# Joint Meeting of The American Society of Parasitologists & The American Association of Veterinary Parasitologists



July 6 — July 10, 1995 Pittsburgh, Pennsylvania

# OF PARASITOLOGISTS

&

# THE AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

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### Announcing a Joint Meeting of

THE AMERICAN SOCIETY
OF PARASITOLOGISTS
(70th Meeting)

THE AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS (40th Meeting)

Pittsburgh, Pennsylvania July 6-10, 1995

### **INFORMATION & REGISTRATION**

Hyatt Regency Hotel, 112 Washington Place

**THURSDAY** 

Regency Foyer, 2nd Floor t

July 6th

Registration Begins, Noon-5:00 p.m.

**FRIDAY** 

Regency Foyer, 2nd Floor t

July 7th

8:00 a.m.—5:00 p.m.

**SATURDAY** 

Regency Foyer, 2nd Floor

July 8th

8:00 a.m.—5:00 p.m.

SUNDAY

Regency Foyer, 2nd Floor

July 9th

8:00 a.m.-Noon

† Items for the Auction may be delivered to this location before 3:00 p.m. on Friday, July 7th.



### **WELCOME RECEPTION**



Thursday, July 6th 7:00-10:00 p.m. **Grand Ballroom** 

### **SOCIAL, MATCH THE FACES & AUCTION**



Friday, July 7th Preview: 6:30-7:30 p.m. Ballrooms 2-3-4

### PFIZER, RECEPTION FOR ASP/AAVP

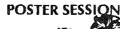


Saturday, July 8th 7:30-11:00 p.m. **Grand Ballroom** 

### STUDENT PARTY



Sunday, July 9th 7:00-10:00 p.m. Ballrooms 4-5

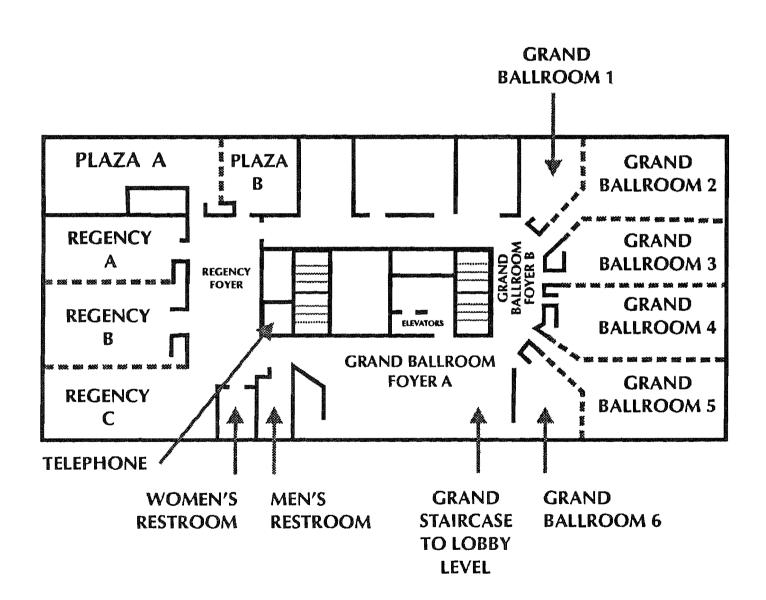




Monday, July 10th 1:00-4:00 p.m. Plaza A-B

# MAP

# HYATT REGENCY HOTEL SECOND FLOOR DIAGRAM



Time	Thurs		Fri.	7th		
8:00			i	OPEN	ING	
9:00	ASP	AAVP	PLENARY SESSION			
10:00	COUNCIL MEETING	COUNCIL	р. 11			
11:00			p. 12	TOLL-STO LECT		)
12:00						
1:00	p. 10	p. 10	√ CY−1	\PY—I	₹Ţ	SIS CF
2:00	STUD	DENT	ECOLOGY/ EPIDEMIOLOGY—	CHEMOTHERAPY—	BIOCHEMISTRY/ PHYSIOLOGY—I	COCCIDIOSIS
3:00	WORK p. 10	SHOP	EC			
4:00			р. 12	р. 14	<b>p.</b> 15	p. 17
5:00		e de la companya de La companya de la co				
6:00				CTION		
7:00	ASP/	AAVP	<u> </u>	ATCH T	HE FACI	ES 
8:00	WELCOME/		ASP AUCTION			
9:00		DENTS' PTION		AUL		
10:00	p. 11		р. 18			

p. = page in Program

Time		Sat. 8	3th		Sı	ın. 9th		Mon. 1	Oth
7:00	A p. 18		PRESID AKFAST	ENTS					
8:00	Ţ	Ţ	EMO II		ENT/	ET ZASTE SHIPS	R R	ł	UEDING VON IAND LECTURE
9:00	ECOLOGY/ EPIDEMIOLOGY—II	MMUNOLOGY	OHEW P. 23	ATICS/	DEVELOPMENT, LIFE CYCLES	C INVERT HOST-PARASITE	MOLECULAR		ASP & AAVP CIAL AWARDS
10:00	EPIDEA	IMMUN	HEW OL_LI	SYSTEMATICS/ PHYLOGENY—	DEV	IMMUNOL II	WO O	ASP BUSINESS	AAVP BUSINESS
11:00	p. 18	p. 21	E BIOCHEM/	p. 24	p. 29	₹ p. 32	р. 33	MEETING p. 41	MEETING p. 41
12:00					•				
1:00	PRESID	ASP/AAN ENTIAL A	/P DDRESSES	5		NOL	LATE BREAKERS	ASP/A	<b>NAVP</b>
2:00	p. 26	B. McGHE	E LECTUF	RE	CA/	TON III p. 37	p. 39 ∰ ⊐ ∰	JOI POS	1
3:00	p. 27		TRIAL LIA	****	ECOLOGY/ EPIDEMIOLOGY III			SESS p. 41-46	ION
4:00	P. 27		MPOSIU!		p. 35	SYSTEMATICS/ PHYLOGENY II			
5:00	CHE p. 28	MOTH	ERAPY-	111		p. 38			
6:00	P. 20								
7:00					A	SP/AAV	P		
8:00		PFIZ RECEP				TUDEN <sup>*</sup> PARTY			
9:00		FC ASP/A		Technology of the		ERYON			
10:00	р. 29	AJF/F	**** L		p. 39	ELCOM	C.		

AUTHOR INDEX pp. 47–54
ADDENDUM: AAVP PRESIDENT'S SYMPOSIUM
& EQUINE PARASITIC WORKSHOP, pp. 55–57
ABSTRACTS pp. 58–end

p. = page in Program

# FOR YOUR INFORMATION

### COFFEE



# NEED TO PRACTICE YOUR TALK?



### **SETTING UP A POSTER?**



### PARKING?



### LATE BREAKERS



- All coffee breaks: Regency Foyer
- Friday, July 7, 3:00–3:15 p.m.
   Saturday, July 8, 10:00–10:15 a.m. & 3:00–3:15 p.m.
   Sunday, July 9, 10:00–10:15 a.m. & 3:00–3:15 p.m.

Ballroom 1 will be available for previewing your slides from 8:00 a.m.-4:00 p.m., Friday through Sunday, and from 8:00-11:00 a.m. on Monday.

If you have a poster, Plaza A-B will open at 8:00 a.m. on Monday, July 10th. Please give yourself enough time to set up before the actual session begins at 1:00 p.m. Thanks for your cooperation.

- Parking in the hotel garage: \$8/day (8 a.m.-5 p.m.)
- Civic Arena, across from hotel: \$4/day.
- Hotel guests, 24-hr. parking: \$10/day self-parking, \$15/day valet parking.

This session is specifically designed for brief presentations of important, new data/discoveries obtained after this Program went to press. LATE BREAKERS will be held on Sunday, July 9th, from 1:00–3:00 p.m. in Regency C. Presentations are restricted to five minutes with five minutes for discussion. Submit your abstract of 200 words or less no later than 5:00 p.m., July 7th to: Dr. Darwin Murrell (Beltsville Agricultural Research Center, Room 223, Bldg. 003, BARC-West, Beltsville, MD 20705; Telephone: 301/504-6070, FAX: 301/504-5474).

### **MATCH THE FACES**





While you're previewing auction items, Match the Faces to the names of "famous" parasitologists. Win a great prize.

Friday, July 7th 6:30–7:30 p.m. Ballroom 2–3–4

### **ASP PAST PRESIDENTS BREAKFAST**



Saturday, July 8th 7:00–8:00 a.m. Ballroom 6

### POLITICAL ACTION NETWORK (PAN) LUNCHEON



Saturday, July 8th Noon Ballroom 6

### STUDENT BUSINESS MEETING



Sunday, July 9th 5:30–6:30 p.m. Ballroom 6

### A MULTIMEDIA ATLAS



Dr. A.K. Prestwood will demon-Friday-Sunday strate a compact-disk she has authored, Regency Foyer "A Multimedia Atlas of Internal Parasites of Horses," lavishly illustrated with animated life cycle diagrams. Peruse at your leisure.

**CHILD CARE** 



A list of state-certified day-care providers is available from the Front Desk at the Hyatt Regency. In addition to the list provided by the Hyatt, AVMA has contracted Kiddie Corp. If interested, call Kiddie Corp. directly (619/455-1718).

# THURSDAY MORNING, JULY 6th

8:00 a.m.-1:00 p.m. ASP COUNCIL MEETING (working breakfast & lunch),

Ballroom 2. Officers, committee chairpersons, past presidents

and affiliate representatives may attend.

8:00 a.m.-1:00 p.m. AAVP COUNCIL MEETING (working breakfast & lunch),

Plaza A. Officers, committee chairpersons, past presidents

and affiliate representatives may attend.

### THURSDAY AFTERNOON

2:00–4:15 p.m. EIGHTH ANNUAL ASP STUDENT WORKSHOP, Regency B.

Presiding: S.D. SNYDER E.J. WETZEL

University of Nebraska Wake Forest University
Lincoln NE Winston-Salem NC

Theme: GETTING STARTED IN ACADEMIC PARASITOLOGY

<u>TIME</u>	PAPER NO.	
2:00		Introduction. E.J. WETZEL.
2:05	1	Getting that teaching job at a smaller comprehensive university. D.D. WITTROCK, Department of Biology, University of Wisconsin, Eau Claire WI.
2:30		Discussion.
2:35	2	Teaching an introductory course in parasitology to undergraduates. H.D. BLANKE-SPOOR, Department of Biology, Hope College, Holland MI.
3:00		Discussion.
3:05	3	Field parasitology at Cedar Point: The design of a course. J. JANOVY, JR., School of Biological Sciences, University of Nebraska, Lincoln NE.
3:30		Discussion
3:35	4	So you have your own lab. M.E. SCOTT, Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, QC, Canada.
4:00		Open discussion and close.

# THURSDAY EVENING

7:00– ⊕ ♬ p.m. ASP/AAVP WELCOMING PRESIDENTS' RECEPTION, BUFFET &

ATTITUDE ADJUSTMENT, Grand Ballroom.

### FRIDAY MORNING, JULY 7th

8:00 a.m.-11:00 a.m. OPENING PLENARY SESSION, Regency A-C.

Presiding: P.M. SCHANTZ L.S. MANSFIELD

Centers for Disease Control Michigan State University

Atlanta GA East Lansing MI

Theme: INTESTINAL HELMINTH INFECTION & GROWTH & DEVELOPMENT: THE CASE

FOR INTERVENTION.

	FOR INTERVENTION.			
TIME	PAPER NO.			
8:15		Introduction. P.M. SCHANTZ, DPD, NCID, CDC, Atlanta GA.		
8:20	5	Intestinal helminth infections inhibit growth and development: The evidence from animal models supports intervention. L.S. MANSFIELD, College of Veterinary Medicine, Michigan State University, East Lansing MI.		
8:50	6	Impact of intestinal helminth infections in humans: Effects, mechanisms and recommendations. L.S. STEPHENSON, Division of Nutritional Sciences, Cornell University, Ithaca NY.		
9:20	7	Allergy, intestinal helminths and nutrition: The good, bad and the ugly. N.R. LYNCH*, I. HAGEL and M. DiPRISCO, Central University of Venezuela, Caracas, Venezuela.		
9:50	8	A healthy body and a healthy mind: The relationship between parasitic helminth infection and cognitive function/educational achievement in school-age children. C. NOKES* and D.A.P. BUNDY, Department of Zoology, Oxford University, UK.		
10:20	9	Intestinal helminths and malnutrition: A global perspective. R.J. STOLTZFUS, Division of Human Nutrition, School of Public Health, Johns Hopkins University, Baltimore MD.		
10:50		Closing remarks, discussion.		

### FRIDAY MORNING (continued)

STOLL-STUNKARD LECTURE, Regency A-C. 11:00 a.m.-noon

G. CAIN Presiding:

> University of Iowa Iowa City IA

**PAPER** 

<u>TIME</u> NO.

11:00 10 Molecular genetics of development and behavior in C. elegans. P.W. STERNBERG,

Division of Biology, Howard Hughes Medical Institute/CalTech, Pasadena CA.

### FRIDAY AFTERNOON

1:00-4:45 p.m. ECOLOGY/EPIDEMIOLOGY-I, Regency A.

Presiding: J. HARLEY H. EURE

> Eastern Kentucky University Wake Forest University

Richmond KY Winston-Salem NC

**PAPER** TIME NO. 1:00 11† Ecological analysis of copepods living up the noses of blue sharks. J.W. KOHL\* and G.W. BENZ, Tennessee Aquarium and University of Tennessee, Chattanooga TN. 1:15 12† Epidemiology of the strongylid parasites of horses in Prince Edward Island, Canada. K. M'ABURI\* and G. CONBOY, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada. 1:30 13+ An artifical life approach to host-parasite interactions. P.G. WILBER\*, Department of Biology, and H.D. SHAPIRO, Department of Computer Science, The University of New Mexico, Albuquerque NM. 1:45 14† Distribution of paratenic infections of *Plagiorhynchus cylindraceus* (Acanthocephala) among co-occurring Nebraskan mammals. N.R. COADY, School of Biological Sciences, University of Nebraska, Lincoln NE. 2:00 15† Altered escape response in cockroaches infected with an acanthocephalan (Moniliformis moniliformis) and its impact on predation. L.D. FLOYD\*, M. FREEHLING, J.

MOORE and F. LIBERSAT, Department of Biology, Colorado State University,

Fort Collins CO and Ben Gurion University of the Negev, Israel.

<sup>†</sup> Student Competition Paper

2:15	16†	Regional and local richness in the parasite communities of Pacific halibut, <i>Hippo-glossus stenolepis</i> . R.B. BLAYLOCK*, Pacific Biological Station, Nanaimo, British Columbia, and Department of Biological Sciences, University of Alberta, Edmonton, Alberta, J.C. HOLMES, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, and L. MARGOLIS, Pacific Biological Station, Nanaimo, British Columbia, Canada.
2:30	17†	The effect of lake size on the determination of the parasite component community of yellow perch, <i>Perca flavescens</i> (Mitchill). D.A. ZELMER* and H.P. ARAI, Department of Biological Sciences, University of Calgary, Alberta, Canada.
2:45	18†	Effect of age on infectivity of cercariae of <i>Halipegus occidualis</i> (Digenea: Hemiuridae) to their second intermediate host. E.J. WETZEL* and G.W. ESCH, Department of Biology, Wake Forest University, Winston-Salem NC.
3:00		BREAK.
3:15	19†	Resource allocation by <i>Helisoma anceps</i> infected with <i>Halipegus occidualis</i> . B.E. KEAS* and G.W. ESCH, Department of Biology, Wake Forest University, Winston-Salem NC.
3:30	20†	On the distribution of aspidogastrids (Trematoda) in freshwater mussels (Bivalvia: Unionidae) from eastern North America. S.S. CURRAN, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT.
3:45	21†	Investigation of the distribution of copepods on shortfin make sharks, with special reference to copepod ecology and life history. J.C. BACKER* and G.W. BENZ, Tennessee Aquarium and University of Tennessee, Chattanooga TN.
4:00	22†	Resource partitioning of scalloped hammerhead sharks by two genera of parasitic copepods. K.L. SMEDLEY* and G.W. BENZ, Tennessee Aquarium and University of Tennessee, Chattanooga TN.
4:15	23	Urban wildlife as reservoirs of cat fleas, Ctenocephalides felis. M.W. DRYDEN*, Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan KS, A.B. BROCE, Department of Entomology, Kansas State University, Manhattan KS, J. CAWTHRA and D. GNAD, Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan KS.
4:30	24	PARABAN-A mathematical model of parasitic infections of cattle. G. SMITH*, School of Veterinary Medicine, University of Pennsylvania, Kennett Square PA, J.A. JACOBSEN, Merck AgVet, Rahway NJ, and J. GUERRERO, MSD AgVet, Merck Sharp and Dohme deEspana, Madrid, Spain.

1:00—4:30 p.m. CHEMOTHERAPY OF PARASITIC INFECTIONS—I, Regency B.

Presiding:	C. MAGOWAN Lawrence Berkeley Laboratory Berkeley CA		H.W. HAINES Merck Research Laboratories Rahway NJ
TIME	PAPER NO.		
1:00	25	goats under extensive managem Agricultural Research Institute, RANJAN, Institute of Parasito J.M. GATHUMA, W.K. MUN	eatment regimes on flock performance of sheep and nent in semi-arid Kenya. P.M. GATONGI, Kenya Kenya, M.E. SCOTT, R.K. PRICHARD*, S. logy, McGill University, Montreal, Quebec, Canada, IYUA, University of Nairobi, Faculty of Veterinary nya, and H. CHERUIYOT, Kenya Agricultural
1:15	26	MOORE, A.M. ZAJAC*, C.D Departments of Biological Scie. Virginia-Maryland Regional Co	ne control of internal parasites of grazing lambs. G.A. THATCHER, D. NOTTER, S. UMBERGER, nces, Pathobiology, Large Animal Clinical Sciences, ollege of Veterinary Medicine and Department of echnic Institute, Blacksburg VA.
1:30	27	efficacy. M.R. CHAPMAN*, D	against equine cyathostomes: Resistance or lack of D.D. FRENCH and T.R. KLEI, Department of tate University Agricultural Center, Baton Rouge LA.
1:45	28	D.K. MILLER* and T.M. CR.	unthelmintic resistance in goats and sheep in Texas. AIG, Department of Veterinary Pathobiology, , Texas A&M University, College Station TX.
2:00	29	nema viteae. D. CHRIST*, A.J. Education, Indiana University	on muscle contractility in the filariid Acanthocheilo-MINARDI, South Bend Center for Medical School of Medicine, and H.J. SAZ, Department of of Notre Dame, Notre Dame IN.
2:15	30	S.S. JOHNSON*, G.A. COND DUTTON, S.J. NELSON, E.M	odepsipeptide PF1022A in <i>in vitro</i> and <i>in vivo</i> models. DER, D.S. NOWAKOWSKI, T.E. BLAKE, F.E. I. THOMAS, J.P. DAVIS, T.G. GEARY and D.P. Discovery Research, The Upjohn Co., Kalamazoo
2:30	31	horses. C.R. REINEMEYER*	etect pyrantel resistance in strongylid nematodes of and A.A. TINEO, Department of Comparative see College of Veterinary Medicine, Knoxville TN.

2:45	32	Benzimidazole resistant <i>Haemonchus contortus</i> of small ruminants in Hawai'i. J.P. TRITSCHLER, II* and B.R. LEAMASTER, Beaumont Research Station and Department of Animal Sciences, University of Hawaii at Manoa, Hilo HI.
3:00		BREAK.
3:15	33	Efficacy of moxidectin pour-on in the treatment of gastrointestinal parasites of cattle. J. DIPIETRO*, R. VALDEZ, D. MORIN, C. LICHTENSTEIGER, A. PAUL and K.S. TODD, JR., Departments of Veterinary Pathobiology and Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana IL, and F. GUERINO, Agricultural Research Division, American Cyanamid Co., Princeton NJ.
3:30	34	The development of a strategic deworming program for horses. W.G. KVASNICKA*, D.H. BLISS and J.B. SHANER, University of Nevada, Reno NV, Midamerica AG Research, Verona IL, and University of Missouri, Columbia MO.
3:45	35	Fasting enhances Albendazole bioavailability in sheep and cattle. C. LANUSSE*, A. LIFSCHITZ, S. SANCHEZ, G. VIRKEL, L. ALVAREZ and M. MASTROMARION, Departamento de Fisiopatologica, Facultad Ciencias Veterinarias, Universidad Nacional del Centro, Tandil, Argentina.
4:00	36	Efficacy of Imidacloprid for control of fleas on dogs. R.G. ARTHER*, Bayer Corp., Agriculture Division, Animal Health, Shawnee KS, T. HOPKINS, Bayer Australia Ltd., Queensland, Australia, J. CUNNINGHAM and R. EVERETT, AgResearch Consultants, Greenbrier AR.
4:15	37	The critical test and efficacy of pyrantel pamoate for <i>Anoplocephala perfoliata</i> in equids. J.O.D. SLOCOMBE, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

# 1:00–5:00 p.m. BIOCHEMISTRY & PHYSIOLOGY—I, Regency C.

Presiding: H. PROFOUS-JUCHELKA T.G. GEARY

Merck Research Labs The Upjohn Company

Rahway NJ Kalamazoo MI

TIME	PAPER NO.	
1:00	38	Dirofilaria immitis alters the behavior of canine pulmonary artery endothelial cells studied in vitro. L. KAISER*, M. MUPANOMUNDA, A.J. SCHWARTZ and J.W. WILLIAMS, Departments of Physiology and Microbiology, Michigan State University, East Lansing MI.

1:15	39	Correlative histochemical observations on intestinal epithelium of <i>Trichuris wis</i> (Nematoda). M. JOHAL, Department of Zoology, Punjabi University, Patiala, India.
1:30	40	Comparative pharmacology of inhibitory nematode FMRFamide-related peptides (FaRPs). A.G. MAULE*, T.G. GEARY, J.W. BOWMAN, K.L. BLAIR, D.P. THOMPSON, The Upjohn Co., Kalamazoo MI, N.J. MARKS, D.W. HALTON and U.C. SHAW, Comparative Neuroendocrinology Research Group, The Queen's University of Belfast, UK.
1:45	41	More nematode FMRFamide-related peptides (FaRPs). N.J. MARKS*, U.C. SHAW, D.W. HALTON, Comparative Neuroendocrinology Research Group, The Queen's University of Belfast, UK, J.P. DAVIES, A.G. MAULE, T.G. GEARY and D.P. THOMPSON, The Upjohn Co., Kalamazoo MI.
2:00	42	Transglutaminase activity in equine strongyles and its potential role in growth and development. U.R. RAO*, M.R. CHAPMAN, R.N. SINGH, K. MEHTA and T.R. KLEI, Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, and Department of Clinical Investigation, M.D. Anderson Cancer Center, University of Texas, Houston TX.
2:15	43	Biochemical characterization of excreted/secreted proteinases of adult <i>Haemonchus contortus</i> . H.D.F.H. SCHALLIG* and D.P. KNOX, Utrecht University, Institute of Infectious Diseases and Immunology, Department of Parasitology, Utrecht, The Netherlands.
2:30	44	The uptake and incorporation of <sup>3</sup> H-leucine labeled hemoglobin by adult <i>Haemon-chus contortus in vitro</i> . R.H. FETTERER* and M.L. RHOADS, Parasite Biology and Epidemiology Laboratory, USDA, ARS, Beltsville MD.
2:45	<b>4</b> 5	Degradation of connective tissue matrices by <i>Haemonchus contortus</i> . M.L. RHOADS* and R.H. FETTERER, Parasite Biology and Epidemiology Laboratory, USDA, ARS, Beltsville MD.
3:00		BREAK.
3:15	46	Are aerobic and anaerobic-specific isozymes present during the development of <i>Ascaris suum</i> ? D. WALKER*, K. HAYTON, E. DURAN, P. KOMUNIECKI and R. KOMUNIECKI, Department of Biology, University of Toledo, Toledo OH.
3:30	47	Potential role of a novel subunit in the reduced sensitivity of the adult muscle <i>Ascaris suum</i> pyruvate dehydrogenase complex to NADH. R. ARNETTE*, D. WALKER and R. KOMUNIECKI, Department of Biology, University of Toledo, Toledo OH.

3:45	48	The pyruvate dehydrogenase complex from the insect trypanosomatid, <i>Crithidia fasciculata</i> : E3 binding protein contains multiple lipoyl domains. F. DIAZ* and R. KOMUNIECKI, Department of Biology, University of Toledo, Toledo OH.
4:00	<b>4</b> 9	Evaluation of serum chemistry values associated with avian malaria infections in African black-footed penguins ( <i>Spheniscus demersus</i> ). T.K. GRACZYK, Department of Molecular Microbiology and Immunology, Johns Hopkins University, Baltimore MD, M.R. CRANFIELD* and E.J. BICKNESE, The Baltimore Zoo, Baltimore MD.
4:15	50	Glycogen metabolism in <i>Giardia intestinalis</i> trophozoite and in differentiation to cysts. D.S. CROSS and J.G. ZALITIS*, School of Biochemistry and Molecular Genetics, University of New South Wales, Sydney, Australia.
4:30	51†	Interisolate heterogeneity of <i>Haemonchus contortus</i> excretory secretory proteases. F.N. KARANU*, F.R. RURANGIRWA, T.C. McGUIRE and D.P. JASMER, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman WA.
4:45	52†	Detection and characterization of a salivary vasodilator from <i>Culicoides variipennis</i> , the North American vector of bluetongue viruses. A.A. PEREZ DE LEON*, Plant, Soil and Insect Sciences Department, University of Wyoming and ABADRL-USDA-ARS, and W.J. TABACHNICK, ABADRL-USDA-ARS, Laramie WY.

# 1:15–4:30 p.m. 31st ANNUAL COCCIDIOSIS CONFERENCE, Plaza A.

Presiding:	A.P. RICKETTS	Theme:	IMMUNITY TO COCCIDIA &
	Pfizer Central Research		RELATED APICOMPLEXANS-
	Groton CT		CONTEMPORARY APPROACHES.

TIME	PAPER NO.	
1:15		Introduction, A.P. RICKETTS
1:20	53	Role of T and B lymphocytes in immunity to <i>Eimeria</i> infection in mice. R.C. FINDLY*, L. WEN, S.J. ROBERTS, A.L. SMITH and A.C. HAYDAY, Central Research, Pfizer Inc., Groton CT, and Department of Biology, Yale University, New Haven CT.
1:45	54	Role of $\alpha/\beta$ and $\gamma/\delta$ T cells in resistance against <i>Toxoplasma gondii</i> . Y. SUZUKI*, O. LIESENFELD and J.S. REMINGTON, Research Institute, Palo Alto Medical Foundation and Stanford University School of Medicine, Palo Alto CA.

<sup>†</sup> Student Competition Paper

2:10	55	T. gondii induces CD4+ T cell unresponsiveness in mice. I. KHAN*, T. MATSUURA and L.H. KASPER, Dartmouth Medical School, Hanover NH.
2:35	56	A Toxoplasma gondii superantigen: Biological effects and preliminary characterization. E.Y. DENKERS*, P. CASPAR, S. HEINY and A. SHER, Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda MD.
3:00		BREAK
3:10	57	The γδ T cell immunity to blood-stage malaria. W.P. WEIDANZ*, H. VAN DER HEYDE, M. ELLOSO and D. MANNING, Medical School, University of Wisconsin, Madison WI.
3:35	58	Development of CD8+ T cell mediated immunity against liver stages of malaria parasites. F. ZAVALA, New York University Medical Center, New York NY.
4:00	59	Multi-gene DNA vaccination against malaria: Evasion of MHC restriction? D.L. DOOLAN*, M. SEDEGAH, R.C. HEDSTROM, M. MARGALITH, P. HOBART and S.L. HOFFMAN, Naval Medical Research Institute, Bethesda MD and Vical Inc., San Diego CA.
4:25		Closing Remarks, A.P. RICKETTS.

# FRIDAY EVENING

6:30-7:30 p.m. SOCIAL, AUCTION PREVIEW, and introducing a new event,

"MATCH THE FACES," Ballrooms 2-3-4.

7:30-10:00 p.m. SEVENTH ANNUAL PARASITOLOGY AUCTION, Ballrooms 2-3-4.

Presiding W.E. FOOR Auctioneering: The DYNAMICTRIO of

University of Pittsburgh J.R. BRISTOL, W.M. KEMP

Johnstown PA & J.A. OAKS

# SATURDAY MORNING, JULY 8th

7:00–8:00 a.m. ASP PAST PRESIDENTS BREAKFAST, Ballroom 6.

8:00 a.m.-noon ECOLOGY/EPIDEMIOLOGY—II, Regency A.

Presiding: S. HENDRIX G.W. ESCH

Gettysburg College Wake Forest University
Gettysburg PA Winston-Salem NC

TIME	PAPER NO.	
8:00	60†	Energy storage and host-finding strategies of nematode larvae. D.L. MEDICA*, Department of Animal Sciences, Rutgers University, New Brunswick NJ, M.R. CHAPMAN, T.R. KLEI, Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, and M.V.K. SUKHDEO, Department of Animal Sciences, Rutgers University, New Brunswick NJ.
8:15	61†	A temporal comparison of the prevalence of intestinal parasites in fecal specimens collected from public parks in metropolitan Toronto, Ontario. R. BERMAN*, J. YANG and R. HANSELL, Department of Zoology, University of Toronto, Ontario, Canada.
8:30	62	Coccidian guilds (Apicomplexa: Eimeriidae) in white-tailed and black-tailed prairie dogs ( <i>Cynomys leucurus</i> and <i>C. ludovicianus</i> ) in Wyoming. R.S. SEVILLE* and J. SHIVELY, Department of Zoology and Physiology, University of Wyoming, Casper WY.
8:45	63	The effect of nematophagous fungi fed to cattle, sheep and horses on the development of infective larvae. J. BIRD* and R.P. HERD, Department of Veterinary Preventive Medicine, The Ohio State University, Columbus OH.
9:00	64	The effect of predacious fungi on free-living preparasitic nematode larvae. M. LARSEN, P. NANSEN*, S.A. HENRIKSEN, J. GRØNVOLD, J. WOLSTRUP and R.M. WARUIRU, Danish Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.
9:15	65	Eels from the heart of a mako shark: A conundrum from 500 fathoms. J.N. CAIRA*, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT, G.W. BENZ, The Tennessee Aquarium, Chattanooga TN, J. BORUCINSKA, Department of Pathobiology, University of Connecticut, Storrs CT, N.E. KOHLER and J.G. CASEY, National Marine Fisheries Service, Narragansett RI.
9:30	66	Postmortem migration of <i>Hymenolepis diminuta</i> (Cestoidea: Cyclophyllidea) in outbred Sprague Dawley rats. T.R. PLATT* and S.D. VILLANUEVA, Department of Biology, Saint Mary's College, Notre Dame IN.
9:45	67	Variation in nematode infection levels after the first grazing season in dairy farms in The Netherlands. J. POOT, M. EYSKER* and T.J.G.M. LAM, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

<sup>†</sup> Student Competition Paper

10:00		BREAK.
10:15	68	The distribution and pathobiology of Neoechinorhynchus cylindratus in the intestine of green sunfish, Lepomis cyanellus. M. ADEL-MEGUID, G.W. ESCH and H.E. EURE*, Department of Biology, Wake Forest University, Winston-Salem NC.
10:30	69	National prevalence of canine parsites based on centrifugal sucrose flotation examination of fecal specimens. B.L. BLAGBURN*, D.S. LINDSAY, J.L. VAUGHAN, N.S. RIPPEY, R.C. LYNN, W.G. KELCH, G.C. RITCHIE and D.L. HEPLER, Department of Pathobiology, Auburn University, Auburn AL, and CIBA Animal Health, Greenboro NC.
10:45	70	Realized niche structure and autecological community structuring mechanisms in the gregarine assemblage parasitizing <i>Tenebrio molitor</i> . R.E. CLOPTON, Department of Entomology, Texas A&M University, College Station TX.
11:00	71	Parasite burdens of two bison herds in Kansas. R.K. RIDLEY*, A. MELLI, P. STEWART, T. WERKMEISTER and M. O'DONNELL, Kansas State University, Manhattan KS.
11:15	72	Preliminary observations on the epidemiology of gastrointestinal helminths of free-ranging scimitar horned oryx in Texas with evidence for summer arrest of Camelo-strongylus mentulatus. T.M. CRAIG* and J. JENSEN, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station TX.
11:30	73	Distribution of helminth parasites of native gobioid and introduced poeciliid fishes in streams of Hawai'i Island. W.F. FONT, Department of Biological Sciences, Southeastern Louisiana University, Hammond LA.
11:45	74	Isolation and characterization of <i>Trypanosoma cruzi</i> from dogs in Virginia. S.C. BARR*, Department of Clinical Sciences, Cornell University, Ithaca NY, O. VAN BEEK, Westwood Animal Hospital, Staunton VA, M.S. CARLISLE-NOWAK, Department of Pathology, Cornell University, Ithaca NY, J.W. LOPEZ, Department of Diagnostic Laboratory, Cornell University, Ithaca NY, L.V. KIRCHHOFF, Department of Internal Medicine, University of Iowa, Iowa City IA, N. ALLISON, Pathology Reference Laboratory, Department of Agriculture and Consumer Services, Richmond VA, A.M. ZAJAC, Department of Parasitology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA, A. DE LAHUNTA, Department of Anatomy, Cornell University, Ithaca NY, D.H. SCHLAFER, Department of Pathology, Cornell University, Ithaca NY, and W.T. CRANDALL, Ardmoor Veterinary Hospital, Vernon VA.

8:00 a.m.-noon IMMUNOLOGY---I, Regency B.

Presiding:	R. LEVEN Hershey M Hammelsto	edical College	J.F. WILLIAMS Michigan State University East Lansing MI
TIME	PAPER NO.		
8:00	75	0 1 , 0	of <i>Trichinella spiralis</i> revealed by immunocytochemical L. WU and T. YAMADA, Department of Parasitol-Medicine, Gifu, Japan.
8:15	76	process antigen in swine whipw College of Veterinary Medicine	ture in the colon: Lymphoglandular complexes form infections. L.S. MANSFIELD*, Microbiology, c, Michigan State University, East Lansing MI, and obiology Laboratory, USDA, Beltsville MD.
8:30	77†	KELLMAN*, A.M. ZAJAC an	ions from pigs infected with <i>Trichuris suis</i> . M.F. d J.F. URBAN, Virginia Polytechnic University, nmunobiology Laboratory, USDA, ARS, LPSI,
8:45	78†		selenium on the course of experimental Chagas' D. DAVIS, Biology Department, Western Kentucky
9:00	<i>7</i> 9†	· - · · -	ma cruzi infection in mice held at elevated environand C.D. DAVIS, Department of Biology, Western Green KY.
9:15	80†		lity to <i>Toxoplasma gondii</i> infection in mice: Cellular NERJEE* and F. MONROY, Department of Life ity, Terre Haute IN.
9:30	81†	M.L. SCHITO*, J.R. BARTA, College, University of Guelph,	ular responses to Eimeria papillata in BALB/C mice. Department of Pathology, Ontario Veterinary Guelph, Ontario, Canada, and B. CHOBOTAR, ws University, Berrien Springs MI.

9:45	82	In vivo and in vitro studies of irradiated Eimeria tenella. M.C. JENKINS*, M.S. ABRAHAMSEN, H.D. DANFORTH, EH. LEE and H.S. LILLEHOJ, Parasite Immunobiology and Parasite Biology and Epidemiology Laboratories, ARS, Beltsville MD, Department of Pathobiology, University of Minnesota, St. Paul MN, and VeTech Laboratories, Rockwood, Ontario, Canada.
10:00		BREAK.
10:15	83	Characterization of immune effector cells mediating protective immunity to <i>Eimeria acervulina</i> . H.S. LILLEHOJ* and J.A. TROUT, Parasite Immunobiology Laboratory, LPSI, USDA-ARS, Beltsville MD.
10:30	84	Characterization of a low molecular weight antigen of <i>Eimeria tenella</i> . J.R. BARTA*, Department of Pathology, University of Guelph, Guelph, Ontario, Canada, S.A. TENNYSON, Tropical Diseases Unit, The Toronto Hospital, Toronto, Ontario, Canada, and D.S. MARTIN, Department of Pathology, University of Guelph, Guelph, Ontario, Canada.
10:45	85	Efficacy of Eimeria maxima oocyst immunization with day-old broiler chickens. H.D. DANFORTH*, Parasite Biology and Epidemiology Laboratory, USDA, ARS, Beltsville MD, K. WATKINS, Elanco Animal Health, Indianapolis IN, M. DEKICH, Perdue Farms Inc., Salisbury MD.
11:00	86	Development of resistance to <i>Eimeria boris</i> in young calves while on lasalocid. B.E. STROMBERG*, S.M. PROUTY, G.A. AVERBECK, J.F. ANDERSON and D.L. HAGGARD, Department of Veterinary Pathology, College of Veterinary Medicine, University of Minnesota, St. Paul MN.
11:15	87	IL12 inhibits development of <i>Cryptosporidium parvum</i> (Cp) in neonatal BALB/C and SCID mice. J.F. URBAN*, R. FAYER, W. GAUSE, M. GATELY and F. FINKELMAN, PIL, LPSI, ARS, USDA, Beltsville MD, and Hoffman-LaRoche Inc., Nutley NJ, and the Departments of Medicine and Microbiology, USUHS, Bethesda MD.
11:30	88	Stability and safety of the attenuated vaccine strain, <i>Toxoplasma gondii</i> T-263, in pregnant cats. I. POPIEL*, Paravax Inc., Fort Collins CO, L. FORESMAN, Department of Laboratory Animal Resources, A. FREYRE, Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City, KS.
11:45	89	Protective action to mice infected with <i>Plasmodium berghei</i> by spleen immune RNA. Z. LU, C. YANG and Z. XU, Jiangsu Institute of Parasitic Diseases, PR China.

### 8:30—10:00 a.m. CHEMOTHERAPY OF PARASITIC INFECTIONS—II, Regency C.

Presiding:	J.A. HAWKINS	G.A. CONDER
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MSO AgVet Pfizer Animal Health

Starkville MS Groton CT

TIME	PAPER NO.	
8:30	90	Efficacy of decoquinate on ovine coccidiosis. T.K. MILLER* and D.D. BOWMAN, Department of Microbiology, Immunology and Parasitology, College of Veterinary Medicine, Cornell University, Ithaca NY.
8:45	91	Efficacy of atovaquone against <i>Babesia microti</i> infection in hamsters. H.S. OZ* and W.T. HUGHES, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis TN.
9:00	92	Development of a microtiter ELISA for rapid screening of pharmaceuticals against <i>Cryptosporidium in vitro</i> . K.M. WOODS, M.V. NESTERENKO and S.J. UPTON*, Division of Biology, Kansas State University, Manhattan KS.
9:15	93	Infection dynamics associated with <i>Cryptosporidium parrum</i> in an ICR outbred suckling mouse model. S.J. UPTON*, Division of Biology, Kansas State University, Manhattan KS.
9:30	94	Safety of moxidectin in mares and their unborn/newborn foals. R.L. ASQUITH*, J. KIVIPELTO and E.L. JOHNSON, Horse Research Center, University of Florida, Gainesville FL.
9:45	95	Efficacy evaluation of the new insect growth regulator, pyriproxifen. B.L. BLAG-BURN, College of Veterinary Medicine, Auburn University, Auburn AL, and T.A. MILLER*, Virbac Inc., Ft. Worth TX.

### 10:15 a.m.-noon BIOCHEMISTRY & PHYSIOLOGY—II, Regency C.

Presiding: J.A. STARLING P.R. KOMUNIECKI

University of Missouri University of Toledo

St. Louis MO Toledo OH

TIME	PAPER NO.	
10:15	96	Purification and characterization of adult <i>Hymenolepis diminuta</i> mitochondrial NADPH-NAD transhydrogenase. C. FU* and C.F. FIORAVANTI, Department of Biological Sciences, Bowling Green State University, Bowling Green OH.
10:30	97	Relationship of mitochondrial NADPH-NAD transhydrogenase and transmembrane proton translocation in adult <i>Hymenolepis diminuta</i> . A.M. WATSON* and C.F. FIORAVANTI, Department of Biological Sciences, Bowling Green State University, Bowling Green OH.
10:45	98	Preparation of a solubilized form of ATPase from adult <i>Hymenolepis diminuta</i> mitochondria. M.S. JANES* and C.F. FIORAVANTI, Department of Biological Sciences, Bowling Green State University, Bowling Green OH.
11:00	99	Catalysis of an NADPH-NAD transhydrogenation reaction by adult <i>Ascaris suum</i> mitochondria. D.V. UPITE* and and C.F. FIORAVANTI, Department of Biological Sciences, Bowling Green State University, Bowling Green OH.
11:15	100	Distribution and characterization of a novel cestode neuropeptide related to vertebrate neuromedin. U.C. SHAW*, G. McMURRAY, D.W. HALTON, Comparative Neuroendocrinology Research Group, The Queen's University of Belfast, UK, P. VERHAERT, Zoological Institute, University of Leuven, Belgium, and A.G. MAULE, The Upjohn Co., Kalamazoo MI.
11:30	101	Immunocytochemical mapping of putative neuronal messenger molecules associated with the reproductive apparatus in flatworms. D.W. HALTON*, G. McMURRAY, N.J. MARKS, R.N. JOHNSTON, C.F. JOHNSTON, C. SHAW, Comparative Neuroendocrinology Research Group, The Queen's University of Belfast, UK, and A.G. MAULE, The Upjohn Co., Kalamazoo MI.
11:45	102	RFamides in Fasciola hepatica: A role in feeding activity? S.C. SUKHDEO* and M.V.K. SUKHDEO, Department of Animal Sciences, Rutgers University, New Brunswick NJ.

# 8:30 a.m.-noon SYSTEMATICS & PHYLOGENY—I, Plaza A.

Presiding: L. PETERS S.L. GARDNER

Northern Michigan University University of Nebraska

Marquette MI Lincoln NE

TIME	PAPER NO.	
8:30	103	Comparative studies on phenotype and genotype of <i>Blastocystis</i> strains isolated from different hosts. H. YOSHIKAWA*, Department of Biology, Faculty of Science, Nara Women's University, Nara, Japan, I. NAGANO, Department of Parasitology, Gifu University School of Medicine, Gifu, Japan, E.H. YAP, M. SINGH, Department of Microbiology, Faculty of Medicine, National University, Singapore, and Y. TAKAHASHI, Department of Parasitology, Gifu University School of Medicine, Gifu, Japan.
8:45	104	Evolutionary relationships of anuran trypanosomes as inferred from 18S ribosomal RNA gene sequences. D.S. MARTIN*, AD.G. WRIGHT and J.R. BARTA, Department of Pathology, University of Guelph, Guelph, Ontario, Canada.
9:00	105	Phylogenetic relationship of the Myxozoa Henreguya exilis, Ceratomyxa shasta and Aurantiactinomyxon sp. as determined by rRNA sequence comparison. D.L. LIN, L.M. POTE* and L.A. HANSON, College of Veterinary Medicine, Mississippi State University, Mississippi State MS.
9:15	106	Marine Acanthocephala of eastern United States. O.M. AMIN, Institute of Parasitic Diseases, Arizona State University, Tempe AZ.
9:30	107	Systematics of the tetraphyllidean genus <i>Anthocephalum</i> . T.R. RUHNKE* and R.A. KNAAK, Department of Biological Sciences, East Stroudsburg University, East Stroudsburg PA.
9:45	108†	Using parasites to answer a systematic question in the stingray genus <i>Urobatis</i> . K. JENSEN* and J.N. CAIRA, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT.
10:00		BREAK.
10:15	109†	On the cestode genus <i>Echinobothrium</i> : Host records, distribution and fine structure. G. TYLER* and J.N. CAIRA, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT.
10:30	110†	A revision of the genus <i>Platybothrium</i> with discussion of its host associations. C.J. HEALY* and J.N. CAIRA, Ecology and Evolutionary Biology, University of Connecticut, Storr CT.

<sup>†</sup> Student Competition Paper

10:45	111†	On the status of the genus <i>Pinguicollum</i> (Tetraphyllidea: Onchobothriidae). C.P. KEELING* and J.N. CAIRA, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT.
11:00	112†	On the identity of <i>Phoreiobothrium</i> from sharks of the order Carcharhiniformes. J.L. SWANSON*, C. RICHMOND and J.N. CAIRA, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT.
11:15	113†	Systematics of the genus <i>Litomosoides</i> —host parasite coevolution or parasite transfer? S.V. BRANT* and S.L. GARDNER, Division of Parasitology, H.W. Manter Laboratory of Parasitology, University of Nebraska State Museum, University of Nebraska, Lincoln NE.
11:30	114†	Molecular systematics of <i>Plasmodium</i> species and related apicomplexan parasites. R.A. CARRENO*, D.S. MARTIN and J.R. BARTA, Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.
11:45	115†	Phylogenetic analysis of the acanthocephala using complete 18S ribosomal DNA sequences. T.J. NEAR* and S.A. NADLER, Department of Biological Sciences, Northern Illinois University, DeKalb IL.

# SATURDAY AFTERNOON

1:00–2:30 p.m. ASP/AAVP PRESIDENTIAL ADDRESSES, Regency A–B–C.

Presiding: L. MARGOLIS P.M. SCHANTZ

Pacific Biological Station Center for Disease Control

Nanaimo, British Columbia, Canada Atlanta GA

ASP President
S.S. DESSER
AAVP President
C.H. COURTNEY

Department of Zoology Department of Infectious Diseases

University of Toronto University of Florida
Toronto, Ontario, Canada Gainesville FL

"Peeling the Cosmic Onion" "AAVP in the Information Age"

### **SATURDAY AFTERNOON (continued)**

### 2:30-3:30 p.m. R. BARCLAY McGHEE LECTURE, Regency A-B-C.

Presiding: S.A. NADLER

Northern Illinois University

DeKalb IL

PAPER TIME <u>NO.</u>

2:30

116 Prospects for controlling animal parasitic nematodes by predacious microfungi. P.

NANSEN, Danish Center for Experimental Parasitology, Royal Veterinary &

Agricultural University, Frederiksberg, Denmark.

3:30-4:25 p.m. INDUSTRIAL LIAISON SYMPOSIUM: ALLIANCES FOR KNOW-LEDGE & TECHNOLOGY, Regency A-B-C.

Presiding: R.S. REW **G.A SHAD** 

> Pfizer, Inc. University of Pennsylvania

New York NY Philadelphia PA

**PAPER TIME** <u>NO.</u> 3:30 Introduction. G.A. SHAD. 3:35 117 Abolition of industrial research: Would it be a good thing for parasitology? W.C. CAMPBELL, Charles A. Dana Research Institute, Drew University, Madison NJ. 3:50 118 Pharmaceutical and academic research: Where's the common ground?. J.F. WIL-LIAMS, Department of Microbiology and Public Health, Michigan State University, East Lansing MI. 4:05 119 Synergies which result from academic and industry relationships. R.B. GRIEVE,

Paravax Biopharmaceuticals Inc., Fort Collins CO.

4:20 Concluding remarks. R.S. REW.

# **SATURDAY AFTERNOON (continued)**

4:30–6:15 p.m.	CHEMOTHERAPY OF PARASITIC INFECTIONS—III,
	Regency A-B-C.

