part to the difficulty of testing drugs against these slow-growing pathogens. Recent advances in *in vitro* drug screening methodology have focused on indirect measurements of bacterial viability resulting in much faster and simpler assays. Established and experimental systems include radiorespirometry (BACTEC and biphasic systems), luciferase, and redox/colorimetric assays. Using radiorespirometry, several thousand substances are being screened for activity against virulent strains of *Mycobacterium tuberculosis, M. avium,* and *M. leprae*. These include analogs of existing anti-mycobacterial agents and drugs developed for other infections, as well as novel synthetic and natural products. Plants used traditionally for tuberculosis and leprosy, as well as those chosen randomly from diverse geographical regions are being extracted and screened. Active principals are

then isolated by bioassay-directed fractionation. A number of promising leads have been identified and are being pursued in macrophage and mouse models and in clinical trials.

S54 MICROSPORIDIOSES: PREVALENCE AND PROSPECTS FOR TREATMENT. Canning EU*, Hollister WS, Colbourn NI, and Silveira H. Department of Biology, Imperial College of Science, Technology and Medicine, London, UK.

Ten species of microsporidia have been found in humans causing ocular, intestinal, renal, hepatic, pulmonary or generalized infections. In immunocompromised patients, especially those with AIDS, the infections are severe and life threatening. Now that it is well established that microsporidia are common opportunistic pathogens, there is a need for data on their susceptibility to drugs which are registered for human use. We have examined the drugs albendazole and propamidine isethionate (brolinc) which are reported to have antimicrosporidial activity. Albendazole was tested *in vitro* on *Encephalitozoon cuniculi* and *Nosema corneum* by estimating the percentage of infected cells in cultures exposed to drug, as compared with controls and the effects were monitored by ultrastructural studies. The *in vitro* system was also used to determine the effect of propamidine on *N. corneum*. The burden of infection with *N. corneum* in different organs in albendazole- or propamidine-treated athymic mice was also investigated. Albendazole had a greater effect on *E. cuniculi* than on *N. corneum in vitro*. Inhibition of division gave rise to grossly enlarged intravacuolar stages of *E. cuniculi*, in which abnormal tubular structures were formed. Propamidine had a greater effect on *N. corneum* than albendazole *in vitro* but the effects of both drugs on *N. corneum in vivo* are equivocal. The *in vitro* results confirm the anti-microsporidial activity of the two drugs. Albendazole ameliorates the diarrhoea due to *Enterocytozoon bieneusi* but does not eliminate the parasite. *Encephalitozoon* spp. and *Septata intestinalis* are highly susceptible to albendazole: the drug may become concentrated in parasitophorous vacuoles. Methods are needed to enhance the uptake of albendazole into host cells and achieve increased efficacy against *E. bieneusi*. Propamidine is administered topically and further work is required to assess its effect on ocular infections.

S55 NEW THERAPIES FOR OPPORTUNISTIC PATHOGENS: NIAID RESOURCES AND OPPORTUNITIES. Laughon BE*, Fairfield AS, and Lambros C. Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda MD.

The discovery and development of new therapies for AIDS and its associated opportunistic infections (OIs) is a national health priority. The Developmental Therapeutics Branch (DTB) of the Division of AIDS at NIAID promotes research to stimulate the preclinical discovery and development of new drugs for clinical trials. The preclinical phase includes 1) drug discovery - the identification of potential new targets and new agents, and 2) drug development - a systematic process to generate efficacy and safety data prior to Investigational New Drug Applications to the FDA. A major component of the drug discovery effort within DTB is the National Cooperative Drug Discovery Groups for Opportunistic Infections (NCDDG-OI). The NCDDG-OI program supports multidisciplinary groups of investigators from academia, industry, and government to promote the rapid discovery and development of new treatments for | Pneumocystis, Cryptosporidium, Toxoplasma, Mycobacterium avium, M. tuberculosis |, several pathogenic fungi, and cytomegalovirus. Traditional research project grants such as R01s are encouraged and supported. In addition, the Division of AIDS has resources available for testing new therapies utilizing *in vitro* and *in vivo* models, particularly for promising new therapies that are seeking corporate sponsorship. Other resources available to assist in drug development include chemical resynthesis, formulation, toxicology, pharmacokinetics and combination testing of therapies. DTB also maintains a database of chemical structures with known or potential activity against OIs and HIV. Individuals in corporate, government, or academic settings are encouraged to contact the NIAID to explore potential opportunities for discovery or development of new OI compounds. Capacity and availability of *in vitro* testing varies according to the organism. Priority for *in vivo* testing will be given to defined