Presiding:	R.S. REW Pfizer, Inc. New York N	F.S. SY University of South Carolina Columbia SC
TIME	PAPER NO.	
4:30	120	Efficiency of doramectin in the treatment of induced infections of gastrointestinal nematodes in young calves. C.E. COUVILLION, L.M. POTE*, C. SIEFKER and A.S. LITTLE, College of Veterinary Medicine, Mississippi State University, Mississippi State MS, and Pfizer Animal Health Product Development, Groton CT.
4:45	121	Efficacy of injectable doramectin against natural gastrointestinal nematode infections of weanling beef calves. J.A. STUEDEMANN*, H. CIORDIA, C.P. WILKINS and D.H. SEMAN, USDA, ARS, Watkinsville GA, The University of Georgia, Experiment GA, and Pfizer Animal Health Product Development, Terre Haute IN.
5:00	122	Efficacy of injectable doramectin against inhibited Ostertagia ostertagi of naturally infected yearling calves. J.A. STUEDEMANN*, H. CIORDIA, C.P. WILKINS and D.H. SEMAN, USDA, ARS, Watkinsville GA, The University of Georgia, Experiment GA, and Pfizer Animal Health Product Development, Terre Haute IN.
5:15	123	Field efficacy of doramectin in the treatment of gastrointestinal nematode infections of cattle. F.E. PHILLIPS*, N.B. LOGAN and R.M. JONES, Pfizer Animal Health Product Development, Groton CT.
5:30	124	Efficacy of doramectin against eyeworms ( <i>Thelazia</i> spp.) in naturally and experimentally infected cattle. M.J. KENNEDY* and F.E. PHILLIPS, Animal Health Laboratories Branch, Alberta Agriculture, Edmonton, Alberta, Canada, and Pfizer Animal Health Product Development, Groton CT.
5:45	125	Field efficacy of doramectin in the treatment of lice infestations of cattle. J.E. LLOYD*, F.E. PHILLIPS, N.B. LOGAN and R.M. JONES, Entomology Section, University of Wyoming, Laramie WY, and Pfizer Animal Health Product Development, Groton CT.
6:00	126	Doramectin efficacy against cattle grubs (Hypoderma lineatum and Hypoderma bovis) and cattle lice (Bovicola bovis, Linognathus vituli, Solenopotes capillatus and Haematopinus eurysternus) in Wyoming. J.E. LLOYD*, R. KUMAR, J.W. WAGGONER and F.E. PHILLIPS, Entomology Section, University of Wyoming, Laramie WY, Animal Science Department, University of Wyoming, Laramie WY, and Pfizer Animal Health Product Development, Groton CT.

# SATURDAY EVENING

7:30–11:00 p.m. PFIZER RECEPTION FOR ASP/AAVP, Grand Ballroom.

# SUNDAY MORNING, JULY 9th

8:00 a.m.-12:15 p.m. DEVELOPMENT & LIFE CYCLES, Regency A.

Presiding:	M.L. ADAMSON University of British Columbia		A.D.W. ACHOLONU
		of British Columbia, Canada	Alcorn State University  Lorman MS
<u>TIME</u>	PAPER NO.		
8:00	127†	Etheostoma rufilineatum. V.R.	ructure of <i>Myxobolus</i> sp. from the Redline Darter, DIDERRICH* and S. PATTON, Department of sity of Tennessee College of Veterinary Medicine,
8:15	128†	A.L. WILLINGHAM*, The D Veterinary and Agricultural Ui	mosis japonica: A possible "self-cure" phenomenon. Panish Centre for Experimental Parasitology, Royal niversity, Frederiksberg and The Danish Bilharziasis Denmark, and H.O. BØGH, The Danish Bilharziasis Denmark.
8:30	129†	quences in larval and adult form	ticillatum: Comparison of ribosomal subunit sens. S.E. LITTLE*, R.A. BULLIS, M.L. SOGIN and asitism Course, Marine Biological Laboratory, Woods
8:45	130†		contortus: A role for volatile fatty acids. L.  SUKHDEO, Department of Animal Sciences, swick NJ.
9:00	131†	stercoralis. F.E. THOMPSON*	mmune-damaged and transplanted Strongyloides, V.M. BHOPOLE and G.A. SCHAD, Graduate ment of Biomedical Science, University of Pennsylva-
9:15	132		ion of autoinfection in the gerbil. T.J. NOLAN*, HAD, Department of Pathobiology, University of ary Medicine, Philadelphia PA.

<sup>†</sup> Student Competition Paper

9:30	133	The homogonic life cycle of <i>Strongyloides robustus</i> . R.P. ECKERLIN, Natural Sciences Division, Northern Virginia Community College, Annandale VA.
9:45	134	The number of moults in the egg of sealworm, <i>Pseudoterranova decipiens</i> . L.N. MEASURES*, Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, Quebec, and H. HONG, Department of Zoology, University of Toronto, Toronto, Ontario, Canada.
10:00		BREAK.
10:15	135	Uniformity in the development and life history of ascaridoid nematodes. HP. FAGERHOLM*, Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Frederiksberg, Denmark, and Institute for Parasitology, Department of Biology, Åbo Akademi University, Finland, M. KØIE, Marine Biology Laboratory, Helsingør, University of Copenhagen, Denmark, A. ROEPSTORFF and P. NANSEN, Centre for Experimental Parasitology, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.
10:30	136	In vitro production of first generation Schistosoma mansoni daughter sporocysts. T.P. YOSHINO* and J.R. LAURSEN, Department of Pathobiological Sciences, University of Wisconsin, Madison WI.
10:45	137	Complete development of <i>Cryptosporidium parvum</i> in bovine fallopian tube epithelial cells. S. YANG*, M.C. HEALEY, C. DU and J. ZHANG, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan UT.
11:00	138	Immunoblot analysis of wildlife sera using cultured <i>Sarcocystis neurona</i> merozoites. D.E. GRANSTROM*, J.M. DONAHUE, Z. XIAOMIN, K.K. POONACHA, N.M. WILLIAMS, S. STAMPER, S.M. SCHNEIDER and C.K. FENGER, Department of Veterinary Science, University of Kentucky, Lexington KY, J.P. DUBEY, USDA, ARS, LPSI, ZDL, Beltsville MD, J. PATTERSON, J. MARTENIUK, Departments of Pathology and Large Animal Clinical Science, Michigan State University, East Lansing MI, and B.D. SNYDER, College of Veterinary Medicine, University of Georgia, Athens GA.
11:15	139	Comparative pathology of avian Sarcocystis falcatula infection. J.H. SMITH, Department of Pathology, University of Texas Medical Branch, Galveston TX.
11:30	140	Which factors determine a species of <i>Hepatozoon</i> (Apicomplexa: Adeleina)? T.G. SMITH*, S.S. DESSER and S.H. KOPKO, Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

11:45	141	Veterinary aspects of "bumper car" disease caused by Anophryoides sp. (Scuticociliatida) in the lobster fishery. R. CAWTHORN, L. HAMMELL, B. HORNEY, F. MARKHAM, M. NOVOTNY*, D. SPEARE and K. BROWN, Faculty of Veterinary Medicine, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada.
12:00	142	Acute fulminating babesiosis in hamsters with <i>Babesia microti</i> : An experimental model. H.S. OZ* and W.T. HUGHES, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis TN.

# 8:30–10:00 a.m. INVERTEBRATE HOST-PARASITE RELATIONSHIPS, Regency B.

Presiding:	L.A. COOPER University of Maryland College Park MD		E.S. LOKER The University of New Mexico Albuquerque NM
TIME	PAPER NO.		
8:30	143		nanges in response to Echinostomatid trematode and D.J. MINCHELLA, Department of Biology, ette IN.
8:45	144	capable of interacting with trem	-induced plasma protein from <i>Biomphalaria glabrata</i> atode antigens. L.A. HERTEL*, C.M. ADEMA and iology, The University of New Mexico, Albuquerque
9:00	145	Investigations of cell mediated of	een Biomphalaria glabrata and Schistosoma mansoni: cytotoxicity using an in vitro assay. S.E. FRYE* and cology, Oregon State University, Corvallis OR.
9:15	146†	cation, characterization and effe	productive system of <i>Biomphalaria glabrata</i> : Identifict of schistosome infection. G. BAI*, J. LI, M. SHINO, Department of Pathobiological Sciences, on WI.
9:30	147†	2	the salivary glands of Anopheles gambiae sensu SINA, Department of Entomology, University of

9:45

10:45

11:15

### **SUNDAY MORNING (continued)**

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		japonicum. B. YAO*, J. ZHAO tural University, Wuhan, PR C	, L. MA, Q. WANG and Z. YU, Huazhong Agricul- hina.	
10:00		BREAK.		
10:15 a.ı	m.–noon	IMMUNOLOGY—II, Regency B.		
Presiding:	R.B. GRIE	VE	H.R. GAMBLE	
	Paravax, Ind	<b>.</b>	USDA, API, HDL	
	Fort Collins	s CO	Beltsville MD	
	PAPER			
TIME	<u>NO.</u>			
10:15	149†	during Taenia crassiceps cysticere ROMANO, T. GOVEZENSK	ne response by androgen reconstitution of mice coids. J. MORALES*, L.I. TERRAZAS, M.C. Y and C. LARRALDE, Department of Immunology, omédicas de la UNAM, México.	
10:30	150†	juvenile Fasciola hepatica. J. CR	cathepsin protease expression by newly excysted EANEY*, L.W. WILSON, R.M. SANDEMAN, SONS, Department of Immunoparasitology, Victoria, Australia.	

Efficiency of crystal toxin of Bacillus thuringiensis against schistosomula of Schistosoma

Louisiana State University, Baton Rouge LA.

GA.

The efficacy of serology, blood smear evaluation, and polymerase chain reaction in the diagnosis of ehrlichial infections in dogs. S.A. EWING\*, J.C. FOX, J.S. MATHEW, G.L. MURPHY, K.M. KOCAN and E.F. BLOUIN, Departments of Veterinary Parasitology and Veterinary Pathology, Oklahoma State University, Stillwater OK.

Characterization of 16-28 kilo Dalton Fasciola hepatica immunodiagnostic

Toxicology, and J.B. MALONE, Veterinary Microbiology and Parasitology,

coproantigen. S.M. ABDEL-RAHMAN\*, K.L. O'REILLY, Veterinary Microbiology and Parasitology, D.H. SWENSON, Veterinary Physiology, Pharmacology and

151†

<sup>11:00 152†</sup> Canine lyme disease (LD): Safety, efficacy and duration of immunity of an OspA vaccine. J.C. JARECKI-BLACK\* and R.E. WIKLE, Rhone Merieux Inc., Athens

<sup>†</sup> Student Competition Paper

11:30	154	Antigenic analysis of Encephalitozoon cuniculi (CDC:V282) isolated from the urine of a patient with AIDS. G.P. CROPPO*, G.S. VISVESVARA, Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta GA, G.J. LEITCH, Moorehouse School of Medicine, Atlanta GA, S. WALLACE, Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta GA, M.A. DE GROOTE, Division of Infectious Diseases, University of Colorado Health Sciences Center, Boulder CO, and R. REVES, Denver Disease Control, Denver CO.
11:45	155	Cytokine production in BALB/C mice infected with Giardia muris. K. DJAMIATUN* and G.M. FAUBERT, Institute of Parasitology, McGill University, Montreal, Quebec, Canada.

# 8:30 a.m.-noon MOLECULAR & CELL BIOLOGY, Regency C.

			. 0 ,
Presiding:	D.E. SNYI Lilly Resear Greenfield	rch Labs US	S. ZARLENGA SDA, ARS, LPSI, BPL Itsville MD
TIME	PAPER NO.		
8:30	156	technique for synthesizing competit	nd its applications in developing a simple tor molecules for RT-PCR. D.S. ZARLENGA*, E, Parasite Immunobiology Laboratory, Beltsville , ARS, USDA, Beltsville MD.
8:45	157	diagnostics of parasite vectors. J. TA	assay in population genetics and molecular ANG*, K. PRUESS and T.R. UNNASCH, University of Alabama, Birmingham AL, and essity of Nebraska, Lincoln NE.
9:00	158	with abnormal erythrocyte membra MORONNE and M. NARLA, Life	em erythrocyte membrane-associated proteins enes. C. MAGOWAN*, R. COPPEL, M. e Sciences Division, Lawrence Berkeley Labora- eley CA and Monash University, Melbourne,
9:15	159		Coccidia). G. ZHU* and J.S. KEITHLY, e Department of Health, Albany NY.
9:30	160		m on invasion of cells by Eimeria adenoeides arasite Biology and Epidemiology Laboratory,

9:45	161	Detection of Sarcocystis neurona DNA in blood, cerebrospinal fluid and feces by polymerase chain reaction. C.K. FENGER*, D.E. GRANSTROM, J.L. LANGE-MEIER, S. STAMPER, Z. XIAOMIN, Department of Veterinary Science, University of Kentucky, Lexington KY, A.A. GAJADHAR, Health of Animals Laboratory, Agriculture Canada, Saskatoon, Saskatchewan, Canada, J. PATTERSON, J. MARTENIK, Departments of Pathology and Large Animal Clinical Sciences, Michigan State University, East Lansing MI, and J.P. DUBEY, Parasite Biology and Epidemiology Laboratory, USDA, Beltsville MD.
10:00		BREAK.
10:15	162†	Molecular identification of <i>Enterobius vermicularis</i> larvae as a cause of human eosino-philic ileocolitis. L.X. LIU*, J.Y. CHI, M.P. UPTON, Departments of Medicine and Pathology, Harvard Medical School, Boston MA, and L.A. ASH, Department of Epidemiology, University of California School of Public Health, Los Angeles CA.
10:30	163	Dirofilaria immitis cDNA clones identified with immune dog sera. C. TRIPP*, M. MIKA-GRIEVE, M. RUSHLOW, G. FRANK, R. FRANK, M. STORY and S. BLEHM, Paravax Inc., Fort Collins CO.
10:45	164	Reactivation of hypobiotic hookworm larvae during pregnancy and lactation. P. ARASU, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute, Blacksburg VA.
11:00	165	Ancylostoma secreted protein: A novel protein associated with the transition to parasitism by infective hookworm larvae. J.M. HAWDON*, B.F. JONES and P.J. HOTEZ, Yale University School of Medicine, New Haven CT.
11:15	166	Two morphologically distinct secretory mechanisms are present at the free tegumental surface of cestodes. J.A. OAKS*, Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison WI, and J.M. HOLY, Department of Anatomy and Cell Biology, University of Minnesota, Duluth MN.
11:30	167	Molecular cloning and characterization of SMAK, the homologue of MAK16 from Schistosoma mansoni. J.L. MILHON*, T.J. ALBERT, E.A. VANDE WAA and J.W. TRACY, Departments of Comparative Biosciences and Pharmacology and the Environmental Toxicology Center, University of Wisconsin, Madison WI.

11:45 168

Genetic variation of the nuclear genomes of *Schistosoma japonicum* from Taiwan and Mainland China by random amplified polymorphic DNA (RAPD). K.M. LEE\*, L.C. SHEN, C.T. LO, Institute of Parasitology, National Yang-Ming University, Taiwan, M.J. PAN, Department of Veterinary Medicine, National Taiwan University, Taiwan, and C.L. YU, Section of Allergy, Immunology and Rheumatology, Department of Medicine, Veterans General Hospital-Taipei, National Yang-Ming University School of Medicine, Taiwan, Republic of China.

### SUNDAY AFTERNOON

1:15–5:00 p.m. ECOLOGY/EPIDEMIOLOGY—III, Regency A.

Presiding: W.J. KOZEK L. COUCH

University of Puerto Rico The University of New Mexico

San Juan PR Albuquerque NM

	San Juan PR	Albuquerque NM
<u>TIME</u>	PAPER NO.	
1:15	169	Human parasitic and mycotic infections in Mississippi. A.D.W. ACHOLONU, Department of Biological Sciences, Alcorn State University, Lorman MS.
1:30	170	Capture as a plausible mechanism of sympatric speciation in parasites. M.L. ADAM-SON, Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada.
1:45	171	Mathematical models of African horse sickness and Cullcoldes imicola. C.C. LORD*, M.E.J. WOOLHOUSE, Department of Zoology, University of Oxford, Oxford, UK, and P.S. MELLOR, Institute for Animal Health, Pirbright, UK.
2:00	172	Fatal cerebral coenurosis in a cat. B.T. HUSS, M.A. MILLER, R.M. CORWIN*, E.P. HOBERG and D.P. O'BRIEN, Department of Veterinary Medicine and Surgery, Veterinary Medical Diagnostic Laboratory and Department of Veterinary Microbiology, University of Missouri, Columbia MO and USDA, ARS, Beltsville MD.
2:15	173	Significance of parasitologic examination of stools in a tertiary-care hospital in the southern United States of America. J.H. SMITH, Department of Pathology, University of Texas Medical Branch, Galveston TX.
2:30	174	Computerized decision analysis for the diagnosis and treatment of canine heartworm disease. R.A. HOLMES*, R.D. SMITH, B. DUNAVENT, M.E. HUGH-JONES and M.E. KEARNEY, Veterinary Microbiology and Parasitology, School of Medicine, Louisiana State University, Baton Rouge LA.

# **SUNDAY AFTERNOON (continued)**

2:45	175	The combined effect of a move nine weeks after turnout and treatment with fenbendazole or moxidectin on lungworm infections in dairy calves. M. EYSKER*, J.H. BOERSEMA, F.N.J. KOOYMAN, T.J.G.M. LAM and T. VAN WERVEN, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.
3:00	176	A comparative assessment of direct fluorescence antibody, modified acid fast stain and sucrose flotation techniques for detection of <i>Cryptosporidium serpentis</i> oocysts in snake fecal specimens. T.K. GRACZYK*, Department of Molecular Microbiology and Immunology, John Hopkins University, Baltimore MD, M.R. CRANFIELD, The Baltimore Zoo, Baltimore MD, and R. FAYER, USDA, LPSI, ARS, Beltsville MD.
3:15		BREAK.
3:30	177	Comparison of direct and indirect methods for the detection of trichinellosis in horses. H.R. GAMBLE*, A.A. GAJADHAR and M.B. SOLOMON, Parasite Biology and Epidemiology Laboratory, USDA, ARS, Beltsville MD and Health of Animals Laboratory, Agriculture Canada, Saskatoon, SK, Canada.
3:45	178	Long-term immunity to shedding of <i>Toxoplasma gondii</i> oocysts by cats. J.P. DUBEY, Parasite Biology and Epidemiology Laboratory, USDA, ARS, Beltsville MD.
4:00	179	Effect of low temperatures on survival of Cryptosporidium parrum oocysts. R. FAYER*, Parasite Immunobiology Laboratory, USDA, ARS, Beltsville MD.
4:15	180	The effect of rotational grazing on the dynamics of sheep parasite fecal egg counts. B.E. STROMBERG*, G.A. AVERBECK, C. CHRISTIANS and C.C. SHEAFFER, Department of Veterinary Pathology, College of Veterinary Medicine, University of Minnesota, St. Paul MN.
4:30	181	Chemical stimulation of host recognition by <i>Diplostomum spathaceum</i> cercariae. A. BANSEMIR*, Department of Animal Sciences, Rutgers University, New Brunswick NJ, E. SCHÖNAMSGRUBER and W. HAAS, Institut für Zoologie, Universität Erlangen-Nürnberg, Erlangen, Germany.
4:45	182	Prediction of mature Fasciola hepatica by fecal sedimentation from yearling calves in Louisiana. K.L. SMITH*, J.B. MALONE and D. SCHOLL, Department of Veterinary Microbiology and Parasitology and Department of Epidemiology and Community Health, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA.

1:00–3:15 p.m. IMMUNOLOGY—III, Regency B.

Presiding:	B. HAMMERBERG North Carolina State University Raleigh NC		M.L. SCHITO University of Guleph Guelph, Ontario, Canada
TIME	PAPER NO.		
1:00	183	mansoni. K. NYAME, Universit City OK, J.B. PILCHER, V. T.	in monkeys and humans infected with Schistosoma by of Oklahoma Health Sciences Center, Oklahoma SANG, Centers for Disease Control and Prevention, NGS*, University of Oklahoma Health Sciences
1:15	184	with naked DNA. S.G. KAYES TRACY, J.S. LATTENDRESS Cellular Biology and Biological	vers of Schistosoma mansoni-infected mice vaccinated *, J.S. JACKSON, J.J. O'BRIEN, J. POWELL, J.W. E and J.A. WOLFF, Departments of Structural and Sciences, University of South Alabama, Mobile AL ve Biosciences and Pediatrics, University of Wiscon-
1:30	185	elevated TNF-α and histamine r	in canine popliteal lymph node cells leading to elease <i>in vitro</i> . S. ORTON*, D. SCHEURER and B. Veterinary Medicine, Raleigh NC.
1:45	186	ectopic infections in cats and do	antigen test kits for effectiveness in detecting induced gs. J.W. McCALL*, P. SUPAKORNDEJ, M.T. repartment of Parasitology, College of Veterinary L. Athens GA.
2:00	187	O'CONNOR*, P. HILLMAN,	nent free adult heartworm antigen test kits. T. B. BARTOL, IDEXX Laboratories Inc., Westbrook versity of Florida, Gainesville FL.
2:15	188	polyproteins and solubilized by JASMER*, L.E. PERRYMAN	surface membrane proteins: Encoded as related phosphatidylinositol specific phospholipase C. D.P. and T.C. McGUIRE, Department of Veterinary Vashington State University, Pullman WA.
2:30	189	native sheep. J.E. MILLER*, S. ment of Epidemiology and Com	ssion and nematode infection in resistant Gulf Coast S. KHALAH-ALLAH and S.R. BARRAS, Departmental Health, School of Veterinary Medicine, A&M College, Baton Rouge LA.

2:45	190	Parasitologic and immunologic definition of cattle selected for enhanced or diminished resistance to gastrointestinal nematode infection. L.C. GASBARRE*, A CANALS and D.S. ZARLENGA, USDA, ARS, LPSI, PIL, Beltsville MD.
3:00	191	H-2 genes in mice and resistance to <i>Heligmosomoides polygyrus</i> . S. ZHONG and C. DOBSON*, The Department of Parasitology, The University of Queensland, Queensland, Australia.

# 3:30–5:15 p.m. SYSTEMATICS & PHYLOGENY—II, Regency B.

Presiding:	R.S. SEVIL University of Casper WY		T.R. RUHNKE East Stroudsburg University East Stroudsburg PA
TIME	PAPER NO.		
3:30	192	, ,, ,	stomatoida (Copepoda)—the most successful rebrates. G.W. BENZ, Tennessee Aquarium,
3:45	193	COI. M.V.K. SUKHDEO*, S.	ingylida based on nucleotide sequencing of mtDNA C. SUKHDEO, D.L. MEDICA, Department of and R. VRIJENHOEK, Center for Theoretical and ersity, New Brunswick NJ.
4:00	194		res in <i>Trichostrongylid</i> nematodes. J.R. LICHTEN- JRPHY and P.A. PILITT, USDA, ARS, BARC,
4:15	195	BANNA*, S.L. GARDNER and	genus <i>Pratylenchus</i> (Nematoda: Tylenchida). L. ALd V.M. WILLIAMSON, Department of Fornia, Davis CA and University of Nebraska State
4:30	196	Heterorhabditidae) from analysi DNA polymorphisms. S.L. GAI and E.P. CASWELL-CHEN, D	nematodes of the genus <i>Heterorhabditis</i> (Nematoda: s of 26s rDNA sequences and random amplified RDNER*, F. WU, W.K. THOMAS, H.K. KAYA ivision of Parasitology, H.W. Manter Laboratory of raska State Museum, University of Nebraska, Lincoln

4:45 197 A lungworm in Umingmak, the muskox, from the Canadian Arctic. E.P.

HOBERG\*, Biosystematics and National Parasite Collection Unit, USDA, ARS, Beltsville MD, L. POLLEY, Department of Pathobiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada, and J. NISHI, Renewable Resources, Government of the Northwest Territories, Coppermine,

Canada.

5:00 198 New evidence of hystricognath rodents monophyly from the phylogeny of their

pinworms. J.P. HUGOT\*, Biologie Parasitaire-Helmintholige, Muséum National d'Histoire Naturelle, Paris, France, S.L. GARDNER, Manter Laboratory of Parasitology, Division of Parasitology, University of Nebraska State, Lincoln NE, S. MORAND, Université de Perpginan Laboratoire de Biologie Animale, France, and

C. SUTTON, Facultad de Ciencias Naturalés y Muséo de La Plata, La Plata,

Argentina.

1:00–3:00 p.m. LATE BREAKERS, Regency C.

Presiding: D. MURRELL

Beltsville Agricultural Research Center

Beltsville MD

#### SUNDAY EVENING

7:00- @ fl p.m. ASP/AAVP STUDENT PARTY, Ballrooms 4-5.

# **MONDAY MORNING, JULY 10th**

8:00–10:00 a.m. ANNOUNCEMENT & PRESENTATION OF ASP & AAVP SPECIAL

AWARDS, Regency A-B-C.

8:00-9:00 a.m. THE SIXTH BUEDING-VON BRAND LECTURE

Presiding: W.E. FOOR

University of Pittsburgh

Johnstown PA

The Recipient of the Sixth BUEDING-VON BRAND AWARD will be announced at the meeting.

# **MONDAY MORNING (continued)**

PAPER

TIME NO.

8:10 199 Bueding-von Brand Lecture, to be announced at the meeting.

9:00 HENRY BALDWIN WARD MEDAL AWARD

Presiding: R. FAYER

USDA, ARS, LPSI, ZDL

Beltsville MD

The Recipient of the 1995 H.B. WARD MEDAL will be announced at the meeting.

**PAPER** 

TIME NO.

9:10 200 H.B. WARD MEDAL LECTURE, to be announced at the meeting.

THE BEST STUDENT PRESENTATION AWARDS

Presiding: D. RITTER

San Carlos CA

CLARK P. READ NEW INVESTIGATOR AWARD, to be announced at the

meeting.

Presiding: C.F. FIORAVANTI

Bowling Green State University

Bowling Green OH

MARC DRESDEN STUDENT TRAVEL GRANT AWARDS, to be an-

nounced at the meeting.

Presiding: J.P. DUBEY

USDA, ARS Beltsville MD

AAVP DISTINGUISHED VETERINARY PARASITOLOGIST AWARD, to

be announced at the meeting.

Presiding: J.P. DUBEY

USDA, ARS Beltsville MD

#### MONDAY MORNING (continued)

10:00–12:00 a.m. ASP BUSINESS MEETING, Regency A.

10:00-12:00 a.m. AAVP BUSINESS MEETING, Regency B.

#### MONDAY AFTERNOON

1:00-4:00 p.m. POSTERS, Plaza A-B.

#### **BIOCHEMISTRY, PHYSIOLOGY**

- The effect of Giardia lamblia enteritis on intestinal transit and smooth muscle contractility. L. DESELLIERS\*, M.E. OLSON and R.B. SCOTT, G.I. Research Group, University of Calgary, Calgary, Alberta, Canada.
- 202 Effects of giardiasis on growth and development in the young. M.E. OLSON\*, T.A. McALLISTER, L. DESELLIERS, K.-J. CHENG and D.W. MORCK, G.I. Research Group, University of Calgary, Calgary, Alberta, Canada.
- A histochemical study of vitelline cells and egg-shell in the cyclocoelium mutabile. ZH.K. SHAYMARDANOV\* and K. AHMETOV, Department of Zoology, Pavlodar University, Pavlodar, Republic of Kazakhstan.
- Chemical nature and egg-shell formation of some trematodes. ZH.K. SHAYMARDANOV, Department of Zoology, Pavlodar University, Pavlodar, Republic of Kazakhstan.
- 205 Localization of aerobic- and anaerobic-specific enzymes in Ascaris suum larvae. B. MEI\*, P. KOMUNIECKI and R. KOMUNIECKI, Department of Biology, University of Toledo, Toledo OH.
- Arginine phosphate in *Haemonchus contortus* and *Steinernema carpocapsae*. E. PLATZER\*, S.N. THOMPSON, D.B. BORCHARDT and H.R. GAMBLE, Departments of Nematology, Entomology and Chemistry, University of California, Riverside CA, and Helminthic Disease Laboratory, USDA, ARS, Beltsville MD.
- 207 Brugia pahangi depresses the frequency of spontaneous contractions in bovine mesenteric lymphatics studied in vitro: A role for filarial factors in the development of lymphedema? L. KAISER\*, M. MUPANOMUNDA and J.F. WILLIAMS, Departments of Physiology and Microbiology, Michigan State University, East Lansing MI.
- Longevity and nutrient acquisition in isolated nurse cells of *Trichinella spiralis*. J. MONTGOM-ERY\* and G.L. STEWART, Center for Parasitology, The University of Texas, Austin TX.

#### **ECOLOGY, EPIDEMIOLOGY**

- Helminth communities in the Northern Spring Peeper, *Pseudacris C. crucifer* Wied, and the Wood Frog, *Rana sylvatica* Le Conte, from southeastern Wisconsin. H.R. YODER\* and J.R. COGGINS, Department of Biological Sciences, University of Wisconsin, Milwaukee WI.
- 210 Retrospective report on gastrointestinal and ectoparasites from gray wolves (*Canis lupis*) submitted to the National Wildlife Health Center, 1988-1994. R.A. COLE\*, N.J. THOMAS, C.L. RODERICK and C.U. METEYER, National Wildlife Health Center, National Biological Service, Madision WI.
- Parasites in Georgia farm pigs. T.B. STEWART\*, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, L.A. STEUDEMANN, USDA Agriculture Service, Watkinsville GA, H. CIORDIA, University of Georgia, Experiment GA.
- Incidence of intestinal protozoan parasites in *X. vigilis* and *B. attenuatus* of California mountainous regions. I. Syntopy influence on cross-parasitism. A.R. SANCHO\*, Pharmaceutical Science Department, University of Southern California, Los Angeles CA, and D. SOLEYMANI, Biology Department, California State University, Dominguez Hill, Carson CA.
- 213 Cryptosporidum infections and other intestinal parasites in Saimadoyi, a Bari Indian community from western Venezuela. L. CHACIN-BONILLA\* and Y. SANCHEZ-CHAVEZ, Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela.
- Detection of *Giardia* cysts in foods using immunofluorescence microscopy and flow cytometry. B.R. DIXON\*, M. PARENTEAU and G. SANDERS, Health Protection Branch, Health Canada, Ottawa, Ontario, Canada.
- Cryopreservation of pathogenic *Acanthamoeba* and *Naegleria*. D.T. JOHN\*, P.L. EDDY and R.A. JOHN, Oklahoma State University, Tulsa OK.
- Detection of antibodies of schistosomiasis by micro-filter-paper blood radio-immuno PEG precipitation. Z. LU, Jiangsu Institute of Parasitic Diseases, PR China.
- Prevalence and evolution of antibody in natural bovine hypodermosis in Spain. J. MARTINEZ\*, I. NAVARRETE, A. MARTINEZ, V. JIMENEZ and S. HERNANDEZ, Cátedra Parasitología, Facultad de Veterinaria, Universidad de Côrdoba, Côrdoba, Spain.
- Monthly fluctuations and hypobiosis phenomenon of gastrointestinal nematodes in calves in the Pyrenees (Spain). S. ALMERIA\*, Parasite Immunbiology Laboratory, LPSI, ARS, USDA, Beltsville MD, and J. URIARTE, Unidad de Sanidad Animal, Parasitologia, Servicio de Investigacion Agraria (DGA), Zaragoza, Spain.

- Kinetics of pasture contamination by gastrointestinal nematode larvae in the Pyrenees (Spain). S. ALMERIA\*, Parasite Immunbiology Laboratory, LPSI, ARS, USDA, Beltsville MD, J. URIARTE and M. LLORENTE, Unidad de Sanidad Animal, Parasitologia, Servicio de Investigacion Agraria (DGA), Zaragoza, Spain.
- The effect of selection for ivermectin resistance on the fitness of *Heligmosomoides polygyrus* (Nematoda). J.M. NJOROGE and M.E. SCOTT\*, Institute of Parasitology, McGill University, Montreal, Quebec, Canada.
- 221 Effect of albendazole selection and/or rapid passage on life history traits of *Heligmosomoides* polygyrus (Nematoda). A. CHEHRESA, M.E. SCOTT\* and R.N. BEECH, Institute of Parasitology, McGill University, Montreal, Quebec, Canada.
- Prevalence of *Dirofilaria tenuis* in raccoons in Georgia. P. DAVIS\*, W. IRBY and O. PUNG, Department of Biology and Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro GA.
- Defining seasonal limits of *Dirofilaria immitis* transmission in the USA. J.B. LOK\*, D.H. KNIGHT, M. O'BRIEN and G. SMITH, Departments of Pathobiology and Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia PA.
- An ecological study of the overwintering of Taxocara canis eggs in Toronto, Ontario. R. BERMAN\*, R. HANSELL and J. YANG, Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

#### INVERTEBRATE HOST-PARASITE RELATIONSHIPS

Peptide antigen common to *Schistosoma mansoni* and *Biomphalaria glabrata* found in snail cerebral ganglion. S. FILE\*, A. CHEN, A. FERNANDEZ and J. JIMENEZ, Department of Biology, University of Puerto Rico, San Juan PR.

#### **CHEMOTHERAPY OF PARASITIC INFECTIONS**

- Intestinal permeability properties of the 7-14-day-old chicken. D.P. THOMPSON\*, N.F. HO, J.S. DAY, B.A. OELSLAGER and T.G. GEARY, The Upjohn Laboratories, Kalamazoo MI.
- Efficacy of decoquinate against *Neospora caninum* in cell cultures. D.S. LINDSAY\*, J.M. BUTLER, M.A. TOIVIO-KINNUCAN and B.L. BLAGBURN, Department of Pathobiology, Auburn University, Auburn AL.
- 228 Efficacy of triclabendazole against Fasciola hepatica in experimentally infected goats. S. HERNANDEZ\*, V. JIMENEZ, J. MARTINEZ and A. MARTINEZ, Cátedra Parasitología, Facultad de Veterinaria, Universidad de Córdoba, Córdoba, Spain.
- Prepatent period, mean fluke burdens and percent take in sheep experimentally infected with metacercaria of the liver fluke, *Fasciola hepatica*. D.E. SNYDER, Animal Science Discovery and Development Research, Eli Lilly & Co., Greenfield IN.

- Does RM340 depress relaxation less than thiacetarsamide in pulmonary artery from heartworm infected dogs? D.S. MAKSIMOWICH\*, J.F. WILLIAMS and L. KAISER, Departments of Physiology and Microbiology, Michigan State University, East Lansing MI.
- Correlation between *in vitro* and *in vivo* potency and onset kinetics for selected anthelmintics. E.M. THOMAS\*, S.S. JOHNSON, T.J. VIDMAR, G.A. CONDER, N.F. HO and D.P. THOMP-SON, Animal Health Discovery Research, The Upjohn Co., Kalamazoo MI.
- Efficacy of azadirachtin and pyriproxyfen against developing stages of the cat flea, *Ctenocephalides felis*. A.A. MARCHIONDO\*, S.W. FOGT, D.L. HEIMBICHNER and S.L. STROH, Fermenta Animal Health Co., Kansas City MO.
- Development and utilization of a DNA probe assay for quantitation of Eimeria parasite load in in vivo studies. H. PROFOUS-JUCHELKA\*, M. HOZZA, J. ONDROF, M. KREIDER and D. SCHMATZ, Department of Parasite Biochemistry and Cell Biology, Merck Research Laboratories, Rahway NJ.

#### **DEVELOPMENT & LIFE CYCLES**

- Pathological changes in the jejunum of calves naturally infected with *Giardia* and *Cryptosporidium*. N. RUEST\*, C. GIRARD, Y. COUTURE, Médecine Vétérinaire, University de Montréal, Montréal, Quebec, Canada, and G.M. FAUBERT, Institute of Parasitology, McGill University, Montréal, Quebec, Canada.
- 235 Plasmodium falciparum malaria parasites imaged by soft x-ray microscopy. C. MAGOWAN\*, M. MORONNE and W. MEYER-ILSE, Life Sciences Division and The Center for X-ray Optics, Lawrence Berkeley Laboratory, University of California, Berkeley CA.
- Growth and developmental surface ultrastructure of *Plagiarchis muris* from rat. H.-C. WOO and S.-J. HONG\*, Department of Parasitology, Gyeongsang National University, Chinju, Korea.
- Metacestode of canine origin in mice. E.G. PLATZER\*, W.J. HERNANDEZ and W. BOYCE, Department of Nematology, University of California, Riverside CA, and Department of Veterinary Microbiology and Immunology, University of California, Davis CA.
- Development of the tapeworm *Diphyllobothrium alascense* from the Kuskokwim region of Alaska. A.M. ADAMS\*, Seafood Products Research Center, U.S. Food & Drug Administration, Bothell WA, and R.L. RAUSCH, Department of Comparative Medicine, University of Washington, Scattle WA.
- Comparison of sodium azide and refrigeration for preservation of hookworm eggs for fecal thick smear examination. G.J. GREER\* and N.A. NIX, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta GA, and Universidad del Valle de Guatemala, Guatemala City, Guatemala.

- Surface ultrastructure and encystment of *Paragordius varius* larvae (Nematomorpha, Gordioidea). P.J. MIKLOS, P.D. LEWIS, JR.\*, and D.F. BRAY, Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada.
- Helminths of *Rattus* sp. in southern and central Puerto Rico. C. MALDONADO\* and W.J. KOZEK, Department of Environmental Health, School of Public Health, and the Department of Microbiology and Medical Zoology, Medical Sciences Campus, University of Puerto Rico, San Juan PR.

#### **IMMUNOLOGY**

- Chicken line differences in *Eimeria tenella*-induced changes in splenic T-lymphocyte subpopulations. K. ZYAN\* and H. LILLEHOJ, Parasite Immunobiology Laboratory, Livestock and Poultry Sciences Institute, USDA, ARS, Beltsville MD.
- Cytokine and antibody isotype profile of immune responses induced by iscoms containing Toxoplasma gondii antigen. A. LUNDÉN\*, Department of Parasitology, and A. SJÖLANDER, Department of Virology, National Veterinary Institute, Uppsala, Sweden.
- 244 Cell transfer of resistance to repeat *Trypanosoma cruzi* infection in mice. A. SLEEPER and E.C. ROWLAND\*, Department of Biological Sciences and College of Osteopathic Medicine, Ohio University, Athens OH.
- Isolation and immunogenicity of phylogenetically conserved intestinal antigens from *Haemonchus placei*. C. SIEFKER\*, L.R. BALLWEBER and C.P. COYNE, College of Veterinary Medicine, Mississippi State University, Mississippi State MS.
- Partial characterization of stage related proteins of Capillaria hepatica and their immune recognition in experimentally infected muskrats. J. BORUCINSKA\* and A. GARMENDIA, Department of Pathobiology, University of Connecticut, Storrs CT.
- 247 Effects of a phosphorylcholine-containing filarial excretory-secretory products on lymphocyte signalling pathways. W. HARNETT\*, M.R. DEEHAN and M.M. HARNETT, Department of Immunology, University of Strathclyde, Glasgow, UK.
- Immune response in mice infected with a Tasmanian Devil isolate of *Trichinella pseudospiralis*. K. ALFORD\*, D.L. OBENDORF, T.M. FREDEKING, E. HAEHLING and G.L. STEWART, Center for Parasitology, The University of Texas, Arlington TX, Animal Health Laboratory, Tasmania, Australia, and Antibody Systems Inc., Bedford TX.
- Analyses of immune responses of SLA<sup>a</sup> minipigs that react against encysted *Trichinella spiralis* muscle larvae. J.K. LUNNEY\*, J. BRYANT and S. HYATT, Parasite Immunobiology Laboratory, ARS, USDA, Beltsville MD, Abbott Park, Abbott Park IL, and Department of Animal Science, University of Maryland, College Park MD.

#### SYSTEMATICS AND PHYLOGENY

- Contradicting primitive assumptions: Evidence for a more advanced placement of the accelomate phylum platyhelminthes. J.A. CURTIS\*, K. LEE, D.J. MINCHELLA and P.T. LoVERDE, Department of Biology, Purdue University, West Lafayette IN and Department of Microbiology, School of Medicine, State University of New York, Buffalo NY.
- Molecular phylogeny of some free-living and parasitic nematodes based on the 28S ribosomal RNA gene. L.X. LIU, Department of Medicine, Harvard Medical School, Boston MA.

#### **MOLECULAR & CELL BIOLOGY**

- Molecular karyotyping of anuran trypanosomes. Z.-R. LUN\* and S.S. DESSER, Department of Zoology, University of Toronto, Toronto, Ontario, Canada.
- Expression of piroplasm proteins of *Theileria sergenti* (Korean isolate) and its immunogenicity in laboratory animals. S.W. KANG\*, C.H. KWON, E.J. CHOI and Y.D. YOON, Parasitology Division, National Veterinary Research Institute, Anyang City, South Korea.
- Effect of brefeldin-a (BFA) on the Golgi complex of *Tritrichomonas foetus*: A laser scanning confocal and electron microscopic study. J.C. ALMEIDA\*, M. BENCHIMOL and W. de SOUZA, Rio de Janeiro, Brazil.
- Cloning of two putative calcium channel cDNAs from triclad flatworm *Bdelloura candida*. R.M. GREENBERG and P.A.V. ANDERSON\*, Whitney Laboratory, University of Florida, St. Augustine FL.
- Differentiation between the human hookworms *Ancylostoma duodenale* and *Necator americanus* using PCR-RFLP. J.M. HAWDON, Yale University School of Medicine, New Haven CT.
- Cloning and sequencing of a kunitz-type protease inhibitor from the hookworm *Ancylostoma* caninum. J.M. HAWDON, B.F. JONES\*, M. CAPPELLO and P.J. HOTEZ, Yale University School of Medicine, New Haven CT.



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# **ADDENDUM**

# Tuesday, July 11, 1995 Pittsburgh PA

In Conjunction with the 1995 Meeting of American Association of Veterinary Parasitologists American Society of Parasitologists American Veterinary Medical Association

For those of you who are staying on to attend part or all of the AVMA Meeting, the following two sessions may be of interest to you.

# AAVP/AVMA PRESIDENT'S SYMPOSIUM

Location: To Be Announced at the Meeting

9:00	Introduction. C.H. COURTNEY, AAVP President.
9:05	Drug residues and antiparasitic drugs. S.F. SUNDLOF, Director, Center for Veterinary Medicine, U.S. Food & Drug Administration.
9:45	Adverse reactions encountered with the use of antiparasitic drugs. M.J. BLACKWELL, Deputy Director for Post-Marketing Surveillance & Compliance, Center for Veterinary Medicine, U.S. Food & Drug Administration.
10:25	Resistance to antiparasitic drugs. R.K. PRICHARD, Professor of Parasitology & Dean for Research & Graduate Studies, McGill University.
11:05	Environmental impact of antiparasitic drugs. R.P. HERD, Professor of Parasitology, The Ohio State University.
11.45	Concluding remarks, C.H. COURTNEY.

# EQUINE PARASITE WORKSHOP: A FOCUS ON SMALL STRONGYLES

Location: To Be Announced at the Meeting

1:00 p.m. OPENING. T.R. KLEI, Department of Veterinary Science &

Veterinary Microbiology and Parasitology, Louisiana

State University, Baton Rouge LA.

1:05–1:45 p.m. BIOLOGY OF SMALL STRONGYLES

Moderator: E.T. LYONS, Department of Veterinary Science,

University of Kentucky, Lexington KY.

Presenter: M. EYSKER, Veterinary Medicine, Department of

Infectious Disease & Immunity, University of Utrecht,

The Netherlands.

1:45–3:00 HOST RESPONSE/PATHOPHYSIOLOGY

Moderator: D.E. GRANDSTROM, Department of Veterinary

Science, University of Kentucky, Lexington KY.

Presenter: S. LOVE, Department of Veterinary Medicine,

University of Glasgow, Scotland.

3:00–3:30 Coffee

3:30–6:00 EPIDEMIOLOGY & CONTROL

Moderator: J.A. DiPIETRO, Department of Pathology, University of

Illinois, Urbana IL.

Presenters: J. DUNCAN, Department of Veterinary Medicine,

University of Glasgow, Scotland.

R.P. HERD, Department of Veterinary Preventive Medicine, Ohio State University, Columbus OH.

P. NANSEN, Danish Center for Experimental Parasitology, Royal Veterinary & Agricultural College,

Frederiksberg, Denmark.

C.R. REINEMEYER, Department of Comparative Medicine, University of Tennessee, Chattanooga TN.

6:00 SUMMARY & CLOSE. T.R. KLEI

Rapporteurs: D.D. FRENCH, Department of Veterinary Science, Louisiana State

University, Baton Rouge LA.

G.E. HACKETT, Department of Animal Science, California Polytechnic

University.

1

Getting that Teaching Job at a Smaller Comprehensive University. DARWIN WITTROCK\*

As a chair of a biology department at a smaller four-year university, I have evaluated hundreds of job applications and interviewed many candidates. Several good candidates have been unsuccessful in gaining a position due to incomplete applications, missed deadlines, ineffective seminars, and/or underestimation of the importance of teaching. I will discuss the methods by which teaching jobs are formulated within a department and will provide the graduate students with some tips on how to be successful in getting that teaching job. Specific topics addressed will include acquiring teaching experience, how to find open positions, whether or not post-doctoral experience is needed, writing the letter of application, importance of letters of reference, significance of deadlines, and what to expect at and how to prepare for an interview. Information will also be provided on what to expect during one's first year in a teaching job, typical teaching assignments/workload, start-up money, and development of one's research program.

2

Teaching an Introductory Course in Parasitology to Undergraduates. HARVEY D. BLANKESPOOR\*

Animal parasites can be a fascinating and challenging group for undergraduates to study. Complex life cycles, sophisticated host-parasite interactions, behavior and ecology of parasites are just some of the exciting topics for students to pursue. During this presentation, several aspects of teaching an introductory course in Parasitology will be discussed. Some of these include: breadth of subject material, lecture and lecture outlines, audiovisual aids, live material, hands-on activities, investigative labs, outside readings, use of equipment, testing and course evaluations. In addition, various attributes of a good teacher such as enthusiasm, concern for students, background experiences and one-on-one interactions between professor and student will be delineated. This talk should provide many practical suggestions for organizing and teaching an introductory course in Parasitology at the undergraduate level.

3

Field Parasitology at Cedar Point: The design of a course. J. JANOVY, JR.

Field Parasitology at the Cedar Point Biological Station in Keith County, Nebraska, is a course designed to integrate all of the experiences of a professional parasitologist into a 5-week program of field work, research, collection, identification, use of original literature, data analysis, and written and oral presentation, using parasites from a very diverse set of hosts The course can be viewed as one in ecology, diagnosis, biodiversity, microscopy, public health, as well as one in which the student learns to deal with complexity and acquires a certain amount of patience. Teaching devices include video quizzes, daily question sets, class field problems, as well as a more standard series of exercises in parasite ecology. In 20 years of teaching this course, two student projects have been published in J. Parasitol., and a third, started in this course, was finished and published later as an undergraduate research project. A good time is generally had by all.

4

So You Have Your Own Lab. MARILYN E. SCOTT Life changes dramatically on the day you are offered an academic position. To get a lab "up and running" requires space and money to create the physical facility, appropriate planning to choose research orientation and set priorities, and good interpersonal skills to ensure that you establish an appropriate working relationship with your colleagues and students. Even prior to a job interview, you need to consider your requirements for space and facilities. Are the specialized facilities or equipment that are necessary for your research available within the department and accessible to you? During the interview, and certainly before you accept a job offer, you need to clarify what start-up funds will be provided to you, and what "strings" may be attached. Does the university have an Equipment Grant competition? What other sources of funds exist for setting up the lab? Planning continues when you arrive. Can you bargain with supply companies? Should your equipment be the basic model or should it come with all the frills? As your lab begins to take shape, where do you place your priorities? Should you accept graduate students immediately? Do you have realistic expectations of your first graduate student? What about collaboration? Should you demonstrate your independence by working alone, or should you pursue collaborative research? How do you choose an appropriate collaborator? Should you try to start many projects or just one? Should you try to get "mega" money or a modest research grant? To make the most of your new life, be ready for the challenge, and always remember the love you have for research and discovery.

5

Intestinal Helminth Infections Inhibit Growth and Development: The Evidence From Animal Models Supports Intervention. LINDA S. MANSFIELD\*

Intestinal helminthiasis has long been recognized as a significant cause of wasting and stunting in animals. *Ascaris, Trichuris* and hookworm infection have been linked to poor rates of growth and delayed development in domesticated animal species. Multiple mechanisms contribute to suboptimal acquisition and utilization of nutrients including anorexia, direct mucosal damage, diarrheas of various types, malabsorption, protein losing enteropathies, nutrient subversion, proteinase destruction of tissues, anemia, chronic inflammation, etc., resulting in growth failure. Effective health regimes for food and companion animal species include management schemes and anthelminthic therapies designed to prevent or ameliorate the effects of chronic helminth exposure. These schemes rely on strategic timing which is based on intimate knowledge of parasite life history, disease pathogenesis and infection dynamics.