chemical entities with demonstrated bioavailability and supporting activity data. Evaluations may be performed under a confidentiality agreement.

S56 USE OF TRANSFECTION TECHNIQUES TO STUDY ORGANELLE BIOGENESIS IN *TRYPANOSOMA BRUCEI*. Parsons M *, Peterson GC, Sommer JM, Wang CC. Seattle Biomedical Research Institute, Seattle, WA; and Department of Pharmaceutical Chemistry, University of San Francisco, San Francisco, CA.

Many parasites show novel compartmentalization of metabolic processes. For example, trypanosomes compartmentalize glycolysis within a unique organelle called the glycosomes, while in virtually all other organisms glycolysis is cytoplasmic. Since bloodform *Trypanosoma brucei* derive all their energy from glycolysis, an understanding of the compartmentalization process has important implications. Accordingly we have used used transfection as a tool to identify glycosomal targeting signals within the living cell. For these studies, we have used the phosphoglycerate kinase (PGK) isozymes, since they exist as two glycosomal forms and one closely related cytoplasmic form. The entire coding regions of the genes, or fragments thereof, were appended to the coding region of luciferase. The constructs, placed under the PARP promoter, and with appropriate trans-splicing signals, were transfected into *T. brucei* procyclic forms and the subcellular localization of the luciferase reporter enzyme was ascertained. These experiments allowed us to determine that the major glycosomal isozyme of PGK has a single glycosomal targeting signal, which is located at the C terminus of the molecule and which ressembles the targeting signal found on many peroxisomal proteins. The minor glycosomal isozyme lacks this signal, and instead appears to have an internal targeting signal. Thus at least two distinct types of glycosomal targeting signals exist in these organisms.

S60 TARGETTED GENE DELETION AND COMPLEMENTATION OF SURFACE GLYCOPROTEIN 72 OF *TRYPANOSOMA CRUZI*. Nozaki T*, Cooper R, de Jesus AR, Epinosa M, Gomes JE, Garcia ES, Paul S, and Cross GA. Laboratory of Molecular Parasitology, The Rockefeller University, New York, NY; Centro de Investigacion Y De Estudios Avanzados Del IPN, Mexico City; Department of Biochemistry and Molecular Biology, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

A 72kDa glycoprotein, GP72, is a surface antigen of *Trypanosoma cruzi* expressed primarily in the epimastigote and metacyclic trypomastigote stages of the life cycle. GP72 has been suggested to be involved in the control of differentiation within the insect vector. GP72 is also the major acceptor site for complement component 3. To study function of GP72, null mutants of GP72 were created by targetted gene replacement. Targetting plasmids were constructed in which neomycin and hygromycin phosphotransferase genes were flanked by GP72 sequences. These plasmids were sequentially transfected into epimastigotes. Southern blots indicated that precise replacement of the genes had occurred. Western blots confirmed that GP72 is not expressed in the null mutant. The morphology of the null mutant is dramatically different from the wild-type throughout the life cycle. Normal attachment of the flagellum to the cell body is lost in epimastigotes and metacyclic trypomastigotes of the null mutant. Intra-cellular amastigotes of the null mutant do not transform to trypomastigote as efficient as the wild type; instead, round extra-cellular parasites with a short flagellum, which remain antigenically amastigotes, were observed. Metacyclogenesis of the null mutant was similar to the wild-type as measured by complement sensitivity and sialidase activity. Infectivity of tissue cultURe-derived parasites of the null mutant was similar to the wild-type. To unequivocally demonstrate that the defects displayed in the null mutant are solely due to loss of GP72, complementation with an episomal expression vector harboring GP72 is being undertaken, which requires the use of a new selective marker.

S62 THE *LEISHMANIA* GENOME PROJECT. A TOOL FOR MOLECULAR BIOLOGISTS. Cruz A. Universidade de Sao Paolo, Faculdade de Odontologia de Ribeirao, Ribeirao Preto-SP, Sao Paolo, Brasil.

Our goal is the construction of a physical map of the *Leishmania major* chromosomes. The physical map will ultimately consist of a fully overlapping collection of DNA fragments cloned in a cosmid shuttle vector. To generate contigs (i.e. identification of overlapping clones) we have chosen a fragment size matching method; the comparison of digestion patters obtained with two different restriction enzymes with every clone. Instead of attacking the entire genome as a whole, we are proceeding in a chromosome by chromosome basis for generating the contigs. The cosmid library was constructed with sheared parasite DNA from a clonal infective line (LV39, clone 5) at the laboratory of S. Beverley (Harvard Medical School). The cosmid shuttle vector, cL-HYG, contains the necessary sequences for selection and replication in bacteria and Leishmania. Hybridization of the arrayed clones with chromosomal DNA probes was carried out to map clones to a specific chromosome. The completed physical map will simplify genetic analysis and provoke scientific projects considering the products of newly located genes and their biological role. The combination of a shuttle vector that permits immediate genetic tests through transfection, with a chromosome-specific approach for contig generation will lead to more rapid answers--and then new questions--concerning the biology of *Leishmania*.

S73 PEDIATRIC MORTALITY AND MORBIDITY IN SUB-SAHARA AFRICA: BURDEN OF DISEASE AND DEMOGRAPHIC TRENDS. Mosley H*. Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.

Diarrhea, pneumonia, malaria, and malnutrition cause over three quarters of deaths among children in Sub-Saharan Africa. Simple health interventions are available to prevent death from each of these conditions. Currently, health care services are over-burdened, poorly equipped, and often have have inadequately trained and supervised health workers. The challenge of correcting these problems will be compounded by persistent high fertility and rapid population growth. The estimated 28-million births in 1990 will increase to 44-million by the year 2020. Improvements in child survival will require major advances in the technical bases of disease management and re-allocation of resources within the health sector.

S74 EXPERIENCE WITH DISEASE-SPECIFIC CONTROL PROGRAMS - RESPIRATORY DISEASES. Fagbule D*. University of Ogun, Nigeria.

The recognition that simple clinical signs could identify children with pneumonia and that most pneumonias were caused by bacteria kindled the development of ARI control programs throughout the world. Community intervention trials have demonstrated repeatedly the efficacy of early treatment of children with signs of pneumonia in reducing child mortality. Translating these research findings into national programs has been problematic; limited financial resources, difficulties in training health workers and educating mothers, have all hindered program success. Nevertheless, since the mid-1980's many developing countries have confronted the ARI problem and developed control programs based on the most recent science.

S75 EXPERIENCE WITH DISEASE-SPECIFIC CONTROL PROGRAMS - MALARIA. Steketee R*. Malaria Branch, Centers for Disease Control, Atlanta, GA.

Understanding the diverse manifestations of malaria has challenged health workers and those charged with developing interventions to control malaria. Malaria is now understood to cause acute disease in children, to cause chronic sometimes subclinical disease, and to cause placental infection

during pregnancy. Each of these manifestations of malaria requires a different strategy for recognition and treatment. Malaria control programs throughout Africa are now developing guidelines for the management of each of these manifestations.

S76 DISEASE SYNDROME OVERLAP: IMPLICATONS FOR PROGRAMS. Kazembe P*. Kamuzu Central Hospital, Lilongwe, Malawi.