For example, weaned swine given increasing doses of Trichuris suis (Urban et al., 1992) or Ascaris suum (Hale et al., 1985) had higher feed per gain ratios, lower average daily gains, lower average daily feed intakes, and lower final weights compared to uninfected controls. The degree of these effects increased proportionally to the dose of worms given. Growth stunted, Ascaris-infected pigs had heavier intestinal tracts with increased size of the tunica muscularis (Stephenson et al., 1980) and a significant decrease in lactase activity, fat digestion and nitrogen retention compared to uninfected controls (Forsum et al., 1981). Pigs which acquired Ascaris and Trichuris naturally during the growing phase without intervention had permanent loss of growth potential (Urban et al, 1989). Here, rate, but not efficiency, of gain was significantly improved by strategic anthelminthic treatment. In a concrete management environment, strategic treatment of pigs with anthelminthics following repeated inoculation with A. suum had no effect on rate of gain. However, treatment during the growing phase did reduce adult worm burdens following the finishing phase of growth. In a related study, weaned pigs infected with a single low level dose of T. suis and kept in confinement had stunted growth, diarrhea, anemia, eosinophilia, and colonic pathology. Clinical signs and pathology were attributable to opportunistic bacteria exacerbated by subclinical worm infections and could be eliminated either by antibiotic treatment or vaccination with a T. suis crude adult excretory/secretory extract (Mansfield and Urban, 1995; Hill et al., submitted).

6

Impact of Intestinal Helminth Infections in Humans: Effects, Mechanisms, and Recommendations. LANI S. STEPHENSON\* Intestinal helminth infections are among the most common infections worldwide: an estimated 1.4 billion persons harbor Ascaris lumbricoides, 1.2 billion suffer from hookworm (Necator americanus and Ancylostoma duodenale), 1 billion have Trichuris trichiura, and at least 100 million harbor Strongyloides stercoralis. Most of these cases occur in developing countries where approximately 192.5 million preschool children are underweight, 229.9 million children are stunted, 49.5 million children are wasted and 56% of pregnant women and high percentages of

children are anemic. These parasitic infections cause or aggravate conditions that inhibit societal development including decreased appetite, poor growth, decreased fitness, activity, work capacity, and cognitive and school performance; and in the case of hookworm, poor reproductive performance, partly by causing anemia and/or decreasing food intake. Nutritional stunting alone is estimated to cause an annual loss in productivity on the order of \$8.7 billion, not to mention unmeasurable but widespread human suffering. Studies in areas where polyparasitism and malnutrition are common show that entire groups of children improve in the above measures after treatment with broadspectrum anthelminthics, even though many initially had low worm burdens. New recommendations for treatment and control of intestinal helminths in children and women of child bearing age will be presented.

7

Allergy, Intestinal Helminths and Nutrition: the Good, Bad and the Ugly.

NEIL R. LYNCH\*, ISABEL HAGEL, and MARIA DIPRISCO.

The Good: IgE mediated allergic reactions are important components of the protective mechanisms against helminthic infection.

The Bad: In industrialized countries, where helminthic infection has been controlled by adequate sanitary measures such reactions are generally directed against "inoffensive" environmental allergens, and can cause disease.

The Ugly: i) Helminths can induce the polyclonal stimulation of IgE synthesis, that suppresses allergic reactivity, and increases susceptibility to infection. (The not-so-ugly: this also suppresses allergic reactivity to environmental allergens, reducing the prevalence of allergic conditions in endemic areas). ii) This polyclonal stimulation is potentiated, and becomes irreversible, in a situation of malnutrition.

Mixed Blessings: Anthelmintic treatment re-activates allergic reactivity against the parasites (good) and environmental allergens (bad).

8

A Healthy Body and a Healthy Mind - the relationship between parasitic helminth infection and cognitive function/educational achievement in school-age children. C. NOKES\*, and D.A.P. BUNDY.

If we begin with the assumption that ill health adversely affects a child's physical as well as mental health status then we need to find out what aspects of mental health are affected, whether these affects are measurable and whether or not they are of any functional significance. If helminth infection is shown to compromise a child's potential for learning, then treating children to remove these infections could have important developmental consequences which may be particularly relevant in developing countries where children may receive very few years of schooling. There are three types of evidence which suggest helminth infection may affect a child's potential for learning. (i)

Implied evidence comes from the fact that the morbidity associated with helminth infection includes undernutrition and iron deficiency both of which are known to adversely affect cognition and educational achievement (ii) Correlational evidence dates from the early 1900's and consistently shows that children infected with helminths score significantly worse than uninfected children on a wide range of cognitive and educational tests even after controlling for confounding variables such as socioeconomic and nutritional status. These studies imply therefore, that preventing infection could be important. (iii) Intervention studies where improvements in cognitive function are examined following treatment in terms of improved cognition and educational achievement than children who are adequately nourished and with light worm loads. The Cognition Panel of the Partnership for Child Development is exploring which specific aspects of cognition are adversely affected by helminth infection and what possible mechanisms may explain these effects.

9

Intestinal Helminths and Malnutrition: A Global Perspective. REBECCA J. STOLTZFUS

At least one billion people worldwide are malnourished, and a great many on these people have intestinal helminth infections. The most common forms of malnutrition globally are protein-energy malnutrition and iron deficiency, each affecting 30-50% of women and children in the developing world. Vitamin A and iodine deficiencies affect lesser but still impressive numbers. The consequences of these most common forms of malnutrition include increased risk of death from infectious disease, delayed mental and physical development, decreased physical fitness and work capacity, and blindness. Malnourished women are more likely to die in childbirth and bear growth-retarded babies, creating an intergenerational cycle of malnutrition.

The potential impact of helminth control on the global burden of malnutrition has not been defined. However, it is almost certain that helminth control could help to alleviate protein-energy and iron deficiencies, and very likely vitamin A deficiency. The evidence for this will be reviewed. There is an urgent need for our disciplines to collaborate to define this impact because helminth control is affordable, feasible, and acceptable relative to many nutrition interventions. It is useful to approach the problem separately for women, young children and school-age children, because each group is unique in its nutritional risks and consequences, and the available avenues for helminth control. Ironically, a strategy for helminth control is best defined for school children, whereas recent nutrition research has largely neglected the nutritional risks and consequences for this group. Malnutrition in women and young children has been prioritized in nutrition research, but public health strategies for helminth control and its benefits in these groups are not well-defined. Several priority research areas will be put forth.

10

Molecular Genetics of Development and Behavior in C. elegans. PAUL W. STERNBERG

11

Ecological Analysis of Copepods Living Up the Noses of Blue Sharks. JOSH W. KOHL\* and GEORGE W. BENZ

The locations of 1623 Kroeyerina elongata collected from the left olfactory sacs of 14 blue sharks were analyzed. Numbers of K. elongata per olfactory sac ranged from 0-228 (x =  $115.92 \pm 82.82$ ), and a weak relationship  $(r^2=0.213)$  existed between the number of copepods per olfactory sac and shark fork length. The number of olfactory lamellae per olfactory sac did not vary with shark fork length. Female copepods typically outnumbered males (mean number of males per female =  $0.487 \pm 0.263$ ). Both males (96.84%)  $\pm 0.034$ ) and females (99.00%  $\pm 0.013$ ) appeared positively reotactic with respect to water flow through the olfactory sac, and adults of each sex seemed to prefer different locations within the olfactory sac. Ovigerous females typically attached at the base of the rachis or within the first third of the excurrent water channel, while males tended to attach randomly about the secondary lamellae of the olfactory sac. It is interesting that the few subadult females that were collected (some of them paired with males) were distributed in male-like fashion. Together these results suggest some mechanisms through which the realized niche of K. elongata might be achieved. (Partially supported by Tennessee Aquarium Intern Research Program)

12

Epidemiology of the Strongylid Parasites of Horses in Prince Edward Island, Canada.

KAREGA M. M'ABURI\* and GARY CONBOY

Detailed knowledge of the seasonal patterns of transmission is important for the design of effective, efficient parasite control programs for livestock. Climatic factors have a major impact on these patterns, hence the need for investigation of the specific patterns for each region. The rate of development and longevity of equine strongyles on pasture was studied. Each month for one year, twelve, fresh positive fecal samples were placed on an ungrazed pasture in marked 1.5m<sup>2</sup> plots. The fecal samples were monitored for egg development and longevity. Grass from the plots was monitored for L3 counts (first appearance, peak recovery and longevity). The results indicated seasonal patterns of egg development and larval recovery. The minimum time for larvae to reach infective stage was seven days during July to September. Maximum recovery of L3 was in 11-21 days. First appearance of L3 in grass was 7 days in July and up to six months for samples deposited between October to February. Maximum recovery from the pasture

was attained in five weeks on average. Maximum longevity of L3 occurred in samples put out in August and September. Infective larvae were recovered in low levels up to eleven months later.

#### 13

An artificial life approach to host-parasite interactions. P.G. WILBER\* and H.D. SHAPIRO.

Computer simulations that model individuals over space and time are rare. We developed an artificial host-parasite "universe" that incorporates these parameters as well as host density, host immune response, parasite life-history parameters and weather effects on both host and parasite. Graphical output of the host-parasite universe allows visualization of the system over time. In order to examine interactions between the parameters listed above, we chose high and low values for each parameter and tested all possible combinations. We found that under constant weather conditions, various combinations of the other parameters produced remarkably similar temporal patterns for prevalence of infection. However, prevalence became much less predictable when real weather conditions were used. We also found that the relationship between host density and prevalence of infection can be non-linear under many conditions. When compared with a field-generated rodent-coccidia data set, the model behaved in a biologically reasonable way.

# 14

Distribution of Paratenic Infections of *Plagiorhynchus* cylindraceus (Acanthocephala) Among Co-Occurring Nebraskan Mammals, N.R. COADY

Acanthocephalans from many taxonomic families are known to encyst in the viscera of vertebrate hosts in which maturity is not attained. In many instances it does not appear that paratenic occurrences contribute to continuance of the life cycle. *Plagiorhynchus cylindraceus*, a common parasite of birds, does not require a paratenic host for completion of the life cycle, but worms of this species are known to occur in viscera of *Blarina brevicauda*. Distribution of visceral infections of *P. cylindraceus* in small Nebraskan mammals was investigated to determine if this worm

infects ecologically similar mammals. Of co-occurring species of mammals trapped at two study sites, only *B. brevicauda* and *Sorex cinereus* were infected with visceral forms. This constitutes the first report of paratenic infection of *Sorex cinereus* by *P. cylindraceus*. Laboratory infections of *Peromyscus maniculatus* resulted in one visceral infection with *P. cylindraceus* 21 days postinfection. This visceral cystacanth appeared viable, but no attempt was made to infect a bird with it. When fed orally, cystacanths from wild-caught *B. brevicauda* did not infect robins, the usual definitive host. It appears that the paucity of reports from non-insectivorous mammals may be due to ecological rather than to physiological constraints.

#### 15

Altered Escape Response in Cockroaches Infected with an Acanthocephalan (*Moniliformis moniliformis*) and its Impact on Predation. L.D. FLOYD\*, M. FREEHLING, J. MOORE, AND F. LIBERSAT

Some parasites alter the behavior of their hosts thereby facilitating passage into their next host. This alteration of behavior often involves increasing the intermediate host's chance of being preyed upon by the definitive host. The acanthocephalan Moniliformis moniliformis has been shown to have an effect on the escape response of its cockroach (Periplaneta americana) intermediate host. In wind puff experiments, infected cockroaches were less likely to exhibit an escape response when stimulated and tended to turn away from the stimulus less often than their uninfected counterparts. The latency period and threshold for the escape response were lower in infected than uninfected cockroaches as well. We attempted to discover if this effect on escape response translated into an increased predation risk for the roach. Mammalian predators were placed in a circular, walled arena into which two roaches, one infected and one uninfected, were simultaneously introduced. Pursuits, pursuit duration, captures, and escapes were recorded for each predator and prev type. Preliminary results from these predators indicate that infected roaches tend to be captured more quickly than uninfected and may, in fact, be captured more often.

#### 16

Regional and Local Richness in the Parasite Communities of Pacific halibut, *Hippoglossus stenolepis*. R.B. BLAYLOCK\*, J.C. HOLMES AND L. MARGOLIS

Ecologists are increasingly aware that the interpretation of community patterns is dependent on the scale of the

observations, and have debated the importance of various processes that produce pattern at different scales. We are examining patterns in regional species richness (RSR) and local species richness (LSR) in the metazoan parasite communities in Pacific halibut, an important commercial species off the North American Pacific coast. Analysis of 225 halibut of similar size and age from 20 localities ranging from California to the Bering Sea indicates three regions (Bering Sea/western Aleutians, Gulf of Alaska, and Oueen Charlotte Islands/south) based on parasite presence/absence patterns. RSR peaks in Aleutians/western Gulf of Alaska. LSR calculated from 175 fish from 11 localities (each with  $n \ge 10$ ) is not correlated with sample size and is positively (and curvilinearly) correlated with RSR (regression of log<sub>e</sub>LSR on  $\log_{r} RSR$ : r = .796,  $r^{2} = .634$ , p = .003). Maximum LSR averages 65-75% of RSR. LSR of common species (prevalence > 50%) is not correlated with RSR; RSR appears to reflect the presence of the less common species. Mean LSR per locality tends to be higher in the Gulf of Alaska and appears to reflect the present and, possibly, historical center of halibut abundance. These results generally agree with previous studies highlighting the importance of local processes in determining parasite communities, but in this marine system, regional processes appear to play a larger role than in previous studies.

with autogenic species dominating perch parasite component communities that are at or near equilibrium levels.

#### 18

Effect of age on infectivity of cercariae of Halipegus occidualis (Digenea: Hemiuridae) to their second intermediate host. E.J. WETZEL\* and G.W. ESCH. The effect of age on the infectivity of cercariae of the hemiurid trematode Halipegus occidualis to their second intermediate host was examined. For 2 days, individual ostracods (Cypridopsis sp.) were each exposed to 6 cercariae that were either 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, or 25 days old. Surviving ostracods were then examined for infection, and the number of dead hosts recorded. The number of cercariae ingested by the ostracods remained constant throughout the experiements. However, the number of mesocercariae recovered from the ostracods declined significantly as the cercariae increased in age. Cercariae 25 days old were not infective to ostracods. The number of deaths among infected hosts was significantly higher than those in uninfected controls. Examination of variance to mean ratios suggested that host mortality was parasite-induced. The results indicate that the opportunity of transmission for these cercariae is considerably smaller that previously suggested.

17

The effect of lake size on the determination of the parasite component community of yellow perch, Perca flavescens (Mitchill). D.A. ZELMER\* and H.P. ARAI.

Perca flavescens from 5 Alberta lakes perch known to contain natural populations were examined for metazoan parasites. The species richness, number of autogenic parasite species, and the number of larval parasites harbored by perch in a given lake were found to be significantly correlated with surface area by regression analysis. determine whether these regression lines could be taken as equilibrium values, perch from 10 lakes that had been stocked from these natural populations were examined. The predictions held for all equations in the central and northern lakes, but only the relationship between the number of autogenic parasite species and lake surface area was supported by the data from the southern lakes. The combined data support a succession of colonizing parasites from those that are allogenic to those that are autogenic,

19

Resource Allocation by <u>Helisoma anceps</u> Infected with Halipegus occidualis.

BRIAN E KEAS\* and GERALD W ESCH Gigantism is exhibited in the planorbid snail, Helisoma anceps when infected by the hemiurid trematode, Halipegus occidualis, only when infected prior to reproductive maturity (IPR) and when fed a High quality diet. Snails infected after reproductive maturity (ÎAR) were not significantly different in size from uninfected (UNI) controls. The hypothesis that gigantism is a parasite adaptation is rejected because the number of cercariae shed per snail was no different for snails exhibiting gigantism (IPR) or not (IAR). Gigantism cannot be ruled out as an adaptation by the snail and seems to be controlled by the snails' consumption and assimilation of food. UNI snails were better able to compensate for lower food quality by increasing consumption. IPR and IAR snails fed the low quality diet generally consumed less food than UNI controls resulting in lower assimilation. Probably because of this lack of resources ingested by the snail and made available to the parasite, 50% of IPR and IAR snails on the low diet lost their infection by the end of the study. This is the first study we know of that rules out gigantism as a parasite adaptation and suggests that a host, by consuming less, can actively cause a loss of infection by depriving the parasite of resources.

#### 20

On the Distribution of Aspidogastrids (Trematoda) in freshwater mussels (Bivalvia: Unionidae) from Eastern North America. STEPHEN S. CURRAN\*

Aspidogastrid trematodes have been reported to parasitize freshwater mussels from sporadic localities over a huge range in North America while at the same time exhibiting low host specificity. Unfortunately, their distributional limits have not been well documented. The examination of potential hosts from a broad range of localities is necessary to provide information leading to the better understanding of the biogeographical limits of the aspidogastrids. Three hundred seventeen unionid mussels representing 15 species in 7 genera were examined for aspidogastrids from five localities including: two in Tennessee, one in Mississippi, one in Connecticut, and one in Maine. Eleven species hosted Aspidogaster conchicola from four of the five localities. These hosts included: Amblema plicata, Elliptio complanata, Fusconaia cerina, F. ebena, F. flava, Lampsilis teres, Quadrula apiculata, Q. metanevra, Q. pustulosa, and Q. quadrula. Overall prevalence of A. conchicola was high with 209 (65.9%, n = 317) of the mussels parasitized. The highest prevalence and intensity values of A. conchicola occurred in Q. quadrula; 74.4% of the individuals examined were infected. This species had a mean intensity of 8.6 worms per host (range = 1 - 40). Two unionid species, L. teres (n = 2) and Pygodon grandis (n = 1), hosted Cotylaspis insignis in Mississippi. The mean intensity in both unionid species was 1.0 worms per host. Elliptio complanata hosted Cotylogasteroides occidentalis in Connecticut. The prevalence of *C. occidentalis* in this host was low with 3 (3.4%, n = 87) mussels parasitized. The mean intensity of infection was 1.66 worms per host. Aspidogastrids were absent from mussels examined from Maine, indicating a potential northern boundary to their distribution in New England.

### 21

Investigation of the Distribution of Copepods on Shortfin Mako Sharks, with Special Reference to Copepod Ecology and Life History.

JENNIFER C. BACKER\* and G. W. BENZ

The ecology of the parasitic copepod Dinemoura latifolia was studied on 112 shortfin mako sharks captured in the North West Atlantic. In all, 1411 D. latifolia were collected, with females typically outnumbering males on individual sharks. prevalence of infection for the sample was 70.5%, with infected sharks hosting from 1 to 78 copepods. The mean intensity of infection was 12.59 ± 19.29. Dispersion of D. latifolia throughout the shark sample was well described by a negative binomial model, these parasites clumped indicating Linear (overdispersed) amongst these sharks. regression revealed a poor overall relationship between the density of infection and shark fork length (r<sup>2</sup>=0.162), indicating that a simple surface area model could not account for copepod density. Most female D. latifolia (51.46%) attached about the caudal keel. Males also preferred this body location (76.76% attached there), however, males exhibited a wider overall distribution about the bodies of examined sharks. Comparison of sharks infected solely with *D. latifolia* and others infected also with *Pandarus* spp. revealed the distribution of *Dinemoura latifolia* to be unaltered by the presence of *Pandarus* representatives and vice versa. This finding is contrary to a previous report in the literature. Overall, results of this study can be interpreted in light of our current understanding of the life history of pandarid copepods. (Partially supported by Tennessee Aquarium Intern Research Program)

#### 22

Resource Partitioning of Scalloped Hammerhead Sharks by Two Genera of Parasitic Copepods. KRISTY L. SMEDLEY\* and GEORGE W. BENZ

The spatial distribution of representatives of two parasitic copepod genera (Alebion and Pandarus) on the external body surface of scalloped hammerhead sharks was investigated using data collected from 16 sharks captured off North Carolina. The number of copepods per shark ranged from 3-181 (x=22.875 ± 41.252; median =14), with Pandarus individuals (range = 2-174) typically outnumbering Alebion individuals (range = 0-14). A weak relationship ( $r^2$  = 0.427) between total number of copepods per shark and shark fork length was largely determined by individuals of the genus Pandarus. Alebion and Pandarus seemed to prefer different body regions for attachment. Most Alebion females (86.8%) attached just below the trailing free edge of the first dorsal fin. while most Alebion males (85.2%) attached on the ventral body surface between the pelvic fins and the origin of the caudal fin. Most Pandarus females and males were collected from the ventral surface of the head (76.7% and 52.8% respectively). however, were more locally concentrated about the lower jaw and were additionally better represented about the rest of the entire body surface. There did not appear to be any negative interspecific covariation between numbers of Alebion and Pandarus individuals on the studied sharks. Together these data suggest some possible mechanisms through which the realized niches of these two copepod genera might be achieved. (Partially supported by Tennessee Aquarium Intern Research Program)

# 23

Urban Wildlife as Reservoirs of cat fleas, Ctenocephalides felis. MICHAEL W. DRYDEN\*, A.B. BROCE, J. CAWTHRA, and D. GNAD

A study was undertaken to determine if small and medium sized wild mammals living in close association with humans and their pets serve as reservoir hosts of cat fleas. Small and medium sized wild mammals were live-trapped

within the city limits of Manhattan Kans., and in rural areas surrounding this city. Trapping was initiated June 20, 1994 and continued through January 8, 1995. Animals were sedated and fleas were removed and identified to species. Cat fleas were recovered from 67.2 (39/58) and 31.4% (16/51) of urban trapped opossums, Didelphis virginiana and raccoons, Procyon lotor, respectively. Whereas, cat fleas were recovered from only 5.2 (1/19) and 0% (0/22) of rural trapped opossums and raccoons, respectively. Flea numbers and the percentage of urban trapped opossums and raccoons infested with fleas increased during the fall and early winter. Predominant flea species were Pulex sp. and Ctenocephalides felis. Percentage of rural trapped opossums infested with fleas and the number of fleas per animal also increased during the fall and early winter, however, those increases were not due to cat fleas nor Pulex sp. Rural trapped raccoons had very low rates of flea infestation (4.5%;1/22). Overall, 10 different flea species were recovered from opossums and 7 from raccoons. Cat fleas were also recovered from one Eastern cottontail, Sylvilagus floridanus (1/7). Other mammal species trapped included; Scuirus niger (16), Neotoma floridana (54), Sigmodon hispidus (6), Rattus norvegicus (5), and Vulpes vulpes (3).

# 24

PARABAN-A Mathematical Model of Parasitic Infections of Cattle. G. SMITH\*, J.A. JACOBSEN and J. GUERRERO PARABAN is a mathematical model for the population biology of parasitic infections of cattle. It is used to devise, investigate and explain parasite control strategies based on a combination of chemoprophylaxis and pasture management. Previous versions of PARABAN focused only on the population biology of the common gastrointestinal trichostrongylid nematode parasites of cattle. To help investigate the likely benefit of plausible control strategies for other common parasites of cattle, additional models have been developed to explain the overall effect of those strategies to veterinarians and cattle producers. Modelling of parasites is a tractable proposition since the patterns observed in field investigation can be explained in terms of a small number of very influential mchanisms. Adequate model function depends upon an accurate representation of the processes that regulate parasite density; these processess vary according to the parasite being modelled. The validity of PARABAN can be illustrated by the extent to which model output matches actual data from independent field trials.

#### 25

Effects of Three Anthelmintic Treatment Regimes on Flock Performance of Sheep and Goats Under Extensive Management in Semi-arid Kenya.

P.M. GATONGI, M.E. SCOTT, R. K. PRICHARD\*, S. RANJAN, J.M. GATHUMA, W.K. MUNYUA and H. CHERUIYOT.

A study was undertaken in a semi-arid area of Kenya between August 1991 and June 1993 to evaluate the effects of anthelmintic treatment using ivermectin before or during the rains on performance of mixed sheep and goat flocks, in comparison with an untreated flock. Performance parameters measured included body weights, growth rates, birth weights, parturition intervals, age and weight at first parturition and mortality rates. Among these parameters birth weights growth rates were found to be significantly improved by the treatment administered before the rains. Mortality was significantly lower in lambs and kids with high birth weights. Treatment, either before or during the rains significantly reduced the faecal egg output and impoved body weight, packed cell volume and flock fertility. Liveweight was confirmed to be a better measure of sexual maturity than age. It was further shown that lambs and kids, born of dams at their first lambing or kidding, experienced higher mortality rates than lambs and kids born of dams in their second and subsequent parturitions. Overall, flock performance was significantly better when treatment was administered before the onset of the rains than when it was administered during the rains.

#### 26

Use of Diatomaceous Earth in the Control of Internal Parasites of Grazing Lambs. G.A. MOORE, A.M. ZAJAC\*, C.D. THATCHER, D. NOTTER, S. UMBERGER.

Interest in the antiparasitic effects of diatomaceous earth (DE) has recently grown, particularly amongst producers undertaking organic livestock production. However, there has been little testing of this product to determine its efficacy. To evaluate whether DE would be beneficial in a parasite control program for sheep, its effect against trichostrongyle parasites in grazing lambs was investigated.

Thirty-six mixed breed weaned lambs were treated with ivermectin (200 µg/kg), divided into 3 groups and placed on 3 approximately equivalent mixed grass pastures. Group 1 was treated monthly with ivermectin and given standard trace mineral salt. Group 2 was treated every 8 weeks with ivermectin and was exposed to free choice feed grade DE. Group 3 was given no anthelmintic treatments and free access to DE. Lamb weights, packed cell volume, and fecal egg counts were determined every 2 weeks from April 24th to August 31st.

Fecal egg counts in all groups increased during the grazing season. However, trichostrongylid fecal egg counts in Group 3 rose most rapidly and were consistently higher than those of the other 2 groups until the end of

July when the clinical condition of the Group 3 animals deteriorated and anthelmintic treatment was required. Group 2 lambs also required additional anthelmintic treatment before the end of the grazing season. Weight gain in the 2 groups receiving DE was severely depressed compared to Group 1, with differences appearing early in the grazing season. These results do not indicate that DE provides control of trichostrongyles and suggest that it may cause additional detrimental effects when levels in the diet are not carefully controlled.

#### 27

Evaluation of Pyrantel Pamoate Against Equine Cyathostomes: Resistance or Lack of Efficacy? M.R.CHAPMAN\*, D.D.FRENCH, and T.R. KLEI.

Resistance of equine cyathostomes to pyrantel pamoate (PP) has been reported but evidence for this is minimal. The objective of these studies was to test the effectiveness of this drug against equine cyathostomes on a well managed Thoroughbred stud farm in Louisiana where a loss of efficacy of PP suspected. Three fecal eqq reduction assays (FECR) were conducted over a period of 2 years using yearlings and mares bred and housed on the farm. Efficacy of PP based on FECR varied from 26% in mares to 83% in yearlings. Second treatments of PP 2 wk following initial treatment failed to reduce EPG. critical trial was performed determine the cyathostome species resistant to PP. Three strongyle-naive ponies which subsequently acquired infections on the farm were used for this purpose. Following treatment with PP at the recommended dose, 11 species of cyathostomes remained in the gut of the tracer ponies. Reduced efficacies (12%-88%) were noted for 7 species. Resistance to oxibendazole (OBZ), which was >90% effective on this farm in 1982, was also evaluated and found to exist. The results of one experiment indicate that dual resistance of parasites to PP and OBZ also exists. Screening for PP cyathostomes resistant is warranted during evaluations of equine parasite control programs.

# 28

Naturally Occurring Nematode Anthelmintic Resistance in Goats and Sheep in Texas. D.K. MILLER\* and T.M. CRAIG

Four ranches on which naturally occurring ivermectin resistance was suspected were evaluated by the fecal egg count reduction test (FECRT). The anthelmintics tested were Ivermectin (IVM 0.2 mg/kg), Albendazole (ABZ 10 mg/kg), and Levamisole (LEV 8 mg/kg), when compared to untreated controls. The percent FECRT results are as follows:

	LEV	ABZ	IVM
Sheep ranch 1	93	100	100
Sheep ranch 2	30	100	78
Goat ranch 1	56	100	97
Goat ranch 2	41	96	33

The results in light of the individual ranch management and environmental conditions are discussed.

#### 29

Effects of calmodulin inhibitors on muscle contractility in the filariid <u>Acanthocheilonema viteae</u>. DARYL CHRIST\*, ANDREW J. MINARDI and HOWARD J. SAZ

The functional role of calmodulin in filarial muscle was investigated by observing the effects of the calmodulin inhibitors on muscle contractility. Adult, female A. viteae were isolated from infected hamsters, slit open longitudinally and mounted in a tissue bath that was filled with a warmed (37°C). aerated (95% N2-5% CO2) physiological solution. Isotonic contractions were recorded from the preparation. The calmodulin inhibitors, trifluoperazine and W-7, enhanced the spontaneous contractions at concentrations of 10<sup>-5</sup> and 3 x 10<sup>-5</sup> M. At 10<sup>-4</sup> M each drug induced an initial contraction of the parasite followed by a reduction of the spontaneous activity. Concentration-response curves for ACh were determined. In the presence of TFP (3 x 10<sup>-6</sup>, 10<sup>-5</sup> M) or W-7 (3 x 10<sup>-5</sup> M), the ACh concentration-response curves were shifted in a downward direction, indicating a non-competitive mechanism for the antagonism. The effects of trifluoperazine and W-7 on the KCl concentration-response curves were very similar to those on the ACh contractions, indicating that cholinoceptor blockade was not inducing the antagonism. Finally, trifluoperazine or W-7 relaxed preparations that were pre-contracted by 10<sup>-5</sup> M ACh or 100 mM KCl, which indicates that calmodulin is involved in sustaining the contraction, as well as in initiating the contraction. These results support a role for calmodulin in the generation of contractions from ACh and KCl. In this regard, the nematode body-wall muscle is similar to mammalian smooth muscle. (Supported in part by NIH Grant Al 09483)

### 30

Anthelmintic Profile of the Cyclodepsipeptide PF1022A in *In Vitro* and *In Vivo* Models. SANDRA S. JOHNSON\*, GEORGE A. CONDER, DEAN S. NOWAKOWSKI, TROY E. BLAKE, FRED E. DUTTON, STEPHEN J. NELSON, EILEEN M. THOMAS, JOHN P. DAVIS, TIMOTHY G. GEARY, and DAVID P. THOMPSON

A novel cyclodepsipeptide of fungal origin, PF1022A, was recently reported to have anthelmintic activity. To supplement published reports and to determine the potential utility of PF1022A as a ruminant anthelmintic, the compound was synthesized and examined using in vitro and in vivo models. Assays used measured motility of Haemonchus contortus (intrinsic drug potency), ATP levels (parasite death), and activity against *H. contortus* and *Trichostrongylus* colubriformis in the jird (spectrum, potency and efficacy by various routes). The potency of PF1022A in reducing motility of H. contortus is 4-10X greater than ivermectin, and at least 300X greater than levamisole, two widely used commercial anthelmintics. Examination of ATP levels in PF1022A-paralyzed H. contortus indicates that the worms are not killed, suggesting the compound acts as a neurotoxin in nematodes. In the jird, PF1022A has activity orally against each of the parasites studied and at doses comparable to all commercial anthelmintics, except the macrocyclic lactones which are more potent. activity of the compound in vivo appears to be highly sensitive to formulation and route of delivery.

#### 31

A Modified Egg Hatch Assay to Detect Pyrantel Resistance in Strongylid Nematodes of Horses. CRAIG REINEMEYER\*, and AL A. TINEO

Pyrantel-resistance has been reported infrequently in strongylid nematodes of horses, but in-vitro assays to detect altered susceptibility to this drug class have not been adapted for equine testing. A candidate assay which measured strongylid egg hatchability in 96-well ELISA plates was evaluated for horses. Columns of wells contained either distilled water (control) or serial dilutions of pyrantel tartrate (ranging from 1.5 to 50 mcg/ml); each drug concentration was replicated in 6 to 8 wells. Each well was inoculated with approximately 100 unembryonated strongylid eggs and plates were incubated at 74°F for 22 to 26 hours. When maximal hatching had occurred in the control column, further development was halted by the addition of dilute Lugol's iodine solution to all wells. Plates were examined with an inverted microscope at 100x magnification. Within each well, free larvae were counted and compared with the sum of a) unhatched eggs plus b) partially emerged larvae. Control wells were corrected to 100% hatching. Hatching percentages of treated wells were calculated for each drug concentration and converted to probit values. A dose-response slope was calculated for each plate and the concentration of pyrantel required to prevent complete hatching in 50% of exposed eggs (LC<sub>50</sub>) was derived from the slope. This candidate egghatch assay demonstrated excellent intra-assay variation, but differences in inter-assay variation

were occasionally significant. This assay was used clinically to monitor drug susceptibility in strongylid populations with different levels of selection pressure, and to confirm pyrantel-resistant strongyles in two horses.

#### 32

Benzimidazole Resistant *Haemonchus contortus* of Small Ruminants in Hawaii. J. P. TRITSCHLER II\* and B. R. LEAMASTER.

On the Big Island of Hawaii, groups of goats or sheep were treated with different classes of anthelmintic. Fecal egg depression tests were conducted by comparing fecal egg output prior to treatment with that of 10-14 days post treatment. Strong resistance to benzimidazoles was indicated with fecal egg reductions of only 7.9%, 12.2% and 56.8% to fenbendazole (5 mg/kg bd wt), fenbendazole (15 mg/kg bd wt), and albendazole (10 mg/kg bd wt). Following benzimidazole treatment, eggs were culture to infective larvae, which were identified as Haemonchus contortus. In contrast, levamisole (8 or 11.88 mg/kg bd wt) and ivermectin (0.2 mg/kg bd wt) were >99% effective. To confirm benzimidazole resistance, sheep were treated with thiabendazole (132 mg/kg bd wt), and feces were collected after 13 days for a benzimidazole egg hatch test. Eggs were collected by rinsing onto a 45 micron screen following centrifugation in saturated sucrose. Eggs were incubated for 48 hours in thiabendazole solutions ranging from 0 ppm to 3.1 ppm. The dosage of anthelmintic to inhibit 50% embryonation and hatching (LD50) was determined to 1.6 to 1.8 ppm. LD50 values from 1.15 to 1.75 ppm were found in the literature for benzimidazole resistant strains of H. contortus, while susceptible reference strains had LD50 values form 0.023 to 0.25 ppm. The results of these trials indicate that there are H. contortus populations present on the Big Island of Hawaii which have developed resistance to benzimidazoles.

# 33

Efficacy of Moxidectin Pour-on in the Treatment of Gastrointestinal Parasites of Cattle. J. DIPIETRO\*, R. VALDEZ, D. MORIN, C. LICHTENSTEIGER, A. PAUL, F. GUERINO and K.S. TODD, JR. A controlled efficacy study of 0.5% moxidectin (MXN) pour-on was carried out utilizing 30 mixed-breed beef cattle with natural nematode infections. The cattle were housed indoors and were rank ordered by parasite egg count and allocated to treatments by replicate. Treatments were assigned randomly within each replicate as follows: Group I-0.25 mg/kg MXN; Group II-0.5 mg/kg MXN; Group III-controls treated with pour-on vehicle. Animals were observed for reactions to treatment 1 to 2 hours

posttreatment and daily thereafter until necropsy. Cattle were necropsied on days 14 or 15 posttreatment for nematode recovery. Fecal samples were collected at necropsy to evaluate nematode egg counts and the pour-on sites examined. No pretreatment differences (P>0.05) in mean number of trichostrongyle eggs present in fecal samples were observed. Fecal samples taken at necropsy indicated that both 0.25 and 0.5 mg MXN/kg reduced (P<0.05) trichostrongyle egg output by 99.1% and 99.9%, respectively, as compared to control animals. No difference in the reduction of trichostrongyle eggs occurred between the MXN pour-on treated groups. Adult Haemonchus contortus, adult H. placei, adult Ostertagia ostertagi, Ostertagia spp. 4th stage larvae, adult Trichostrongylus axei, adult T. colubriformis, adult Bunostomum phlebotomum, adult Cooperia oncophora, adult Cooperia punctata, adult Nematodirus helvetianus, Trichostrongylus spp 4th stage larvae, adult Oesophagostomum radiatum, and adult Trichuris spp. were present in sufficient numbers in control animals to evaluate the efficacy of MXN pour-on. In all cases, treatment with MXN pour-on resulted in significant reduction in the numbers of recovered nematodes as compared to vehicle-treated control animals. There were no differences (P>0.05) in the number of these nematodes recovered between the two MXN pour-on treated groups. Efficacy of both doses was greater than 99.9% for these nematodes. No general adverse reactions were observed in any of the animals nor were there adverse reactions observed at the pour-on site. (Supported in part from a grant from American Cyanamid Company.)

#### 34

The Development of a Strategic Deworming Program for Horses, W.G. KVASNICKA\*, D.H. BLISS and J.B. SHANER

A three-year study was conducted in Nevada to determine if strategically timed dewormings given at three montly intervals in the spring, starting in April, and three montly intervals in the fall, starting in October, could be used to reduce environmental parasite contamination and to maintain low parasite exposure in treated horses. A total of 200 privately owned horses from 51 cooperators located in and around the Las Vegas, NV area were used for the study. The horses were selected on the basis of treatment, which was divided into four categories: non-treated horses, infrequently treated horses (treatment given at random one to four times a year), horses treated six times a year (treatment given every 60 days), and a strategic treatment (treatment given at 30-day intervals in April, May, June, and again in October, November and December). The non-treated control horses and the infrequently treated horses demonstrated a high parasite challenge throughout the year in Nevada. The monthly average worm egg count per 3 gm sample was: 125 for control horses, 66 for the infrequently treated horses, 24 for horses treated six times per year, and 11 for the strategically treated horses.

#### 35

Fasting Enhances Albendazole Bioavailability In Sheep and Cattle. C. LANUSSE\*, A. LIFSCHITZ, S. SANCHEZ, G. VIRKEL, L. ALVAREZ, M. MASTROMARINO

Feeding management may influence the absorption rate and the resultant systemic availability of benzimidazole anthelmintics in ruminants. This study investigated the influence of fasting on the

disposition kinetics and bioavailability of albendazole (ABZ) and its metabolites in sheep and cattle. Two different experiments were performed. Experiment I: fifteen parasite-free Corriedale sheep (50-60 kg) grazed on pasture were divided into three groups. ABZ was orally administered at 7.5 mg/kg (Group A) and at 11.3 mg/kg (Group B). Animals in Groups A and B were fed ad libitum prior and during the experimental period. Sheep in Group C were fasted during 24 h and subsequently treated with ABZ at 7.5 mg/kg. Experiment II: six parasite-free Holstein calves (150-160 kg) were treated intraruminally with ABZ (10 mg/kg) in three different experimental phases. There was a 4-week wash-out period between phases. Animals in phase 1 were fed ad libitum with high-quality alfalfa hay. In phases 2 and 3. calves were fasted during 24 and 48 h, respectively, prior to ABZ treatment. Abomasal fluid via cannula (calves) and jugular blood (sheep and calves) samples were taken over 120 h post-treatment. Plasma and abomasal samples were analyzed by HPLC. ABZ sulphoxide (ABZSO) and ABZ sulphone (ABZSO2) were the metabolites found in plasma. ABZ, ABZSO and ABZSO2 were the analytes recovered in abomasal fluid. Fasting induced marked modifications to the kinetics of ABZ metabolites in both species. Significantly higher AUC and Cmax values were obtained for both ABZ metabolites in fasted compared to fed sheep. The administration of ABZ at a 50% higher dose rate (11.3 mg/kg) resulted bioequivalent to the treatment with ABZ at 7.5 mg/kg in fasted animals. ABZSO AUC values resulted 132% (24 h fasting) and 310 % (48 h fasting) higher in fasted compared to fed calves. Delayed elimination for both ABZ metabolites was also observed in sheep and cattle subjected to fasting. Starvation decreases digesta flow rate, which may have facilitated an enhanced ABZ absorption by delaying the rate of passage of the drug down the gastrointestinal tract.

#### 36

Efficacy of Imidacloprid for Control of Fleas on Dogs. R.G. ARTHER\*, T. HOPKINS, J. CUNNINGHAM and R. EVERETT

Imidacloprid is a new chlorinated heterocycle insecticide currently under worldwide development for use on field crops, ornamental plants and for structural pest control. This compound has broad insecticidal activity, low mammalian toxicity and is environmentally safer than most currently registered insecticides. Preliminary studies have demonstrated this compound also has insecticidal activity when applied to animals. A total of 32 adult mixed -breed dogs were randomly assigned to 4 study groups (8 dogs/group). Three groups received a single treatment (study day 0) with an imidacloprid solution to provide either 3.75, 7.50 or 10.0 mg/kg active ingredient. Treatment was applied by parting the hair of the dog between the shoulders and applying the solution to one spot on the skin. Group 4 dogs served as controls and received solution without active ingredient. Each dog was experimentally infested with 100 adult fleas one day prior to treatment and again on days 6, 13, 20, 27 and 33. Post-treatment live flea counts were performed on each dog on post-treatment days 1, 7, 14, 21, 28, and 34. All 3 dosages provided greater than 96% flea control one day after treatment. The 3.75 mg/kg treatment provided 92-97% flea control for days 7-34. The 7.50 and 10.0 mg/kg treatments provided 97-100% flea control during the same peroid. The mean flea counts on the control dogs ranged from 69.1-146.4 fleas/ animal during the study.

37

The Critical Test and Efficacy of Pyrantel Pamoate for *Anoplocephala perfoliata* in Equids. J.O.D. SLOCOMBE\*

Thirteen equids were given orally pyrantel pamoate (Strongid-P®) at 13.2 mg pyrantel base/kg bodyweight, euthanised 48 hr later and examined for tapeworms. After treatment, all feces from each equid was examined for tapeworms. Three untreated equids were necropsied to determine normal position of tapeworms in the intestine. Only worms with scolices were counted in assessing efficacy and were deemed abnormal if disintegrating or had a brown discolouration. At necropsy, worms were noted as attached to or detached from the intestine.

In untreated equids 694 normal worms were in the cecum with 148 detached. The percentage of detached worms in the 3 equids were 0, 8 and 27. In treated equids 97 worms were recovered; 24 attach- ed to the cecum and one to the ventral colon and 72 free in the intestine. All attached worms and one detached (cecum) were normal. All detached worms including one in the cecum were abnormal. There were 859 abnormal tapeworms in the feces. Mean efficacy of pyrantel pamoate was 96.6%; in one equid the efficacy was 75.3%, for all others it was > 92%, for 8 of 13 it was 100%.

A Critical Test with a 24-hr post-treatment period, no examination of feces and worms classified as removed by treatment if found distal to the cecum has been recommended (Vet Med 81:280, 1986). If in this trial those procedures were followed, efficacy of the drug would have been underestimated. The 2 modifications of the Critical Test will be compared.

Supported by Pfizer Canada Ltd.

38

Dirofilaria immitis alters the behavior of canine pulmonary artery endothelial cells studied in vitro LANA KAISER\*, MARIA MUPANOMUNDA, AJSCHWARTZ, JEFFREY F WILLIAMS

In canine heartworm disease, alterations of pulmonary vascular reactivity have been attributed to the physical presence of the adult parasites and/or the hosts' immunologic response to the parasites. However, biologically active factors released by adult D immitis circulate and are capable of altering vasoactivity in vivo, suggesting another mechanism whereby heartworms could influence pulmonary vasoreactivity. Experiments were designed to test the hypothesis that endothelium-dependent relaxation is

altered in the in vitro pulmonary artery from heartworm infected dogs when compared to control. Dose-response relationships to methacholine, the calcium ionophore A23187, substance P, histamine, & bradykinin were compared. While relaxation to bradykinin was not different between groups, relaxation to methacholine, A23187, and substance P was significantly depressed in heartworm pulmonary artery. Interestingly, histamine caused constriction in control, but an endothelium-dependent relaxation in heartworm. Since relaxation responses in heartworm pulmonary artery are not globally depressed, it is unlikely that physical damage can explain the change in reactivity. These data suggest that infection with D immitis alters the behavior of pulmonary artery endothelial cells. (Supported by NIH Grants AI #35757 & AI #01082 and the WHO)

39

Correlative Histochemical Observations on Intestinal Epithelium of Trichuris ovis (Nematoda). M. JOHAL The intestinal epithelium of Trichuris ovis is anisocytous and lobular, leaving an irregular luminal space in the center. The cells are uninuculeate and, unlike other nematodes, the nuclei are situated near the microvillous border. Glycoproteins form the main structural component of the cell walls. Proteins and RNA are concentrated near the nuclear area, suggesting the synthesis of proteins at that site. The tips of microvilli are distended with carbohydrate and lipoidal substances, which probably are enzymatic in nature. The microvillar border contains a large amount of acid mucopolysaccharides, forming a permeability barrier against the proteolytic enzymes of host origin.

40

Comparative pharmacology of inhibitory nematode FMRFamide-related peptides (FaRPs).

A.G. MAULE\*, T.G. GEARY, J.W. BOWMAN, N.J. MARKS, K.L. BLAIR, D.W. HALTON, C. SHAW, and D.P. THOMPSON

Nematode FaRPs influence tension and contractility of neuromuscular strips isolated from *Ascaris suum* body wall. Previous results have indicated that the inhibitory effects of SDPNFLRFamide (PF1) are mediated by nitric oxide (NO). These studies reveal that the effects of PF1 are also dependent on external Ca<sup>++</sup>. Inhibitory actions have been identified for the nematode FaRPs KSAYMRFamide (PF3) and KPNFIRFamide (PF4);

however, none of these appears to operate through the release of NO. PF3 is only inhibitory on dorsal muscle strips; it is excitatory on muscle strips prepared from the ventral half of the worm. The inhibitory actions of PF3 are dependent on the main nerve cords and are attenuated in medium containing high K<sup>+</sup> or low Ca<sup>++</sup>. The effects of PF4 are independent of nerve cords and are Cl<sup>-</sup>-dependent. Inhibitory actions have also been identified for the KNEFIRFamide (AF1)-analog, KNAFIRFamide (A<sup>3</sup>AF1). The effects of this peptide are independent of nerve tissue, NO, Ca<sup>++</sup> and Cl<sup>-</sup>. Using anatomical and ionic dependence, these data reveal at least 4 distinct mechanisms underlying the pharmacology of inhibitory nematode neuropeptides.

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More nematode FMRFamide-related peptides (FaRPs). N.J. MARKS\*, C. SHAW, J.P. DAVIES, A.G. MAULE, D.W. HALTON, T.G. GEARY, and D.P. THOMPSON

Numerous FaRPs have been isolated and sequenced from extracts of free-living and parasitic nematodes. The most abundant FaRP identified in ethanolic/ methanolic extracts of the parasitic forms, Ascaris suum and Haemonchus contortus and from the freeliving nematode, Panagrellus redivivus, was KHEYLRFamide (AF2). Analysis of the nucleotide sequences of cloned precursor genes from Caenorhabditis elegans and, more recently, C. vulgaris identified a series of related FaRPs which did not include AF2. These data indicate either that AF2 does not occur in C. elegans or, if it does, then C. elegans possesses more than one FaRP gene. To investigate this possibility, an acid-ethanol extract of C. elegans was screened radioimmunometrically for the occurrence of FaRPs. A number of peptides were isolated using sequential analytical reverse-phase HPLC techniques, the most abundant of these was identified by Edman degradation as AF2. These results confirm the widespread occurrence of the neuropeptide AF2 in nematodes, and demonstrate that C. elegans contains at least 2 FaRP genes.

#### 42

Transglutaminase Activity in Equine Strongyles and its Potential Role in Growth and Development. U.R. RAO\*, M. R. CHAPMAN, R. N. SINGH, K. MEHTA, and T. R. KLEI.

Transglutaminases are a family of Ca2+-dependent enzymes that stabilize protein structure by catalyzing the formation of isopeptide bonds. A novel form of transglutaminase has been identified characterized in adult of filarial nematodes and seems to play an important role in microfilarial growth and development. The aim of this study was to identify this enzyme and to measure its significance to the in vitro growth and development of Strongylus spp. Activity of this enzyme was identified in extracts of adults and larvae of Strongylus vulgaris and S. edentatus. The significance of transglutaminase in the early growth and development of S. vulgaris and S. edentatus was tested adding by inhibitors monodansylcadaverine (MDC), and cystamine (CS) to in vitro cultures of L3 and L4. The viability, growth and molting of L3 of both spp. was effected in a dose dependent manner by both inhibitors. Cystamine promoted abnormal development of S. edentatus L3 resulting in an aberrant expansion of the anterior end. Addition of these inhibitors to cultures of L4 also reduced growth of both spp. Transglutaminase activity was also identified in extracts of Parascaris equorum, Cylicocyclus insigne. The results indicated that transglutaminase is present in a wide array of nematode spp. and may be important in growth and development process.

### 43

Biochemical characterization of excreted/secreted proteinases of adult *Haemonchus contortus*. HENK D.F.H. SCHALLIG\* and DAVID P. KNOX.

The excreted/secreted (ES) proteinases which might be associated with the blood feeding habits of the adult parasite were defined. Proteinase pH optima and inhibitor sensitivities were studied in a spectrophotometric assay with azocasein as substrate or by 7.5% non-reducing gelatin (0.1%) substrate gel analysis. ES proteinases were optimally active at pH 5.5, 7.5 and 8.5. ES proteinase activity between 45 and 55 kDa was stimulated by DTT and strongly inhibited by E64, indicative for cysteine proteinase activity. Activities around 65-85 kDa and at approximately 110 kDa were sensitive to the serine proteinase inhibitor PMSF. The ability of adult ES proteinases to degradate natural protein substrates (fibrinogen, plasminogen, haemoglobin, immunoglobin and albumin) was assessed using reducing 10% SDS-PAGE. All these substrates, with the exception of haemoglobin, were hydrolysed. Fibrinogen and plasminogen degradation indicate a possible anticoagulant role. IgG degradation suggests an immune evasion mechanism, although Ig might also serve as a nutrient source. The Nterminal amino-acid sequences of two ES products (52 & 84 kDa) have been determined with Edmann degradation. Oligonucleotide primers based on these sequences were used

in combination with oligo dT in PCR in order to obtain cDNA fragments encoding these ES products. These cDNA fragments will be used to screen a *H. contortus* cDNA library and the subsequent sequencing of the genes encoding for these proteinases will take place.

(This study received financial support (travel grants to Dr. H.D.F.H. Schallig) from the British Council and the Netherlands Organization for Scientific Research, N.W.O.)

#### 44

The Uptake and Incorporation of <sup>3</sup>H-Leucine Labeled Hemoglobin by Adult <u>Haemonchus</u> <u>contortus</u> In Vitro. RAYMOND H. FETTERER\* and MARCIA L. RHOADS

Adult Haemonchus contortus is presumably a blood feeding organism causing significant anemia in the parasitized host. Adult H. contortus secrete and contain within their gut a cysteine protease which may play a role in uptake and digestion of blood proteins. To test the hypothesis that this enzyme functions in the digestion of hemoglobin, the incorporation of <sup>3</sup>Hhemoglobin (HGB) into parasite proteins was investigated. <sup>3</sup>H-HGB was isolated from reticulocytes obtained from phenylhydrazine treated sheep metabolically labeled with <sup>3</sup>H-leucine. Parasites (100-200 parasites/well) were cultured in 1.5 ml of media in 24 well plates and 2  $\mu$ Ci of <sup>3</sup>H-HBG added to each well and incubated at 37C for 24 or 48 hrs. After 48 hrs, significant amounts of radioactivity were incorporated into TCA-precipitable parasite protein. The protein synthesis inhibitor puromycin (200 µg/ml), caused a 50% inhibition in the incorporation of radioactivity into proteins while the specific cysteine protease inhibitor Z-phe-ala-FMK (100 µM) had no effect on incorporation of radioactivity. These results indicate that although adult H. contortus take up hemoglobin in vitro and incorporate amino acids into their protein, the cysteine protease may not have a direct role in hemoglobin digestion.

# 45

Degradation of Connective Tissue Matrices by Haemonchus contortus. MARCIA L. RHOADS\* and RAYMOND H. FETTERER

Adult <u>Haemonchus</u> contortus secrete a cysteine protease(s). To better understand the in vivo function of this enzyme, the ability of live parasites and excretory/secretory products (ESP) to degrade connective tissue proteins was investigated using

radioactively labeled extracellular matrices produced by smooth-muscle cells (R22). The matrix was composed of glycoproteins, elastin, and collagen biosynthetically labeled with [3H]proline in a complex, cross-linked structure resembling connective tissue and anchored to 16-mm culture wells. During 24 hr in vitro cultivation of live adult worms in matrix-containing wells (120 worms/well), the entire matrix was degraded. The presence of Z-phe-ala-FMK (100  $\mu$ M), a specific cysteine protease inhibitor, blocked matrix degradation but did not affect parasite motility. ESP (0.1 mg protein/matrix-containing well) degraded 70% of the extracellular matrix; degradation was inhibited by Z-phe-ala-FMK. Thus the secreted cysteine protease of H. contortus is able to degrade the major components of connective tissue in a model system simulating their structure in vivo, and may indicate a role for this enzyme in penetration of abomasal tissue.

#### 46

Are Aerobic and Anacrobic-Specific Isozymes Present During the Development of Ascaris suum? DANIEL WALKER\*, KAREN HAYTON, EMILIO DURAN, PATRICIA KOMUNIECKI and RICHARD KOMUNIECKI

The development of A. suum is characterized by a marked aerobic/anaerobic transition in its energy metabolism. Early larval development from unembryonated egg (UE) to the second stage (L2) is aerobic and involves initial glycogen utilization followed by glycogen resynthesis from stored triglycerides. In contrast, in the fourth-stage larvae and the adult, energy metabolism is anaerobic and results in the formation of branched-chain fatty acids (BFA) which make up a significant fraction of the stored triglycerides. To examine the regulation of these processes, we have immunoblotted homogenates of early ascarid larval stages (UE through L2) with affinity-purified polyclonal antisera prepared against subunits of the adult muscle pyruvate dehydrogenase complex (PDC) and the 2methyl branched-chain enoyl CoA reductase (ER). The PDC is a key enzyme complex involved in both anaerobic metabolism and the entry of glycolytically generated pyruvate into the TCA cycle and the ER is the terminal cnzyme involved in BFA formation and presumably similar to the acyl CoA dehydrogenase responsible for the conversion of triglycerides to glycogen during early larval development. In summary, the immunoblots demonstrate that the PDC is more abundant during early development when glycogen utilization is maximal, the presence of potential acrobic and anacrobic specific isozymes, based on different mobilities during SDS-PAGE, and the presence of a potential acyl CoA dehydrogenase, whose apparent Mr differs slightly from the ER and whose abundance increases markedly during development from the UE to the L2, coincident with the increase in triglyceride utilization. These studies are continuing to

more definitively identify these putative stage-specific isozymes at the molecular level. (Supported by NIH Grant AI 18427)

#### 47

Potential Role of a Novel Subunit in the Reduced Sensitivity of the Adult Muscle Ascaris suum Pyruvate Dehydrogenase Complex to NADH. ROBIN ARNETTE\*, DANIEL WALKER and RICHARD KOMUNIECKI

The pyruvate dehydrogenase complex (PDC) occupies a key position in the anaerobic mitochondrial metabolism of adult A. suum muscle. Its regulatory properties are modified to function under the reducing conditions present in the host gut. For example, PCD activity is much less sensitive to inhibition by NADH. The mechanism of reduced sensitivity to NADH is unclear. It could involve an anaerobic-specific dihydrolipoyl dehydrogenase (E3) or, alternatively, an anaerobic specific E3-binding protein (E3BP). E3 has been purified from both aerobic second stage larva (L2) and adult muscle and, based on the amino terminal 56 amino acids, which includes the redox active thiol domain, E3s from both stages appear to be identical. In contrast, E3BP, which contains a high affinity binding site for E3 in yeast and mammalian PDCs, is absent in the PDC isolated from adult muscle. However, the adult muscle PDC does contain a protein (p45) of unknown function, which is present in amounts similar to E3BP. p45 is not acetylated during incubation in [2-14C]pyruvate, as is E3BP, and its amino terminal sequence bears no similarity to that of E3BP. In addition, p45 is not present in L2. More importantly, p45 remains tightly associated with the core of the complex during treatment with high salt or the selective removal of the lipoyl domains of dihydrolipoyl transacetylase by limited proteolysis with V8. These data suggest that p45 may play a role in E3 binding similar to that of E3BP in complexes from aerobic organisms. These studies are continuing with the cloning and expression of p45 with the intention of testing its ability to restore normal assembly of the PDC in E3BP-deficient Saccharomyces cerevisiae mutants. (Supported by NIH Grant AI 18427)

# 48

The Pyruvate Dehydrogenase Complex from the Insect Trypanosomatid, Crithidia fasciculata: E3 Binding Protein Contains Multiple Lipoyl Domains. FRANCISCA DIAZ\* εnd RICHARD KOMUNIECKI
The pyruvate dehydrogenase complex (PDC) has been purified from the insect trypanosomatid, C. fasciculata, a member of the most primitive eukaryotic group to contain mitochondria. The purified PDC yielded 5 bands of 70 (p70), 60 (p60), 55, 46 and 36.5 kDa, after separation by SDS-PAGE, which appeared to correspond to E3 binding protein (E3BP), E2, E3, E1α, and E1β, respectively. p70 was less abundant than p60 and both remained associated

with the core of the complex even after chromatography on Superose 12 in 1.5 M NaCl. Both p70 and p60 contained similar amino terminal sequences and the MPALSP motif present in both E3BP and E2 from other sources. Incubation of the purified PDC with [2-<sup>14</sup>C]pyruvate in the absence of CoA resulted in the acetylation of both p70 and p60, suggesting that both proteins contained lipoyl domains, but the specific incorporation of label into p70 was greater than into p60. Polyclonal antisera raised against p70 did not cross react with p60, and antisera raised against p60 did not cross react with p70, suggesting that p70 and p60 were different proteins and that p60 did not arise from proteolysis of p70. Limited proteolysis of the acetylated complex with trypsin yielded one major acetylated fragment of p70 of 57 kDa and two major fragments of p60 of 35 and 30 kDa, which corresponded to E2L and E2]. Therefore, given its apparent M<sub>r</sub>, amino terminal sequence and total acetylation, these results suggest that p70 is E3BP, but in contrast to E3BPs of yeast and mammals, the crithidial protein contains multiple lipoyl domains. Confirmation of these results awaits the cloning and sequencing of p70. This work was supported by NIH Grant AI 18427 to RWK.

#### 49

Evaluation of Serum Chemistry Values Associated with Avian Malaria Infections in African Blackfooted Penguins (Spheniscus demersus).
THADDEUS K. GRACZYK, CRANFIELD M.R.\*, and ELIZABETH J. BICKNESE

The value profiles of 5 intracellular enzymes, 15 metabolites (with 2 associated ratios), and 3 electrolytes were monitored over time in 9, captivereared African black-footed penguins (Spheniscus demersus) with different avian malaria clinical status: uninfected, subclinically infected, and clinically infected with fatal outcome. Fatal infections were caused by <u>Plasmodium relictum</u>. The reference ranges of 23 serum clinical chemistry parameters and 2 ratios were established for S. demersus. The mean values of 8 of 23 parameters of infected penguins were significantly different from those recorded for the uninfected birds indicating impaired renal function, hepatic dysfunction, and nonspecific tissue damage related to the infestation with exoerythrocytic schizonts. Analysis of sensitivity, specificity, negative and positive predictive values (PPVs) showed that gamma glutamyltranspeptidase (GGTP), alanine aminotransferase (ALT), and creatinine reached PPVs, and a specificity over 57% for avian malaria infections in penguins. Creatinine, ALT, and

GGTP values should be consulted in evaluation of the clinical malaria status of <u>S. demersus</u>. (Supported by the AKC Fund of New York and the Maryland Zoological Society.)

#### 50

Glycogen Metabolism in *Giardia intestinalis* Trophozoites and in Differentiation to Cysts. DAVID S. CROSS and J. GEORGE ZALITIS\*

The survival of Giardia depends on their ability to form impermeable cysts with a low metabolic rate which contain glycogen as their energy reserve. Trophozoites have glycogen (1.5 umoles/mg protein) the enzyme activities (as milli units/mg protein) were. glycogen synthase (25), UDP-glucose pyrophosphorylase (200), phosphoglucomutase (120) and glycogen phosphorylase (30). None of the above enzymes responded to known and potential allosteric effectors. Studies with <sup>14</sup>C glucose in trophozoites showed net synthesis of glycogen. Differentiation to cysts was initiated by exposing trophozoites to growth medium containing 1% bile w/v. This resulted in inhibition of cell division and a 2-fold increase in glycogen within 24 hours. The activities of enzymes of glycogen metabolism were not significantly affected. After 24 hours encystation and a further 24 hours in growth medium, the cysts were separated from trophozoites by hypotonic lysis and purified. The cysts did not metabolise 14C glucose. Freshly prepared cysts contained similar amounts of glycogen and the enzymes UDPglucose pyrophosphorylase and phosphoglucomutase to trophozoites. The cysts have no glycogen synthase and barely detectable phosphorylase activity. Cyst extracts did not contain inhibitors of glycogen phosphorylase or synthase. The low phosphorylase activity does not respond to allosteric effectors like 5'-AMP. Control may be exerted through protein phosphorylation.

# 51

Interisolate Heterogeneity of Haemonchus contortus Excretory Secretory Proteases. F.N. KARANU\*, F.R. RURANGIRWA, T.C. McGUIRE, and D.P. JASMER

Proteases in excretory secretory (ES) products of Haemonchus contortus are potential targets for immune control. However, preliminary evidence suggested variations in these proteases between Kenya and USA isolates of H. contortus. Here, we show variations in Mr, pH optima and sensitivity to protease class specific inhibitors among proteases from two different Kenya isolates of H. contortus. Predominant proteases also varied among different cohorts of worms, raised in separate lambs. representing the first generation from a field isolate.

variation was observed among cohorts representing a second generation from this field isolate. However, profile of predominant ES proteases from second generation cohorts differed from that of first generation cohorts. The analysis also confirmed differences observed between the Kenya and USA isolates of H . contortus. results indicate a laboratory selection process that leads to expression of ES proteases that are not representative of field isolates.

### 52

Detection and Characterization of a Salivary Vasodilator from <u>Culicoides variipennis</u>, the North American Vector of Bluetongue Viruses. ADALBERTO A. PEREZ DE LEON\*, and W.J. TABACHNICK

Blood-feeding by Culicoides variipennis caused a lesion in naive hosts characterized by a central petechial hemorrhage with peripheral erythema. No swelling was observed at the feeding site, and the erythema lasted more than 24 hrs. It was hypothesized that the erythema was induced by a salivary vasoactive factor present in <u>C. variipennis</u>. We reproduced the erythema by injecting homogenized salivary glands intradermally into sheep and rabbits. A dose-response relationship was observed when serial dilutions of homogenized salivary glands were injected into rabbits. As little as 0.125 of a gland pair induced erythema. Injections of controls, consisting of saline alone, gave a negative response. Biochemical analyses indicated that a peptide may be responsible for the vasodilatory activity. The stability of the putative vasodilator peptide after incubation in acetonitrile (40%) and trifluoroacetic acid (0.1%) suggested that it is amenable to purification by reverse phase high performance liquid chromatography. The vasodilatory activity present in the salivary glands of C. variipennis could facilitate feeding by increasing blood flow from superficial capillaries surrounding the biting site. The apparent ability of C. variipennis to manipulate the host response to tissue injury during blood-feeding suggests that vector-host interactions may have implications in the salivary transmission of bluetongue viruses.

### 53

Role of T and B Lymphocytes in Immunity to Eimeria Infection in Mice. R. CRAIG FINDLY\*, LI WEN, SCOTT J. ROBERTS, ADRIAN L. SMITH, and ADRIAN C. HAYDAY Infection of intestinal epithelial cells by Eimeria induces specific resistance to subsequent challenges, and

mechanisms involving both cellular and humoral responses have been implicated in the development of this protective immunity. However, the precise role of each component of the immune response in conferring protection is not clearly defined. In order to determine more rigorously the mechanisms leading to protection, we are using mice with defined genetic deletions. Infection studies have been carried out using mice containing gene knockouts that delete αβ or γδ T cells or B lymphocytes. Our previous studies demonstrated that of T cells are important for both primary and secondary immune responses. We have now shown that mice lacking the  $\alpha$  chain of the T cell receptor (TCR $\alpha$ -/-) fail to develop immunity to challenge infections, even after multiple challenges and over long time courses. To ask whether  $\alpha\beta$  T cells act by inducing a local humoral response, we examined the levels of mucosal immunoglobulin post primary and secondary infection. Following primary infection, little increase in gut lavage IgA or IgG antibody was found in either TCR  $\alpha$  +/- or TCR  $\alpha$  -/- mice. Essentially the same was true for secondary infection. Moreover, immunoglobulin µ chain -/- mutant mice, which lack B lymphocytes, were fully resistant to secondary infection. In contrast to the TCR α -/- mice, mice lacking yo T cells (TCR  $\delta$  -/-) are fully resistant to secondary infection. Transcriptional responses of cytokine genes in lymphocytes isolated from these mice over the course of infection have been studied using competitive PCR. Similar levels of IFNy and IL-4 mRNA expression were detected at different time points post infection.

#### 54

Role of α/β and γ/δ T Cells in Resistance against Toxoplasma gondii. YASUHIRO SUZUKI\*, OLIVER LIESENFELD and JACK S. REMINGTON

Cell-mediated immunity plays a critical role in protective immunity against infection with T. gondii. We have examined the role of  $\alpha/\beta$  and  $\gamma/\delta$  T cells in protective immunity against this parasite.

Role of  $\alpha\beta$  T cells: Athymic nude mice (which lack thymus-dependent  $\alpha\beta$  T cells) all died of acute toxoplasmosis following peroral infection with the avirulent ME49 strain whereas control mice all survived. These results suggest the critical role of thymus-dependent  $\alpha\beta$  T cells in resistance. To define the role of  $\alpha\beta$  T cells, mutant mice deficient in  $\alpha\beta$  T cells were infected with the ME49 strain. Mutant mice all died of acute toxoplasmosis whereas wild-type control mice all survived. These results clearly indicate the requirement of  $\alpha\beta$  T cells for survival of mice following infection.

Role of  $\gamma/\delta$  T cells: Following infection with the ME49 strain, mutant mice deficient in both  $\alpha/\beta$  and  $\gamma/\delta$  T cells died markedly and significantly earlier than mutant mice deficient in only  $\alpha/\beta$  T cells. Histological studies revealed remarkably greater numbers of tachyzoites in the brains, lungs, hearts, livers, spleens and small intestines of the mutant mice deficient in both  $\alpha/\beta$  and  $\gamma/\delta$  T cells than in these same organs of mutant mice deficient in only  $\alpha/\beta$  T cells following infection. These results indicate that  $\gamma/\delta$  T

cells can inhibit proliferation of tachyzoites and confer partial but significant resistance against the parasite.

#### 55

T. gondii Induces CD4+ T cell Unresponsiveness in mice. IMTIAZ KHAN\*, TADASHI MATSUURA and LLOYD H. KASPER

The induction of T cell anergy by infectious agents. expressing superantigens is not well understood. Here evidence is presented that Toxoplasma gondii, a major opportunistic pathogen of the newborn and those with AIDS is able to induce an anergic-like condition in the infected murine host. We have examined the changes in the CD4+ T cell population that occur during acute infection in an experimental mouse model. Seventy six percent of the CD4+ T cell population increased in volume by day 7 post infection and express T cell maturation markers (CD44hi, IL-2Rhi, Mel-14lo). Further noted and consistent with superantigen activity was a clonal expansion of CD4+ T cells expressing the VB5 chain of the T cell receptor. Although partial reduction of all CD4+ T cells in response to mitogen or parasite antigen stimulation was observed, the VB5 T cell subset specifically failed to proliferate in the presence of exogenous stimulation. Addition of rlL-2 partially restored the CD4+ T cell proliferative response in vitro. The T cell activation marker CTLA-4 could not be detected and the co-stimulatory molecule, CD28 was down regulated. Electrophoretic and morphologic analysis of these cells post culture demonstrated a DNA fragmentation pattern and chromatin condensation consistent with apoptosis. These studies provide the first description of parasite driven T cell anergy-like condition by a protozoan pathogen. The unresponsive T cells involved in this response appear to be stimulated by a superantigen present in the parasite.

# 56

A *Toxoplasma gondii* Superantigen: Biological Effects and Preliminary Characterization. ERIC Y. DENKERS\*, PATRICIA CASPAR, SARA HEINY and ALAN SHER

Culture of whole tachyzoites or soluble tachyzoite antigen (STAg) with nonimmune mouse spleen cells results in strong proliferation and release of IFN- $\gamma$ . The response appears to be superantigen (SAg) driven based on the following criteria. 1. CD8+ lymphocytes bearing the V $\beta$ 5 chain of the T cell receptor are preferentially expanded. 2. The effect is mediated through class II molecules on antigen presenting cells (APC), but is not haplotype restricted. 3. Conventional Ag processing is not required, since fixed APC stimulate proliferation when incubated with STAg. Studies on cells from chronically infected mice reveal that T cells bearing the reactive T cell receptor V $\beta$  chain are rendered nonresponsive as determined by proliferation and IFN- $\gamma$  release following stimulation with

anti-VB5 mAb in vitro. The latter inhibition does not reflect nonspecific immunosupression since stimulation with anti-VB8 or CD3 mAb results in normal levels of proliferation and cytokine production. In addition, culture of cells form chronically infected mice with tachyzoites fails to result in preferential expansion of Vβ5-bearing cells. Using a panel of MHC class II-transfected fibroblasts, we have identified a candidate SAg of 60-65 kDa that binds to transfectants expressing IA or IE, but not the IA-/IEparent line. Furthermore, binding is inhibited by inclusion of anti-class II mAb during incubation of STAg with the fibroblasts. We propose that this molecule is involved in early production of IFN-y and the consequent induction of a protective Th1 response, and furthermore that potentially detrimental immunopathology associated with cytokine overproduction is avoided by anergizing Vβ5+ lymphocytes during chronic infection.

### 57

The  $\gamma\delta$  T Cell Immunity To Blood-stage Malaria. W.P. WEIDANZ\*, H. VAN DER\_HEYDE, M. ELLOSO, and D. MANNING

Acute infections with Plasmodium chabaudi adami (Pca) resolve in B cell deficient mice but not in athymic nude mice. Adoptive transfer of immune T cells, antigen-reactive lines and clones to nude mice enable these reconstituted mice to resolve acute infection with Pca. The CD3+ T cells responsible for protection have the CD4+, CD8-, TCRαβ. THI phenotype. During Pca infection, splenic γδ T cells increase in number maximally shortly after peak parasitemia and remain elevated for prolonged periods of time. In B cell deficient "knockout" mice, which resolve Pca infection, the numbers of splenic  $\gamma\delta$  T cells increase dramatically so that the  $\gamma\delta$  T cells account for 30 to 50% of the CD3+ T cells present. Our finding that antibody depletion of the CD4<sup>+</sup> T cell population inhibits the expansion of the γδ T cell subset in response to Pca infection, suggests that the expansion of the γδ T cell subset is dependent upon the prior activation of CD4<sup>+</sup>  $\alpha\beta$  T cells. Depletion of the  $\gamma\delta$  T cell subset prevents mice from resolving Pca malaria but has no effect on the CD4<sup>+</sup> T cell response. Together, these finding suggest that CD4<sup>+</sup> T cells alone are not sufficient for the resolution of acute Pca malaria but that both CD4<sup>+</sup> T cells and νδ T cells are essential. Moreover, the observation that B cell deficient mice depleted of γδ T cells fail to clear acute Pca malaria indicates a crucial role for these cells in mediating CMI to this parasite. Although the mechanisms by which these cells suppress parasitemia remain unknown, we have observed that cloned human γδ T cells inhibit the replication of P. falciparum in vitro by a contact dependent mechanism. (Supported by NIH Grant AI12710.)

# 58

Development of CD8+ T Cell Mediated Immunity Against Liver Stages of Malaria Parasites. FIDEL ZAVALA\* Protective immunity against sporozoite and liver stages of malaria is a multifactorial phenomenon involving several immune mechanisms. It includes antibodies that inhibit sporozoite infectivity and  $\alpha\beta$  and  $\gamma\delta$  T cells that inhibit the intrahepatocytic development. The character of this immunity evolves with time and conditions of immunization. Thus after a single immunization,  $\gamma$ -IFN dependent T cell responses are the predominant antiparasite mechanism. After repeated imunizations,  $\gamma$ -IFN independent immune-mechanisms such as antibodies and possibly other T cell mediated mechanisms become predominant.

Among T cells, CD8+ T cells are particularly efficient at eliminating or severely limiting the development of the intrahepatocytic stages. They appear early after immunization with sporozoites, are readily detected in spleen and liver of mice after a single immunization, and last for several weeks. These anti-malaria CD8+ T cells can be induced in large numbers after immunization with a combination of recombinant viruses expressing a malaria epitope. These viruses can also boost T cells previously primed by parasite immunization. The virus-induced CD8+ T cells display a strong anti-malaria parasite activity and confer protection against sporozoite infection.

# 59

Multi-gene DNA vaccination against malaria: Evasion of MHC restriction? D.L. DOOLAN\*, M. SEDEGAH, R.C. HEDSTROM, M. MARGALITH, P. HOBART AND S.L. HOFFMAN

Construction of a sub-unit vaccine incorporating defined CD4+ and CD8+ T cell and B cell epitopes is one strategy for vaccine development. This approach requires that specific targets of protective immune responses be identified at the epitope level for each candidate antigen. MHC restriction of immune responses to these epitopes poses a significant obstacle. We have used an alternate strategy: DNA vaccination utilizing complete or partial genes encoding target malarial antigens, including the P. yoelii circumsporozoite protein (CSP) and a newly defined antigen, P. yoelii Hepatocyte Erythrocyte Protein 17 (HEP17). Mice of five strains differing in their H-2 haplotype (H-2 a, b, d, k, q) were studied. The CSP DNA vaccine protected 69% of Balb/C mice (H-2d) against development of blood-stage parasitemia but only 25% of B10.BR (H-2k) and 20% of AJ (H-2a) mice and did not protect B 10.Q (H-29) or C57 BL/6 (H-2b) mice. In contrast, the HEP17 DNA vaccine protected 70% of A/J mice, 38% of B 10.BR, 23% of Balb/C, 17% of B 10.Q and did not protect C57 BL/6 mice. Significantly, the combination of the two vaccines was able to protect 88-100% of Balb/C, 77-90% of A/J and 71% of B 10.BR mice. Protection was CD8+ dependent. Data demonstrate that individual vaccines predominantly protected mice of a single genetic background while multi-gene DNA vaccination protected on at least three backgrounds. This protection appeared to be additive. The potential of

multi-gene DNA vaccination to overcome the genetic restriction of protective immune responses is demonstrated.

# 60

Energy Storage and Host-finding Strategies of Nematode Larvae. D.L. MEDICA\*, M.R. CHAPMAN, T.R. KLEI, and M.V.K. SUKHDEO

This study was designed to test the hypothesis that nematodes with similar host-finding strategies have similar energy storage patterns. Lipid is the primary energy reserve of infective L<sub>3</sub> nematode larvae. The fatty acid compositions of the horse bloodworms Strongylus vulgaris, S. edentatus, and S. equinus (Suborder Strongylidae), Haemonchus contortus, an abomasal worm of sheep, and Heligmosomoides polygyrus, a small intestinal parasite of murines (Suborder Trichostrongylidae) were compared using gas chromatography. Strongylus spp. and H. contortus larvae have very similar fatty acid compositions which include a small amount of 14:0 (5%), and consist primarily of other medium and long chain length saturated fatty acids (16:0-18%; 18:0-13%; 20:4-5%; and 24:0-20%). The similar pattern of energy storage in these larvae may reflect their similar host finding strategies (ingested during host grazing), despite the taxonomic separation between Strongylus spp. and H. contortus. H. polygyrus larvae are composed of medium chain length saturated fatty acids, primarily 14:0 (80%), with small amounts of 16:0 (10%) and 18:0 (7%). This energy storage pattern may reflect the host finding strategy of H. polygyrus larvae (ingested during grooming). These data suggest that larval energy storage and metabolism may be related to host-finding strategies. In addition, current taxonomic separation of these nematodes suggests convergent evolution of behavioral mechanisms and energy storage patterns in the members of the Trichostrongylidae and Strongylidae. Supported by SES Special Initiative Fund; NJAES #K-9948-1-95

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A Temporal Comparison of the Prevalence of Intestinal Parasites in Faecal Specimens Collected from Public Parks in Metropolitan Toronto, Ontario. REVA BERMAN\*, J. YANG AND R. HANSELL

In 1975, examination of faecal samples from 8 parks in Metropolitan Toronto revealed <u>Toxocara</u> spp. (24.2%), hookworm-like spp. (11.3%), <u>Trichuris</u> spp. (9.7%) and <u>Toxascaris</u>

leonina (2.7%). As well, eggs of Toxocara spp. were found in 10.3% of children's sandboxes in public parks. Subsequent changes in sanitation bylaws led to a presumed change in the behaviour of dog owners, with concurrent public health implications. Sampling has been repeated in 1994-95. A total of 282 samples were taken from seven of the Faecal samples and original sites. soil samples from children's sandboxes were collected. A temporal comparison in the prevalence of intestinal parasites as evidenced from faecal samples and children's sandboxes in public parks of Metropolitan Toronto is reported here.

### 62

Coccidian guilds (Apicomplexa: Eimeriidae) in White-tailed and Black-tailed Prairie Dogs (Cynomys leucurus and C. ludovicianus) in Wyoming. R. SCOTT SEVILLE\* and JEANICE SHIVELY.

During the summer of 1994 populations of Cynomys leucurus and C. ludovicianus were sampled biweekly to identify and monitor the prevalences of *Eimeria* spp. Overall, 61 C. leucurus (97 total captures) and 52 C. ludovicianus (124 captures) were marked and monitored for the three months of the study. Seven species were found occurring in the two hosts: Eimeria adaensis (50.5% C. leucurus, 25.8 C. ludovicianus), E. beecheyi (27.8%, 4.0%), E. bilamellata (7.2%, 0%), E. callosi ermoj hili (71.1%, 50.8%), E. lateralis (6.2%, 1.6%), E. morainensis (7.2%, 12.9%), E. j suedosj ermoj hili (4.1%, 1.0%) and E. sj ermoj hili (1.0%, 1.0%). Comparison of our results with those of other studies indicate that while the species present in different spermophiline hosts are consistent the prevalences vary. Factors important in determining prevalence levels likely vary between host populations but include differences in host response to the eimerian species, differing host densities, and the different environmental conditions in which each host exists.

63

The effect of nematophagous fungi fed to cattle, sheep and horses on the development of infective larvae. J. BIRD\* and R.P. HERD

Six-month-old heifers, adult ewes and adult mares were fed spores from two species of nematophagous fungi, Arthrobotrys oligospora and A. flagrans. For each host group of five fungi-fed animals, feces were collected, pooled and mixed with nematode ova harvested by a

filter technique from feces of naturally-infected donor animals. Feces from naturally-infected donor animals were the controls. For each species of fungus, eight experiment and eight control fecal pats of uniform weight were formed for each animal group and randomly deposited 2 m from each other on a clean pasture. The number and genera of infective larvae (L3s) found on herbage in a 20 cm diameter semi-circle around each pat were determined four week and eight weeks after deposition. The dominant species of parasites present were Ostertagia and Cooperia (cattle), Haemonchus (sheep) and cyathostomes species (horse). For A. oligospora, reductions in the number of L3s were 47.3% and 37.3% in the cattle study, zero in the sheep study, and 38.1% and zero in the horse study, at four and eight weeks, respectively. For A. flagrans, reductions in the number of L3s were 93.2% and 72.4% in the cattle study, 36.0% and 60.1% in the sheep study, and 55.1% and 17.0% in the horse study, at four and eight weeks, respectively. Overall, A. flagrans exhibited superior ability to A. oligospora in reducing pasture infectivity.

### 64

The effect of predacious fungi on free-living preparasitic nematode larvae. M. LARSEN, PETER NANSEN\*, S.A.HENRIKSEN, J. GRØNVOLD, J. WOLSTRUP and R.M. WARUIRU.

A wide range of predacious microfungi may grow in faecal material where they produce trapping devices that capture live nematode larvae. In vitro and in vivo screening procedures have been developed for selecting suitable fungal candidates which both have a strong nematode-killing effect and the ability to maintain viability after passage torough the gastro-intestinal tract of host animals. Duddingtonia flagrans, possibly due to its high ability to produce chlamydospores, is a particularly promising candidate for biological control of nematodes, since it survives gut passage of both cattle, sheep, horse and pig and subsequently destroys nematode larvae in the faeces of these species. Apparently, the entrapment of larvae is not very discriminative, since many different nematode species are killed, provided they are present as larvae at the time when the fungus is operative. The nematodekilling effect is dependent on fungus dose level, but also on the number of developing parasitic nematode larvae in the faecal material. The simultaneous presence of free-living soil nematodes seems to enhance killing of the parasite larvae.

# 65

Eels from the Heart of a Mako Shark: A Conundrum from 500 Fathoms. J. N. CAIRA\*, G. W. BENZ, J. BORUCINSKA, N. E. KOHLER, and J. G. CASEY A 395 kg shortfin mako shark (*Isurus oxyrinchus*) landed at Montauk, New York in June of 1992 was found to harbor

two immature female individuals of the cel Simenchelys parasitica (Synaphobranchidae) within the lumen of its heart. The stomachs of both eels were filled with blood cells indicating that these eels had been within this shark for at least enough time to feed on its blood. Histological sections were prepared from tissue taken at the junction of the auricle and the ventricle as well as the junction of the conus arteriosus and the ventricle of the infested shark. Comparable sections were prepared from tissues from three control make sharks weighing 56 to 262 kg, also landed at Montauk. Examination of the tissue sections revealed the presence of medial hyperplasia of the capillaries, and arteriosclerosis in the infested shark; neither phenomenon was seen in the control sharks. In addition, the infested shark exhibited significantly higher numbers of capillaries per unit area in both regions of the heart than the control sharks. No breach of the external surface of the shark or its heart were found, thus the portal of entry was not determined. Previous natural history data for S. parasitica eliminate the possibility of this species being an obligate parasite. But, based on our findings it is likely that this species is a facultative parasite, which if the opportunity arises, is perfectly capable of living for extended periods of time within, for example, the heart of a shark.

# 66

Postmortem Migration of <u>Hymenolepis diminuta</u> (Cestoidea: Cyclophyllidea) in Outbred Sprague Dawley Rats.

THOMAS R. PLATT\* and SUSAN D. VILLANUEVA Postmortem migration is a concern in surveys and parasite ecology where accurate estimates of is crucial. parasite location Yet, quantitative information exists documenting this phenomenon. Twenty seven Sprague-Dawley rats infected with <u>diminuta</u> were each 5 <u>н</u>. cysticercoids. Four weeks post-infection 15 rats were killed and the small intestines of 3 rats were removed immediately and fast frozen. Additional groups of 3 rats (experimentals) were processed at intervals of 30, 60, 120 and 240 min after death (AD). Four groups of 3 rats (controls) were killed and processed at 30, 60, 120, and 240 min to assess changes due to circadian movement. Changes in the position of the scolex and biomass (dry weight) were determined as a percent of small intestine length for: anterior, median and posterior location and range. Within group differences were only found in median biomass position; pair-wise comparisons significant. Between group differences were not evident 30 min AD. Anterior and median scolex position shifted significantly posteriad in the experimentals at 120 and 240 min respectively. Median and posterior biomass shifted anteriorly at 60 and 120 min AD, respectively, in the experimentals. Worm range was significantly reduced at 120 min in the experimentals. There appears to be a 30 min

period following host death in which  $\underline{H}$ .  $\underline{diminuta}$  does not change its position within the rat small intestine.

#### 67

Variation in Nematode Infection Levels After the First Grazing Season in Dairy Farms in The Netherlands. JACQUELINE POOT, MAARTEN EYSKER\* and THEO LAM

In the Netherlands a wide variation in nematode infection levels has been demonstrated in dairy cattle herds (Ploeger, thesis 1989). In some herds exposure during the first grazing season was not sufficient to protect against production losses in the second grazing season. Since these surveys, the use of highly suppressive early season systems of anthelmintic application has increased in the Netherlands. This implies that underexposure to helminth infections during the first grazing season may be even more a matter of concern. The summer of 1994 was warm and dry, probably enhancing the risk of underexposure. Therefore a seroepidemiological study, using crude worm Ostertagia and Cooperia antigen, was performed on 20 dairy calf herds after the end of the first grazing season. As reference four groups with well-defined natural nematode infections were used, one with moderate infections, two with light infections and one without infections. Infection levels on the 20 farms varied from approximately the non-infected reference group to higher infections than the moderate group. On the majority of farms infection levels were low or extremely low. Moderate or high infection levels tended to be found in set stocked herds, even in one herd where a long acting anthelmintic device had been given at turnout. Low infections tended to be found in herds that had been moved regularly. This did not seem to be related with suppressive anthelmintic treatment. Owners of herds with very low infection levels were informed that prophaxis against parasitic gastroenteritis should be extended to the second grazing season.

# 68

The distribution and pathobiology of <u>Neoechinorhynchus</u> <u>cylindratus</u> in the intestine of green sunfish, <u>Lepomis</u> <u>cyanellus</u>. MOHAMED ADEL-MEGUID, GERALD W. ESCH, and HERMAN E. EURE\*

The status of bluegill (Lepomis macrochirus) and green sunfish (Lepomis cyanellus) as homologous hosts for the acanthocephalan Neoechinorhynchus cylindratus was experimentally determined. It was found that the adult parasite did not establish in bluegills, but that these fish could serve as paratenic hosts. In contrast, complete growth and development to the adult stage occurred in the green sunfish. When green sunfish were intubated with 10 cystacanths/fish, the parasite exhibited a clear preference for the anterior half of the intestine; when 50 cystacanths/fish were intubated, the parasites showed a preference for the posterior half of the intestine. With repeated exposure of cystacanths, the parasites were distributed throughout the intestine. The extent of histopathology induced by N. cylindratus was related to the numbers of parasites present. In light infections (10 cystacanths), the parasite penetrated deeply into the intestinal wall and connective tissue

developed around the proboscis. In infections with 50 cystacanths, the proboscis penetration was shallow and little if any connective tissue accumulated. There was also an indication that in crowded areas, the parasites appeared to change their sites of attachment frequently. In both heavy and repeated infections, the parasites evoked significant goblet cell hyperplasia and substantial quantities of mucus covered the intestinal wall. It is suggested that the sticky covering and the presumed presence of antibodies in the mucus combined to create a protective barrier thereby reducing the numbers of parasites that could attach and become established.

### 69

National Prevalence of Canine Parasites Based on Centrifugal Sucrose Flotation Examination of Fecal Specimens. B. L. BLAGBURN, D. S. LINDSAY, J. L. VAUGHAN, N.S. RIPPEY R. C. LYNN, W. G. KELCH, G.C. RITCHIE, and D.I. HEPLER

Internal parasites are among the more common infectious agents that companion animal veterinarians must control. To develop and implement effective diagnostic and control strategies, veterinarians and parasitologists must be aware of prevalences of parasites in their regions. At the 1994 meeting we presented preliminary data on a national survey of canine parasites, based sucrose flotation examination of fecal specimens. Herein we provide complete data for 6,458 specimens. Nation-wide sampling in this study was based on the 1990 human census, assuming that the dog population closely follows the human population. Fecal specimens were collected from dogs housed in animal shelters. Animal shelters were selected from the largest cities in each state. Fresh specimens were collected into 120 ml plastic specimen cups with screw-cap lids. Specimen cups were placed in styrofoam shipping boxes containing "cold pack" inserts, and shipped to Dr. Blagburn's laboratories via overnight courier. Signalment information was provided on an accompanying sheet. Specimens were examined using the centrifugal sucrose flotation procedure. The following parasites (% of total examined in parentheses) were observed: Toxocara canis (14.5%), Toxascaris leonina (0.7%), Ancylostoma caninum (19.2%), Uncinaria stenocephala (1.0%), Trichuris vulpis (14.3%), Capillaria spp. (0.4%), Giardia spp. (0.6%), Isospora spp. (4.8%), Sarcocystis spp. (0.8%), Hammondia spp. (0.06%), Physaloptera spp. (0.05%), Dipylidium caninun (0.09%), Taeniidae (0.6%).

# 70

Realized niche structure and autecological community structuring mechanisms in the gregarine assemblage parasitizing *Tenebrio molitor*. R. E. CLOPTON\*

Communities are often comprised of interactive populations that are united and structured by synecological forces. However, community composition and structure might also evolve and be maintained by mutually independent autecological processes. Three fundamental qualities should be demonstrable in such a structured assemblage: 1) a stable community structure (component species should each posses a unique realized niche that is equivalent to their fundamental niche); 2) the absence of

competitive community structuring mechanisms (minimal niche overlap and displacement among component species); and, 3) an alternative community structuring mechanism (realized niche structure as a direct function of component species' differential responses to autecological conditions). Fundamental and realized niche structures in the gregarine assemblage parasitizing larval T. molitor were studied using protozoan smears of snap frozen and divided host intestines. Autocological structuring mechanisms were examined using in vitro parasite excystation protocols and in vivo host intestinal transit time studies. Each parasite species exhibits a consistent niche breadth and position along the length of the host's intestine and there is no difference among fundamental and realized niche breadths within parasite species. No evidence of competitive displacement or exclusion was observed. Each parasite species also exhibits a unique pattern of excystation synchrony that corresponds to the breadth and position of the parasite's niche along the length of the host's intestine. The excystation rate of each parasite species is a differential function of initial pH levels and combines with the host's intestinal transit rate to provide a consistent pattern of niche separation among gregarine species.

# 71

Parasite Burdens of Two Bison Herds in Kansas. R.K. RIDLEY\*, A. MELLI, P. STEWART, T. WERKMEISTER, AND M. O'DONNELL. KANSAS STATE UNIVERSITY, MANHATTAN, KANSAS 66506

Trichostrongyle parasite burdens of two bison herds grazing under two different management systems were compared by quantitative fecal egg counts (EPG). A 176-head herd grazing 2,150 acres of the Konza Prairie in Riley Co., Kansas was compared to a private 80head herd grazing three different pastures in Clay Co. The Konza herd is maintained without anthelmintic intervention; the privately owned animals are routinely dewormed. Quantitative egg counts of calves out of treated cows ranged from 0 to 220, with an average EPG of 50. Three of those eight calves harbored Nematodirus sp., and Moniezia sp.; half of them had Eimeria sp. oocysts. Cows grazing with the calves had EPGs ranging from 0 to 220, with an average EPG of 15. The average EGP of cows grazing the other two pastures were 73 and 144. The bulls were on the pasture with cows with the heaviest parasite load; the average EPG of the four bulls was 58. The average trichostrongyle EPG of '94 calves grazing the Konza was 117 (Range = 10 - 390). Eleven of the nincteen calves were infected with Moniezia sp. and eleven harbored Eimeria sp. The average EPG of the Konza

females was 158, with a range of 0 to 880. Lungworms (*Dictyocaulus* sp.), *Moniezia benedeni* and *Eimeria* sp. were also present.

### 72

Preliminary observations on the epidemiology of gastrointestinal helminths of free ranging scimitar horned oryx in Texas with evidence for summer arrest of <u>Camelostrongylus mentulatus</u>. T.M. CRAIG \* and J. JENSEN

Tracer lambs were utilized to determine the helminth exposure of a herd of free ranging scimitar horned oryx (Oryx dammah) in the eastern Edwards Plateau of Texas. Groups of 3 lambs each were placed with the bachelor herd of approximately 20 oryx rotated between two approximately 50 ha pastures. Two fallow deer, a red deer and varying numbers of white-tailed deer also utilized these pastures. The internal parasite-free lambs were grazed for periods of 4 to 6 weeks then exchanged with replacement lambs, then held on concrete for 2 to 3 weeks to allow helminth maturation before slaughter. Two nematodes, Camelostrongylus mentulatus and Trichostrongylus colubriforms, were the predominant The summer of 1994 was species encountered. unusually dry and virtually no nematodes were transmitted from July through September. Peak parasite transmission occurred during the periods of rapid grass growth following rains. Early 4th stage ostertanginae (arrested larvae) were recovered during the late winter and early spring, and C. mentulatus was the only species of ostertaginae identified. few Trichostrongylus axei, Cooperia punctata, Oesophagostomum venulosum, and Trichuris sp were also recovered from the tracers.

# 73

Distribution of Helminth Parasites of Native Gobioid and Introduced Poeciliid Fishes in Streams of Hawai'i Island. WILLIAM F. FONT\*

Previous research has documented the prevalence and abundance of the nematode Camallanus cotti, the tapeworm Bothriocephalus acheilognathi and the leech Myzobdella lugubris in 5 species of native gobioid fishes and in introduced swordtails Xiphophorus helleri in Hakalau Stream on the Big Island of Hawai'i but the occurrence of these helminths in other streams of that island has not been reported. C. cotti was by far the most widely distributed helminth, occurring in 14 of 15 aquatic habitats. It was unique in its occurrence in populations of Lentipes concolor

located above high waterfalls like those that exist in Nanue Stream. Maximum abundance of C. cotti was found in the native goby Awaous guamensis in Wai'ula'ula Gulch where a large population of exotic guppies, Poecilia reticulata, and intermittent stream flow may provide ideal conditions for parasite transmission. In contrast, B. acheilognathi and M. lugubris were less widely distributed, occurred at lower prevalence and abundance, and did not infect fishes collected above waterfalls. Additional parasites included cystacanths of Southwellina hispida from several native and exotic hosts and Spirocamallanus sp. from sleepers Eleotris sandwicensis collected from estuarine habitats. spring-fed Seaside Pond, 4 species of exotic poeciliids occurred syntopically with native fishes. The introduced snail Melanoides tuberculata in Seaside Pond was responsible for introducing 3 species of heterophyid trematodes and metacercariae of one of these, Ascocotyle tenuicollis occurred in the conus arteriosus of native sleepers and exotic mosquitofish, Gambusia affinis.

# 74

Isolation and characterization of <u>Trypanosoma cruzi</u> from dogs in Virginia. STEPHEN C. BARR\*, OLGA VAN BEEK, MELISSA S. CARLISLENOWAK, JORGE W. LOPEZ, LOUIS V. KIRKHHOFF, NEIL ALLISON, ANNE ZAJAC ALEXANDER DE LAHUNTA, DONALD H. SCHLAFER, WILSON T. CRANDALL

Clinical cases of canine trypanosomiasis have been reported from Texas, Oklahoma, Louisiana and South Carolina. Here we describe the first isolation and characterization of Trypanosoma cruzi from a Walker hound puppy from Virginia that also had postvaccinal distemper. The mother of the puppy and 7 of its 8 siblings were also found to be infected with T. cruzi, suggesting that the parasite had been transmitted transplacentally or through lactation. Parasitologic, serologic, histologic, and molecular methods were used to establish the diagnosis of T. cruzi infection in these dogs, and compare the isolate to South American isolates. In a serologic survey of 12 dogs (including the father of the puppies) from the area in which the index case occurred, none were found to have antibodies to T. cruzi. However, serum of 2 of a further 52 dogs from different areas to the index case but in the same county in Virginia were positive for anti-T, cruzi antibodies. These findings indicate that canine trypanosomiasis is present in an area of the United States not previously known to be enzootic.

# 75

Two groups of major antigens of Trichinella spiralis revealed by immuno cytochemical studies.

Y. TAKAHASHI\*, Z-L. WU and T. YAMADA After a primary infection with spiralis hosts elicite a strong immune response mobilizing a wide spectrum of antibodies. Such response is biphasic, composed of the early phase (2 wks) and the later phase (4 wks). Immunocytochestudies successfully localized corresponding antigens in the tissue of Sera taken from the host in the early phase recognize a variety of structures of both larvae and adults. Possible source of antigens that trigger the immune response is likely ES products of adult worms. Such antigenicity is related to phosphorylcoline and devoid of species specificity. Sera from the later phase recognize mainly larval structures such cuticle surface, stichosome granules esophagus and occupying substances. Possible source of antigens that trigger the immune response is likely ES products of newly-encysted larvae. Such antigenicity is endowed with species specificity.