Disease specific control programs for malaria and pneumonia were developed without full understanding of the possible relationships between the two diseases. We studied malaria and pneumonia at the outpatient department of our hospital to determine how readily these conditions could be distinguished. The guidelines recommended for identifying children with pneumonia actually identify a group of children who also meet the conditions recommended to receive treatment for malaria. Children meeting the clinical definition for pneumonia were more likely to be parasitemic and had higher density parasitemias than children not meeting the pneumonia definition. At our outpatient department, these two definitions identified many of the same children and appeared to distinguish the more severely ill children from those less severely ill rather than identifying a single specific disease.

S77 THE SICK CHILD INITIATIVE: PUTTING SUBSTANCE TO PHC. Tulloch J*. Division of Control of Diarrheal and Respiratory Diseases, World Health Organization.

Today, at health facilities throughout the developing world, integrated case management is occurring. In many facilities, health workers are practicing skills learned in training courses sponsored by vertical disease control programs for ARI and CDD. Recognizing that ill children frequently have clinical signs that satisfy the criteria for more than one disease and that many health workers would benefit from training in updated management of major diseases of children, UNICEF and WHO have begun developing a set of integrated guidelines for the assessment and treatment at health facilities of children with ARI, diarrhoea, malaria, malnutrition, and measles, diseases that cause the vast majority of deaths in children.

S78 INITIAL FIELD EVALUATIONS OF A "SICK CHILD ALGORITHM" - KENYA. Zucker J* and Otieno J. Malaria Branch, Centers for Disease Control, Atlanta, GA; and CDC/Kenya Medical Research Institute.

WHO has coordinated the development of integrated case management charts to guide health workers caring for sick children. These Sick Child Charts incorporate recommendations for management of major disease presentations in young children, respiratory and diarrheal illness, fever illness, malnutrition, and measles. Results from the initial evaluations in Kenya and The Gambia of the assessment, classification, and treatment guidelines of the Sick Child algorithm will be presented, and the implications for improving the management of pediatric illness will be discussed.

S79 THE CHALLENGES OF INTEGRATED DISEASE MANAGEMENT IN CHILD SURVIVAL PROGRAMS. Foster S*. International Health Program Office, CDC, Atlanta, GA.

The initiative to achieve integrated management of the sick child at health facilities is an important step in making quality health care available to children in the developing world. Improved management of the sick child also requires an understanding of 1) the epidemiology of disease in the community, 2) its recognition or non-recognition in the home, and 3) how decisions on care are made at the family and community level. In addition to upgrading case management at facilities, an

active outreach to and involvement in patterns of care in the home and community are required to reduce child mortality.

S80 THE ROLES OF PARASITOLOGY IN BIODIVERSITY STUDIES. Hoberg EP*. USDA, ARS, Biosystematic Parasitology Laboratory, Beltsville, MD.

Parasitology is becoming an integral component of biodiversity programs. Studies have shown parasites to be elegant markers of contemporary and historical ecological relationships, biogeography and host-phylogeny. Complex life cycles of helminths are strongly correlated with intricate foodwebs. Dependence on a series of intermediate, paratenic and definitive hosts indicates that each parasite species exquisitely represents an array of organisms within a community. Knowledge of the evolution of parasite-host assemblages provides direct estimates of the history of ecological associations and community development, and is indicative of the temporal continuity of trophic assemblages. Parasites constitute probes that can be applied directly to questions of contemporary diversity and the historical development of community structure. The dual phenomena of host-parasite evolution and faunal distribution (endemism) are keystones to examining patterns of diversity. A predictive framework, with parasites as indicators, exists for elucidating the impacts of natural or anthropogenic perturbations to faunas and ecosystems. Biodiversity studies promote a revitaliztion of systematics and appreciation of parasitology as the most integrative of the biological disciplines.

S81 THE ROLE OF PARASITE COLLECTIONS AND DATABASES IN BIOLOGICAL SURVEYS. Hoagland KE*. Association of Systematics Collections, Washington, DC.