# 76

A New Antigen Processing Structure in the Colon: Lymphoglandular Complexes Process Antigen in Swine Whipworm Infections. LINDA S. MANSFIELD\* AND JOSEPH F. URBAN.

Inoculation of weaned swine with low levels of Trichuris suis (whipworm) initiates an interaction with resident bacterial flora to induce mucohemorrhagic enteritis in the colon. These pigs exhibited diarrhea, mucosal edema, inflammatory cell infiltration, bacterial accumulation at the site of worm attachment in the proximal colon, and in some cases intestinal adenomatosis associated with the intracellular Ileal symbiont intracellularis bacteria. Submucosal lymphoglandular complexes in the distal colon were enlarged and contained numerous extracellular and intracellular bacteria, eosinophils, lymphocytes, macrophages, and neutrophils. Control pigs infected with T. suis and treated with antibiotics had lesions localized to the site of worm attachment and histologically normal lymphoglandular complexes with no invasive bacteria in the distal colon. Another control group not inoculated with T. suis but exposed to the same bacterial flora had no disease and no pathology. The size of the lymphoid follicles within the lymphoglandular complexes correlated with the antigenic load. Propria nodules varied from 0.25 to 0.63 cm in diameter.

Light and electron microscopy were used to examine enlarged lymphoglandular complexes. Specialized cells with M cell characteristics were found in the follicle associated epithelium lining the entrapped crypts of Lieberkuhn within enlarged lymphoglandular complexes in *Trichuris*-infected pigs. These cells were among

epithelial cells between the lumen of the crypt and the lymphoid follicle. M cells were connected to adjacent cells by tight junctions. They had invaginations in the basolateral membrane containing lymphocytes and macrophages. Unlike M cells of the Peyer's patch, they had apical microvilli which were less dense, thicker, and more irregular than flanking enteroabsorptive cells. Bacteria were found in these cells at the apical margin and in intracellular sites. Cells with large burdens of intracellular bacteria were undergoing apoptosis as described in Shigella-infected enterocytes (Sansonetti, 1995).

### 77

Antibody Secreting Cell Populations from Pigs Infected with <u>Trichuris suis</u>.

M.F.KELLMAN\*, A.M. ZAJAC, and J.F.URBAN

Parasite antigen-specific antibody levels increased in serum 28 days after inoculation of pigs with T. suis eggs or after natural exposure on a contaminated dirt lot. An ELISPOT assay was developed to determine the frequency of antibody-secreting cells in regional lymph nodes draining the site of infection as well as in peripheral lymphoid tissue. The kinetics of development of antibody responses both locally, by ELISPOT, and systemically, by serum ELISA, were determined weekly after a primary and secondary infection with T. suis eggs. The ELISPOT assay was used to quantitate both total and antigen-specific antibody secreting cells. Explants of the mesenteric lymph nodes located near the ceci and the submandibular lymph nodes were taken. Cell suspensions were added to ImmulonII plates that were coated with either mouse monoclonal anti-pig IgA or anti-pig IgG for analysis of total antibody secreting cells or with T. suis ESP antigen for analysis of antigen-specific secreting cells. Spots were detected after 6 hour incubation of cells and development with isotype specific biotinylated monoclonals, streptavidin-alkaline phosphotase conjugate and BCIP substrate agarose. Total IgA and IgG secreting cells were similar in both the uninfected control pigs and pigs from the two infected groups. Analysis of T. suis ESP antigen specific antibody secreting cells were present only in the infected groups, and located preferentially to regional tissue. They appeared prior to detectable increase in antigen specific antibodies in serum.

# 78

Effect of Dietary Vitamin E and Selenium on the Course of Experimental Chagas' Disease. BRENDA BENNETT\* and CHERYL D. DAVIS

To study the role of diet on the course of experimental Chagas' disease, C3HeB/FeJ mice

were fed pelleted, synthetic food consisting of: no vitamine E and selenium, 800 IU/kg vitamin E, 2ppm selenium, or food containing 800TU/kg vitamin E plus 2ppm selenium. Mice on synthetic food were compared to groups of mice fed a commonly used Purina rodent chow. After diet supplementation for a minimum of 14 days, the mice were infected with 103 blood-form trypomastigotes of <u>Trypanosoma cruzi</u>. The mean peak parasitemia of mice fed synthetic food was  $2.56 \times 10^6$  parasites per ml of blood compared to  $1.12 \times 10^7$  parasites per ml of blood for the control group. By day 70 of infection, mortality in the control group was 100%, whereas the mice fed synthetic diets exhibited 25-50% mortality. Mice fed synthetic diets containing 800 TU/kg vitamin E had the lowest overall mortality rate of 25%. These results demonstrate that diet can influence the survivorship of mice that have been infected with a lethal dose of T. cruzi.

# 79

Antibody Response to *Trypanosoma cruzi* Infection in Mice Held at Elevated Environmental Temperature. LIANYONG GAO\*, CHERYL D. DAVIS

When C3H mice were infected with 103 blood form trypomastigotes (BFTs) of the Brazilian strain of Trypanosoma cruzi and were held at room temperature (RT) of 25±2°C, they developed severe infection with an average peak of parasitemia of 7.36 X 106 BFTs/ml blood and died of acute infection between day39 and day51 of inoculation. However, infected mice held at an environmental temperature of 36±0.5°C survived the entire course of infection until day 103 when they were transferred to RT, with no relapse of parasitemia thereafter. Infected mice held at 36°C showed both delayed and decreased parasitemias with an average peak of 9.34 X 10<sup>5</sup> BFTs/ml blood which is 8-fold lower than that of infected mice held at RT. The protein profile of trypomastigotes metabolically labeled with 35S-cysteine/methionine showed that most proteins were produced at much lower levels at 39°C than at 36°C. However, three proteins with M.Ws. of 89, 75 and 65 kDa were produced more abundantly at 39°C than at 36°C. These three parasite heat shock proteins (Hsp90, Hsp70 and Hsp60, respectively) were strongly recognized by antisera from mice held at both RT and 36°C with continuously increasing reactivity during the entire course of infection. The reactivity of antisera from mice held at 36°C increased sharply after day45 of infection and reached highest levels at day85, suggesting a positive relationship between antibody production and the level of parasitemia. The results clearly show that T. cruzi Hsps are predominant antigens recognized by antisera from infected mice held at 36°C.

80

Cold Stress Enhanced Susceptibility to Toxoplasma gondii Infection in Mice: Cellular and Humoral Responses, SUMAN BANERJEE\* and FERNANDO **MONROY** 

Toxoplasma gondii is a ubiquitous apicomplexan protozoan parasite that can cause clinical syndromes in animals and humans when the immune system is suppressed. Many research areas dealing with opportunistic infectious agents focus on suppression of the host immune responses with immunosuppressive drugs. We have examined the effect of a physical stressor viz. cold stress on the pathogenesis of murine toxoplasmosis, an infection in which cell-mediated immunity is of major importance in host defense. Cold water stress ( $1 \pm 0.5$ °C) was applied daily for five minutes for eight days beginning 1 day after infection with twenty cysts of the low virulent Me49 strain. Infection was monitored for eight weeks, during this period at every seven days interval mice from different groups were sacrificed and processed for immunological, histological and immunocytochemical studies. Cold stress appeared to enhance early dissemination of infection and increased cyst formation throughout the brain. In vitro T lymphocyte responsiveness to T. gondii antigens was measured. In addition, quantitation of IgM anti-toxoplasma antibodies by ELISA was performed. These results showed corresponding alterations in both cellular and humoral responses. Our findings suggest that exposure to cold stress can alter the pathogenicity of T. gondii infection in mice (Supported by IAS Grant.)

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Lymphocyte Dynamics and Cellular Responses to Eimeria papillata in BALB/c Mice. M.L. SCHITO\*, J.R. BARTA, and B. CHOBOTAR

During primary infections, we observed that the immune response to Eimeria papillata as measured by oocyst output and clinical signs did not differ significantly between immunocompetent BALB/c and congenic immunodeficient SCID (Severe Combined Immunodeficent) mice. Re-infection resulted in significant reductions in oocyst output for BALB/c mice but not for SCID mice indicating that lymphocytes were primed during initial parasite exposure in immunocompetent mice. We recently quantified lymphocyte populations during the course of primary and secondary infections in BALB/c mice. Using a panel of monoclonal antibodies, lymphocyte populations within mesenteric lymph nodes (MLN) and sections of the gastrointestinal (G1) tract were enumerated by FACS (fluorescent activated cell analysis) and direct FA (fluorescent antibody) respectively. Total cellularity and the relative quantification of cellular activity by tritiated thymidine incorporation were also assessed for MLN lymphocytes. The data show that cellular responses occurs in both tissues during primary and secondary infections. Lymphocyte activation, as measured by T cell blastogenesis with concanavalin A, occurred earlier and at higher levels for challenge infections than for primary infections. B cells, T cells and their subsets fluctuated in response to E. papillata throughout the course of infection. Our results suggest that T-lymphocyte responses during primary infections were ineffectual in controlling replication of this parasite but were required for inducing subsequent immunity. Secondary infections were controlled more aggressively and in a shorter time frame suggesting that the magnitude and kinetics of the response is important. (Supported by NSERC Grant OGP0106453 to JRB)

In Vivo and In Vitro Studies of Irradiated Eimeria tenella. M. JENKINS\*, M.S ABRAHAMSEN, H. DANFORTH, E.-H. LEE and H.S. LILLEHOJ

Previous studies have shown that 15 kRad-irradiated E. tenella sporozoites can elicit protective immunity against coccidiosis in the absence of merogonic development. Although E. tenella sporozoites exposed in the oocyst stage to a high dose of irradiation (25 kRad) can penetrate epithelial cells of the caecum, they are incapable of eliciting protective immunity. The difference between "protective" and "non-protective" sporozoites appears to be correlated with intracellular metabolic activity. The purpose of the present study was to (1) compare mRNA of non-irradiated, 15 kRad-irradiated, and 25 kRad-irridiated intracellular sporozoites for identifying transcripts unique to each stage that encode protective antigens or proteins involved in intracellular development and metabolism and (2) to develop a practical method of inoculating chickens with low doses of irradiated E. tenella oocysts to confer protective immunity against coccidiosis. The technique of PCRbased differential display was applied to total RNA isolated from uninfected chicken embryo fibroblasts (CEF) or from CEF infected with irradiated or non-irradiated E. tenella sporozoites. Although most mRNA were CEF-derived, several sequences appear to be unique to "protective" sporozoites. Also, a few CEF sequences appeared to be down-regulated after sporozoite infection. A novel technique for immunizing chickens per os with 15 kRad-irradiated E. tenella oocysts was developed that confers protective immunity against weight loss associated with coccidiosis. The oocyst delivery method is an adapted uniform infection with low doses of irradiated parasites.

83

Characterization of Immune Effector Cells Mediating Protective Immunity to Eimeria acervulina. HYUN LILLEHOJ' and JAMES A. TROUT.

interaction The nature of between Eimeria parasites host and immune lymphocytes and macrophages were investigated using two-color immunofluorescence staining of Eimeria acervulina-infected duodenal tissue. At 24 h after infections, sporozoites were seen primarily in CD8<sup>+</sup> intraepithelial

lymphocytes and macrophages. By 48 h primary infection, meront development was observed in most crypts, with infection spreading up the villi. No development of parasite was seen in lymphocytes or macrophages. Many CD8+ cells were seen at all sampling times and are frequently in direct contact with infected epithelial cells suggesting that infected host cells may the target οf immune attack. Furthermore, infected epithelial cells express the major histocompatibility complex class I, but not class II antigen. To further investigate the role of CD8+ cells in coccidiosis, chickens were treated with anti-CD8 or anti-CD4 monoclonal antibody. Depletion of CD8+, but not CD4+ lymphocytes, enhanced the severity of coccidiosis. These results indicate that CD8+ IEL mediate host immunity to Eimeria parasites in chickens (Supported by NRI Grant 91-37204 6358).

# 84

Characterization of a Low Molecular Weight Antigen control birds. TENNYSON AND DONALD S. MARTIN

A low molecular weight (LMW) antigen of Eimeria tenella bands at 2-3 kDa on a western blot preparation of controls. These results indicate that sporozoite antigens separated using tris-tricine SDS-PAGE day-old oocyst immunization used singly and detected using the monoclonal antibody C34F1. This or in combination with anticoccidial LMW antigen was partially characterized by enzymatic and chemical degradation. The antigen is not affected by treatment with proteinase K, α-amylase or denaturation with β-mercaptoethanol but is degraded by mixed glycosidases and sodium periodate. The LMW antigen may therefore be a glycolipid or a polysaccharide with an  $\alpha$ -1,6-linked structure. The antigen was localized by IFA and immuno-electron microscopy using C<sub>3</sub>4F<sub>1</sub> in the following life cycle stages of E. tenella: sporozoites; sporocyst walls; first, second and third generation meronts; gamonts and oocysts. Specifically, it was observed in the cytoplasm and pellicle of the parasite, in the parasitophorous vacuole and in the cytoplasm of the infected host cells. Following the intramuscular injection of dead E. tenella sporozoites into chickens or the oral inoculation of chickens with E. tenella oocysts, plasma IgG antibody response to the LMW antigen was consistently prompt and strong. The C<sub>3</sub>4F<sub>1</sub> monoclonal antibody was able to block the serum antibody from binding to the antigen, but the reverse was not true.

Efficacy of Eimeria maxima Oocyst Immunization with Day-Old Broiler Chickens. H. DANFORTH\*, K. WATKINS, and M. DEKICH

Battery and floorpen immunization studies were run to determine if 3 different strains of day-old male and female broiler chickens would elicit a protective immune response after a single oral gavage inoculation with Eimeria maxima oocysts. Birds in all trials were challenged with the same strain of E. maxima at 2 or 3 weeks of age, and the parameters of average weight gain, feed conversions and middle intestinal lesions scores were measured at 6 to 28 days post-challenge (PC). All 3 strains of birds showed significant protection against challenge for all parameters measured at 6 days PC in battery studies. No sex-linked difference in elicited protection was seen within these bird strains. Floorpen studies demonstrated that both sexes of immunized birds developed significant protection against challenge when compared to unimmunized-challenged These same immunized of Eimeria tenella. JOHN R. BARTA, SHAN A. birds showed no significant difference for any of the parameters measured when compared to anticoccidial medicated immunized-challenged and unchallenged medication would be effective in control of E. maxima infection in broiler birds.

# 86

Development of resistance to Eimeria bovis in young calves while on lasalocid. BERT E. STROMBERG\*, SUSANNE M. PROUTY, GARY A. AVERBECK, JOHN F. ANDERSON and DALE. L. HAGGARD.

Cattle develop immunity to an E. bovis infection as demonstrated by resistance to reinfection and the humoral antibody response. Young calves are often maintained on an ionophore to improve feed efficiency and prevent coccidiosis. Will calves develop immunity to coccidiosis when they are maintained on lasalocid, and repeatedly challenged with E. bovis? Twenty-five calves, < 2 weeks of age were allotted into 5 groups based on weight. Treatments were randomly assigned to the groups; infected control, uninfected environmental control, and 0.5, 1.0 or

2.0 mg/kg/day lasalocid. Three days before the first infection (day 0) the lasalocid treated groups were started on the ionophore. Each calf in the lasalocid treated groups was infected with 50,000 oocysts of E. bovis on day 0, 10, 20 and 30. Seventeen days (day 47) after the last exposure. lasalocid was removed from all calves. Calves in the treatment and infection control groups were then challenged with 150,000 oocysts of E. bovis 3 days later (day 51). Oocyst counts were determined using the modified McMaster technique weekly (days -21 to 20), twice a week (days 21 to 65) and daily from day 66. The condition of the feces was scored on a scale of 1 to 5. A few oocysts were observed in the feces of the 0.5mg/kg group, 21 days after the first infection and few clinical signs were observed (fecal scores) during the time on preventative. After the final challenge calves in the infection control group passed significant numbers of oocysts and the fecal scores suggested clinical coccidiosis compared with all treatment groups.

#### 87

IL12 Inhibits Development of Cryptosporidium parvum (Cp) in neonatal BALB/c and SCID mice.

J. URBAN\*, R. FAYER, W. GAUSE, M. GATELY, and F. FINKELMAN

Treatment of 1 wk old BALB/C mice with >0.lug of IL12 on the day of inoculation with 100,000 Cp oocyts completely inhibited parasite development in intestinal epithelial cells that were examined histologically 3 days later. Inhibition was dependent on the production of IFNg because specific neutralization of IFNg activity in vivo with monoclonal antibodies (mAb) completely reversed the protective effect Protection was also expressed in neonatal SCID mice, suggesting that an Malaria prophylaxis still remains a problem needed to be IFNg-activated effector mechanism induced by IL12 is effective in the absence of functional T-and B-cells. Application of IL12 to both neonatal and adult SCID mice with an established infection was not therapeutic, but in vivo neutralization of either IL12 or IFNg with specific mAbs exacerbated the severity of the infection and increased oocyst shedding. Analysis of cytokine gene expression in both the MLN and gut tissue of Cp-infected BALB/C mice by RT-PCR demonstrated that exogenous IL12 enhanced IFNg and IL10 message, but decreased IL4 message. pattern was reversed when mice were treated with a neutralizing anti-IL12 These results indicate that either developmental stages of Cp are resistant

to IFNg-induced killing or that there is a downregulation of the appropriate effector mechanism in chronic infection.

#### 88

Stability and safety of the attenuated vaccine strain, Toxoplasma gondii T-263, in pregnant cats. IRENE POPIEL\*, LARRY FORESMAN, and ALVARO FREYRE.

The attenuated mutant strain, Toxoplasma gondii T-263, is currently being developed as a vaccine to protect cats from shedding oocysts, and thereby reduce the transmission of T. gondii to intermediate hosts. The purpose of this study was to evaluate the stability of the oocyst negative phenotype and the safety of T. gondii T-263 following administration to pregnant cats. Bradyzoites of T. gondii T-263 were administered to 10 pregnant cats. Two pregnant cats were administered bradyzoites of the wild type strain, T. gondii T-265. Twelve pregnant cats served as non-infected controls. The cats infected with T. gondii T-263 did not shed oocysts. Six of these cats had normal pregnancies, three aborted and one had a premature delivery. Of the 36 products of conception, T. gondii was detected in one kitten. Both of the T. gondii T-265-infected cats shed oocysts, one aborted and one had a utcrine torsion; T. gondii was not recovered from the fetuses. All of the non-infected cats had normal pregnancies.

# 89

Protective Action to Mice Infected with Plasmodium berghei by Spleen Immune RNA. ZHONGLING LU\*. CUNXING, YANG and ZHAOGEN XU

solved. Immune RNA can transmit immune message. This study attempts to find a new malaria vaccine through the transmission of the immune message. The RNA from spleen of SD rats immune to Plasmodium berghei protected NIH mice against a lethal challenge of the blood stages of P. berghei. The RNA was extracted by the cold phenol procedure from freshly removed spleens. Protection was measured by survival as compared to controls. The levels of RNA administered were 200 µg RNA/mouse. A survival rate of 46.46% was observed. The experiment was repeated. RNA was administered at 500 µg RNA/mouse, the survival rate was 58.82%, while the mice in control (saline group) all died (0/11). So it is suggested that immune RNA has the function of partial protection for mice against *Plasmodium* berghei. (Supported by National Foundation for Natural Sciences Grant B3870688, P.R. China.)

90

# Efficacy of Decoquinate on Ovine Coccidiosis. TIMOTHY K. MILLER and DWIGHT D. BOWMAN

In a double-blind study, a daily dose of decoquinate (0.5 mg/kg BW) prevented clinical signs of coccidiosis and greatly reduced oocyst shedding in lambs experimentally infected with field isolates of Eimeria ovinoidalis, E. ahsata, E. bakuensis, E. crandallis, and E. parva. Lambs were hand-raised from birth, were not shedding coccidial oocysts at the time of experimental infection, and were randomly assigned to three groups, housed in three isolated pens. Six lambs in the drug treatment group began receiving a daily oral dose of decoquinate 3 days prior to the single infection with field isolates of coccidia. Daily treatments continued for 28 days after infection when the trial was terminated. The six lambs in the placebo group were dosed and infected the same way, but received cornmeal instead of decoquinate. negative control lambs were not infected with coccidia and received only the cornmeal placebo treatment. Clinical signs were monitored daily and regular fecal examinations were done to enumerate each oocyst species shed. Twelve days after infection, lambs in the placebo group began showing clinical signs of depression, diarrhea, and inappetence. Clinical signs worsened and within 2 weeks after initial signs, all the placebo lambs had either died or were euthanized. Large numbers of oocysts in the feces accompanied the clinical signs. In contrast, the decoquinate treated lambs showed no clinical signs referable to coccidiosis, although they did shed a small fraction of the number of oocysts shed by the placebo group. The negative control lambs showed no clinical signs and no oocysts were identified in their feces during the trial.

# 91

Efficacy of atovaquone against <u>Babesia microti</u> infection in hamsters.

#### HELIEH S.OZ\* and WALTER T. HUGHES

<u>B. microti</u> a rodent hemoprotozoa is transmitted to human by Ixodes. Atovaquone is a 1'4 hydroxynaphthoquinone compound. The prophylactic effect of atovaquone, proguanil and clindamycin plus quinine was evaluated. After 3 days of initial treatment hamsters were infected with <u>B. microti</u> and treatment was continued for additional 7 days. None of the untreated controls or proguanil-treated animals survived longer than 12 days. Whereas 9 of the 10 given atovaquone and 6 out of 10 given clindamycin plus quinine survived beyond 54 days post inoculation.

The therapeutic administration of atovaquone after

parasitemia developed resulted in a dose-related response by day 4 when 80% (mean) of erythrocytes of control animals were parasitized compared to 56%, 25%, 1.2% and .002% of parasitized cells in groups receiving 10, 80, 150, and 300 mg/kg/ day of atovaquone. Survival was 100% for animals treated with 300, 150 and 80mg/kg/day; 40% in those given 10mg/kg/day and 10% of the untreated group on day 35. In conclusion atovaquone has prophylactic and therapeutic effect against babesiosis in hamster model.

(Supported by NIA Grant RO1-AI20673, NIH P30 CA21765 and the American Lebanese Syrean Associated Charities)

# 92

Development of a microtiter ELISA for rapid screening of pharmaceuticals against *Cryptosporidium* in vitro. K.M. WOODS, M.V. NESTERENKO, and S.J. UPTON\*

An in situ enzyme-linked immunosorbent assay (ELISA) was developed to evaluate growth of Cryptosporidium parvum in vitro. Ninety-six well tissue culture microtiter plates were each seeded with 4.0 x 10<sup>4</sup> Human ileocecal adenocarcinoma (HCT-8) cells, then infected with CsCl purified oocysts 24 h later. The growth medium consisted of RPMI 1640 supplemented with 10% fetal bovine serum, 15 mM HÉPES, 50 mM glucose, 1  $\mu$ g/ml folic acid, 4  $\mu$ g/ml 4-aminobenzoic acid, 2  $\mu$ g/ml pantothenic acid and 35  $\mu$ g/ml ascorbic acid. Incubation conditions were at  $37^{\circ}$ C in a 5% CO<sub>2</sub>/95% humidified air incubator. Oocysts were allowed to excyst in situ so that sporozoites could infect cells directly. Monolayers were then washed, new medium added, and infected cells reincubated. Levels of infection were assessed 48 h later using a rat anti-C. parvum polyvalent antiserum directed against purified parasite membranes, followed by a goat anti-rat IgG conjugated to horseradish peroxidase and TMB substrate. Using various parasite inoculating doses and incubation times, optimal results were obtained using a 90 min exposure of host cells to 2.5-3.0 x 10<sup>4</sup> oocysts/well. Evaluation of various concentrations of ionophores, macrolide antibiotics, and sulfonamides in the system resulted in the acquisition of precise dose response curves for each compound.

# 93

Infection dynamics associated with *Cryptosporidium* parvum in an ICR outbred suckling mouse model. S.J. UPTON\*

An inexpensive ICR outbred suckling mouse model was developed to examine the effects of exogenous

pharmaceuticals on the course of cryptosporidiosis in vivo. Forty-four groups of 12 mice each, ranging in age from 4-12 d, each received 10,000 CsCl purified oocysts per os in 5  $\mu$ 1 PBS. At 6 DPI, mice were killed by CO<sub>2</sub> overdose, weighed, the intestines individually homogenized and oocysts quantitated by hemacytometer. Results revealed that both age and weight had a pronounced effect on the numbers of oocysts produced per mouse. Mice 8-9 days of age at the time of inoculation displayed the least weight dependent variability, produced the highest numbers of oocysts, and appeared superior over other age groups for the assays. Significant reductions in numbers of oocysts occurred in mice inoculated at 10 days of age, and few oocysts were found in mice inoculated at 11-12 days of age. A protocol is suggested to help eliminate some of the variability associated with in vivo testing with Cryptosporidium, which includes matching litters by both age and weight and rotating litters among dams. This study also suggests that previous Cryptosporidium data generated from very young, or/and non-age/non-weight matched suckling mice, is unreliable and should be viewed skeptically.

### 94

Safety of Moxidectin in Mares and Their Unborn/Newborn Foals. R. L. ASOUITH\*, J. KIVIPELTO, and E. L. JOHNSON

Moxidectin, a new macrocyclic lactone endectocide, was used in this trial. The objective of the present study is to observe and evaluate the safety of the oral moxidectin gel when administered to adult mares every 2 weeks at a dose level of 1.2 mg/kg body weight (3 x the expected use level). Thirty-nine (39) adult Quarter Horse, Thoroughbred, Saddlebred, Standardbred and Morgan mares that ranged in age from 4 to 26 years were used in this trial and were randomly assigned to either a treatment group receiving moxidectin or a control group treated with a placebo. All mares were observed and monitored for 6 hours following treatment then daily for 1 week. Complete serum chemistry and hematological analyses were determined at 48 hours before treatment and 24 to 48 hours after In 1992 treatment began at 60 days treatment. postfoaling, in 1993 nineteen additional mares were added to the study and received their first and second treatment prior to the first breeding date. Normal herd health procedures were followed, and anthelmintics were administered to the placebo group when the parasite egg count became high. By the last treatment date, 18 May 1994 the first treatment group of mares had received 45 to 54 doses of moxidectin and the second treatment group had received 31 to 36 doses. No adverse effects have been observed on ovarian function, estrus cycle, mating behavior, conception rate, gestation, parturition nor lactation. In addition fetal development and neonatal viability has been normal.

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87

Efficacy Evaluation of the New Insect Growth Regulator, Pyriproxifen. B.L. BLAGBURN and T.A. MILLER\*

A new insect growth regulator, pyriproxifen (Nylar®, Sumitomo Chemical Co.) is close to registration by the Environmental Protection Agency. Technical registration will soon be followed by pyriproxifen's appearance in combination insecticide/acaricide-IGR products for application to pet animals and to their environments. When technical pyriproxifen was first made available almost five years ago for development of formulations and generation of data to support registration applications, there was no information on topical dosage. Pyriproxifen sprays ranging from 0.05% to 0.00004% were applied once to dogs and to cats that were preinfested and were subsequently repeatedly reinfested with fleas. Untreated or placebo-treated control dogs and cats were similarly infested and reinfested. In five experiments, 40 principles and 14 controls were utilized. Flea eggs were collected periodically and incubated. Hatch rate was determined at 5 days and adult flea emergence at not less than 28 days. The data were analyzed using the regression-correlation analysis program in Microsoft® Excel V. 5.0. Residual efficacy at 100% flea egg sterilization after single treatment ranged from 0 days at less than 2 mcg/kg to 150 days at rates of 10 mg/kg body weight. Correlation between dose rate, measured as log mcg pyriproxifen/kg, and duration of 100% residual efficacy was very highly significant (p < 0.0001). There were minor differences in regressions between dogs and cats and between solvent (ethanol) and water-based emulsion formulations. Residual efficacy was longest on dogs treated with water-based formulations and shortest on cats. Regression equations were used to predict the single topical dose rate of pyriproxifen and hence, at specific spray application rates (e.g., 7.5 g/kg for dogs and 15 g/kg for cats), the potencies of sprays (% w/w pyriproxiten) required to provide 100% residual ovisterilant efficacy for up to 90 days on cats and 150 days on dogs.

# 96

Purification and Characterization of Adult Hymenolepis diminuta Mitochondrial NADPH→NAD Transhydrogenase. CHI FU\* and CARMEN F. FIORAVANTI

In adult Hymenolepis diminuta, the mitochondrial inner membrane-associated NADPH—NAD transhydrogenase serves a crucial physiological function in coupling malic enzyme-dependent reduction of NADP with the NADH-requiring, anaerobic electron transport system. Furthermore, initial findings suggest that in catalyzing NADPH-dependent NAD reduction, the transhydrogenase engages in concomitant transmembrane proton translocation. In light of its role as an essential metabolic connector and its apparent function in membrane energization, the cestode transhydrogenase was purified and characterized. Following detergent solubilization from NaCl-washed mitochondrial membranes, the enzyme was purified to homogeneity using DEAE Sepharose and hydroxylapatite chromatography. By SDS-PAGE, the purified enzyme yielded a

single band with an M<sub>r</sub> of 110 kDa. Via immunoblotting, an antibody preparation generated against the 110 kDa protein detected both the isolated protein and a 110 kDa band noted in mitochondrial membranes by SDS-PAGE. The activity of isolated and membrane-associated transhydrogenase was significantly inhibited by this antibody preparation and the monospecificity of the preparation was indicated in that it did not affect other membrane-associated pyridine nucleotide-utilizing activities. Whereas lysophosphatidylcholine inhibited transhydogenase activity of membranes and detergent-extracted material, it markedly stimulated enzyme activity recovered from DEAE-Sepharose and hydroxylapatite in accord with a phospholipid dependency. Of the phospholipids tested, phosphatidylcholine promoted the greatest activity of purified enzyme. In addition, NADPH→NAD activity of the isolated enzyme was inhibited by 5'adenylates as well as N, N'-dicyclohexylcarbodiimide, a known inhibitor of proton translocating systems. Supported by NIH Grant AI-15597.

### 97

Relationship of Mitochondrial NADPH—NAD Transhydrogenase and Transmembrane Proton Translocation in Adult *Hymenolepis diminuta*. AARON M. WATSON\* and CARMEN F. FIORAVANTI

Hymenolepis diminuta mitochondria contain an inner membrane-associated transhydrogenase that catalyzes reversible hydride ion transfer between NADP and NAD. With NADPH-dependent NAD reduction, the transhydrogenase couples intramitochondrial NADPH accumulation with NADH-requiring electron transport. In the direction of NADP reduction, the transhydrogenase can be energylinked, i.e., the reduction of NAD is markedly stimulated by either electron transport-dependent NADH utilization or ATP hydrolysis by ATPase. In turn, this is consistent with the NADH -> NADP reaction being energized by an NADH- or ATP-dependent proton gradient. Furthermore, these findings suggest that in its catalysis of the NADPH →NAD reaction, the transhydrogenase serves as a proton pump that establishes a gradient across the inner mitochondrial membrane. Initial findings suggest that NADPH →NAD transhydrogenation, as catalyzed by submitochondrial particles (SMP), results in intravesicular proton accumulation. Employing SMP, studies were begun to evaluate the relationship of NADPH-NAD transhydrogenation to proton translocation. Under isotonic conditions and with rotenone, the catalysis of NADPH→NAD transhydrogenation was inhibited by N, N'-dicyclohexylcarbodiimide (DCCD). When assessed in the presence of the protonophore, carbonylcyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), the rate of SMP-catalyzed NAD reduction increased, thereby suggesting that intravesicular proton accumulation diminishes the rate of transhydrogenation. In addition, preliminary data suggest that the ratio of hydride transfer to proton translocation for SMP can be quantified by pH electrode and cresol red measurements of proton uptake. Supported by NIH Al-15597.

98

Preparation of a Solubilized Form of ATPase from Adult *Hymenolepis diminuta* Mitochondria. MICHAEL S. JANES\* and CARMEN F. FIORAVANTI

Energetically, adult Hymenolepis diminuta is essentially anaerobic and accumulates succinate as the primary endproduct of carbohydrate utilization. It is now clear that succinate accumulation is the result of the cytosolic formation of malate and its subsequent metabolism by the cestode's anaerobic mitochondria. Within the mitochondria, malate metabolism leads to NADPH accumulation. Via hydride transfer from NADPH to NAD, as catalyzed by the pyridine nucleotide transhydrogenase, the required NADH for anaerobic electron transport is made available. Furnarate, arising from malate, serves as the terminal acceptor for electron transport and its reduction results in a site I-dependent net phosphorylation. Vital to this phosphorylation is the participation of an ATP synthase/ ATPase. However, comparatively little is known of the mitochondrial ATPase of the parasitic helminths generally and of the cestodes in particular. Accordingly, the preparation of a solubilized form of H. diminuta ATPase was pursued. Treatment of isolated mitochondrial membranes with the detergent octyl-β-D-glucopyranoside yielded a solubilized ATPase preparation that withstands chromatographic isolation. Following DEAE-Sepharose treatment, an ATPase preparation was obtained that displayed at least 10 bands by SDS-PAGE. Two intense bands were apparent with an M<sub>r</sub> of 45 kDa and 55 kDa that may correspond to the  $\alpha$ - and  $\beta$ - peptides of the mammalian F1 ATPase subunit. Further chromatography on Sepharose appears to yield a more purified preparation (in excess of 10-fold). Recent findings suggest that n-dodecyl- β-D-maltoside is a more effective detergent in solubilizing ATPase and this is being pursued. Supported by NIH AI-15597.

### 99

Catalysis of an NADPH—NAD Transhydrogenation Reaction by Adult Ascaris suum Mitochondria.

DAVID V. UPITE\* and CARMEN F. FIORAVANTI

In terms of its physiological energetics, adult Ascaris suum is predominantly anaerobic and forms succinate, as well as products derived from succinate, as the result of glucose dissimilation. As is now apparent for numerous parasitic helminths, the anaerobic mitochondrial metabolism of glycolytically derived malate leads to the accumulation of reducing power, in the form of NADH, needed for anaerobic electron transport and concomitant succinate formation. By contrast, in some other helminths, the metabolism of malate leads to intramitochondrial NADPH accumulation despite the need for NADH by the electron transport system. These other systems employ a mitochondrial NADPH→NAD transhydrogenase that catalyzes hydride transfer from NADPH to NAD, producing the required NADH. Apparently, this transhydrogenase also acts as a transmembrane proton shuttle. Given this latter

possibility, it is of interest that *A. suum* mitochondria display an NADPH→NAD transhydrogenation reaction. Initial characterization of this reaction revealed that it is membrane-associated and unaffected by dialysis. Using isolated mitochondrial membranes as the source of activity, the transhydrogenation reaction exhibited its greatest activity under acidic conditions and the thermal lability of the reaction attested to its being enzyme-catalyzed. Furthermore, the nematode transhydrogenation was inhibited by 2'-AMP, 5'AMP, NADP and NAD. Detergent treatment (n-dodecyl-β-D-maltoside) resulted in an active solubilized preparation, thereby making purification attempts possible. Supported by NIH AI-15597.

# 100

Distribution and characterization of a novel cestode neuropeptide related to vertebrate neuromedin U. C. SHAW\*, G. McMURRAY, P. VERHAERT, A.G. MAULE, and D.W. HALTON

The peptidergic component of the platyhelminth nervous system is both highly-developed and complex. To date, two endogenous neuropeptides have been isolated and characterized from extracts of the sheep cestode, Moniezia expansa. These are the 39-amino acid residue peptide, neuropeptide F (NPF) and the FMRFamiderelated peptide (FaRP), GNFFRFamide. Using antisera directed against the conserved C-terminal region of vertebrate neuromedin U (NMU), immunoreactive cell bodies and fiber tracts were demonstrated in tissue sections of M. expansa. The distribution of these immunoreactive elements was different both qualitatively and quantitatively from those immunostained with antisera to NPF and GNFFRFamide, as indicated by dual localisation and cross-preadsorption studies. Radioimmunometric analysis of gel permeation chromatographic fractions of an M. expansa extract detected a single peak of NMU-immunoreactivity eluting in a similar position to synthetic NMU-25, intermediate between NPF and GNFFRFamide immunoreactivity. The NMU-immunoreactive peptide was purified to homogeneity using reverse phase HPLC, and spectrometric profiling indicated a peptide of 20-25 residues with a single tyrosine. Preliminary gas-phase sequencing produced a partial N-terminal structure exhibiting structural similarity to vertebrate NMU-2. Full primary structural characterization is proceeding.

# 101

Immunocytochemical mapping of putative neuronal messenger molecules associated with the reproductive apparatus in flatworms.

D.W. HALTON\*, G. McMURRAY, N.J. MARKS, R.N. JOHNSTON, A.G. MAULE, C.F. JOHNSTON, and C. SHAW

The flatworm reproductive system comprises gonads and a complex series on muscularised ducts and associated glands. All of these structures are innervated to varying degrees by elements of the worm's peripheral nervous system (PNS). Immunocytochemical studies of this portion of the PNS, in conjunction with confocal scanning laser microscopy, have revealed in representatives of both free-living and parasitic flatworms, extensive staining for serotonin (5-HT) and several neuropeptides. notably neuropeptide F (NPF), FMRFamide related peptides (FaRPs), and neuromedin U (NMU). Sex differences in the patterns of immunostaining were evident, with peptide immunoreactivities concentrated largely in the female ducting and egg-forming chamber, and 5-HT associated mainly with the male copulatory apparatus. Dual localization studies at both light and EM level have revealed co-expression between some of the peptides, but not with 5-HT. The implications of these findings in understanding neuronal control of reproductive function in flatworms will be discussed.

# 102

RFamides in Fasciola hepatica: A Role in Feeding Activity? S.C. SUKHDEO\* and M.V.K. SUKHDEO

FMRFamide-related peptide immunoreactivity has been localized in the cerebral ganglion of the trematode Fasciola hepatica. Post-embedding imunogold labelling of FMRFamide shows gold labelling over dense granules contained within the small and giant nerve processes. Giant nerve processes were found innervating the oral sucker where both chemical and electrical synapses occur. Radioimmunoassays of decerebrate worms with filled caecal contents showed significantly higher levels of FMRFamide equivalents (63±14 fmol/mg protein) than worms that were allowed to regurgitate and empty their caecal contents (25± 8; p<0.05). Under the EM, regurgitated caecal contents comprise of an amorphous matrix with large particulate matters; immunogold labelling occur in discrete areas over these particles. When FMRFamide was applied exogenously, it significantly reduced the frequency of oral sucker activity in the intact worms (-62.3±13.4%; p<0.05) when compared to controls, and induced a flaccid paralysis in dissected suckers, suggesting a direct effect on the muscular activity. These data suggest that FMRFamide may have a role in feeding activity.

Supported by Whitehall Foundation; NJAES #K-06188-1-95.

#### 103

Comparative Studies on Phenotype and Genotype of *Blastocystis* Strains Isolated from Different Hosts. H. YOSHIKAWA\*, I. NAGANO, E. H. YAP, M. SINGH, and Y. TAKAHASHI

Since Blastocystis hominis was first reported in 1912, many B. hominis-like organisms had been isolated from various hosts. Most of these organisms were indistinguishable from B. hominis by light and electron microscopy because they lacked consistent morphological characteristics. We have reported polymorphism of Blastocystis strains isolated from different hosts. In this paper, we examined correlation between phenotype and genotype of Blastocystis. The phenotype of strains was investigated by means of IFA using polyclonal antibodies against Nand II strain, which was used as a reference strain of B. hominis (purchased from ATCC #50177). The genotype was evaluated by means of banding pattern of AP-PCR products (RAPD) using genomic DNA from each strain. Five strains were isolated from humans, pig, rat, and python and evaluated by IFA and AP-PCR. The strains that did not cross-react with Nand II by IFA showed different banding pattern from Nand II in AP-PCR products. In contrast, the strains that cross-reacted with Nand II strain by IFA shared same bands with Nand II strain in AP-PCR products. These results indicate that the difference of phenotype of *Blastocystis* isolates are well-correated with genotypic differences.

# 104

Evolutionary Relationships of Anuran Trypanosomes as Inferred from 18S Ribosomal RNA Gene Sequences. DONALD S. MARTIN\*, ANDRÉ-DENIS G. WRIGHT, and JOHN R. BARTA.

The sequence of the 18S ribosomal RNA gene of 5 presumptive taxa of giant trypanosomes infecting anurans was elucidated. The gene was amplified by PCR and purified and sequenced directly or ligated into pUC 18 for subcloning. The gene was sequenced using a modification of the Sanger dideoxy chain termination technique. These

sequences were aligned against sequences from members of the sub-orders Trypanosomatina and Bodonina. A phylogenetic hypothesis was constructed from the aligned sequences using maximum parsimony analyses. The hypothesized genealogical tree indicated the possible paraphyletic nature of the genus Trypanosoma. Phylogenetic affinity of the anuran trypanosomes with Trypanosoma cruzi was also indicated, providing further evidence in support of the hypothesized relationship of giant trypanosomes of poikilothermic vertebrates with the Stercoraria. The hypothesized phylogeny is discussed in the context of parasite biogeography and host/parasite coevolution.

### 105

Phylogenetic Relationship of the Myxozoa Henneguya exilis, Ceratomyxa shasta, and Aurantiactinomyxon sp. as Determined by rRNA Sequence Comparison. D. L. LIN, L. M. POTE\*, and L. A. HANSON

Currently, there are two classes of Myxozoa recognized: Actinosporea, which are primarily found in invertebrates and Myxosporea, which occur in cold-blooded vertebrates. Recent research has indicated that some myxosporeans in fish may have an actinosporean counterpart in an oligocheate, which implies that these Actinosporea and Myxosporea should not exist in separate classes. This research examined the phylogenetic relationship of the Myxozoa using ribosomal RNA.

Comparison of small subunit ribosomal RNA (ssr-RNA) gene sequences was used to elucidate the phylogenetic relationship of the myxosporeans, Henneguya exilis and Ceratomyxa shasta and the actinosporean, Aurantiactinomyxon sp. Genes were cloned coding for the ssr-RNA and sequenced in these myxozoans. This was accomplished by amplifying the genes from lysed H. exilis, C. shasta, and Aurantiactionmyxon sp. spores using polymerase chain reaction. The 2Kb products were cloned into the plasmid pBluescript SK-. Mung bean nuclease-exonuclease III digestion was used to generate sequential deletions of the H. exilis clone. Each cloned deletion was sequenced on double stranded plasmid. Sequence results were compared to known rRNA gene sequences in GenBank. Conserved regions of H. exilis were used as primers to sequence Aurantiactinomyxon and C. shasta. Sequence results showed that H. exilis and Aurantiactinomyxon are 91.6 % homologous and both are 53% homologous to C. shasta in the ssr-RNA coding region.

# 106

Marine Acanthocephala of the Eastern United States. OMAR M. AMIN

The acathocephalan fauna of the Atlantic coast of the United States includes 43 species and 20

genera belonging to three orders: Polymorphida, Echinorhynchida, and Neoechinorhynchida. Adults are exclusively intestinal parasites of vertebrates. This study includes those species found in vertebrates of marine and estuarine environments along the North American Atlantic coast, at least between Maine and Texas. Species with potential presence within that geographical range status as three separate species. This study was undertaken and those that typically infect freshwater fishes but with occasional presence in marine or estuarine hosts are also included. The taxonomy, anatomy, natural history, and ecology of the Acanthocephala as a group are discussed and an illustrated key to the genera is presented. Techniques, an annotated systematic treatment of all 43 species, and a systematic index are included. systematic decisions will be made at this time but areas where such decisions are pending will be pointed out and studied for future reports. This is a contribution to NOAA Technical Report NMFS: Marine Flora and Fauna of the Eastern United States.

# 107

Systematics of the tetraphyllidean genus Anthocephalum T.R. RUHNKE\*, and R.A. KNAAK

The tetraphyllidean tapeworm genus Anthocephalum was recently resurrected by Ruhnke (1994) for 5 species, collected from 4 batoid species. The genus is morphologically diagnosed by the presence of marginal loculi on the both ridial periphery, a both ridial "rim", features of the scolex tegumentary surface, and a posterior genital pore. Tapeworm collections from 2 stingray species in the Gulf of California revealed 3 putatively new species of Anthocephalum. Two of the species were collected from the spiral intestines of Dasyatis longus and 1 species from D. brevis. Study specimens were prepared for light and scanning electron microscopy, and relevant taxonomic literature on existing members of the genus was reviewed. The new species can be identified by morphological differences in worm length, segment number, scolex and bothridial morphology, testes number, and size of mature segments. The 3 new species are at least phenetically similar to 3 existing species parasitic in 2 other species of Dasyatis. The new species also possessed a similar pattern of tegumental microtriche polymorphism to 4 of the existing species, indicating that these features may be taxonomically informative. A phylogenetic analysis indicates that a clade within Anthocephalum shares a co-evolutionary history with stingrays of the genus Dasyatis. Brooks and McClennan (1993) hypothesized that species of Anthocephalum are related to cestodes in the genus Rhinebothroides, cestode species parasitic in South American freshwater stingrays. The systematic position of Anthocephalum is presented in relation to Rhinebothroides and other rhinebothriid taxa.

# 108

Using Parasites to Answer a Systematic Question in the Stingray Genus Urobatis. K. JENSEN\* and J. N. CAIRA

Three species of *Urobatis* have been reported from the Sea of Cortez. These include: Urobatis halleri, Urobatis maculatus and Urobatis concentricus. These stingray species are distinguished from one another primarily on the basis of disc color pattern. However, the fact that individuals with combinations of the three basic color patterns are known, has lead some authors to question their to determine wether differences in parasite faunas are associated with the 3 basic color pattern differences. In 1993, 74 round stingrays were collected from four different localities in the Sea of Cortez. Based on their color patterns, 45 were identified as *U. halleri*, 28 as *U. maculatus* and only one as U. concentricus. Tapeworms were removed form the spiral intestine of each host, fixed in AFA and then transferred to 70% ethanol. Whole mounts were prepared of all cestodes. These were examined using light microscopy. A total of 17 species of cestodes were identified, representing five genera including: Acanthobothrium, Rhinebothrium, Anthocephalum, Oncomegas and at least one other genus of trypanorhynch. Urobatis halleri hosted 12 different species of cestodes, 5 of which were unique to this species of stingray. Urobatis maculatus hosted 8 species; only one of these was unique to this stingray. Seven species of cestodes were found both in *U. halleri* and U. maculatus. The one specimen of U. concentricus hosted 6 species, 4 of which were unique to this stingray examined. These data preliminary support the validity of three distinct species of *Urobatis* in the Sea of Cortez. In addition they suggest that U. halleri and U. maculatus are more closely allied to one another biologically than either is to U. concentricus.

# 109

On the Cestode Genus Echinobothrium: Host Records, Distribution and Fine Structure. G. TYLER\* and J.N. CAIRA

Tapeworm species of the genus Echinobothrium parasitize batoid fishes and benthic sharks, but have not been extensively studied. New collections of parasites from the spiral intestines of Myliobatis californicus, M. longirostris and Rhinoptera steindachneri from the Sea of Cortez, and Dasyatis leylandi from Australia reveal the presence of three new species of Echinobothrium. Worms of all three species were removed from the spiral intestines of infected individuals and fixed in 10% formalin. Whole worms were stained in Gill's hematoxylin and mounted in Canada balsam. Serial sections were cut at 10 µm intervals, stained in Gill's hematoxylin and eosin, and mounted in Canada balsam. Preparations of rostellum hooks were mounted in Berlese's medium. Specimens for scanning electron microscopy were treated with 1% osmium tetroxide and dried in Peldri II (Ted Pella, inc.), mounted in carbon paint, and sputter coated with 100Å of gold. All three new species differ from previously described species in the number and arrangement of rostellum and peduncle hooks, and in segment morphology. All four of the hosts represent new records for Echinobothrium. The Sea of Cortez represents a new geographic record for this

genus. The presence of *Echinobothrium* in the eastern Pacific suggests that the genus may be cosmopolitan in distribution. Scanning electron microscopy revealed the presence of palmate microtriches on the bothridial surfaces of all three species, indicating that this character has a broader distribution among cestode taxa than previously believed.

### 110

A Revision of the Genus *Platybothrium* with Discussion of its Host Associations. CLAIRE J. HEALY\* and J. N. CAIRA

Six genera of sharks in the Family Carcharhinidae are parasitized by members of the genus *Platybothrium*. While several of the species in this genus such as P. auriculatum are well known, a number of species including P. spinulifera and P. parvum are known only from their original and one or two subsequent descriptions. This study was undertaken to improve our knowledge of this genus and its host associations. New collections were made for P. auriculatum, P. cervinum, P. hypoprioni, P. spinulifera, and P. parvum from species of sharks including: Prionace glauca, Negaprion brevirostris, Galeocerdo cuvier, Rhizoprionodon terrae-novae, Sphyrna lewini, Sphyrna zygaena, and eight species in the genus Carcharhinus. All available type material was examined. The hosts were collected from areas including Austrailia, Wood's Hole, the Florida Keys, and Baja California. Specimens were removed from the spiral intestines of the hosts, fixed in 10% formalin, and preserved in 70% ethanol. The parasites were examined and measured with a compound microscope, and their internal anatomy was examined by means of histological sections. The majority of the species were also examined with scanning electron microscopy. Redescriptions of P. auriculatum, P. cervinum, P. hypoprioni, P. spinulifera, and P. parvum were prepared. In addition, two new species, which differ from all previously described species in hook morphology and size, were discovered in Carcharhinus limbatus, Carcharhinus amblyrhynchoides, and Sphyrna zygaena. Analysis of the geographic distribution of *Platybothrium* indicates the genus is cosmopolitan in distribution. A cladistic analysis was performed on the members of the genus, and the results used to investigate the relationships between the species of Platybothrium and their carcharinid hosts.

# 111

On the Status of the Genus Pinguicollum (Tetraphyllidea: Onchobothriidae), C.P. KEELING\* and J. N. CAIRA
The genus Pinguicollum was originally erected in 1955 by
Riser for a unique tapeworm collected from an unidentified skate in Monterey, California in 1927 by Sleggs. Since that time little additional information has appeared about the genus which, because of the paucity of available study material, remains poorly known. Questions exist about the number of bothridial loculi, the nature of the bothridial musculature and "pseudoscolex" and the morphology of the

hooks; few data are available on the segment morphology of this genus. In fact, in his treatment of the family Onchobothriidae, Euzet (1994) synonymized the genus with Acanthobothrium until additional material could be collected and studied. The recent availability of two lots of specimens from the personal collections of Dr. Nathan Riser and Dr. Mike Moser allowed us to address some of the uncertainties associated with the morphology and systematic position of Pinguicollum. Spirit material from both collections was prepared for, and examined with, light and scanning electron microscopy. Hooks were prepared in Berlese's medium. Cross sections were cut through the scolex, "pseudoscolex" and mature segments. Based on this material the following information is now available for Pinguicollum: the 4 bothridia each consist of a single loculus. These bothridia are unusual in that they appear to be closed proximally. Small filiform and spiniform microtriches are present throughout the visible surfaces of the scolex and "pseudoscolex." The "pseudoscolex" is supported internally by 8 distinct muscle bundles anteriorly; these bundles divide into multiple smaller bundles at the level of the strobila. The bifid hooks possess fused bases but only a single pore each. The ovary is bilobed in cross section. Based on these results, we recommend that the genus be retained as a separate taxon within the Onchobothriidae.

# 112

On the Identity of *Phoreiobothrium* from Sharks of the Order Carcharhiniformes. JON L. SWANSON\* C. RICHMOND and J. N. CAIRA

The onchobothriid genus *Phoreiobothrium* currently contains five species, all of which are characterized by bothridia divided into two loculi, the posterior of which is subdivided into multiple subloculi. Host records to date indicate these species parasitize sharks in the families Sphymidae and Carcharhinidae, but the majority of the 38 species in these two groups have not yet been examined for parasites. The primary purpose of this study was to more broadly sample sharks in these two families for *Phoreiobothrium*. Fifteen species of sharks including members of the genera Sphyma (four species), Negaption (two species), and Carcharhinus (nine species) from Australia, the Sea of Cortez, the Florida Keys, and New Guinea were necropsied and examined for parasites. Tapeworms were removed from the spiral intestines of the hosts, fixed in 10% formalin, or alcoholformalin-acetic acid (AFA), and stored in 70% ethanol. Approximately 20 specimens from each host species were prepared as whole mounts and measured using a compound microscope. Scanning electron microscopy and sectioning techniques were also utilized on the tapeworms from eight of the host species. This work led to the discovery of four new species of *Phoreiobothrium*, one from each of the following shark species: Sphyrna lewini, Sphyrna mokarran, Negaprion acutidens, and Negaprion brevirostris. Sharks in the genus Carcharhinus were found to host Phoreiobothrium lasium as well as a group of much smaller tapeworms that appear to represent a fifth new species in this genus. This fifth species is the only species in the genus reported to parasitize more than a single species of shark.

### 113

Systematics of the Genus <u>Litomosoides</u> - Host Parasite Coevolution or Parasite Transfer? SARA V. BRANT\* and S. L. GARDNER

Two new species of nematodes of the genus Litomosoides are being described from subterranean rodents of the genus Ctenomys. As a result of this work questions arose relative to the relationships between members of this genus and representative species of a closely related genus, Litomosa. Filarioid nematodes of the genus Litomosoides occur in representatives of the Rodentia, Chiroptera and Marsupialia only in the Neotropical and Nearctic regions while species of the genus Litomosa occur in old-world bats and rodents. Species of "Litomosoides" described from rodents of the nearctic family geomyidae appear to represent an undescribed genus. From Bolivia, many unidentified forms of Litomosoides have been recovered from bats and we plan to identify and describe all species of filariod nematodes from Bolivian mammals (mostly bats) and to conduct a morphologically based phylogenetic analysis of all described species. These analyses should allow robust tests of recently proposed hypotheses of the origin of these enigmatic forms of the Nemata. (Supported by NSF Grant No. 9024816)

### 114

Molecular Systematics of *Plasmodium* species and Related Apicomplexan Parasites. RAMON A. CARRENO\*, DONALD S. MARTIN, AND JOHN R. BARTA

The phylogeny of Plasmodium species is still not well understood because morphological data have provided few clues regarding evolutionary relationships among the species. In recent years, DNA sequences encoding the 18S ribosomal RNA gene have been used in phylogenetic analyses of Plasmodium and other groups. Several different ways of interpreting sequence data exist however, and this has led to varying views on the patterns of evolution of Plasmodium spp. as determined by sequence information. In this study, the phylogeny of Plasmodium species parasitizing birds, mammals, and saurians was examined using several outgroups presumed closely related to Plasmodium based on morphological and biological characteristics. These outgroup taxa included other haemospororids of the genera Haemoproteus or Leucocytozoon, as well as gregarines, coccidia, and

piroplasms. Aligned sequences were analyzed using parsimony techniques and trees were rooted using the outgroups listed above. The phylogenetic relationships among taxa in the genus *Plasmodium* and other haemospororids were examined.

#### 115

Phylogenetic Analysis of the Acanthocephala Using Complete 18S Ribosomal DNA Sequences. THOMAS J. NEAR\*, and STEVEN A. NADLER

Phylogenetic relationships within the Phylum Acanthocephala have remained unresolved. Past efforts have focused on creating classifications with little phylogenetic consideration. The Phylum Acanthocephala is currently divided into three classes, Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala. The taxonomic division into three classes is based on morphological similarities and ecological considerations of the intermediate and definitive hosts. In this study, nine acanthocephala species representing the three recognized classes were collected from vertebrate or invertebrate intermediate hosts. Nucleic acids were isolated and PCR was used to amplify a 1,766 base-pair region of the 18S rDNA. The PCR product was ligated into the P-GemT (Promega) plasmid vector and cloned into DH5-a Escherichia coli. Sequences were obtained using a two-step cycle sequencing procedure (A Tag kit, United States Biochemical). Sequencing products were separated by electrophoresis in a 6% polyacrylamide/8.3 M urea gel and visualized by autoradiography. Preliminary alignment of sequences was performed using the CLUSTAL V computer program. Characters states inferred as "gaps" were treated as missing data in maximum parsimony (MP) analyses, which were performed using the branch-and-bound algorithm of PAUP (v 3.1). The robustness of inferred trees was assessed by using bootstrap resampling with 1000 replications. Parsimony analysis of the sequence data supports the monophyly of the classes Archiacanthocephala, Palaeacanthocephala, Eoacanthocephala. Monophyly of the acanthocephalan classes supports the current higher-order taxonomic groupings. The amount of sequence differentiation between acanthocephalan taxa reveals considerable genetic divergence among the classes. The analysis reveals that the Palaeacanthocephala represent the most basal taxon. The derived condition of the Archiacanthocephala revealed in this analysis is consistent with the distribution of certain morphological features and the terrestrial habitat of the intermediate and definitive hosts. The monophyly of the Eoacanthocephala cannot be reliably assessed until more species are included in the analysis.

# 116

Prospects for Controlling Animal Parasitic Nematodes by Predacious Microfungi. PETER NANSEN

R. BARCLAY MCGHEE LECTURE

117

Abolition of Industrial Research: Would it be a Good Thing for Parasitology? WILLIAM C. CAMPBELL\*

Is anybody suggesting such a thing? I hope not; but posing the question may force us to sharpen our focus on the value we perceive, or do not perceive, in the research conducted in our industrial laboratories. The research in question may be empirical or rational or a hybrid of the two. In the context of this symposium it should be remembered that research in all three categories (if it is good research) contributes positively to our discipline. Industry, especially in the past, has generally been associated with "applied" research, and much of that research has been empirical. We tend to look to "basic" or "rational" research for scientific enlightenment, but l would argue that industrial empirical research has been fruitful in scientific facts and figures as well as in dollars and ducats. In the future it is likely that the distinction between empirical and rational approaches to such things as drug discovery will become increasingly blurred, and that the research done in academic and industrial labs will become increasingly similar. There will still be differences (in direction, focus etc.) but progress in parasitology will be enhanced if we maintain close liaison between the academic and industrial moieties of our discipline.

# 118

Pharmaceutical and Academic Research: Where's the Common Ground? J.F. WILLIAMS

Mission-oriented research in pharmaceutical settings imposes certain disciplines on collaborative undertakings between industry and academia that need to be appreciated if productive relationships are to be fostered. Pharmaceutical parasitologists engaged in discovery of new entities can be attracted by innovative and rigorous academic studies of basic biological characteristics of parasites. However, these must offer insights that can be exploited in the discovery process as potential targets for biological activities of new chemical or natural products, and/or as testing instruments for high-volume screens. The essence of successful discovery is selectivity; the pursuit of basic cell biology and physiology of parasites is therefore far more important to the pharmaceutical industry than is generally appreciated. It provides the means for exposing and testing for selectivity. Phenomena of most interest and value are those that promise breadth of antiparasite spectrum. Pharmaceutical prosperity is founded on exclusivity of highly tenable data, so a much greater demand for rigor in data collection, handling and preservation can be expected than most academics are accustomed to. Contrary to prevailing belief, acquiring legitimacy for discovery through publication is as important for the industrial scientists as it is for the academic investigator, and restrictive limitations on publication rights are not a common feature of productive collaborative relationships. The latter are most easily and commonly undermined when academics fail to deliver what they say they will do.

119

Synergies Which Result from Academic and Industry Relationships. ROBERT B. GRIEVE\*

There are numerous advantages to the many relationships which exist between academic and commercial institutions. Historically, academia has been viewed primarily as a source of innovative ideas stemming from basic research, whereas industry has been traditionally viewed as a source for research funding and for providing the mechanism for developing innovative ideas into commercial products. These dogmatic distinctions have become blurred, however, and more complex models are becoming appropriate. These models still derive strength from the complementarity of academics and industry. Many of these synergies can be best illustrated through the perspective of a research-driven company.

# 120

Efficacy of Doramectin in the Treatment of Induced Infections of Gastrointestinal Nematodes in Young Calves. C.E. COUVILLION, L.M. POTE,\* C. SIEFKER, and A.S.LITTLE

Three studies were conducted to determine the efficacy of doramectin injectable against induced infections of adult and larval (L<sub>4</sub>) stages of gastrointestinal nematodes in young cattle. In each study, between 20 and 30 young calves were pretreated twice with fenbendazole (days -50 and -7) prior to infection. All animals within a study then received oral inoculations of infective stages of specific nematode species under test. Infections were timed such that, by the day of treatment, the population of each species had matured to the stage to be tested (L4 or adult). On the day of treatment, one group animals (n=10 or 11/two groups in one study) received doramectin subcutaneously at 200 µg/kg and the control animals (n=10 or 11) were treated with saline at the same time. All animals were slaughtered and worm burdens were determined at least 14 days after treatment. Efficacy against the following adult species was 99% or greater: Haemonchus placei, Ostertagia ostertagi, Trichostrongylus colubriformis, Cooperia oncophora, Cooperia punctata, Cooperia zurnabada, Cooperia spp. and Oesophagostomum radiatum. Efficacy against L4 stages of the following species was 99% or greater: H. placei, O. ostertagi, Trichostrongylus axei, T. colubriformis, C. oncophora, C. punctata, C. zurnabada, Cooperia spp. and O. radiatum. Efficacy against the adult and L4 stages of Nematodirus helvetianus was 9.6 and 83.3%, respectively.

# 121

Efficacy of Injectable Doramectin Against Natural Gastrointestinal Nematode Infections of Weanling Beef Calves. J. A. STUEDEMANN\*, H. CIORDIA, C. P. WILKINS, and D. H. SEMAN.

Fourteen days prior to drug treatment, fecal samples were obtained from 26 calves for determination of nematode eggs per gram of On Day -7, the calves were feces (epg). removed from pasture, weaned, and placed in drylot. Three calves were randomly selected for necropsy on Day O. Twenty calves were raandomly allocated to treatments from the remaining 23 calves. On Day 0, 10 calves were subcutaneously injected with 200  $\mu g$  of doramectin/kg body weight while the remaining calves served as untreated controls. group of 10 calves was housed in a separate pen and received free-choice bermudagrass hay and water during the 14-day post-treatment period. Necropsies were performed on all calves for actual worm counts on Day 14 posttreatment. Geometric mean total adults and larvae were 24,611 and 791, respectively, for untreated control calves and 28 and 0, respectively, for doramectin treated calves. The most prevalent (geometric means of adults) species in control animals were Ostertagia ostertagi (5,772), Haemonchus placei (4,777), Cooperia punctata (2,722), Cooperia oncophora (2.393).Cooperia pec<u>tinata</u> (1,648).Trichostrongylus axei (1,425), and Nematodirus spathiger (1,385). Doramectin was 100% efficacious in reduction of adults (except Nematodirus spathiger which was 97.8%) and larvae of all species.

### 122

Efficacy of Injectable Doramectin Against Inhibited Ostertagia ostertagi of Naturally Infected Yearling Calves. J. A. STUEDEMANN\*, H. CIORDIA, C. P. WILKINS, and D. H. SEMAN.

The objective was to determine the efficacy of injectable doramectin against inhibited 0. ostertagi larvae (L4). Twenty-one days prior to drug treatment, 30 yearling calves were removed from pasture and placed in drylot. Necropsies were performed on two contemporary calves and found to average 24,665 inhibited larvae with total adult and larval counts of 59,668. Twenty calves with relatively high (403) eggs per gram of feces (epg) were selected from the 30 calves, and randomly assigned to either a treated or nontreatedcontrol group (n=10). On Day 0, 10 calves were subcutaneously injected with 200  $\mu g$  of doramectin/kg body weight while the remaining 10 calves served as untreated controls. Each group of 10 calves was housed in a separate pen and received free-choice bermudagrass hay and water during the 14-day post-treatment Necropsies were performed on all calves for actual worm counts on Day 14 posttreatment. Treatment was 100% efficacious

among abomasal and intestinal adult worms. The most prevalent (geometric means of adults) species in control animals Trichostrongvlus axei (22,6/1), O ostertagi (8,880), Cooperia punctata (5,227), Cooperia oncophora (1,907), and Haemonchus placei (1.714).Geometric mean adult worm counts were 40,697 for control and 0 for treated calves. Geometric mean inhibited O. ostertagi larval counts were 49,821 and 22 in control and treated calves, respectively, for a 99.957 percent reduction.

#### 123

Field Efficacy of Doramectin in the Treatment of Gastrointestinal Nematode Infections of Cattle. F.E. PHILLIPS\*, N.B. LOGAN, and R.M. JONES

Doramectin is a potent broad spectrum endectocide of the macrocyclic lactone class. Demonstration of its efficacy when given by injection at 200 µg/kg against gastrointestinal nematode infections of cattle in controlled laboratory tests has previously been reported. To confirm efficacy under field conditions, ten studies involving 828 animals were conducted to a common protocol at sites representative of various climatic zones and management practices within the USA and Canada. In each study, naturally parasitized animals were randomly allotted to a treated or a control group in a tiered manner on the basis of fecal egg counts and, in some cases, body weight and then received doramectin injectable solution or saline, respectively, by subcutaneous administration. Efficacy was assessed by comparing nematode egg counts in fecal samples collected before and after treatment. Percentage reduction in geometric mean egg count from treatment day to 21 days posttreatment was calculated for each treatment group for each study and for pooled data across all studies. The effectiveness of doramectin was 100% in nine studies, 98% in the tenth study and 99% overall. Differences between treatment groups were highly significant (p<0.0001) in all cases. Pretreatment coproculture identified Haemonchus, Ostertagia, Cooperia, Trichostrongylus and Nematodirus as the genera represented. No adverse reactions to doramectin treatment occurred. It was concluded from the study program that doramectin injectable at 200 µg/kg is safe and effective in the treatment of gastrointestinal nematodiasis under field conditions.

# 124

Efficacy of Doramectin Against Eyeworms (Thelazia spp.) in Naturally and Experimentally In Infected Cattle. M.J. KENNEDY\* and F.E. PHILLIPS
The anthelmintic efficacy of doramectin was assessed for the control of Thelazia spp. in two studies (one naturally infected and one experimentally infected) using 44 calves. Cattle were

randomly assigned based on weight to either a doramectin treatment group or a saline control group. Treated animals received doramectin at a dosage of 200 µg/kg, subcutaneously in the lateral midline of the neck. Control animals received an equivalent dosage of 0.9% sterile saline. Animals were slaughtered 13-16 days after treatment, and all eyes and associated tissues (including the lacrymal glands and ducts) were removed and examined for total number, species and viability of eyeworms. Two species of eyeworms, Thelazia skrjabini and Thelazia gulosa were found in the control group of naturally infected calves. Only T. skrjabini was found in the control group of experimentally infected calves. No eyeworms were found in any doramectintreated animal. The efficacy of doramectin against Thelazia spp. in both naturally and experimentally infected calves was 100%.

### 125

Field Efficacy of Doramectin in the Treatment of Lice Infestations of Cattle. J.E. LLOYD\*,

F.E. PHILLIPS, N. B. LOGAN, and R.M. JONES Doramectin is a potent endectocide belonging to the macrocyclic lactone class. An injectable formulation of the drug has been shown to be highly active against lice infestations of cattle. In this paper, we report the results of a series of field studies designed to evaluate this formulation under North American use conditions. Eight studies involving a total of 414 animals were carried out, seven at various geographic locations in the U.S. and one in Canada. In each study, animals with confirmed infestations of one or more species of lice were allotted at random to one of two groups and received either doramectin injectable solution at a dose of 200 μg/kg or saline by the subcutaneous route. Lice were enumerated by species for each animal immediately before treatment and two and four weeks after treatment. Efficacy was determined for each species represented by comparison of pretreatment and posttreatment counts. Percentage reduction in geometric mean louse count from the first to last day was calculated for each treatment in each study and for pooled data across all studies. Doramectin was 100% effective in eliminating infestations of Linognathus vituli, Haematopinus eurysternus and Solenopotes capillatus. Infestations of the biting louse Damalinia bovis were reduced overall by 86%. For all species posttreatment louse counts were significantly less (p<0.02) for doramectin-treated cattle than for controls. No treatment-related adverse affects were noted. It was concluded from these results that doramectin injectable solution is safe and efficacious in field use against sucking lice infestations of cattle.

# 126

Doramectin Efficacy against Cattle Grubs (Hypoderma lineatum and Hypoderma bovis) and Cattle Lice (Bovicola bovis, Linognathus

vituli, Solenopotes capillatus, and Haematopinus eurysternus) in Wyoming. J.E. LLOYD\*, R. KUMAR, J.W. WAGGONER, and F.E. PHILLIPS

Six individual trials were conducted in Wyoming to evaluate the therapeutic efficacy of doramectin administered subcutaneously at a dosage of 200 ug/kg against multiple, natural infestations of cattle grubs or cattle lice. Insect species present and the number of trials that included each species were: Hypoderma lineatum, 2; Hypoderma bovis, 1; Bovicola bovis, 5; Linognathus vituli, 5; Solenopotes capillatus, 3 and Haematopinus eurysternus, 1. Examinations for cattle warbles were performed either weekly or every 4 to 5 weeks from time of first appearance through last appearance in the backs of the cattle. Examinations for lice were performed prior to treatment and weekly thereafter for 28 days. No H. lineatum, H. bovis, L. vituli, S. capillatus or H. eruysternus were found on doramectin-treated animals at any time following treatment. By 28 days following treatment, the number of B. bovis was reduced between 58 and 98%. Treatments applied later in the season, i.e., in March, were more efficacious against B. bovis than those applied in January or February.

# 127

Spore Morphology and Ultrastructure of Myxobolus sp. from the Redline Darter, Etheostoma rufilineatum. VINA R. DIDERRICH\* and SHARON PATTON.

The spore of Myxobolus sp. is described from naturally infected redline darters, Etheostoma rufilineatum, from Abrams Creek, Great Smoky Mountain National Park. Plasmodia containing numerous pyriform spores appeared cyst-like structures in the epidermis and Measurements of 100 spores musculature. obtained from a skin cyst were recorded and analyzed using the SAS system for statistical analysis. Variables studied were spore length and width and polar capsule length and width. The mean, standard deviation, minimum and maximum were calculated for each variable: spore L 15.34  $\pm$  1.04*u*; spore W 9.51  $\pm$  1.01 (8-12)u; polar capsule L  $\hat{7}.52 \pm 1.18 (\bar{5}-10)u$ ; and polar capsule W  $2.62 \pm 0.56$  (2-4)u. Measurements of spores from two similar species. M. neurophila and M. scleroperca were not within the 95% confidence intervals of redline darter spores. means Ultrastructurally, two sporoplasm nuclei and two polar capsules were enclosed by 2 valves. At the junction of the two vavles was a prominent flange. The bilaminar valve wall had a filamentous outer coating. (This work

was supported by a Centers of Excellence Venture Grant from the University of Tennessee College of Veterinary Medicine).

# 128

Experimental Porcine Schistosomosis Japonica: A Possible "Self-Cure" Phenomenon. A. LEE WILLINGHAM\* and HENRIK O. BØGH

Schistosoma japonicum, an important zoonotic trematode parasite of Southeast Asia, infects a wide range of mammals including humans. Although swine are considered an important reservoir host, few studies have been conducted on the hostparasite relationship in porcine infection. Schistosome establishment, attrition and fecundity were investigated by experimentally infecting Landrace/Yorkshire crossbred pigs with an Anhui, China isolate of S. japonicum. Pigs were injected i.m. with 2,000 cercariae and divided into three groups (n = 6), which were killed 4, 11 and 17 weeks post-infection (wpi). Infections became patent 6 wpi, with egg excretion reaching a peak 8-10 wpi before rapidly declining. Hepatic egg granulomas were not evident 4 wpi, notably severe at 11 wpi and virtually resolved at 17 wpi. The number of adult worms recovered by perfusion was significantly lower 11 and 17 wpi with a mean of 172  $\pm$  102 s.d. at 4 wpi, compared to 26  $\pm$  16 and  $9.8 \pm 4$  at 11 and 17 wpi, respectively. Following perfusion, residual adult worms were located manually and counted with a mean of  $6 \pm 7$  at 4 wpi and  $101 \pm 59$  and  $99 \pm 79$  at 11 and 17 wpi, respectively. More than 90% of the residual worms 11 and 17 wpi were surrounded by granulomatous material in the mesenteric veins, thus preventing extraction of worms in toto. The results suggest that L/Y pigs undergo a degree of "self-cure" following a certain period of patent infection. This was detectable already 11 wpi on the basis of adult worm numbers perfused and egg excretion. Further pathological and immunological studies are warranted for elucidation of the mechanisms involved. (Supported by the Danish National Research Foundation.)

# 129

Life Cycle of Calliobothrium verticillatum: Comparison of Ribosomal Subunit Sequences in Larval and Adult Forms. S. E. LITTLE\*, R. A. BULLIS, M. L. SOGIN, and S. L. HAJDUK

Calliobothrium verticillatum is a tetraphyllidean cestode found in the spiral valve of the dogfish Mustelus canis. Plerocercoid larvae with morphologic identity to the adults of C. verticillatum were described from the midgut ceca of the hermit crab Pagurus pollicaris by Caira and Ruhnke (1991), and a tentative life cycle established. To investigate this proposed life cycle, specimens were collected from hermit crabs and dogfish and identity of each confirmed by scanning electron microscopy. Genomic DNA was isolated from each stage and 18S ribosomal subunit genes amplified by polymerase chain reaction (PCR) with universal eukaryotic primers.

Inferred platyhelminth-specific primers were constructed and used to amplify a portion of the ribosomal subunit gene. Restriction endonuclease mapping of both the 2000bp PCR product generated with the universal rRNA primers and the 1010bp PCR product from the platyhelminth-specific primers indicates that the rRNA genes are identical in the organisms isolated from dogfish and crab. Sequences of the ribosomal subunit gene fragments from each stage were compared to verify that the DNA was derived from developmental stages of the same species. These results confirm the life cycle for *C. verticillatum* proposed with morphologic features, and offer another approach to elucidation of life cycles in multi-host parasites. (Supported by the MacAuthur Foundation and Burroughs Wellcome Fund).

# 130

Exsheathment of *Haemonchus contortus*: A Role for Volatile Fatty Acids. L. FAKHRZADEH\* and M.V.K. SUKHDEO

The stimuli for exsheathment of L3 infective larvae have been identified in several nematode species. However, the relationship of the stimuli to physiological conditions in the host at the point of infection have not always been clear. In this study, the signals that trigger exsheathing behavior in the larvae of Haemonchus contortus were investigated. Several physical and chemical signals (gases, acids and digestive enzymes), that might be encountered by the parasite during infection were assayed for their effects on the larvae exsheathment was obtained following sequential treatment with CO2, sheep ruminal fluids, and abomasal fluids at host temperature (38.5±2.8%). This effect could be reproduced by replacing ruminal fluid with acetic acid (28.8±4.2%; p>0.05). Other components of volatile fatty acids (VFA) such as n-butyrate did not affect exsheathment (2.9±1.40%) when compared to controls (1.3±1.3%; p>0.05). The production of acetic acid in the sheep rumen increases following the ingestion of grasses, and this supports the suggestion that acetate may play an important role in the exsheathment process of this nematode.

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# 131

Comparative Ultrastructure of Immune-Damaged and Transplanted Strongyloides stercoralis.

FRANK E. THOMPSON\*, VEENA M.

BHOPALE, and GERHARD A. SCHAD.

Strongyloides stercoralis adults were removed from beagle dogs during the occult chronic phase of infection and transplanted by laparotomy into naive dogs for 5 days, 10 to 12-week primary infection worms (p.i.w.) were stunted and essentially barren, larval production having dropped to undetectable levels by fecal examination. After transplanted adults were in naive recipient dogs for 5 days, larvae were detectable in the feces and adult worm length had increased significantly (p<0.001). Ultrastructural evidence for reversal of immune damage was obtained by comparison of normal, immunedamaged and transplanted worms. Immune-damaged worms had small gonads lacking normal ooplasmic content. The intestinal cells were full of lipid and protein bodies and their microvilli were damaged. After 5 days in naive animals the gonads had regained normal size and content. Intestinal cells had lost the accumulated products seen in immunedamaged worms and regeneration of microvilli was apparent. (Supported by NIH Grant AI-22662.)

# 132

Strongyloides stercoralis: Production of Autoinfection in the Gerbil. THOMAS J. NOLAN\*, VEENA M. BHOPALE and GERHARD A. SCHAD

Autoinfection is a hallmark of S. stercoralis infections in humans and dogs treated with corticosteroids. Autoinfection has also been reported in neonatal canines experimentally infected with this nematode, as well as in dogs with an induced decreased intestinal motility. We have examined the induction of autoinfection in the infected gerbil using a variety of methods. Autoinfection was seen in gerbils given any one of the following: prednisolone (a corticosteroid), cyclophosphamide (a non-steroidal immunosuppressant), 3 daily doses of diphenoxylate (an opioid derivative that decreases intestinal motility), or a very large infection (7500 L3) of S. stercoralis. Transfer of adult worms into naive gerbils also gave rise to a short period of autoinfection. Low density infections (5 to 10 adult worms), 1 or 2 daily doses of diphenoxylate, or treatment with indomethacin (a non-steroidal antiinflammatory drug) did not lead to autoinfection. (Supported by NIH Grant AI-22662.)

# 133

The Homogonic Life Cycle of Strongyloides robustus. RALPH P. ECKERLIN\*
The homogonic life cycle of Strongyloides robustus, an intestinal nematode parasite of squirrels, was completed in the laboratory and life cycle stages are described. Eggs from naturally infected gray squirrels, Sciurus carolinensis, were cultured to yield the rhabditiform L1 which hatched in 3 hrs and molted to the L2 after 12 hrs or more at room temperature. Considerable growth and development occurred during the second stage. The esophagus changed from the rhabditiform to the filariform type before the second molt occurred at 46 or more hours. The filariform L3 with a quadrifurcate tail tip was an active swimmer and was the infective stage for the vertebrate host. The L3s penetrated unbroken skin of golden hamsters, Mesocricetus auratus, which served as suitable experimental hosts. Larvae migrated from the skin and were found in the blood and lungs within 48 hrs. They reached the small intestine within 48 hrs. The first parasitic molt occurred in the small intestine at 84 hrs to an L4 with a pointed tail. The reproductive system assumed the adult outline but was not yet functional. The second parasitic molt occurred at 144 hrs also in the host intestine. Adult parthenogenetic females with knobbed tails became ovigerous on day 6 but eggs did not appear in the host feces until day 7.

# 134

The number of moults in the egg of sealworm, <u>Pseudoterranova</u> <u>decipiens</u>. LENA N. MEASURES\* AND HENRY HONG

The number of moults in the egg of sealworm, P. decipiens (Nematoda: Ascaridoidea), and other ascaridoids contentious. Transmission is electron microscopic analysis and free-living larvae eggs sealworm confirmed that only moult occurs in the egg. The firststage larval (L,) cuticle on embryos was first observed in eggs incubated at 15°C in sea water on Day 5 after eggs were dissected from the uterus of sealworm obtained from stomach of grey seals. There was no ecdysis of this L<sub>i</sub> cuticle. second cuticle began to form beneath the L, cuticle between Day 5 to 12. The second-stage larval (L,) cuticle

continued to develop and on Day 12 the  $L_2$  hatched enclosed within the cuticle of the first stage. The  $L_1$  cuticle appeared to be partially resorbed during development of the  $L_2$  cuticle. This study provides the first ultrastructural evidence of the number of moults that occur in eggs of sealworm.

# 135

Uniformity in the development and life history of ascaridoid nematodes. H.-P. FAGERHOLM\*, M. KØIE,

A. ROEPSTORFF, and P. NANSEN

Studies have shown that the ascaridoids, containing more than 50 recognised genera, constitute a structurally uniform nematode group. Current studies also point to a marked uniformity in the early and the late phases of their development. It has been shown that in both terrestrial and aquatic taxa that the first and second ecdysis occurs inside the parasite egg. Consequently it is the third-stage larva, often ensheathed by the second-stage cuticula, which emerges from the egg. In terrestrial forms, the larvae emerge only after reaching a host. In aquatic forms, the eggs often hatch in the water. After transmission to paratenic (optional) and intermediate hosts (in some species a migratory phase in the final host), the third-stage larvae reach the gastrointestinal tract of the final host where the third ecdysis occurs within a few days. Later the adult male and female worms develop after an additional ecdysis. In the present work, phases of the life cycle are analysed experimentally in Ascaris sp. in pigs and in Contracaecum sp. from aquatic hosts. Based on results available, we suggest that the type of development described above occurs generally among the ascaridoids. Exceptions to this pattern are evidently rare. Aberrant variations include, for example, cases of development of adult worms of the parasite Raphidascaris acus in the muscles of the fish Osmerus eperlanus (Fagerholm & Ruotsalainen, unpubl.). The occurrence of the first and second ecdysis inside the ascaridoid egg is considered a synapomorphic feature of evolutionary interest.

# 136

In Vitro Production of First Generation Schistosoma mansoni Daughter Sporocysts. T. P.YOSHINO\* and J. R. LAURSEN

Schistosoma mansoni daughter sporocysts (DS) were produced from miracidium-derived mother sporocysts (MS) by synxenic larval cultivation of MS with cells of the Biomphalaria glabrata embryonic (Bge) cell line. In vitro growth and viability of the MS co-cultured with Bge cells or in Bge cell-conditioned medium were significantly extended beyond that of larvae cultured in fresh medium alone. However, complete DS development and emergence from MS were achieved only in the presence of Bge cells. The timing of in vitro DS

development from culture to culture was somewhat variable. However, in general, early embryo formation (i.e., germ cell aggregation and formation of primitive epithelium) was first detected at 15-20 days postculture (pc), while motile, intra-MS daughter stages were observed at 25-30 days pc and thereafter. Mature, first generation DS, measuring  $136 \pm 46$  um long by  $22 \pm 6$  um wide, emerged from MS starting approximately 30-45 days pc. Although the basic morphology and size of emergent, in vitro-derived DS were comparable to those propagated in vivo, there was a large reduction in the in vitro reproductive capacity of the MS and a temporal delay in DS development in culture. Bge cells provide an acceptable physiological and/or physical environment for in vitro MS-to-DS development, although conditions optimal for MS and DS growth, and those permitting continued DS-to-cercarial stage formation have yet to be defined. (Support by NIH Grant Al 15503)

### 137

Complete Development of <u>Cryptosporidium</u> parvum in Bovine Fallopian Tube Epithelial Cells. SHIGUANG YANG\*, MARK C. HEALEY, CHUNWEI DU, and JIANFEI ZHANG

Cryptosporidium parvum is a coccidian parasite responsible for causing unrelenting diarrheal illness in humans and other animals. Tremendous effort has been devoted to developing in vivo and in vitro models for cryptosporidiosis. This study is the first to demonstrate that C. parvum can complete its entire life cycle (from sporozoite to infective oocyst) in a primary culture of bovine Fallopian tube epithelial (BFTE) cells. Scanning and transmission electron photomicrographs were used to detail the ultrastructure of individual parasitic stages. Successful infections were produced by using either purified sporozoites or oocysts as the cell culture inoculum. Infection of BFTE cells with <u>C. parvum</u> closely paralleled in vivo infections with regard to host cell location and chronology of parasite development. Infecting BFTE cells with sporulated oocysts provided a reproducible and quantitative cultivation system with significantly (P<0.001) higher infection rates than Madin-Darby canine kidney cells. Oocysts produced in BFTE cells were infective for immunosuppressed adult C57BL/6N mice. Cultivation of <u>C. parvum</u> in BFTE cells will enable investigators to further study interactions between the parasite and the host cell and provide a reliable system for evaluating potentially efficacious compounds.

### 138

Immunoblot Analysis of Wildlife Sera Using Cultured Sarcocystis neurona Merozoites. D.E. GRANSTROM, J.P. DUBEY, J.M. DONAHUE, Z. XIAOMIN, K.K. POONACHA, N.M. WILLIAMS, J. PATTERSON, J. MARTENIUK, S. STAMPER, S.M. SCHNEIDER, C.K. FENGER, B.D. SNYDER.

Specimens from 215 wildlife of various species were collected from horse farms in Kentucky, Michigan, and Panama. Histologically-confirmed cases of equine protozoal myeloencephalitis (EPM) had occurred on most farms in the study. Blood, feces, diaphragm, heart, and small intestine were obtained from carnivores and omnnivores. Small intestine was not taken from herbivores. Sera were evaluated for the presence of antibodies directed against proteins considered unique to Sarcocystis neurona by immunoblot analysis. Twenty-two of 37 skunk sera had S. neurona-specific antibodies. Sera from 1 skunk, 6 of 72 raccoons, 5 of 8 rabbits, and I of 5 mice contained antibodies that recognized epitopes shared with other Sarcocystis spp. Sarcocystis-like sporocysts were found in intestinal digests from 4 skunks and 3 raccoons. Sera from 2 skunks with sporocysts also contained S. neurona-specific antibodies.

# 139

Comparative Pathology and Pathogenesis of Avian Sarcocystis falcatula infection. JEROME H SMITH, MD\*

Infections of varying durations in budgerigars, pigeons and canaries with controlled doses of oocysts of Sarcocystis falcatula are studied morphometrically by light and electron microscopy. Parasite morphology, host response and pathologic consequences of varying infective dosage, duration and host species are presented with qualitative and quantitative data. After inital merogony in liver, successive waves of merogony progress from capillaries and venules, then pulmonary veins of the lungs, prior to skeletal muscular encystation. Localization of cysts among various muscles appears to differ with host species. High dose infections often produce death early in the course of the disease while lesser infective loads may compromise locomotion later in the disease course. Implications and unresolved issues for further study are posed.

# 140

Which Factors Determine a Species of Hepatozoon (Apicomplexa: Adeleina)? TODD G. SMITH\*, SHERWIN S. DESSER, and SUSAN H. KOPKO

In the course of investigations of the distribution of the protozoon Hepatozoon sipedon, an intraerythrocytic parasite of Northern water snakes (Nerodia sipedon sipedon) from eastern Ontario, Canada, it has become apparent that more than one species of Hepatozoon infects various species of snakes in Ontario. Determining which of the numerous forms from different hosts and localities in the province actually constitute discrete species has raised some interesting questions about apicomplexan taxonomy. Differences in certain morphological and cytopathological characteristics have been observed amongst the various forms of the parasite, which, taken alone, would suggest a multitude of species of Hepatozoon infecting Ontario snakes. Life cycle and host-specificity data, used extensively to define species of parasitic organisms, have defined at least two groups of species, but have been of limited value for recognizing species within these groups, Investigating the extent of genetic variation among different forms of Hepatozoon by analyzing DNA fingerprints and determining the potential of hybridization between putative species of Hepatozoon in their mosquito hosts provide means for examining more sensitive species-defining criteria. The suitability of all these aforementioned criteria to forms of Hepatozoon will be evaluated in the context of defining a species concept for the genus.

# 141

Veterinary Aspects of "Burnper Car" Disease, Caused by *Anophryoides* sp. (Scuticociliatida), in the Lobster Fishery, R. CAWTHORN\*, L. HAMMELL, B. HORNEY, F. MARKHAM, M. MOVOTNY, D. SPEARE and K. BROWN

The lobster fishery, valued at \$600 million US in 1993-94 in eastern North America, has post-harvest losses which are 10-15% of landed values. "Bumper car" disease, caused by a species of Anophryoides (Scuticociliatida) can cause losses in coldwater impoundments. Outbreaks occur more frequently and with greater severity; epidemiological factors are unknown, A culture of Anophryoides sp. from a wildcaught Maine lobster (Homarus americanus), was infectious and lethal to lobsters after 16 months in vitro at 5 C. Cultured ciliates (in sterile seawater with lobster muscle) required longer and more parasites to kill lobsters, than ciliates transmitted by intrahaemocoelic injection from lobster-to-lobster. The larger the inoculum, the more rapid the death of lobsters. Ciliates were detected sooner, histologically, than by haemolymph examination or culture. Monoclonal antibodies, prepared to sonicated ciliates, were used for indirect fluorescent antibody testing of haemolymph and tissue sections, and for immunoperoxidase staining of tissue sections. Ciliates sequester in gill and muscle tissues. In heavily infected lobsters, haemocyte numbers decrease to zero; arginase kinase (indicative of muscle damage), and an analogue of adrenocorticotrophic hormone are greatly elevated. In vivo, horizontal transmission of Anophryoides sp. likely occurs by

gill penetration of freeswimming ciliates. Disinfectants and therapeutants licensed for use in food-producing animals, are efficacious against *Anophryoides* sp. in vitro. The *Anophryoides* parasite is useful to model health and infectious disease and processes of lobsters.

(Supported by NSERC and NRC-IRAP).

### 142

Acute fulminating babesiosis in hamsters with Babesia microti: an experimental model. HELIEH S. OZ\* AND WALTER T. HUGHES

Human babesiosis (B.microti) in immunocompromized, splenectomized and elderly patients is increasing in frequency. Safe and effective drugs are needed. purpose of this study was to develop an animal model that mimicked the human disease and manifested extensive parasitemia, clinical disease and death due to babesiosis. The B. microti (ATCC30222) maintained in the laboratory mouse was adapted to golden hamsters. The hemoprotozoa was serially passaged to corticosteroid immunosuppressed hamsters and then adapted to normal hamsters. When 30 hamsters were inoculated IP with this strain, parasitemia reached 8% of erythrocytes by day 3 and increased to 72% by day 7 when 70% of hamsters (21/30) died. By day 12 parasitemia extended to 85-90%, with 97% (29/30) mortality. Hearts and kidneys from infected animals weighed respectively 25% and 30% over normal hamsters. Scanning and transmission electron microscopy, direct smears, Giemsa, and acridine orange staining of parasite were compared. (Supported by NIA Grant RO1-AI 20673 Cancer P30 CA21765 and the American Lebanese Syrean Associated Charities)

# 143

Intermediate Host Life History Changes in Response to Echinostomatid Trematode Infections. ROBERT E. SORENSEN\*, AND D. J. MINCHELLA

Considerable attention has been devoted to exploring the relationship between schistosomatid parasitism and intermediate host life history parameters. The paucity of snail life history studies involving other digeneans, specifically those requiring redial intramolluscan stages, limits our understanding of this interaction and potentially skews our interpretation of evolutionary processes. To garner a more clear and comprehensive understanding of the influence that trematode parasitism imposes on snail growth, fecundity, and survival, I employed field surveys and laboratory exposure methodologies with members of the family Echinostomatidae. These results indicate that parasitic castration is a widespread phenomena among freshwater pulmonates infected with echinostomes since none of the more than two hundred, field-collected,

echinostome-infected snails laved eggs when isolated following their collection. The size of these infected snails frequently exceeded their uninfected conspecifics, but this outcome varied strongly and depends upon the trematode and time of year. Likewise, the average lifespan of infected individuals depended upon the snail's size when collected and the infecting fluke. To further assess the impact of trematode parasitism involving redial stages on snail fecundity, experimental infections of Lymnaea elodes and Helisoma trivolvis snails with Echinoparyphium flexum and Echinostoma trivolvis flukes, respectively, presented evidence of a fecundity compensation response only among the L. elodes snails, although both trematode's redial stages effectively castrated their host. Concurrent with castration was evidence of increased snail growth for both H. trivolvis and L. elodes.

### 144

Characterization of a Trematode-Induced Plasma Protein from *Biomphalaria glabrata* Capable of Interacting with Trematode Antigens. LYNN A. HERTEL\*, COEN M. ADEMA and ERIC S. LOKER

We are interested in the structure and function of molecules produced by invertebrates that play a role in recognition of non-self and in initiating protective responses following infection. Exposure of the snail Biomphalaria glabrata to infection with the digenean Echinostoma paraensei has been shown previously to provoke increased production of selected host plasma proteins and thus provides a relevant model for study. Addition of varying quantities of secretory/excretory products (SEP) derived from cultured E. paraensei sporocysts to plasma from E. paraensei-exposed snails results in formation of conspicuous precipitates that are highly enriched in these previously described plasma proteins. The same approach has also permitted the identification of a plasma polypeptide (65 kDa by SDS-PAGE) not previously noted. Formation of precipitates containing these molecules does not occur following addition of SEP to plasma from unexposed control snails or from snails exposed to Schistosoma mansoni infection. The 65 kDa polypeptide forms covalently associated penta- or hexamers in its native configuration and these multimers further aggregate to form complexes of over 1 million kDa. Tryptic digests of the 65 kDa molecule were undertaken and 4 pentides obtained were sequenced and used to design primers for use in PCR. Using M line B. glabrata genomic DNA as template, a specific PCR product of 0.32 kb was obtained and sequenced and portions were found to have homology with vertebrate fibrinogen and tenascin. This study was supported by NIH grant RO1 AI24340.

# 145

Host-parasite interactions between Biomphalaria glabrata and Schistosoma mansoni: investigations of cell mediated cytotoxicity using an in vitro assay. SARAH E. FRYER\* and CHRISTOPHER J. BAYNE

An in vitro cell mediated cytotoxicity assay monitoring interactions between Biomphalaria glabrata hemolymph components and primary sporocysts of Schistosoma mansoni has been modified to allow evaluation of interactions over a 40 hour period. This has enabled us to investigate the rate of parasite killing in hemolymph from molluscan host strains differing in susceptibility, with repeat observations being possible on individual preparations. Sporocysts survive well in hemolymph from susceptible snails, with an average survival of 80% after 40 hours. In contrast, less than 20% of parasites were alive after 40 hours incubation in hemolymph from resistant snails. This assay has been used to investigate the mechanisms of parasite recognition and destruction. The cystein protease inhibitor E64, known to inactivate a protease released by the parasite, did not alter killing by hemocytes from either host strain, indicating that this parasite protease is not involved in determining the outcome of host-parasite encounters. Addition of mammalian cytokines IL-1β and TNF-α similarly had no effect on the rate of parasite destruction. Experiments designed to investigate the role of reactive oxygen species in cytotoxicity revealed that addition of catalase actually increased the rate of sporocyst destruction by hemocytes from resistant snails. Supported by NIH grant AI-16137.

# 146

Phenoloxidase Activity in the Reproductive System of <u>Biomphalaria glabrata</u>: Identification, Characterization and Effect of Schistosome Infection. G. BAI\*, J. LI, B. M. CHRISTENSEN, T. P. YOSHINO

Previous studies have shown that larval Schistosoma man<u>soni</u> infection causes a cessation of egg production in its intermediate host, B. glabrata, called parasitic castration. The involvement of phenoloxidase (PO) in egg formation has been suggested in other invertebrate animals. In this study, PO activities in the reproductive system and egg masses of B. glabrata were identified and partially characterized using an in vitro enzyme assay, high performance liquid chromatography with electrochemical detection and colorimetric techniques. Both albumen gland (AG) and egg mass extracts eatalyzed L-tyrosine hydroxylation (monophenol oxidase activity, MPO) and L-dopa oxidation (diphenol oxidase activity, DPO). However, no PO activity was found in the ovotestis. Both MPO and DPO activities in AG and egg masses were significantly inhibited by 1-phenylthiourea and completely inactivated by boiling. Nacetyl dopamine, a diphenolic compound, climinated the lag period during tyrosine hydroxylation by this enzyme, further supporting its identity as a PO. Temperature and pH optima for this enzyme were found to be 30 C and 7.5, respectively. Two forms of the cuzyme were detected using size exclusion HPLC. To assess the involvement of this enzyme in S. mansoniinduced parasitic castration, PO activities in AG of infected and control snails were measured at 14, 21 and 28 days post-infection (PI). The results showed that both specific and total enzyme activities in AG of infected snails were significantly inhibited at 28 days PI when compared to those of control snails. It is hypothesized that larval <u>S. mansoni</u> infection may exert its inhibitory impact on egg production of <u>B. glabrata</u> through modulation of the PO activities. (Supported by NIH grant AI 28781)

### 147

Lectin Binding Characteristics of the Salivary Glands of Anopheles gambiae sensu stricto. LUCY ANDREWS\* and

#### BARBARA SINA

Malaria sporozoites selectively invade the medial and distal lateral regions of the female salivary glands of vector anopheline mosquitoes. These regions are not found in male salivary glands which do not take a blood meal, and do not transmit malaria. The basal lamina of the different regions of the female salivary glands have structural and histochemical differences which are reflected in the surface carbohydrate residues. Cell surface carbohydrates have been implicated in the recognition of host red blood cells by merozoites. Whether differences in the lectin binding characteristics of the female salivary glands may be associated with sporozoite recognition of specific regions of the salivary gland will be tested. Carbohydrate-containing residues in male and female salivary glands of Anopheles gambiae sensu stricto were analyzed with seven biotin-labeled lectins by western blot. The results showed distinct differences in the lectin binding characteristics of male and female salivary glands, with each lectin having a characteristic binding pattern. Six to eight female specific salivary gland glycoproteins were identified. These lectins could be used in further characterizing female salivary gland glycoproteins to determine their possible role in invasion by sporozoites.