To the public, biological survey implies the itemization of known species of plants and terrestrial vertebrates. However, survey also means new scientific DISCOVERY, and parasitology has a great role to play. Parasitologists are the natural allies of ecologists, biomedical researchers, epidemiologists, and agricultural researchers, whom we should engage to support the inclusion of parasitological research within the framework of national biological surveys. Collections, research upon collections, and databases derived from field work and collections-based research are inseparable. Discovery of new species and study of phylogenetic relationships and biological properties require voucher specimens, for reasons familiar to us. Yet resources for collections are often given low priority, even by scientists. As habitats and species assemblages are increasingly fragmented and destroyed, we need to preserve not only specimens but records of host associations. The Association of Systematics Collections and its Computer and Data Networking Committee have developed a data model to demonstrate how information from systematics collections can by compatibly recorded across taxonomic disciplines, allowing association of host and parasite data. ASC is working with those planning biological survey activities in the US, Canada, and Mexico to achieve holistic support for collections and data basing activities on a long-term basis, much like the US weather bureau supports meteorological research.

S83 PARASITOLOGY AND BIODIVERSITY: RESEARCH AND FUNDING. Yates TL*. Department of Biology, University of New Mexico, Albuquerque, NM.

Interest in research focused on understanding biological diversity has accelerated rapidly during the past decade. Funding opportunities for this work has increased as well and continues to receive high visibility on national and international levels. Although many projects are being conducted worldwide on the flora and fauna of the Earth, few include an extensive parasite component. The potential is currently high for interdisciplinary research in biodiversity among parasitologists and other scientists. A complimentary high potential for funding such projects currently exists as well. The scientific and funding potential of this cross-disciplinary effort in biological diversity will be

discussed in light of recent developments in Washington. The need for a joint effort at data standardization will also be presented.

S84 A MOBILIZATION PLAN FOR PARASITOLOGY IN ALL TAXON BIODIVERSITY INVENTORIES. Brooks DR*. University of Toronto, Department of Zoology, Toronto, Ontario.

All-Taxon Biodiversity Inventories (ATBI's) may enhance the biodiversity resource base of developing countries. Parasites represent a critical interface between humans and their agendas in under-developed areas, so parasitology should participate in any ATBI. In the ongoing war for the preservation of biodiversity, systematic biologists, including parasitologists, would be analogous to medical support units. At the front are triage teams, close-knit groups of field parasitologists, collecting parasites and making them ready for transport to the equivalent of a MASH unit, a centralized collection unit on the periphery of the ATBI site. There, field data are verified and entered into an electronic knowledge base, and specimens are shipped to the equivalent of a military hospital, the national parasite collection of the ATBI host country. Parasites would be identified to major groups there, the knowledge base updated accordingly, and specimens sent to specialists throughout the world for final identification and systematic work-up. Parasitologists need to be involved in ATBI's at many levels: training specialists and parataxonomists, participating in field collections, helping establish and maintain museum collections in host countries, and helping with the monumental task of identifying and classifying the material collected. This represents an opportunity to renew systematic parasitology throughout the world and to provide a service for mankind at the same time.

S102 MOLECULAR BASIS OF *LEISHMANIA* VIRULENCE. Chang KP*. Microbiology/Immunology, UHS/Chicago Medical School, North Chicago, IL.

How *Leishmania* spp. interact with their respective sandfly vectors, reservoir animals and exposed human populations determines their virulence in leishmaniases. "Invasive determinants" of the parasites are most crucial because they mediate successful invasion of host/vector and evasion of their immunity. Manifestation of virulence presumably requires additional "pathogenic determinants", which are produced by parasites. These determinants interact with "immune factors" of the mammalian hosts, resulting in immunopathology as the principal symptoms in human leishmaniases. Independent up- and down-regulations of the "tripartite" determinants may explain the disparity of virulence, ranging from subclinical infections to fatality observed in leishmanial infections. Recent advances in molecular genetics of Leishmania offer some hope to identify their "invasive" and "immunopathogenic" determinants. Discussion will be focused on attempts to identify candidate determinants for leishmanial invasion of macrophages by genetic means.

S106 THE WALTER REED ARMY INSTITUTE OF RESEARCH: A ONE HUNDRED YEAR LEGACY. Kelley PW*. Division of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC.