# 148

Efficacy of Crystal Toxin of Bacillus thuringiensis against Schistosomula of Schistosoma japonicum. YAO BAOAN\*, ZHAO JINLONG, MA LIHUA, WANG QIANLAN and YU ZINIU.

The schistosomula of Schistosoma japonicum were transformed from cercariae released from the snails by syringe method and incubated in the RPMI-1640 medium, which contained 10% rabbit serum maintained in monoclonal plate in the incubator at

37°C, 5% CO<sub>2</sub>. The crystal toxin from the bacterium *Bacillus thuringiensis* strain YBR-1955. Exposure of about 1,000 schistosomula to 200 µg total protein/ml of *B. thuringiensis* in the hole of plate at 37°C, 5% CO<sub>2</sub> in vitro. The activity of the schistosomula became low and some of the worms died after being incubated for 12 h as observed under microscope. The mortality increased with the time of incubation. The mortality of the schistosomula was 98.7% after 24 h. Crystalline toxin from *B. thuringiensis* strain YBR-1955 was lethal to schistosomula of *S. japonicum in vitro*. (Supported by Office of Schistosomiasis & Endemic Diseases Prevention & Treatment, Hubei Province, China.)

#### 149

Enhancement of cellular immune response by androgen reconstitution of mice during Taenia crassiceps cysticercosis J.MORALES , L.I.TERRA ZAS, T.GOVEZENSKY, M.C.ROMANO AND C.LARRALDE. Susceptibility to the infection in mice by Taen la crassiceps cysticerci is associated with sex: in early infections females carry larger parasi te loads than males althought, later on, males also become massively parasitized. The males develop great endocrinological changes: serum estra diol is increased to levels 200 times their normal values whilst those of testosterone are 90% decreased and the weight of the seminal vesicles is significantly diminished. Female mice do not undergo marked endocrinological changes. In gona dectomized mice the parasite loads increase in males and decrease in females whilst the steroids levels are not detectable in serum. The cellular immune response is decreased whilst the humoral has not change. The aim of this work was to deter minate if androgens mediate resistance to Taenia crassiceps and if any immunological mechanisms are involved in this effect. Here we show that testosterone and dihidrotestosterone decreases 50 percent parasite loads in gonadectomized mice. The cellular immune response (measured as con-A response of the lymphocytes of this reconstituted animals), the IL-2 and IFN-gama production are recovered at normal levels by each treatment. By other way, the humoral immune response to the cysticerci has not change. This results suggest than the androgens are protectors against metacestode infection, and this protection is trought the cellular immune response, whilst humoral response has not change. This progect was supported by CONACyT and DGAPA.

# 150

Radiation-induced Reduction in Cathepsin Protease Expression by Newly Excysted Juvenile Fasciola hepatica.

JENETTE CREANEY\*, L. R. WILSON, R. M. SANDEMAN, T. W. SPITHILL and J. C. PARSONS

Radiation has been successful in the attenuation of infective stage parasites for their use as vaccines against a number of parasites including Fasciola spp. The mechanisms of action of radiationattenuated vaccines, however, are not clearly understood. In this study, we examined the effect of 3, 10 and 40 krad of y-irradiation on the expression of candidate fascioliasis vaccine antigens by newly excysted juvenile Fasciola hepatica (NEJ) derived from nonirradiated and irradiated metacercariae. Cathepsin proteases are a major component of liver fluke excretory/secretory material (ES) and can cleave host immunoglobulin (Ig). Protease activity of ES samples collected over a 24 h period from irradiated and nonirradiated NEJ cultured in vitro, were tested using a rabbit Ig cleavage assay. The proteolytic activity of ES from 10- and 40-krad-irradiated NEJ was reduced during the initial six hours in culture and between 12 - 24 h when compared to ES from non-irradiated controls. Irradiated and non-irradiated fluke remained viable during 24 h in vitro culture. Cathepsin protease was localised in non-irradiated NEJ to within the gut lumen and to secretory granules within the gut epithelia. Irradiation of fluke with 3, 10 and 40 krad of γ-rays significantly reduced the tissue expression of cathepsin at 8 h postirradiation (PI) in an apparently dose-dependent manner. After a further 24h culture in vitro, tissue expression of cathepsin protease was significantly reduced in 10- and 40-krad-irradiated NEJ. The morphological integrity of the intestinal epithelium was unaltered in 3-, 10- and 40-krad-irradiated fluke at 8 h PI. No alteration in the tissue expression of glutathione S-transferase, paramyosin or the serine protease homologue, FhKTM, was observed in irradiated NEJ. The relationship between a radiation-induced reduction in both proteolytic activity and tissue expression of cathepsin protease and the action of radiation-attenuated vaccines is not clear. Reduced cathepsin activity may either be detrimental to the ability of the parasite to invade and induce pathology in the host, thus enabling the host to mount a protective immune response, or, may simply be one of the first indications of a general failure of parasite function following irradiation.

### 151

Characterization of 26-28 KiloDalton Fasciola hepatica Immunodiagnostic Coproantigen. SALMA M ABDEL-RAHMAN\*, KATHY L. O'REILLY, DAVID H, SWENSON, and JOHN B. MALONE Standard fecal sedimentation procedures for quantitating fecal egg counts are time consuming and prone to technical error. As a first step toward the development of a rapid diagnostic assay we have developed a quantitative Western blot that detects a 26-28 KD coproantigen of Fasciola hepatica using monoclonal antibodies (Mab). reports the initial biochemical characterization of this antigen. Differential staining of the separated coproantigen on SDS gel under reducing and non-reducing conditions indicates that the coproantigen is a monomeric highly glycosylated glycoprotein. We have determined that these Mab do not bind the native antigen, we therefore purified the antigen using molecular sieving followed by Western blot, and have confirmed the 26-28 Kd size. The effect of a variety of denaturing agents on antigenicity were examined and revealed that all four

Mab, bind the 8 KD protein core of a stable 25-28 KD Oglycosylated glycoprotein present in the feces of infected animals. Potential protease activity of this antigen was also assayed using 1% gelatin SDS; no protease activity was detected Using these Mab in indirect immunofluorescence, the antigen was localized to gut cells and tegument of the adult fluke. Current experiments include the determination of the sensitivity of Western blot as well as development of rapid ELISA assay for coproantigen.

# 152

Canine Lyme Disease (LD): Safety, Efficacy, and Duration of Immunity of an OspA Vaccine.

#### JC JARECKI-BLACK\* and RE WIKLE

A variety of mammals are susceptible to infection with the spirochete Borrelia burgdorferi. In dogs such infection may be inapparent, or manifested by clinical signs typical of acute arthritis and arthralgia. Vaccination, as an aid in the control of canine LD, currently relies upon the use of commercial bacterins. However, concerns about the safety and efficacy of such whole cell vaccines have been raised. since some of the superfluous antigens may actually have a deleterious effect upon the immune system. Previous reports have suggested that significant protection against LD may be elicited by a vaccine containing the major outer surface protein (OspA) of B. burgdorferi. The purpose of this study was to determine the safety and duration of immunity of such vaccine-induced protection. Fortyone beagles (9 to 10 weeks of age) were vaccinated subcutaneously with two doses of an OspA vaccine, while 25 dogs served as untreated controls. All dogs were challenged by exposure to naturally-infected ticks. Antibody levels were determined at regular intervals by ELISA, and vaccine efficacy was assessed using spirochete reisolation at monthly intervals following challenge. Dogs were also monitored for clinical signs. Results showed that the vaccine induced seroconversion in ≥90% of recipients after the second injection. Significant protection against infection was also evident, as assessed by a reduction in spirochete proliferation (95%) and prevention of clinical disease (100%) in vaccinates as compared to controls. Protection provided by the OspA vaccine was still evident even when challenge was 5 to 6 months postvaccination. No adverse reactions following vaccination were evident in any of the puppies in this study. Other safety trials have shown that this vaccine is safe even when administered in large doses to dogs exhibiting active clinical disease. The results of these studies show that OspA vaccination is both safe and efficacious, as evidenced by a reduction in spirochete reisolation and the prevention of clinical disease in recipient dogs.

#### 153

The Efficacy of Serology, Blood Smear Evaluation, and Polymerase Chain Reaction in the Diagnosis of Ehrlichial Infections in Dogs. S. A. EWING\*, J. C. FOX, J. S. MATHEW, G. L. MURPHY, K. M. KOCAN, AND E. F.BLOUIN

Canine chrlichiosis is recognized to be a complex tickborne disease characterized by acute, subclinical and chronic phases, and its protean nature presents diagnostic difficulties. Accurate diagnosis is essential for planning treatment and control measures. A study utilizing experimental infections was conducted to compare the efficacy of serologic tests, light microscopic evaluation of blood smears, and polymerase chain reaction (PCR) in diagnosis. Data suggest that serology is appropriate for identifying subclinical and chronic cases but may not be useful in the acute phase of disease. Blood smear evaluation, using light microscopy, revealed morulae during the acute phase of infection. PCR proved useful in diagnosis as early as eight days post exposure, several days before morulae were detected in peripheral blood; subclinical and chronic infections were also detectable by PCR.

### 154

Antigenic Analysis of Encephalitozoon cuniculi (CDC:V282) Isolated from the urine of a patient with AIDS. G.P. CROPPO\*, G.S.VISVESVARA, G.J. LEITCH, S. WALLACE, M. A. DE GROOTE, and R. REVES

Microsporidia spores, isolated from the urine of an AIDS patient with sinus and blurred dry eyes, congestion, vision, were established in continuous culture on monkey kidney cell cultures. Based on TEM, IIF, and PCR analysis of the cultured parasites, they were identified as Encephalitozoon cuniculi (De Groote M., et al., JID, in press). Immunoblot studies using the patient serum (dilution 1:400) and the rabbit sera made against E. cuniculi (CDC: V282), E. hellem (CDC: 0291: V213) and Septata intestinalis (CDC: V297) revealed that CDC: V282 and the rabbit isolate (ECLD) reacted intensely with patient serum and the rabbit anti-CDC: V282, and produced a number of bands ranging in molecular weight from 200 to 15, kDa whereas the heterologous reacted minimally. Both antigens and E. CDC: V282 ECLD strains cuniculi reacted minimally with the rabbit anti-E. hellem and the rabbit intestinalis sera. These anti-S. confirm the further studies identification of CDC: V282 cuniculi. We conclude that human and rabbit strains of E. cuniculi have similar antigenic profiles but differ hellem considerably from E . S.intestinalis.

# 155

Cytokine Production in Balb/c Mice Infected with Giardia muris.

KIS DJAMIATUN' and G.M. FAUBERT.

In a previous study it was found that IgA, macrophages and T-helper cells, played a role in controlling Giardia infections. However the role of T-helper cells which is important in eliminating Giardia infection is not known. It has been shown that macrophages treated with recombinant Interferon-gamma (rIFN-y) and bacterial lypopolysaccharide ingested a higher number of in vitro-grown G. lamblia trophozoites than untreated cells. We have examined the levels of IL-5, IL-4 and IFN-y released by spleen and Peyer's patches from G. muris infected mice. Balb/c mice infected with 1000 G. muris cyst were killed on days 5, 15 and 35 which represents the latent, acute and elimination phases, respectively. Spleen and pooled Peyer's patch cells were stimulated with Con A in 48 hour cultures and cytokine levels were measured by ELISA. Spleen cell cultures showed an increased production of IL-5, IL-4 and IFN-y during latent and acute phases. Pooled Peyer's patch cells showed an undetectable level of IL-5 in the latent phase. However, IL-5 was detectable in acute and elimination phases. In contrast IFN-y levels were comparable to control mice during the latent and elimination phase, but it decreased in the acute phase. Peyer's patch cell cultures also exhibited low level of IL-4 throughout the infection. These result suggested that IL-5, IL-4 and IFN-y are likely to play a role in controlling G. muris infection. The cytokine pattern released by spleen and Peyer's patches were different during the latent, acute and elimination phases.

# 156

Cloning of Bovine Interleukin 12 and Its Application in Developing a Simple Technique for Synthesizing Competitor Molecules for RT-PCR.

D.S. ZARLENGA\*, A.CANALS AND L.C. GASBARRE

cDNA generated from stimulated abomasal lymph node cells was used to amplify and clone the 35 kDa and 40 kDa subunits of bovine interleukin 12 (IL-12) using primers derived from semi-conserved regions between human and mouse IL-12 sequences. The deduced amino acid sequence of the 40 kDa subunit demonstrated 84.4% and 67.6% homology with human and mouse sequences, respectively. The deduced sequence of the 35 kDa subunit exhibited comparable similarities to the human 35 kDa subunit (82.2%) but differed significantly (58.6%) from mousederived sequences. A simple and rapid technique was subsequently developed for the generation of IL12 subunit competitor molecules that can be utilized as exogenous controls during competitive RT-PCR. This technique, which requires a single PCR amplification and a previously cloned sequence, uses internal primers separated by a predefined distance and designed to amplify in opposite directions such that the entire vector is coamplified with the cloned insert. The resultant product is a competitor molecule which contains a region of sequence deletion

relative to the native cDNA and therefore is smaller than the naturally-derived cDNA sequence. The development of this methodology has allowed studies on IL-12 gene expression in cattle infected with gastrointestinal nematodes.

#### 157

Application of DNA Heteroduplex Assay in Population Genetics and Molecular Diagnosis of Parasite Vectors. JIANMING TANG\*, KENNETH PRUESS, and THOMAS R. UNNASCH

DNA heteroduplex assay (HDA) and directed heteroduplex analysis (DHDA) have been applied to classify members of Simulium black flies (Diptera: Simuliidae), which serve as vectors for human and bovine onchocerciasis. Samples collected from West Africa and North America are readily identified using a number of mitochondrial DNAs including subregions. of the large (16S) and small (12S) subunit (16S) of rRNA genes, the NADH dehydrogenase subunit 4 (ND4), and cytochrome c oxidase II (COII). In general, protein coding genes are more informative than the rRNA genes. Both tests are highly sensitive. being able to detect polymorphic sequences that differ only by 1 base pair. Moreover, the tests are simple and cost-effective to perform when compared with other types of DNA hybridization methods. Here we report the recent use of HDA in studies of population genetics, molecular diagnosis as well as phylogenetic analysis. Our results indicate that HDA will open another avenue to explore molecular characteristics of parasite vectors and other organisms alike.

# 158

Interactions of *Plasmodium falciparum* Erythrocyte Membrane-Associated Proteins with Abnormal Erythrocyte Membranes CATHLEEN MAGOWAN\*, ROSS COPPEL, MARIO MORONNE, and NARLA MOHANDAS

Previous studies had indicated that abnormal erythrocytes, deficient in skeletal protein 4.1, do not support intraerythrocytic development of *P. falciparum* malaria parasites as well as normal erythrocytes. We used protein 4.1 deficient parasitized cells to investigate three parasite encoded proteins that associate with the host erythrocyte membrane. Knob-associated histidine

rich protein (KAHRP) associates with spectrin and is involved in formation of knobs, 100 nm protrusions of the red cell membrane. The cytoskeletal target of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) has not been identified, but expression of this malarial protein is correlated with new adhesive properties of the infected red cell. Mature parasite-infected erythrocyte surface antigen (MESA) associates with erythrocyte skeletal protein 4.1, but the consequences of this binding are unknown.

We have demonstrated that expression and localization of KAHRP are not influenced by the absence of protein 4.1. Likewise, the cytoadherence phenotype of infected erythrocytes is independent of protein 4.1, indicating that 4.1 is not a target for PfEMP1. Most significantly, protein 4.1 deficiency affects targeting of MESA, which in the absence of 4.1 accumulates in the erythrocyte cytosol, leading to poor parasite survival.

We infected 4.1 deficient erythrocytes with spontaneous parasite mutants that do not express MESA. The expression of KAHRP and PfEMP1 were not affected in this system. Surprisingly, in contrast with MESA(+) parasites, MESA(-) parasites were able to grow as well in 4.1 deficient cells as in normal erythrocytes.

# 159

# β-Tubulin Gene of Eimeria tenella (Coccidia). GUAN ZHU\* and JANET S. KEITHLY

A fragment of Eimeria tenella \beta-tubulin genomic DNA was obtained by PCR with a pair of degenerate primers based on conserved amino acids of the protein. The product was cloned and used for screening a cDNA library of E. tenella. Several cDNA clones of the gene were obtained and sequenced. The coding region of the gene was characterized by the high GC content (60%) and extremely low usage of dinucleotide TA. The codons of TTA, CGA, CTA, ATA, GTG and TAT were infrequently used by E. tenella in the  $\beta$ -tubulin gene as well as other reported genes. Primers designed according to the sequences of 5' and 3' UTRs were used for the amplification of the coding region of the gene from genomic DNA of E. tenella, which revealed that there were three introns present in the β-tubulin gene. All introns of the gene are defined by 5' GTGPuG. .(Py)nNCAG 3'. The deduced protein has 449 amino acids with a molecular weight of 49.9 kDa. The amino acid at position 200 related to the binding of benzimidazoles is Gln instead of Phe, suggesting that the organism might be insensitive to benzimidazoles. Another amino acid at position 239 is Ser, instead of Cys present in other colchicine sensitive organisms, predicting that E. tenella is resistant to colchicine and its analogues, too. Southern blot, quantitation assay and PCR analyses show that \( \beta \)-tubulin is a single copy gene of E. tenella, Both unsporulated oocysts and free sporozoites of E. tenella contained similar levels of  $\beta$ -tubulin mRNA as shown by Northern blot analysis.

# 160

Effect of conditioned culture medium on invasion of cells by <u>Eimeria adenoeides</u> sporozoites. P. C. AUGUSTINE\*

When included as 50% of the inoculation medium, conditioned medium from cultured turkey cecal cells (TCC) significantly increased invasion of several cell types by Eimeria adenoeides sporozoites as compared with invasion in fresh medium (FM). If the incubation period was reduced from 3 hr to 30 min postinoculation, there was no significant difference in invasion between the TCC and FM groups. Retentates from TCC subjected to microconcentrators having a molecular weight cutoff of 300 kDa had invasion-enhancing activity similar to that of the whole medium. Sporozoites pretreated with TCC or FM for up to 3 hr before inoculation showed no significant difference in invasion. In contrast to the TCC, medium from cultured hamster kidney cells (BHK) did not cause an increase in invasion. Silver stained gels of TCC, BHK, and FM culture media electrophoresed under reducing conditions suggest a quantitative difference in two bands migrating well above the 200 kDa molecular weight standard.

# 161

Detection of <u>Sarcocystis neurona</u> DNA in Blood, Cerebrospinal Fluid and Feces by Polymerase Chain Reaction. C.K. FENGER\*, D.E. GRANSTROM, J.L. LANGEMEIER, S. STAMPER, Z. XIAOMIN, A. GAJADHAR, J. PATTERSON, J. MARTENINK, J.P. DUBEY.

The small ribosomal subunit gene (SSURNA) of S. neurona was amplified using universal primers, and sequenced. A unique region of the gene was identified by comparison of this sequence to the SSURNA gene of S. muris, S. cruzi, and T. gondii, and used for unique primer This primer was employed in a nested PCR protocol using DNA of S. neurona and other sporozoan species (S. muris, S. cruzi, S. fayeri, S. rileyi, S. campestris, Toxoplasma gondii, Besnoitia spp., and Eimeria spp.) and found to be specific. Sensitivity was found to be less than 100 copies of the gene in 10 µl of solution. The nested PCR reaction was applied to whole blood samples and the cerebrospinal fluid samples. Sarcocystis neurona could be detected in peripheral blood of foals by PCR over the course of one year, indicating transient detectable parasitemia. Foals were commonly positive two months in succession, indicating a prolonged

parasitemic phase. Parasite DNA could be detected in foals as early as one month of age. Preliminary results comparing nested PCR for detection of S. neurona in the CSF to necropsies show specificity of 100% and sensitivity of 83%. The nested PCR method has been applied to sporocyst DNA from fecal samples from opossums, raccoons, cats and skunks in an attempt to positively identify possible carrier animals. Bands of the appropriate length were identified by ethidium bromide staining from amplification of sporocysts found in opossum feces.

# 162

Molecular Identification of *Enterobius vermicularis* Larvae as a Cause of Human Eosinophilic Ileocolitis. LEO X. LIU\*, JONATHAN Y. CHI, MELISSA P. UPTON, LAWRENCE R. ASH

Clinically significant indigenous helminthic infections are uncommon in the continental United States. We recently evaluated a young Massachusetts native with severe abdominal pain and hemorrhagic eosinophilic ileocolitis. An extensive clinical workup was negative except for numerous nematode larvae in diarrheal stool, which could not be identified initially. The patient's symptoms resolved following anthelminthic treatment alone. Subsequent morphological examination revealed immature larvae ~1.0 mm in length, with an oxyurid esophagus and pointed tail suggestive of female Enterobius vermicularis; no reproductive organs or cephalic inflations were discernable. A molecular speciation of the unknown nematode was performed by cloning the nematode 28S ribosomal RNA and 5S rRNA spacer genes. PCR was used to amplify genomic DNA extracted from individual worms of the unidentified nematode species and those of other common intestinal nematode species. The ~300 bp DNA sequence of the 28S rRNA gene target from the unknown nematode was identical to that of E. vermicularis, and differed from the 28S rRNA sequences of other parasitic nematodes. Amplification of the 5S rRNA spacer region generated different sized major PCR products from various intestinal nematode parasites. The 5S rRNA spacer DNA sequences of the unknown nematode and E. vermicularis were identical in size (893 bp) and 99% identical in nucleotide sequence. Together these data identify the recovered parasite as the E. vermicularis, and implicate the common pinworm as a cause of human eosinophilic ileocolitis. Since the prevalence of occult E. vermicularis infection is very high, enterobiasis should be considered in the differential diagnosis of eosinophilic enterocolitis.

# 163

Dirofilaria immitis cDNA Clones Identified with Immune Dog Sera. CINDY TRIPP\*, MARCIA MIKA-GRIEVE, MICHELLE RUSHLOW, GLENN FRANK, REXANN FRANK, MINDY STORY, and SCOT BLEHM

Dogs receiving chemically abbreviated D. immitis infections are immune to challenge with infectious third stage larvae (Grieve, et al., 1988, Am. J. Trop. Med. Hyg. 39:373). Passive transfer experiments with the immune dog sera demonstrated significant larval killing and stunting compared to controls as assessed by a diffusion chamber system in a murine model (Abraham and Grieve, 1991, J. Parasitol. 77:254). Sera from these dogs were used to immunoscreen a third stage larval cDNA library to identify clones expressing proteins uniquely recognized by these sera and not sera from infected nonimmune dogs. The clones have been expressed in both prokaryotic and eukaryotic expression systems. Antibodies made to the recombinant proteins have been used to identify the size of the native protein encoded by each recombinant. The clones have been further characterized by nucleotide sequence analysis.

### 164

Reactivation of hypobiotic hookworm larvae during pregnancy and lactation. PREMA ARASU

During oral or cutaneous infection of definitive or aberrant hosts, a percentage of Ancylostoma caninum infective larvae migrate into the somatic tissues of the host and undergo developmental arrest. Immuno-physiological and hormonal changes associated with late pregnancy and lactation appear to facilitate larval re-activation and subsequent transmammary transmission of infection to the next generation of hosts. To determine if parasitic nematodes have endogenous receptor molecules that could directly respond to the flux of host hormones during the periparturient period, polymerase chain reaction (PCR) analyses were performed with degenerate primers to the conserved regions of mammalian hormone receptor (HR) sequences for estrogen, progesterone, prolactin and growth hormone. Several nematode PCR fragments were detected in southern blot analyses with internal primer probes and probes from cloned mammalian HR sequences. Sequence analysis, cDNA cloning and expression for ligand binding assays are underway.

# 165

Ancylostoma Secreted Protein: A Novel Protein Associated with the Transition to Parasitism by Infective Hookworm Larvae, J.M. HAWDON\*, B.F. JONES and P.J. HOTEZ The developmentally arrested third-stage infective larva (L<sub>3</sub>) of hookworms resumes development upon entry into the definitive host. This transition to parasitism can be modeled in vitro by stimulating L<sub>3</sub> with a low MW ultrafiltrate of host serum together with glutathione analogues. In vitro activated L<sub>3</sub> of the hookworm Ancylostoma caninum released a 40 kDa protein, termed Ancylostoma secreted protein (ASP), as the major protein in their excretory/secretory products (ESP). Based on a partial amino acid sequence of purified ASP, degenerate oligonucleotide primers were used to amplify a PCR product, which was used to isolate a clone from an A. caninum L<sub>3</sub> cDNA library. The full-length ASP cDNA encoded a 424 amino acid protein homologous to the Antigen 5 (Ag5) protein of Hymenopteran venoms. Heterologous antibody prepared against Ag5 from the yellowjacket Vespula squamosa recognized both native ASP from ESP of activated L3 as well as recombinant ASP expressed in E. coli. The antibody also detected antigen in other soil-transmitted nematodes. ASP is released within 30 min of stimulation, and is released continuously following activation. 4,7-Phenanthroline, an activation inhibitor, prevented the release of ASP. The specific, rapid release by activated L<sub>3</sub> suggests that ASP occupies a critical and central role in the transition to parasitism.

# 166

Two Morphologically Distinct Secretory
Mechanisms are Present at the Free Tegumental
Surface of Cestodes.
JOHN A. OAKS\*, and JON M. HOLY

Evidence is growing that tapeworms control the behavior of host organ systems, as well as the size of individual adult tapeworms within a single host. These regulatory events require that tapeworms communicate with their host and among themselves. Using rapid freezing-freeze substitution (rf-fs) or alternatively, low aldehyde-room temperature fixation to preserve the tapeworm's tegument, the external surface of the tegument was examined for morphological evidence of secretion. Evidence of two secretory mechanisms were observed: 1. Omega profiles  $(\Omega)$  at the free surface plasma membrane indicate that merocrine exocytosis occurs by vesicular fusion.  $\Omega$  were frequently observed with low aldehyde fixation, but not with fixatives of standard aldehyde concentrations, suggesting that slower fixation process with low aldehydes allows the accumulation  $\Omega$ , and that the process of vesicular fusion with the plasma membrane is relatively infrequent and rare. 2. Plasma membrane blebbing, preserved with rf-fs, releases uniform (0.03-0.75  $\mu$ m)

vesicles into spaces between the tegumental microvilli. This extremely rapid stabilization process preserves elements of very short lived structures or those which wash away during the fixation process. Earlier work by us indicates that endogenously synthesized molecules of the tegumental perikarya move into the tegumental ectocytoplasm. The fate of these molecules is still uncertain; however, these observations suggest that one route is their release to the external environment where they could serve as signal molecules.

#### 167

Molecular Cloning and Characterization of SMAK, the Homologue of MAK16 from Schistosoma mansoni. JON L. MILHON\*, THOMAS J. ALBERT, ELIZABETH A. VANDE WAA and JAMES W. TRACY

Adult Schistosoma mansoni express least five at S-transferase (EC 2.5.1.18; SmGST) glutathione isoenzymes that aid in cellular protection. A 708-bp cDNA (pGT11.7) was isolated by screening an adult S. mansoni expression library with rabbit antiserum to affinity purified SmGST, pGT11.7 is truncated at its 5' end and bares little resemblance to previously characterized SmGSTs. The single open reading frame of pGT11.7 lacks certain characteristic amino acids common to other GSTs, but contains a putative nuclear localization signal and a consensus phosphorylation motif. Moreover, the encoded protein displays 41% sequence identity to MAK16, a yeast protein implicated as a checkpoint in G<sub>1</sub> of the cell cycle. Repeated attempts to clone the 5' end of SMAK (schistosome homologue of MAK16) using a variety of methods, including rapid amplification of cDNA ends (5'-RACE), direct inRNA sequencing, and additional library screening, failed. Polymerase chain reaction (PCR) analysis of S. mansoni genomic DNA with pGT11.7-specific primers suggested that the SMAK gene introns are very small or nonexistent. This fact allowed us to use inverse PCR to determine the sequence of the 5'-end of the SMAK gene, including the in-frame initiation codon. PCR amplification of genomic DNA with primers specific to the 5' and 3' untranslated regions produced a fragment that was cloned and sequenced to confirm that indeed the SMAK gene is intronless. Northern analysis showed pGT11.7 hybridizes with a single 1.2-kb message. Hybrid select translation of that mRNA revealed SMAK is a 28-kDa peptide that reacts with the rabbit antiserum to affinity purified SmGST. It remains to be determined whether SMAK has GST activity. Its other possible biochemical functions are under investigation. (Supported by NIH grant AI22520. JLM was supported by NIH grant T32 Al 07414.)

### 168

Genetic Variation of the Nuclear Genomes of <u>Schistosoma japonicum</u> from Taiwan and Mainland China by Random Amplified Polymorphic DNA(RAPD). K.M. LEE\*, L.C. SHEN, C.T.LO, M.J. PAN, and C.L. Yu

Genetic diversities of nuclear genomes of Schistosoma japonicum from Taiwan and Mainland China were studies by RAPD . S. mansoni from Puerto Rico and S. haematobium were included for comparison. Twenty-five primers were used in this study, each consisted of 10 nucleotides. Some of the primers, such as 5'-TGCGCCCTTC, produced polymorphic band patterns which could be used as DNA fingerprints for intraspecies identification of S. japonicum. Others, such as 5'-TAGCCAACGC, produced polymorphic band patterns for inter-species identification of schistosome populations. The different RAPD band profiles exhibited by the Taiwan and Chinese strains show that they belong to two distinct populations. The application of RAPD is an extremely useful tool for the identification of schistosome strains and species and it may open the way to genetic identification of other species of parasites.

# 169

Human Parasitic and Mycotic Infections in Mississippi. A.D.W. Acholonu\* This study was conducted to highlight the existence and prevalence of human parasitic and mycotic infections in the State of Mississippi. A review of the records of the Mississippi Public Health Laboratory covering the period from 1989 to 1993, shows that human parasitism is not a thing of the past and that mycotic infections are not uncommon. The following intestinal parasites and dermatophytes were recorded: hookworm, Enterobius vermicularis, Trichuris trichiura, Ascaris lumbricoides, Strongyloides sterocor-alis (helminths); Giardia intestinalis (protozoan); Trichophyton sp. and Candida albicans (mycotic flora). The overall prevalence of helminthiasis was 2.2%; giardiasis, 4.7%; and mycosis, 24.2%. Of all the counties in the State, Union had the highest hookworm infection (84.0%), trichuriasis (24.0%), ascariasis (9.6%), and strongyloidiasis (32.0%). Hancock had the highest enterobiasis (96.0%). DeSoto had the highest giardiasis (40.0%) Clarke, and Marion had the highest Trichophyton infection (100% each). Stone, George, Sunflower, Clay, Hancock, and Pearl River had the highest candidiasis (100% each) followed by Oktibbeha (86.8%) but the sample sizes examined were mini-

mal. This study shows that human parasitism is still a public health problem in the State of Mississippi and that mycosis is rampant.

### 170

Capture as a plausible mechanism of sympatric speciation in parasites. ADAMSON, M. L.

In the course of evolution, parasites acquire new hosts in two ways: cospeciation, where a parasite is inherited by newly formed daughter host species, and capture, where parasite speciation involves a host switch. Theoretical mechanisms of cospeciation are unproblematic but those involved in capture have not been examined and some invoke sympatric speciation. Sympatric speciation requires that populations diverge genetically in the absence external barriers to gene flow. Most models do this by invoking a niche polymorphism concomitant with assortative mating. This possibility has been recognized for 20 years in microherbivore systems but the mechanism has not been explicitly examined in parasite-host systems. of salmonids. Our studies on western North American Philonema (Dracunculoidea) nematodes suggest that P. oncorhynchi in anadromous hosts may have arisen through sympatric speciation from forms in resident freshwater hosts. The mechanism involves 4 stages: 1. Initially a single species occurs in freshwater salmonids; worms mate in the body cavity of the host and females enter the reproductive tract to be released into the external environment when fish spawn. 2. Origin of anadromous salmon with an extended freshwater residence (e.g., sockeye, Oncorhynchus nerka), creates a new host resource for invasion. 3. Worms must have slower development to be successful in this host because of its protracted marine migration: slower development is selected against in freshwater resident hosts, which spawn annually. This developmental constraint acts to create assortative mating among forms that are successful in only one of the hosts. 4. Modifier genes are selected that enhance separation of stocks in freshwater resident hosts from those in anadromous hosts.

# 171

Mathematical Models of African horse sickness and *Culicoides imicola*. C. C. LORD\*, M.E.J. WOOLHOUSE AND P.S. MELLOR

African horse sickness, a viral disease of equines, is transmitted primarily by the biting midge *Culicoides imicola*. The virus is endemic to sub-Saharan Africa, but epidemic when introduced to naive equine populations (such as occurred in Spain in 1987-1991). There is little information available on many aspects of the system, and

which factors are involved in epidemics are poorly understood. A mathematical model was developed to examine these questions, parameterized to reflect southern Spain. The model included two host species (horses and donkeys), seasonality in the midge population dynamics and a temperaturedependent virus development rate. Sensitivity analysis was used to explore where uncertainty in parameter values affects the uncertainty in the outcome (epidemic or no epidemic). This analysis indicated that the uncertainty in the vector to host ratio, the time of year of the introduction of the virus, the rate of loss of infected horses, and the inter-bloodmeal interval of the midges contributed most to the likelihood of an epidemic. This suggests priorities for future field research. There appears to be a threshold effect in the vector to host ratio and the rate of loss of infected horses. Epidemics were more likely to occur if the virus was introduced when the midge population was increasing. However, the range of possible parameter combinations allowed epidemics in all seasons.

## 172

Fatal Cerebral Coenurosis in a Cat. B.T. HUSS, M.A. MILLER, R.M. CORWIN\*, E.R. HOBERG, and D.P. O'BRIEN

A 6-year-old cat with behavioral abnormalities referred to the University of Missouri Veterinary Teaching Hospital went into respiratory arrest and died. At necropsy a 1.5 cm fluid-filled cyst was found in the white matter of the left cerebrum at the juncture of the occipital, parietal and temporal lobes. The membranous lining of the cyst contained numerous invaginated scolices and was identified as a coenurus of Taenia serialis, a tapeworm with a natural canid-lagomorph life cycle. Domestic cats have been recognized as accidental intermediate hosts but with a coenurus rarely observed in the brain of cats or of other accidental intermediate hosts. Neurologic signs were consistent with a left cerebral lesion with early midbrain stage of herniation. This cat resided in the suburbs and had access to a nearby open field. The short duration of clinical signs and morphologic features of the coenurus suggested rapid development. Although this and other taeniids are relatively rare zoonotic parasites, there is the potential for serious disease in humans and other accidental intermediate hosts.

# 173

Significance of Parasitologic Examination of Stools in a Tertiary Care Hospital in the Southern United States of America. JEROME H. SMITH, MD\*

Previous studies of stool examination for parasites have indicated that a single stool examination for "O & P" identifies less than 3/4 of actually parasitized patients, and that 3 stool samples (Days 1,3 and 5) are necessary to detect 98+ % of parasitized patients. Recent studies have suggested that almost all patients "sick with a parasites (except for Giardiasis) examined by laboratory in the Northern USA have all 3 specimens positive, making the last 2 specimens redundant. Since procedures and extra days of hospitalization costs money, these studies advocate analyzing a single stool specimen. Between January 1, 1991 and December 31, 1993, there were 8961 stool examinations for parasites in the laboratory of the University of Texas Medical Branch in Galveston, Texas; 377 or 4.21 % of these were "positive." The distribution of positives among encountered parasitisms, the frequency of positives in 1, 2 or 3 specimens, cost-benefit analysis and cost / positive case are presented and the validity of this and other data are discussed with regard to future policy on laboratory diagnosis of parasitism in this and other countries.

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Computerized decision analysis for the diagnosis and treatment of carine heartworm disease. R.A. HOLMES\*, R.D. SMITH, B. DUNAVENT, M.E. HUGH-JONES and M.E. KEARNEY

The diagnosis and treatment of canine heartworm (Dirofilaria immitis) disease requires a complex procedure for each process. As the clinical presentation for each case can be extremely varied and the decision to treat the adults based on the current state of health of the patient, there is an inevitable amount of uncertainty. Uncertainty can be the result of a general lack of knowledge of a disease process, the level of experience of the clinician, or unknown facts about the case. As we learn more of the biology of Dirofilaria immitis, some of that uncertainty is diminished. By the same token, as we learn more of the response of the host to parasite invasion, uncertainty in diagnosis and treatment decreases. The experience level of the clinician can be enhanced by the dissemination of knowledge and the use of tools to access and use that knowledge base. The computerized decision analysis program which has been developed attempts to present and make useful a base of knowledge which will help decrease some of the uncertainties encountered when attempting to diagnose and treat heartworm disease. The program is written in Visual Basic 3.0 and incorporates standard decision analysis mathematical tools.

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The Combined Effect of a Move 9 Weeks After Turnout and Treatment with Fenbendazole or Moxidectin on Lungworm Infections in Dairy Calves. M. EYSKER\*, J.H. BOERSEMA, F.N.J. KOOYMAN, T.J.G.M. LAM and T. VAN WERVEN

Since 1991 the control of lungworm disease in calves by a Dose and Move (DM) is evaluated at Utrecht University. Studies from 1991 to 1993 demonstrated that timing of DM and the drug used are important. DM 7 weeks after turnout controls lungworm disease but can be too early for development of immunity; this problem probably does not occur with DM after 9 weeks. The use of drugs with a long persistant effect, like moxidectin (MOX),

may hamper development of immunity through lack of reinfection. On the other hand treatment with a non-persistant drug, like fenbendazole (FBZ), will probably result in reinfection, and possibly in lungworm disease from two months after treatment. This possible difference between MOX and FBZ was evaluated in a trial with a DM after 9 weeks. This trial also included a Pasture Control (PC) group and a Housed Control (HC) group; the latter for evaluating establishment of challenge infections of 5,000 lungworm larvae/calf after the six month grazing season. Though high lungworm burdens established before DM, disease only occurred in PC after DM. Indeed patent reinfections reoccurred in FBZ but not in MOX. The worm burdens after challenge were 72, 7, 1.4 and 845 for the Mox, FBZ, PC and HC groups, respectively. Thus all three groups developed immunity but the degree of immunity was lower in the MOX group. The results over four years indicate that DM probably is a viable option to control lungworm in high prevalence regions like the Netherlands, either using a drug with or without persistent effect. In 1995 the scheme will be further evaluated on a number of commercial farms.

# 176

A Comparative Assessment of Direct Fluorescence Antibody, Modified Acid Fast Stain and Sucrose Flotation Techniques for Detection of <u>Cryptosporidium serpentis</u> Oocysts in Snake Fecal Specimens THADDEUS K. GRACZYK\*, CRANFIELD M.R., and RONALD FAYER

Modified acid fast stain (Mod AFS) of a direct wet smear (DWS), unstained DWS (UDWS), and Sheather's sugar coverslip flotation (SSCF) techniques were compared with commercial monoclonal antibody (MAb) test MERIFLUOR<sup>TM</sup> Cryptosporidium/Giardia for direct fluorescence detection of C. serpentis oocysts in feces from 40 snakes without clinical signs of infection. Absolute sensitivity of MAb detection of purified C. serpentis oocysts was compared with detection of purified C. parvum oocysts. The oocysts of C. serpentis showed the same intensity of fluorescence reaction (majority, 3 + to 4 + ) as did C. parvum. Sensitivity of the MERIFLUOR<sup>TM</sup> test was 100% for SSCF-derived material, and 88% for DWS against 45% for Mod AFS DWS, 35% for SSCF, and 21% for UDWS. The threshold of MAb detection  $(4.7 \times 10^3 \text{ oocyst/ml})$ , the same for of C. serpentis and C. parvum oocysts, was 16 times higher than the absolute sensitivity of the listed conventional techniques. Conventional techniques underestimated an average of 5.3 times the number of C. serpentis oocysts, and produced false-negative results an average of 66%; false-negatives were 100% when the concentration of oocyst was  $< 7.5 \times 10^4$ /ml. The range of concentration of fecal C. serpentis oocysts (0.4 x 10<sup>3</sup>/ml - $1.4 \times 10^{5}$ /ml, mean;  $5.0 \times 10^{4}$ /ml) was an average of 3 times lower than those reported for C. parvum from humans and other mammals. The MAb detection proved to be more sensitive for C. serpentis oocysts than conventional techniques, and it eliminated false-negative outcomes and subjectivity associated with those methods.

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Comparison of Direct and Indirect Methods for the Detection of Trichinellosis in Horses. H. RAY GAMBLE\*, ALVIN A. GAJADHAR and MORSE B. SOLOMON

Outbreaks of human trichinellosis resulting from the ingestion of raw or undercooked horsemeat have been reported regularly over the last decade in France, Belgium and Italy. In several cases, the source of the infected meat has been traced to the United States or Canada, resulting in temporary embargoes and additional inspection requirements. The present study was initiated to determine optimal methods for the detection of trichinae-infected horses. A group of twelve horses was inoculated with 1,000 - 40,000 Trichinella spiralis L1, bled on a weekly basis, then slaughtered after three months. Tissue samples were obtained from 27 muscle groups and compared for intensity of infection. Pooled sample digestion methods using 1, 5 or 10 gram samples and an enzyme immunoassay were compared for efficacy in determining trichinae-positive status. Highest numbers of larvae per gram (LPG) of muscle tissue were always recovered from the tongue and masseters, with the diaphragm and other tissues harboring considerably fewer worms. The tongue harbored the most larvae in light to moderate infections (<100 LPG) while in heavy infections (>200 LPG) the masseters had the highest numbers of worms. As expected, sensitivity of the pooled sample digestion method increased with intensity of infection. One gram samples were unreliable when infection levels were <3 LPG. Enzyme immunoassay detected infected horses beginning two to five weeks following infection.

# 178

Long-Term Immunity to Shedding of <u>Toxoplasma</u> gondii Oocysts by Cats. J.P. DUBEY\*

Cats that have shed  $\underline{Toxoplasma}$  gondii oocysts are considered to be immune to re-shedding. To investigate if this immunity persists in cats for 6 yr, ten 4-to-6-mo-old cats without  $\underline{T}$ . gondii antibodies were inoculated orally with tissue cysts of the ME-49 strain (4 cats) and the TS-2 strain (6 cats) of  $\underline{T}$ . gondii. All of them shed  $\geq 20$  million oocysts between 4 and 13 days after feeding tissue cysts. On day 39 after primary infection, 5 cats (2 infected with ME-49 strain and 3 infected with the TS-2 strain) were challenged orally with tissue cysts of the ME-49 strain. None of the challenged cats shed oocysts. One cat died due to causes unrelated to toxoplasmosis. Seventy-seven mo

after primary infection, the remaining 9 cats were challenged orally with tissue cysts of the P89 strain of T. gondii. Four of these 9 cats re-shed T. gondii oocysts; 3 of them had been challenged also at 39 days after primary infection. Two control cats housed together with chronically infected cats for 6 yr remained seronegative for T. gondii; both of these shed oocysts after challenge with the P89 strain.

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EFFECT OF LOW TEMPERATURES ON SURVIVAL OF CRYPTOSPORIDIUM PARVUM OOCYSTS

#### R. FAYER\*

Previous attempts to cryopreserve oocysts or sporozoites of Cryptosporidium parvum (Cp) have been unsuccessful. Despite the presence of cryopreservative agents and various timed freezing protocols, recovery of infectious organisms from storage at -70 C was not achieved. The present study examined infectivity for neonatal BALB/c mice of 500  $\mu$ l suspensions CP oocysts placed in freezers at -70 C or -20 C for periods of 1, 3, 8, and 18 hr, then thawed at room temperature. All oocysts frozen at failed to infect mice based on histological examination of ileum, cecum and colon. Oocysts frozen at -20 C for 1 and 3 hr survived and were infectious for mice whereas those frozen for 8 hr were not infectious. These findings suggest that ice made from water contaminated with Cp oocysts and ingested shortly thereafter may contain infectious organisms.

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The effect of rotational grazing on the dynamics of sheep parasite fecal egg counts. BERT E. STROMBERG\*, GARY A. AVERBECK, CHARLES CHRISTIANS and CRAIG C. SHEAFFER.

This study was conducted in order to compare the effect of grazing sheep continuously or rotationally on parasitism as shown by fecal egg counts. Thirty-two ewe lambs were allotted into 8 groups based on weight. Continuous or rotational grazing on Kura clover were compared in 0.05 ha paddocks in 4 replicates, utilizing a randomized complete block design. The rotationally grazed paddocks were subdivided into 3 plots and animals were moved to a new plot every 7 days (returning every 21 days). Lambs were put out on pasture May 27, 1994 and the study was terminated September 11, 1994. All lambs had free access to water and were fed 1 lb of supplement per day. Rectal fecal samples were collected every 21 days and lambs were

weighed at the start and termination of the study. The initial weight was 39.75 and 38.25 kg for the continuous and rotationally grazed lambs, respectively. At the end of the study the groups weights were 51.81 and 51.63, respectively. There was no difference in weight gain or average daily gain between treatments. The average fecal egg counts were 0 for both groups at turn-out. The egg counts peaked in mid August with an average of 337.44 and 350.63 for continuous and rotational groups, respectively. Egg counts had declined at the end of the experiment. Parasitism as indicated by epg's increased more rapidly in the rotationally grazed groups and were always higher. Egg counts were not significantly different at any time during the study. Fecals contained trichostrongyle type, *Trichuris*, *Nematodirus* and *Moniezia* eggs.

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Chemical Stimulation of Host Recognition by Diplostomum spathaceum Cercariae. A. BANSEMIR\*, E. SCHÖNAMSGRUBER, and W. HAAS

Recognition of the fish host by Diplostomum spathaceum cercariae is characterized by 3 distinct behaviors: attachment, enduring contact, and penetration. Attachment is a nonspecific response triggered by water-turbulence and CO<sub>2</sub>. Specific recognition of the host by fish-invading cercariae is generally elicited by different chemical cues from fish skin surface mucus for each successive behavior pattern. The objective of this study was to characterize the chemical components of fish skin mucus which stimulate the enduring contact and penetration behaviors of D. spathaceum cercariae. These behaviors were analyzed in vitro by offering mucus extracts in agar substrates. The stimulating component for both behaviors is contained in a fish mucus fraction of molecular weight >30 kDa and enduring contact is enhanced by a fraction between 1 and 10 kDa. stimulating effects are resistant to digestion with proteases and chondroitinase ABC. Alkaline cleavage of O-glycosidic protein-carbohydrate linkages and digestion neuraminidase eliminates both behaviors. However, penetration and not enduring contact is abolished after digestion with lysozyme and the stimulating component for the behaviors is contained in different fractions after anion exchange separation. These results suggest that specific recognition of the fish host by D. spathaceum cercariae occurs during enduring contact and penetration behaviors and that two distinct chemical components of fish skin mucus are necessary for successful recognition of and invasion into the host. This may reflect an adaptive strategy which is critical for the transmission into specific fish hosts. [Supported by DFG and DAADL

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Prediction of Mature Fasciola hapatica by Fecal Sedimentation From Yearling Calves in Louisiana.

KIMOTHY L. SMITH\*, JOHN B. MALONE, and DANIEL SCHOLL

The Quantitative Fecal Sedimentation test is used to determine the number of <u>Fasciola hepatica</u> eggs per 2 grams of feces in cattle. Results of this test are then used to make treatment recommendations to veterinarians and cattle producers. Prior to this study, there has not been an analysis of the relationship of the number of eggs per 2 grams of feces and the number of mature <u>Fasciola hepatica</u> present in cattle being tested in the state of Louisiana. This study addresses that relationship for cattle in the state of Louisiana. Data consisting of total mature flukes and eggs per 2 grams of feces wascollected from 114 calves raised in Louisiana. Analysis of these data include correlation and regression. A correlation value of .99 and standard error value of ±1.59 were obtained. Further, the positive and negative predictive value of the Quantitative Fecal Sedimentation are examined.