The Walter Reed Army Institute of Research has played a central role in preventive medicine and tropical medicine research for 100 years. Major contributions were evident from its earliest days when Walter Reed performed his landmark work on typhoid and yellow fever epidemiology, Frederick Russell manufactured and tested a life saving typhoid vaccine, and Carl Darnell developed a means to purify water with anhydrous chlorine. Later years saw Institute researchers describe key aspects of dengue and Japanese encephalitis transmission, develop numerous vaccines and the jet injector gun, and create one of the world's leading antimalarial drug development efforts. Major activities in the epidemiology, prevention, and therapy of human immunodeficiency virus infections have been

prominent in recent years. This talk will offer an overview to this legacy of scientific accomplishment.

S107 THE ARMY BOARD FOR THE STUDY OF TROPICAL DISEASE IN THE PHILLIPINES 1900-1926. Joy RJ*. Section of Medical History, Uniformed Services Univ. of the Health Sciences, Bethesda, MD.

The work of the "Walter Reed Yellow Fever Board" of June 1900 is well-known. Almost unknown but more important for the United States, its Army and Medical Department, and for medicine in general, was the work of three successive Boards for the Study of Tropical Disease in the Philippines. New and valuable work in ciarrhea, cholera, dengue, amoebiasis, and beri-beri came from the research done by the Army medical officers assigned to the Boards. The political, military, and medical context in which the Boards worked and discussion of the men and their studies are the topics of this presentation.

S108 WALTER REED'S LEGACY: THE GLOBAL IMPACT OF VIROLOGY RESEARCH AT THE WALTER REED ARMY INSTITUTE OF RESEARCH. Hoke CH* and Binn LN. Dept. of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

Over the past century, investigators at the Walter Reed Army Institute of Research have contributed immeasurably to the understanding and control of viral diseases. This work has fulfilled the primary mission--Research for the Soldier--and has resulted in a greatly reduced impact of viral diseases on the ability of our soldiers to defend their country and has had an important impact on disease in the global community. Many scientists have applied considerable talents to a wide range of diseases caused by an even wider range of pathogens. Disease categories studied include respiratory infections, hepatitis, arboviral infections, encephalitis, and fevers of unknown origin. Etiologic agents that are better understood because of work at WRAIR include smallpox, yellow fever, adenovirus, rubella, influenza, Rift Valley fever, Japanese encephalitis, dengue, and hepatitis A, B, C, and E. Although the impact of many diseases has been lessened as a result of activities at WRAIR, many diseases remain to be controlled. Some, no doubt, remain to be discovered. We cannot say with certainty what the challenges of the future will be, but we know that such challenges will emerge. It is our hope that these challenges will be met by teams of WRAIR virologists of the future who will have the successes of the virologists of the past in producing knowledge and products useful both for soldiers and in the global arena.

S110 THE WRAIR MALARIA VACCINE DEVELOPMENT PROGRAM. Ballou WR*. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC.

The Malaria Vaccine Program at the Walter Reed Army Institute of Research had its origins during the 1970's with the increasingly serious threat of malaria for military personnel in Southeast Asia. Beginning with the discovery of a method to maintain long term *in vitro* cultures of falciparum parasites, to the development of the world's first *E. coli* expressed recombinantly engineered vaccine to be tested in humans, to its leading role in the development and testing of new adjuvant strategies, WRAIR and its collaborators have made major contributions to the field. Sporozoite vaccines targeting immune responses against the CS protein have been developed which consistently and safely raise high levels of antibodies against parasite epitopes and which induce cytolytic T cell (CTL) responses against exoerythrocytic schizonts in the liver. WRAIR pioneered the use of attenuated live vectors suitable for use in humans for malaria vaccines, including Salmonella, BCG, and vaccinia, and is a leader in the identification, cloning and characterization of asexual blood stage antigens, some of which have emerged as leading vaccine candidates. A number of vaccine approaches are or will soon be in clinical trials, including an attenuated vaccinia engineered to deliver seven malaria

antigens from various stages of the *Plasmodium falciparum* life cycle. This process of establishing and following new scientific leads to develop vaccines is one of WRAIR's great legacies.

S111 ANTIMALARIAL DRUG DEVELOPMENT, THEN AND NOW. Schuster BG*. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC.

Following World War II, after military downsizing, collaborative efforts between military and civilian sectors to develop new antimalarial drugs were curtailed. In 1962 the military situation in South Vietnam pointed to increased involvement of US military personnel, and a medical research unit was assigned from WRAIR. This unit confirmed that chloroquine-resistant malaria was a major potential problem and gave it highest priority. In 1963 a drug development program was funded and implemented at WRAIR. The original program was organized around a large synthesis effort with over 140 contracts providing thousands of compounds for evaluation. The current program is smaller and is organized around drug discovery more focused on biology of the parasite and mechanisms of drug resistance. Screening of large numbers of compounds is no longer affordable. Requisite evaluation of a lead compound's preclinical pharmacology and toxicology as well as clinical studies and trials necessary for regulatory approval remain essentially the same, although increasing regulatory demands by FDA and evolution of technology have increased the cost and time. In 1962 program directors recognized that antimalarial drug development would require a sustained effort, that currently useful drugs would have a limited span of utility because of the development of resistance, and that it was much more difficult and expensive to start a program from scratch than sustain an ongoing one. As we again face a malaria threat, the nucleus of a drug development program is in place.

S112 A TALE OF TWO VIRUSES: HEPATITIS A VIRUS, HEPATITIS E VIRUS, AND THE FIGHT TO CONTROL CAMPAIGN JAUNDICE. Innis BL*. Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC.

Campaign jaundice historically refers to the acute viral liver disease that invariably appears in war zones. Linked to fecal contamination of food and water, the syndrome has prompted the WRAIR to search for control measures for more than six decades. In the modern era of virology, that search has focused on vaccine development because in a major trial during 1967-69, WRAIR investigators formally established the high efficacy of passive immunity to prevent hepatitis in American soldiers in Korea. Hepatitis A virus was identified in 1973; the first vaccine administered to volunteers was made and tested at the WRAIR in 1986. Since then, the US Army has enrolled thousands of volunteers to characterize candidate vaccines; the aim has been to provide vaccine protection to military and civilian populations. Hepatitis E virus, the only other recognized agent of campaign jaundice, was identified in 1983. Molecular virology has accelerated vaccine development; although the virus has not been propagated to high yield, candidate vaccines of expressed hepatitis E virus proteins are being tested in animals. The lessons learned by the WRAIR during the development of hepatitis A vaccine are being applied to the fight against hepatitis E.

S113 WRAIR TROPICAL DISEASE EPIDEMIOLOGY IN THE FIELD: VIETNAM 1966-8, SOMALIA 1992-3. DeFraites RF*, Sanchez JL, Smoak BL, Magill AJ, Longer CF, and Legters LJ. Walter Reed Army Institute of Research, Washington, DC; and Uniformed Services University of the Health Sciences, Bethesda, MD.

From December 1992 through April 1993, epidemiologists and infectious disease specialists from the WRAIR accompanied U.S. forces in Somalia during Operation Restore Hope. A disease surveillance system covering more than 20,000 U.S. troops was established. Working with a Joint Forward Laboratory deployed from the U.S. Navy Medical Research Institute, rapid on-site diagnosis of most

infectious diseases among U.S. troops was possible. This coordinated effort allowed early identification of disease outbreaks and appropriate intervention. The deployment of a WRAIR epidemiology team to Somalia recalls a tradition established by a 26-man WRAIR-U.S. Army Special Forces Field Epidemiology Survey Team (FEST) during the Vietnam conflict. This team deployed with Special Forces units and performed field investigations on diseases such as malaria, scrub typhus, plague, tropical sprue, and human schistosomiasis. The WRAIR continues to play an active role in the timely definition of disease threats to U.S. troops in the tropics and the development of means to prevent them or to minimize their impact.