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Autoimmunity to Le\* Antigens in Monkeys and Humans Infected With Schistosoma mansoni KWAME NYAME, JOY B. PILCHER, VICTOR TSANG AND RICHARD D. CUMMINGS\*

We reported previously that adult S. mansoni synthesize a group of high molecular weight glycoproteins with complextype N-linked oligosaccharides bearing the fucose-containing Le<sup>x</sup> antigen (Srivatsan et al., J. Biol. Chem. 267:20196-20203, 1992). Oligosaccharides containing the Le<sup>x</sup> structure bound specifically to immobilized sera from S. mansoni infected hamsters. We now report our discovery that antibodies to Le<sup>x</sup> antigens are present in sera from  $\underline{S}$ . mansoni infected mice, hamsters, rhesus monkeys and The presence of anti-Lex antibodies was humans. determined by an ELISA using the Lex containing neoglycoprotein, lacto-N-fucopentaose-III-BSA (LNFP-III-BSA) as target. We observed that infected animals had high titers of both IgM and IgG antibodies reactive to LNFP-III-BSA. Sera from S. mansoni infected humans contained only IgM antibodies reactive to LNFP III-BSA and the titer was lower than observed for infected animals. antibodies to Lex were lytic and mediated specific complement lysis of the human promyelocytic leukemia cell line HL-60, which bear surface Lex determinants. These results demonstrate that an autoimmune disorder based on reactivity to Le<sup>x</sup> antigens accompanies infection with S. mansoni. Supported by NIH Grant AI26725 to R.D.C.

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Reduced Egg Accumulation in Livers of Schistosoma mansoni-infected Mice Vaccinated with Naked DNA. S.G. KAYES\*, J.S. JACKSON, J.J. O'BRIEN, J. POWELL, J.W. TRACY, J.S. LATTENDRESSE and J.A. WOLFF

There are major obstacles to the use of conventional proteinaceous vaccines against tropical diseases including production and isolation of the putative antigen(s), requirement for toxic adjuvants, and inappropriate immune responses. Intramuscular injection of plasmids carrying viral genes has been used successfully to vaccinate mice, cows, chickens and primates against viral infections. We synthesized a eukaryotic expression plasmid encoding an S. mansoni glutathione S-transferase (SmGST) gene under the transcriptional control of a cytomegalovirus (CMV) promoter. This plasmid was used to vaccinate mice and the results compared to mice vaccinated with purified SmGST protein and alum adjuvant. Groups of Balb/c mice were unvaccinated, or vaccinated with SmGST/alum, a control plasmid expressing the luciferase gene, or the plasmid encoding SmGST. Two weeks later all mice were infected with 200 cercariae. After 6-1/2 wk the mice were sacrificed and adult worms recovered by perfusion. The livers were fixed and prepared for histologic examination. Worm burdens were not affected by any of the regimens. As compared to unvaccinated mice, protein/alum vaccination reduced hepatic egg content by 23% (60 v 46 eggs/100 mm<sup>2</sup>) while SmGST DNA reduced it by 89% (60 v 6.7 eggs/100 mm<sup>2</sup>). The luciferase plasmid persisted within the injected muscle and the gene was still being expressed 8.5 wk after injection. This report is the first demonstration of polynucleotide vaccination against a medically important trematode and suggests that the technique can be effectively used against parasitic helminths.

# 185

Brugia pahangi Induced Changes in Canine Popliteal Lymph Node Cells Leading to Elevated TNF -∞ and Histamine Release in vitro. SUSAN ORTON\*, DAMIEN SCHEURER, AND BRUCE HAMMERBERG.

Brugia pahangi infection in the canine rear limb results in marked lymphatic duct and popliteal lymph node pathological changes. Limb edema is variably associated with infection and does not correlate well with duct or node lesions. To understand the mechanism of limb edema we have collected lymph node cells by sequential biopsy following infection and examined production of inflammatory mediators such as histamine, tumor necrosis factor-alpha (TNF- $\propto$ ), and prostaglandin E-2 (PGE-2). A litter of dogs with a high incidence

of edema formation demonstrated spontaneous histamine release levels well above those of dogs not predisposed to edema formation. These dogs also showed elevated release of bioactive TNF- $\propto$  when lymph node cells were cultured for 24 hours with <u>Brugia</u> antigen. The kinetics of TNF- $\propto$  release and the cellular composition of the lymph node biopsies suggest the possibility that mast cells may be the source of TNF- $\propto$ , thus incriminating this cell as a major contributor to the high risk of limb edema during *Brugia* infection.

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Evaluation of Adult Heartworm Antigen Test Kits for Effectiveness in Detecting Induced Ectopic Infections in Cats and Dogs. J.W. McCALL\*, P. SUPAKORNDEJ, M.T. DZIMIANSKI and J.J. JUN.

Ten (3M, 7F) heartworm-naive adult beagles and 10 (4M, 6F) heartworm-naive adult domestic shorthair cats were used to determine whether or not induced ectopic infections of Dirofilaria immitis could be detected using a commercially available adult heartworm antigen (Ag) test kit. Adult heartworms were taken from infected donor dogs under thiamylal sodium (6-8 mg/lb) anaesthesia. Two groups (IP cats) of 3 cats each were given and 2 female worms, respectively, by IP transplantation; similarly, 2 groups (IP dogs) of 3 dogs each were given 1 and 2 female worms, respectively, by IP transplantation. One group of 2 cats (SC cats) was given 1 female heartworm by SC transplantation; 2 dogs received 1 or 2 females SC (SC dogs). Under thiamylal sodium (6-8 mg/lb, for dogs) and ketamine hydrochloride (10-15 mg/lb, for cats) anesthesia, 2 dogs (IV dogs) and 2 cats (IV cats) were given various numbers of female and/or male worms by [V transplantation via a jugular vein to control survival of the worms during handling. For IP and SC transplantation, dogs were given xylazine (0.5 ml/20 lb IV) with local lidocaine/epinephrine block and yohimbine (0.05 mg/lb IV) was given after transplantation to reverse the effects of the xylazine; ketamine was used for cats. All dogs and cats were bled prior to transplantation (Day 0) and at 2, 4, 6, and 7 weeks posttransplantation (PTP) (cats were also bled at 7 weeks PTP) for microfilarial (modified Knott's) and adult heartworm Ag (Snap®, IDEXX Corp., Portland, MÉ) tests. Animals were necropsied at 6 (dogs) and 7 (cats) weeks PTP.

All IP cats were Ag-positive from 2 through 7 weeks PTP, except for 1 cat that was not positive at 7 weeks. Although none of these IP cats had live worms at necropsy, 4 had dead worms and/or fragments. None of the SC cats was Ag-positive, but 1 had 1 live female worm and the other had fragments at necropsy. Both IV cats had 1 live female worm at necropsy, but only 1 cat was Ag-positive during the study. Only 1 (an IV cat) of the 10 cats had microfilariae (MF).

Only 4 of the 6 IP dogs were Ag-positive, and this was at 2 weeks PTP; none of these dogs had live worms or fragments at necropsy. Neither of the 2 SC dogs was Ag-positive and neither had live worms or fragments at necropsy. The 2 IV dogs were Ag-positive beginning at 2 and 4 weeks PTP, respectively, and most of their transplanted worms were alive at necropsy. Both of the IV dogs and 1 of the IP dogs had MF.

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The performance of pre-treatment free adult heartworm antigen test kits (PetChek® PF and SNAP™ PF from IDEXX Laboratories, Inc. and VetRED® from Rhone Merieux. Inc.) were evaluated, and assay results were compared to necropsy findings and results from test kits requiring sample pre-treatment (PetChek® from IDEXX Laboratories, Inc. and DiroCHEK® from Symbiotics Corp.). The PetChek PF assay was compared to the unmodified PetChek assay by testing a total of 1,047 samples (923 canine and 124 feline). The PetChek PF test sensitivity and specificity were 98.4% (196/199) and 99.5% (846/850) respectively, when compared to the standard PetChek assay. The PetChek PF and DiroCHEK assays were compared by testing 231 samples utilizing necropsy results as the reference standard. The sensitivity of each assay was 97.2%. (70/72) for dogs containing one or more gravid females. Knott's test sensitivity for this population was 66.6%. In dogs containing one or more adult worms (N=97), the sensitivities of the assays were 85.6% for PetChek PF, 87.6% for DiroCHEK and 51.5% for the Knott's test. Although the magnitude of color development for positive samples was substantially higher for PetChek PF, the sensitivities of PetChek PF and DiroCHEK were statistically equivalent at all worm burdens. The tests were all 100% specific. The SNAP PF and VetRED assays were compared by testing 143 whole blood samples. Whole blood test results were evaluated by comparison to consensus assays performed using PetChek PF and DiroCHEK with paired plasma samples. A single PetChek PF/DiroCHEK discrepant result was confirmed by necropsy. The sensitivities and specificity of the whole blood tests were, 87.2% (34/39) and 100% (104/104) respectively for SNAP PF, and 61.5% (24/39) and 93.9% (92/98) respectively for VetRED. In the VetRED assay, results from 6 of the 104 consensus negative samples were interpreted as indeterminate.

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Haemonchus contortus GA1 Gut Surface Membrane Proteins: Encoded as Related Polyproteins and Solubilized by Phosphatidylinositol Specific Phospholipase C. DOUGLAS P. JASMER, L.E. PERRYMAN, and T.C. McGUIRE.

Monoclonal antibody 42/53.3.5 was shown to bind the gut surface of H, contortus and recognized 46 and 100 kDa gut proteins. This mAb isolated 46, 52 and 100 kDa proteins when used in immunoaffinity chromatography, and these proteins induced significant protection in goats against challenge infections of H. contortus, p46, p52 and p100 are now referred to as GA1 proteins. Analysis of the gene encoding GA1 proteins indicates that p100 is a polyprotein which is processed into p46 and p52. Unexpectedly, p46 and p52 have 47% identity. The carboxyl terminus of p52 resembles a GIPL anchor addition sequence. Both p46 and p52 are released from the gut membrane and rendered insoluble in triton X-114 by PIPLC; release is associated with decreased electrophoretic mobility of p52, but not p46. This evidence indicates that p52, but not p46, is GIPL anchored membrane protein, and that p46 membrane

association occurs via binding to another GIPL anchored protein. Both p52 and p46 were detected by immunoblot in gut membrane fractions, but only p46 was detected in excretory-secretory products. The results have implications for gut membrane organization and antigen presentation of GA1 gut surface proteins. (Supported by USDA 91-20001).

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Dexamethasone Immunosuppression and Nematode Infection in Resistant Gulf Coast Native Sheep.

J.E. MILLER\* S.S. KHALAH-ALLAH, and S.R. BARRAS Previous studies have shown that Gulf Coast Native (Native) sheep are relatively resistant to Haemonchus contortus infection and do not require any intensive anthelmintic treatment therapy. Elucidation of an immunological mechanism for resistance to H. contortus infection has been shown in a recently selected line of resistant Merino sheep. Dexamethasone was used to immune suppress the resistant line which then responded like the susceptible line of Merino sheep to infection. The objective of this study was to evaluate whether a similar response would be present in the Native breed whose resistance has developed over a long-term host-parasite relationship. Ten age-matched Native lambs were selected at four weeks of age and randomly assigned to two treatment groups. One group (Group 1) of five lambs immediately started receiving dexamethasone (0.5 mg/kg) treatments twice a week, and at ten weeks of age the dosage was increased to 1.0 mg/kg. The other group (Group 2) of five lambs remained untreated. Fecal egg count (FEC), blood PCV, and WBC differential data was collected at biweekly intervals through September. There was no significant (p>0.05) difference in overall mean FEC between Group 1 (2,610±1326 EPG) and Group 2 (1,567±553 EPG). There was a significant (p<0.05) difference in overall mean PCV and percentage of lymphocytes between Group 1 (26.0±1.2 and 44±6%, respectively) and Group 2 (29.0±1.3 and 67±2%, respectively). Lymphopenia did result from dexamethasone treatment, and this did not appear to have any affect on infection level based on FEC. However, the drop in PCV in dexamethasone treated lambs did indicate that there were more H. contortus present. Results of this study indicate that resistance in Native sheep may not have a major immunological component.

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Parasitologic and Immunologic Definition of Cattle Selected for Enhanced or Diminished Resistance to Gastrointestinal Nematode Infection.

L.C. GASBARRE\*, A. CANALS, and D.S.ZARLENGA
Angus cattle selectively bred to produce a herd that is
both homozygous and identical for class I and class II
major histocompatibility loci alleles, have been
secondarily bred for enhanced or diminished resistance
to gastrointestinal nematodes. Selection for parasite
resistance is based upon fecal EPG values and serum

pepsinogen levels of calves exposed, for a minimum of 120 days, to pastures contaminated with a nematode flora that is predominately Ostertagia ostertagi and Cooperia oncophora. To date, extensive immunologic and parasitologic data has been collected on 77 animals over 4 different exposure trials in a 2 year time frame. Natural exposure to the parasites indicate that there are 3 basic phenotypes associated with EPG values: 1) low EPG throughout the test, 2) EPG values that peak, and then decrease to levels similar to the low EPG calves, and 3) high EPG calves. These phenotypes occur at approximately a 1:2:1 ratio respectively. At the end of the trial, the calves appear to have 2 phenotypes: 1) low EPG, and 2) high EPG, at a 3:1 ratio. The decrease in EPG values is likely due to immune responses that reduce either or both parasite numbers, and parasite fecundity. As the number of Ostertagia increase there is an apparant: 1) marked enlargment in the draining lymph nodes, 2) decrease in the percentage of CD2 to cells, and 3) increase in immunoglobulin-bearing cells. Preliminary results indicate that the decrease in T cell percentages corresponds to decreased amounts of IL2 and gamma-IFN mRNA's demonstrable from mitogen stimulated cells from the draining lymph nodes.

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H-2 Genes in Mice and Resistance to Heligmosomoides polygyrus. SU ZHONG and COLIN DOBSON\*

Resistance to Heligmosomoides polygyrus has been associated with II-2<sup>a</sup> and H-2<sup>q</sup>, whereas mice with susceptible phenotype may carry H-2<sup>k</sup> and H-2<sup>b</sup> haplotypes. Here we did the opposite experiment and selected mice as resistance and susceptible to infection, assessed their H-2 haplotypes, and correlated the distribution of the H-2 haplotype with the levels of resistance. Putative correlations detected between the MHC in inbred mice and their level of resistance to metazoan parasites were not substantiated when mice were selected successfully to show the extremes of resistance and susceptibility to infection with H. polygyrus. Genes for resistance and susceptibility become fixed in inbred strains by chance and inbreeding exerts a deleterious effect on the general fitness of the strain in terms of their capacity to react against pathogens. (Supported by a grant from the Australian Research Council.)

# 192

Evolutionary Ecology of Siphonostomatoida (Copepoda) – the Most Successful Crustacean Taxon Parasitic on Vertebrates GEORGE W. BENZ

Eighteen of 40 siphonostome families hold species considered exclusive parasites of fishes and together represent the most successful crustacean group

parasitic on vertebrates. If a hypothesis of monophyly for these siphonostomes is used to investigate various ecological and life history characteristics, interesting evolutionary trends emerge. Data suggest the branchial chamber was the first vertebrate body region to be colonized, and that olfactory sacs may have originated from some premanidular gill arch which caused an evolutionary split in the copepod fauna formerly infecting the branchial chambers of noseless and jawless vertebrates. General body surfaces of vertebrates were probably colonized by gill and olfactory sac infecting taxa, and perhaps this was facilitated by a new type larva possessing a frontal filament. Adults of these larvae seem to have developed two modes of extending attachment security into later life; one involving new methods of permanent attachment of mature females, the other allowing both powerful swimming and efficient suctoral attachment. Reduction in molt numbers is exhibited by some lineages, and is achieved through amalgamation of free living nauplius and/or parasitic copepodid stages. Evolution of two host life cycles by a few species perhaps was facilitated by highly mobile young adults capable of transferring to another host, and by the close ecological association of intermediate and definitive hosts.

# 193

Phylogenetic analysis of the Strongylida based on nucleotide sequencing of mtDNA COI. M.V.K. SUKHDEO\*, S.C SUKHDEO, D.L. MEDICA, M.B. BLACK and R. VRIJENHOEK.

Metabolic pathways, energy resources and energy storage patterns are thought to reflect the host-finding strategies used by infective stages of strongylid nematodes. study was initiated by the observation that members of the Strongylidae (Strongylus edentatus and S. vulgaris) and Trichostrongylidae (Haemonchus contortus) share complex but similar fatty acid profiles (a measure of energy storage patterns determined by gas chromatography) and similar host-finding strategies. Other members Trichostrongylidae (Heligmosomoides polygyrus) distinct fatty acid profiles and distinct host-finding strategy. Nucleotide sequencing of mtDNA Cytochrome C Oxidase subunit I (COI) genes amplified by PCR was used to resolve the phylogenetic relationships among members of these A preliminary analysis with Caenorhabditis elegans, Ascaris suum, Strongylus spp. and H. polygyrus, using neighbor-joining suggest that H. polygyrus is the most phylogenetically divergent of the group of species. Bootstrap values at each node (97% and higher for 1000 replicates) indicate that this hypothesis is highly consistent with this preliminary sequence data. These results are inconsistent with current taxonomic classification and the study will be expanded to include representatives from all major groups in Nematoda.

Supported by the Busch Biomedical Research Support Fund.

### 194

Bilateral, Perivulval Cuticular Pores in Trichostrongylid Nematodes. J.R. LICHTENFELS\*, W.P. WERGIN, C. MURPHY and P.A. PILITT.

A new hypodermal gland was discovered in female nematodes of the family Trichostrongylidae. Because the new structure appears to be associated with the vulva, it was named the perivulval pores. It is similar, based on light and scanning electron microscopy, to phasmids which are located laterally on the tails of nematodes of the Class Secernentea. Like phasmids perivulval pores are paired and bilateral, with cuticular ducts to the surface in the area of the lateral chords. They are located slightly posterior to the vulva in Haemonchus contortus, H. placei, H. similis, Mecistocirrus digitatus, Mazamastrongylus odocoilei, Cooperia oncophora and Ostertagia ostertagi, but in Trichostrongylus colubriformis they are slightly anterior to the vulva. Similar hypodermal glands have been found recently in a new lungworm from muskoxen. Postdeirids described previously in Caenorhabditis elegans, Ascaris lumbricoides and Parascarus equorum may be homologs of the perivulval pores. In plant parasitic nematodes of the family Tylenchidae similar pores referred to as extra phasmids have been used as a systematic character at the species level. Because of the location near the vulva and the similarity in structure to phasmids which are, at least in part, secretory, the perivulval pores should be considered as a possible source of a female attractant for males.

# 195

Phylogeny of Nematodes of the Genus Pratylenchus (Nematoda: Tylenchida). LUMA AL-BANNA\*, S. L. GARDNER, AND V. M. WILLIAMSON.

In our holistic view of parasitology, there is no distinct field of plant or animal parasitology. In this light, we studied the phylogenetic relationships of plant parasitic nematodes in the genus Pratylenchus. These nematodes are migratory endoparasites that infect a wide various of plants causing obvious damage to roots. Phylogenetic relationships among selected species of Pratylenchus were estimated using data

derived from studies of both classical morphology and DNA sequences. Phylogenetic trees were estimated using maximum parsimony analysis of morphological data, molecular data, and a combination of both. characters used in the analysis consisted of sequence data derived from PCR-amplified D-3 loop region of 26S rDNA. Species of Pratylenchus that are very similar at the morphological level show relatively high levels of sequence variation in the D-3 region. Analysis of the DNA-sequence variation supported the general conclusions the morphology-based o f analyses. Phylogenetic analysis of the D-3 region of rDNA helped to resolve some of the polytomies that resulted from analysis of only morphological characters. Concordance or congruence estimates among morphological and molecular phylogenies were also conducted. The phylogenetic hypotheses of relationships among species of the genus Pratylenchus that we developed will contribute to understanding of the biogeographic coevolutionary relationships among these nematodes and their host plants.

### 196

Phylogeny of Insect Pathogenic Nematodes of the Genus <u>Heterorhabditis</u> (Nemata: Heterorhabditidae) from Analysis of 26s rDNA Sequences and Random Amplified DNA Polymorphisms. SCOTT L. GARDNER\*, F. WU, W. K. THOMAS, H. K. KAYA, and E. P. CASWELL-CHEN

We analyzed the phylogenetic relationships of nematodes of the genus Heterorhabditis because they are excellent laboratory model organisms, they are extremely easy to culture, either in-vivo or in-vitro, the described species are morphologically distinct, phylogenetic relationships among the recognized species of Heterorhabditis are unresolved and morphologically-based phylogenetic analyses have not yet appeared in the literature. To gain insight into the evolutionary history of this monophyletic group of nematodes, we applied two different molecular techniques (Random Amplified Polymorphic DNA [RAPD] and DNA Sequencing) to estimate the phylogenetic relationships among the 5 described species and 2 "strains" of Heterorhabditis. The region of the DNA used for the RAPD analysis was from the "whole genome" and the sequence analysis was conducted on a 300 base pair region of 26s rDNA, the d-3 region. Sequences were aligned using the CGC package and phylogenetic analyses were conducted using maximum parsimony techniques. (Supported by NSF Grant 9024816)

### 197

A lungworm in Umingmak, the muskox, from the Canadian Arctic.

E.P. HOBERG\*, L. POLLEY and J. NISHI Muskoxen, Ovibos moschatus, are a relict of the mammalian megafauna which dominated the Holarctic during the Pleistocene. They became extinct in the Palearctic 3,000 years ago, and were largely extirpated from their range in the Nearctic by the end of the 19th century. Parasitological studies have been few and reports of lungworms other than Dictyocaulus have been difficult to confirm. In the 1980's work in the region of the Coppermine River, NWT, Canada documented the localized occurrence of a highly pathogenic lungworm in muskoxen. Collections in 1994 resulted in the recognition of a remarkable new protostrongylid. Adults in the lung parenchyma occur in massive cysts, up to 40 mm in diameter; females are 468 mm in maximum length and lack a provagina; males are up to 171 mm long, possess a bilobate bursa, and unique gubernaculum; first stage larvae have a dorsal spine and three cuticular folds on the tail. Such characters justify recognition of a new genus similar to Cystocaulus. Similar protostrongylids are unknown in Rangifer tarandus, the only large ruminant currently sympatric with muskoxen in the Arctic. Extreme pathogenicity of this parasite suggests caution in future translocations and reintroductions of muskoxen across their historic range in the Holarctic.

#### 198

New Evidence of Hystricognath Rodents Monophyly from the Phylogeny of their Pinworms. J.P. HUGOT\*, S.L. GARDNER, S. MORAND and C. SUTTON

The evolutionary relationships among hystricognath rodents and the geographical origin of the Caviomorpha (which had been endemic to the Neotropics for most of the Tertiary) have generated considerable debate. Some of the arguments used concerned the distribution of the parasitic pinworms of these rodents. New descriptions of the parasites of both African and Neotropical hystricognath rodents reveal the presence of a very specialized spermatheca, which, after dissection of hundreds of specimens, can be assessed to be exclusively encountered in the parasites of the sole hystricognath rodents. This is a very important and quite decisive argument for close relationships between Phiomorpha and Caviomorpha: As this pattern is certainly not closely linked with host/parasite interactions, it is very improbable that its apparition will be the result of a convergence and will be precisely restricted to the parasites specific to these host groups. Using this new information, we have built a data set of morphological characters that allow us to propose a clado-

gram showing phylogenetic relationships of oxyurids parasitic in hystricognath rodents. Character analysis was performed with PAUP computer program.

### 199

The Sixth Bueding-Von Brand Lecture

Recipient and title of the lecture to be announced at the meeting.

### 200

The Henry Baldwin Ward Medal Lecture

Recipient and title of the lecture to be announced at the meeting.

#### 201

The Effect of Giardia lamblia Enteritis on Intestinal Transit and Smooth Muscle Contractility. LYSE DESELLIERS\*, M.E. OLSON, and R.B.SCOTT

First, to determine whether Giardia lamblia has an effect on the contractility of jejunal longitudinal smooth muscle, weanling Mongolian gerbils were divided into a control group (n=8) and an infected group (n=8). Experimental animals were infected orogastrically with 2x105 trophozoites at time 0. Five days later animals were sacrificed and longitudinal smooth muscle segments were obtained from the jejunum. Isometric tension of jejunal segments was recorded in tissue baths perfused with oxygenated Krebs solution. The development of active tension with stretch as well as the dose-response curve to bethanechol were significantly increased in the infected animals compared to controls. This significant increase was still seen in the presence of TTX but was not observed in response to KCL depolarization. This increased contractility of the infected tissues may reflect receptor-dependent changes in smooth muscle function. Secondly, to determine if this altered contractility was associated with changes in gastrointestinal transit, control and infected animals were infused either orogastrically or intraduodenally with Na251CrO4. The isotope was allowed to progress along the GI tract then the radioactivity of the blood, six equal segments of small intestine, the stomach, the cecum and the colon was determined by gamma counting. Using the geometric centre of transit as a marker, gastrointestinal transit in both the fasted and fed

states, and intestinal transit in the fasted state, were all significantly (p<0.05) greater in the infected compared to control animals. In this model, giardiasis is associated with a faster GI transit.

## 202

Effects of Giardiasis on Growth and Development in the Young

M.E. OLSON\*, T.A. MCALLISTER, L. DESELLIERS, K.-J. CHENG, D.W. MORCK

It is believed that infections with Giardia lamblia influence the development and growth in humans and animals. Aim: To determine if giardiasis affects growth and development in an animal model. Methods: Lambs which were free of intestinal parasites were obtained by delivery into iodine solution. They were removed from the ewe, provided colostrum and an intestinal bacterial inoculation. At 5 weeks of age animals were randomly allocated by sex and weight into 2 treatment groups which were housed separately for the study duration. At 6 weeks of age 23 lambs were infected with 10<sup>6</sup> Giardia lamblia trophozoites by intraduodenal inoculation while 24 control lambs received saline. Results: Giardia infected lambs passed cysts from 7 to 16 weeks of age while control lambs were free of Abnormal stools were more frequently infection. observed in infected lambs (P < 0.05) for 5 weeks following the challenge. Giardiasis was associated with a reduction in weight and rate of gain from week 5 to week 16 compared to control animals (P < 0.05). There was no difference in feed intake but from week 5 to 16 feed efficiency was significantly impaired (P < 0.05) between infected (3.77 + .11) and control (3.44)+ .11) lambs. Carcass weight was significantly elevated in control (21.91  $\pm$  .48 kg) over infected lambs (20.60  $\pm$  .41 kg). Conclusion: This study clearly demonstrates that giardiasis has a significant impact on growth and development in the young.

# 203

|A histochemical study of vitelline cells and |egg-shell in the Cyclocoelium mutabile. |ZH.K.SHAYMARDANOV\*, K.AHMETOV

Histochemistry of vitelline cells and egg-shell formation of trematoda <u>Cyclocoelium mutabile</u> (<u>Cyclocoeliidae</u>) have been researched. Mature worms have been collected from air bags of naturally infected ducks (<u>Fulica atra</u>). The vitelline cells have been observed by various histochemical procedures for basic proteins, tyrozine, phenols and phenolase. Vitellaria of <u>C.mutabile</u> are presented in numerous

follicles each of them is containing a large number of vitelline cells of different stages at maturity. Immature vitelline cells are 2.6 x 6.3 µm in diameter the nucleus of which measure 2.3 x 3.9 µm. Their nucleus to cytoplasm ratio are quite high. As the formation and growth rates of vitelline cells increase and the size of mature cells reach to 10.4 x 13.6 µm, their nuclei increase to 2.5 x 2.6 µm in diameter. The beginning of synthetic activity and the appearance of shallow egg-shell granules that gradually grow and fuse in globules are notted. As the vitelline cells mature their egg-shell granules increase in size and number. The presence of basic proteins and proteins containing tyrozine have been indicated. Histochemical methods for proteins containing -NH, and - SH groups have showed their moderate contents in vitelline cells. Reactions for proteins containing - S-S groups, phenols and polyphenol oxidase have given negative results.

### 204

Chemical nature and egg-shell formation of some trematodes. ZH.K.SHAYMARDANOV\* The process of egg-shell formation in Hypoderaeum conoideum and Liorchis scotiae has been studied. Mature worms have been collected from intestines naturally infected ducks and scar of cow. Ten histochemical tests, have been used for detection of precursors of the egg-shell, basic proteins, phenols and phenolase. Vitelline follicles of H.conoideum and L.scotie are formed of cells in three stages of development conditionally: immature, maturing and mature cells. They are varied by size, levels of differentiation, secretional activity and presence of egg-shell globules. Reactions for basic proteins, proteins containing tyrosine were moderately positive in the developing vitelline cells, shell of eggs from proximal duct, but intensive positive in the mature cells. However, they are negative in eggs from distal duct of uterus. This trematodes are characterized by certain diversities: 1) tests for proteins containing - NH, and - SH groups were intensive positive in H.conoideum and weakly positive in L. scotie; 2) intensive positive reaction for S-S groups in L.scotie and negative - in H.conoideum were seen; 3) phenols and phenolase presented in mature vitelline cells of H.conoideum, but they were absent in L.scotie. Thus, this tremotodes differ in various nature of egg-shell stabilization. The presence of all three precursors of sclerotin proves quinon-tanned lineage of egg-shell in H.conoideum. The egg-shell in L.scotie is a keratin type of protein as at most paramphistomatides.

### 205

Localization of Aerobic and Anaerobic Specific Enzymes in *Ascaris suum* Larvae, BAISONG MEI\*, PATRICIA KOMUNIECKI and RICHARD KOMUNIECKI

Ascaris suum undergoes an aerobic to anaerobic transition in its energy metabolism during development. Early larval stages (L1, L2, L3) are aerobic and contain a functional tricarboxylic acid cycle. In contrast, L4 and adult body wall muscle exhibit predominantly anaerobic energy-generating pathways and oxidize glucose incompletely to a mixture of reduced organic acids. However, both the L3 and cultured L4 contain key enzymes characteristic of both aerobic and anaerobic pathways. For example, the L3 contains significant amounts of the 2-methyl branched-chain enoyl CoA reductase (ER), a key enzyme involved in branched-chain fatty acid (BFA) formation in adult muscle, and the L4 still contains substantial cytochrome oxidase (COX) activity, even after they become cyanide-insensitive and begin to synthesize BFAs. Therefore, the current project was designed to localize these key enzymes in L3 and L4 to determine whether they are present in aerobic and anaerobic specific mitochondrial populations or muscle COX activity was identified using DAB (diaminobenzidine) staining under TEM, and the ER was localized immunohistochemically at both light and TEM levels using affinity purified polyclonal antisera prepared against the ER purified from adult muscle. COX staining was observed in mitochondria of hypodermis, muscle and intestine in the L2, L3 and L4, but not in mitochondria of any adult tissue. In contrast, ER immunoreactivity was observed only in muscle mitochondria of the L3, L4 and adult. No selective staining of individual muscle fibers was observed for either enzyme. Collectively, these data suggest that muscle mitochondria of the L3 and L4 contain both enzymes. Current work is focused on the colocalization of these enzymes in a single muscle preparation using antisera against ER and COX.

# 206

Arginine Phosphate in Haemonchus contortus and Steinernema carpocapsae. EDWARD PLATZER\*, S.N. THOMPSON, D.B. BORCHARDT, and H.R. GAMBLE Early biochemical attempts to identify phosphagens in nematodes were unsuccessful. We developed an in vivo flow <sup>31</sup>P NMR technique to examine phosphorus metabolites in infectious larvae of Haemonchus contortus and Steinernema carpocapsae. Long-term viability was maintained by continuous circulation of oxygenated suspensions of larvae through a NMR spectrometer. Phosphorus resonances consistent with phosphoarginine, ATP, and other phosphorus metabolites were readily apparent in in vivo 31P NMR spectra.

The level of phosphoarginine quickly declined when nematode suspensions were purged with nitrogen and was restored quickly by oxygenation. Saturation transfer NMR demonstrated forward and reverse exchange of phosphorus between phosphoarginine and ATP. These studies demonstrate functional phosphagen kinase in two nematode orders.

### 207

Brugia pahangi depresses the frequency of spontaneous contractions in bovine mesenteric lymphatics studied in vitro: A role for filarial factors in the development of lymphedema?

L KAISER\*, M MUPANOMUNDA, JF WILLIAMS

Although physical obstruction of lymphatic vessels may result in lymphedema, the physical presence of the parasites cannot be responsible for all the clinical manifestations of filarial diseases, and alternative mechanisms may play a role in the pathogenesis of filariasis. Filarial factors are known to alter vascular responses in vivo and in vitro, and could be involved in altered lymphatic function seen in filariasis. Experiments were designed to test the hypothesis that spontaneous contractions of bovine mesenteric lymphatics studied in vitro are altered by the filarial parasite Brugia pahangi. Rings of bovine mesenteric lymphatics were suspended in tissue baths at optimum tension and spontaneous contractions were evaluated for rate, rhythm, & amplitude. Rings were used only if they met rigid inclusion criteria determined by preliminary experiments. Eighteen of 75 rings met the criteria (rate >1.8/minute; regular rhythm; and amplitude >500 mg) and 12 were randomly assigned to Brugia or control. Parasites were added to the bath at time zero and changes in rate. rhythm. & magnitude of contractions were evaluated every 10 minutes. Comparisons were made within groups over time & between groups. Brugia significantly depressed frequency of spontaneous contractions and altered the rhythm of contractile activity. In control rings there were no changes in rate, rhythm, or amplitude. Since spontaneous contractile activity is likely important in the propulsion of lymph, alterations of contractile activity could result in lymphedema. Thus, filarial factors may be responsible, in part, for altered lymphatic function seen in lymphedema, and pharmacological intervention aimed toward influencing metabolism of the adult parasites, may in this complicated scenario, prove useful. (AI #35757, AI #01082, & WHO)

### 208

Longevity and Nutrient Acquisition in Isolated Nurse Cells of *Trichinella spiralis*.

J. MONTGOMERY\* and G.L. STEWART

Trichinella spiralis nurse cells recovered with a recently designed nurse cell isolation unit were maintained for over one month in RPMI 1640 (L-glutamine and sodium bicarbonate) supplemented with 10% fetal calf serum and

antibiotic/antimycotic solution at 37°C in a CO<sub>2</sub> incubator. Movement of larvae allowed easy assessment of parasite viability, while nurse cell viability required application of a fluorescent vital stain. To determine the molecular weight range of molecules allowed to pass freely into the nurse cell we used fluorescent-labeled dextrins of varying molecular weight (4000-2,000,000). Regardless of molecular weight, all dextrins were taken up by what appeared to be pinocytosis. Uptake was inhibited by cytochalasin (pinocytosis inhibitor) and confocal microscopic analysis strongly suggested acquisition of dextrin by nurse cells via pinocytosis. Fluorescent label moved from the nurse cell membrane into the cytoplasm of the nurse cell, appearing in the intestine of the enclosed larva by 18 hr postexposure. Nurse cells recovered mechanically as well as those recovered by exposure to trypsin in the nurse cell isolation unit showed similar patterns of uptake of labeled dextrin. The importance of the collagenous capsule and nurse cell membrane in protecting the enclosed cell and worm was illustrated in experiments in which nurse cells and larvae remained viable following 1hr exposure to 5% sodium azide or 10% formalin.

# 209

Helminth Communities in the Northern Spring Peeper, *Pseudacris c. crucifer* Wied, and the Wood Frog, *Rana sylvatica* Le Conte, from Southeastern Wisconsin.

H.R. YODER\* and J.R. COGGINS Fifteen (Pseudacris Spring Peepers crucifer) and 21 Wood Frogs (Rana sylvatica) were collected by dip net from a temporary pond in Ozaukee County Wisconsin in April, 1993. Necropsy revealed that only two of fifteen Spring Peepers were infected with adult helminths. One adult Rhabdias ranae was in one host and one Glypthelmins pennsylvaniensis was found in the other frog. Multiple Spring Peepers were infected with the diplostomula of Fibricola texensis and numerous encysted helminth larvae were recovered from Spring Peepers. Oswaldocruzia pipiens was the most prevalent helminth in Wood Frogs (38%) followed by *Haematoloechus* varioplexus (24%), Rhabdias ranae (14%) and Cosmocercodies dukae (14%). Several Wood Frogs were infected with the diplostomula of F. texensis. Of the helminths found in the Wood Frog population, R. ranae had the highest mean intensity (3.7  $\pm$  3.1) followed by O. pipiens  $(2.75 \pm 4.2)$ , H. varioplexus  $(2.4 \pm 1.3)$  and C. dukae  $(1.5 \pm 0.7)$ . Despite small sample sizes, low intensities and low species richness indicate

depauperate, isolationist helminth communities in these two populations of frogs.

### 210

Retrospective Report on Gastrointestinal and Ectoparasites From Gray Wolves (<u>Canis lupus</u>) Submitted to the National Wildlife Health Center, 1988-1994. R.A. COLE\*, N.J. THOMAS, <u>C.L. RODERICK</u>, and <u>C.U. METEYER</u>.

We conducted a retrospective survey of parasitological results reported in necropsy records for 48 gray wolves (Canis lupus) submitted to the National Wildlife Health Center from 1988-1994. Twenty-five females, eight of which were immature, and 23 adult males were collected from Wisconsin (N=17), Minnesota (N=23), Michigan (N=3), USA and Ontario, Canada (N=5). Ninety-four percent of the wolves had The most common nematode was parasites. Oslerus olseri in the lung and trachea from 25% of wolves. Uncinaria stenocephala and Ancylostoma caninum were found in 17% and 10% of the wolves, respectively. Two genera of cestodes, Taenia sp. (44%) and Echinococcus granulosus (27%) were reported. The trematode, Alaria sp. was recovered from 35% of the carcasses. Three ectoparasites were common; Trichodectes canis (33%), Dermacentor sp. (15%), and Ixodes sp. (10%). Oocysts and/or sporocysts of Sarcocystis sp. were reported from 10% of wolves with an additional 6% having sarcocysts in cardiac or striated muscle. Intensity of infections was not described in necropsy reports.

# 211

Parasites in Georgia Farm Pigs. T.B. STEWART\*, J.A. STUEDEMANN and H. CIORDIA.

Parasites were recovered from a total of 40 market pigs at two slaughterhouses and 125 fecal samples were examined from growing pigs and sows on nine middle Georgia farms. Infection rates of market pigs were: Ascaris suum, 47%; Oesophagostomum spp., 30%; and Trichuris suis, 22%. Other parasites found in one or two pigs were: Stephanurus dentatus, Ascarops strongylina, Hyostrongylus rubidus, Metastrongylus sp. and Sarcoptes scabiei. All diaphragms were negative for Trichinella spiralis. The average number of parasites per infected pig from well managed farms with parasite control programs was 64 compared to 258 for pigs from poorly managed farms without specific parasite control programs. Likewise, the average infection rate of pigs was 18% with good management

and 43% with poor management. Some fecal samples from all nine farms were positive for parasite eggs: A. suum and Eimeria spp. in 8, T. suis in 7,

Oesophagostomum spp. in 6, Strongyloides ransomi in 4 and Metastrongylus spp. in 1. The percent of positive fecal samples were: Oesophagostomum spp., 61%; Eimeria spp., 53%; A. suum, 38%; S. ransomi, 12%; T. suis, 10%; and Metastrongylus spp., 2%.

### 212

Incidence of intestinal protozoan parasites in X. vigilis and B. attenuatus of California mountainous regions. I. Syntopy influence on cross-parasitism. ALFREDO R. SANCHO<sup>1\*</sup>, DAVOOD SOLEYMANI<sup>2</sup>

We investigated the incidence of protozoan intestinal parasites and infection rates in California indigenous species of Xantusia vigilis (Desert night lizard) and Batrachoseps attenuatus (California slender salamander). These host-species are phenotypically different, yet they share common habitats, diets, and certain anatomical features. Both are found coinhabiting the Foothill Woodland communities of the lower elevations within the Pinnacles National Monument, San Benito and Monterey Counties. Three collection sites were selected within 378 to 758 meters in altitude, and each particular site had its own microhabitat characteristics. A total of 21 and 22 specimens of X. vigilis and B. attenuatus respectively, were collected. Surgical extraction of stomach and intestinal contents from both host species, included ants (Phormakytes sp.), small termites (Kalothermes sp.) and varied vegetation. After careful microscopic examination of fecal material, on fresh and stained slide mounts, the following intestinal protozoan parasites were identified: Proteromonas sp., Hexamita sp., Tritrichomonas sp., Chilomastix sp., Balantidium sp., and Nyctotherus sp. Four out of the six parasites were found in both hosts, and one solely in each host. Tritrichomonas augusta had the highest incidence rate in both host species, 59 and 95 percent respectively. Morphological differences between T. augusta found in either host were minimal. Variances in infection rates and incidence were mainly due to the individual host and not due to their biodistribution, since they were both found inhabiting in the same three collection sites. These findings substantiate the importance of co-inhabitance and the influence of syntopy on cross-parasitism between two phylogenetically different species.

# 213

<u>Cryptosporidium</u> Infections and other Intestinal Parasites in Saimadoyi, a Bari Indian Community from Western Venezuela. LEONOR CHACIN-BONILLA' and Y. SANCHEZ-CHAVEZ.

The indian populations in Venezuela are among the most vulnerable groups for intestinal parasites, especially those that live in en-

vironments with little access to public health programs. The venezuelan Bari population is represented by 1503 individuals. A point prevalence survey for <u>Cryptosporidium</u> and other intestinal parasites was conducted in 303 subjects one to 65 years of age in Saimadoyi, a Bari indian population at the southwest of Zulia State, Venezuela. Single stool specimens were collected. Modified Ziehl-Neelsen carbolfuchsin staining of 10% formalin preserved stool was used to identify <a href="mailto:Cryptosporidium">Cryptosporidium</a>
oocysts. Direct wet mounts, iron-hematoxylin stain smears and formalin ether concentrates were examined to detect other intestinal parasites. Cryptosporidium infections were observed in 31 individuals (10.2%) with a high percentage of asymptomatic carriers (19 of 31, 61.2%). Cryptosporidium was the single detectable potential pathogenic parasite in only eigth (25.8%) of the patients. Infection rates with one or more parasites and multiple parasitoses were very high (95.3% and 71.9% respectively) Ascaris lumbricoides (52.1%) and Trichuris trichiura (34.6%) were the most common parasites. Among the protozoa, Entamoeba coli was observed most frequently (43.2%) and Giardia lamblia was the predominant pathogen (28.3%). Entamoeba histolytica infection rate was 17.8%. The high level of parasite infection reflects the low socioeconomic status of the population. Our findings suggest that although Cryptosporidium is an important cause of diarrhea, the proportion of asymptomatic carriers may be high in areas of low socioeconomic status in developing countries.

# 214

Detection of <u>Giardia</u> Cysts in Foods using Immunoflucrescence Microscopy and Flow Cytometry. BRENT R. DIXON\*, MONIQUE PARENTEAU, and GREG SANDERS

Several foodborne outbreaks of giardiasis have been reported in North America within the last fifteen years. In the majority of cases, the suspected 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 foods 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, neither immunofluorescence microscopy nor flow cytometry has been previously evaluated for the detection of cysts in Toods. The present study, therefore, represents a direct comparison of the sensitivity and efficiency of immunofluorescence microscopy with that of immunofluorescence flow cytometry in the detection of Giardia cysts in spiked foods. Freliminary results indicate that flow

cytometry is a more sensitive and rapid technique and may be useful in positively identifying the source of <u>Giardia</u> infection in any future foodborne outbreaks.

### 215

Cryopreservation of Pathogenic *Acanthamoeba* and *Naegleria*. DAVID T. JOHN\*, PENNY L. EDDY and REBECCA A. JOHN.

Pathogenic free-living amebae belonging to the genera Acanthamoeba and Naegleria cause serious infection of the eye and the central nervous system. The purpose of this study was to evaluate a variety of conditions of cryopreservation to develop a single procedure for freezing pathogenic Acanthamoeba and Naegleria. The amebae used in this study were A. castellanii (EI-212), N. australiensis (PP-397) and N. fowleri (LEE). Amebae were cultivated axenically in Mix ameba medium at 37°C, washed in fresh Mix medium and suspended in the freezing medium which varied with each experiment. One-ml quantities of amebae in freezing medium were dispensed in cryogenic vials and frozen at -70°C. Amebae were rapidly thawed by placing the cryovials in a 37°C waterbath and viability was determined by exclusion of 0.4% Congo red prepared in deionized water and mixed with equal volumes of amebae in freezing medium. The average best conditions for freezing the three species studied were: 1x10<sup>6</sup> exponentially growing amebae/ml of freezing medium consisting of 12% dimethylsulfoxide, 20% heat-inactivated bovine calf serum, 4% glucose, in Mix ameba medium; 30 min equilibration at 23°C (room temperature), followed by 60 min at -20°C with storage at -70°C. After 12 months of freezing, viability was 39% for A. castellanii, 47% for N. fowleri, and 53% for N. australiensis. (Supported by EPA Grant R-818106)

# 216

Detection of Antibodies of Schistosomiasis by Micro-Filter-Paper Blood Radio-Immuno Precipitation PEG Assay (RIPEGA), ZHONGLING LU

Schistosomiasis patients and farming cattle, the host reservoir of Schistosoma japonicum, still exist widely in the rural areas of the developing countries. In the past, blood serum was taken for disease examination but it brought much difficulty to the blood taking and serum separation in the field. This study takes capillary blood spotted on filter papers for blood sample and adopted the improved method of radio-immuno assay. This method is to

use isotopic I labelling the egg antigen of <u>Schistosoma japonicum</u> to antibody level in host sera. assess to PEG is used as a separate agent. The total amount of sample blood is only 40-80 ul. average blood precipitating rate (X) + 2SDcriteria, higher is used as the positive and lower for negative. 4 groups of infected rabbits (n=62) were used in the experiment, the positive rate is 100%. 3 batches of people (n=148) were tested for Sephadex G100 chromatographic antigen the positive rate is 100%. Of which 63 for samples were examined by serum control, the positive rate was 98.57%. Our experiment proved the filter paper blood can replace the serum from venous blood. Though the method is used mostly in the investigation of human epidemioligical schistosomiasi, but also applicable in the especially schistosomiasis, veterinary suitable for the rural areas in the developing countries.

### 217

Prevalence and Evolution of Antibody in Natural Bovine Hypodermosis in Spain. JAVIER MARTINEZ\*, IGNACIONAVARRETE, ALVAROMARTINEZ, VICTORIA JIMENEZ and SANTIAGO HERNANDEZ.

The evolution of antibodies anti-Hypoderma in infested hosts is related to the endogenous life-cycle of the parasite, which is dependent of the climate and different for each country. Antibody levels throughout the life cycle of the parasite in South-West of Spain are studied, using the ELISA technique. Animals examined were divided in two groups by age: more than one year and less than one year. The profile of the antibody curve is similar for two groups, and one great peak is detected in Autumn, when first instar larvae are arriving to the "winter resting site" and release of antigen is higher. There is another peak in Spring, related to the arrival, maturation and fell of the third instar larvae from the back of the host. Both peaks coincide with elevated values of prevalence by direct and serologic methods, but in this case is higher the presence in the back. Comparison of the curve of the two groups showed higher levels in older animals. Prevalence by direct examination and immunoenzimatic techniques is also studied in each group. General prevalence by direct methods (examination of different larval stages in "winter resting site" and back) is very similar, but slightly higher in younger animals (18,6%) and 17,7%. On the other hand, the use of the ELISA technique revealed more seropositive animals in the older group (39,7% and 42,4%), probably due to the acquired resistance to the parasite, because there are less warbled animals.

(Supported by CICYT project AGF93-0593-01-02)

### 218

Monthly Fluctuations and Hypobiosis Phenomenon of Gastrointestinal Nematode in Calves in The Pyrenees (Spain). SONIA ALMERIA\*, J. URIARTE.

Monthly fluctuations of worm burdens and arrested development of gastrointestinal nematodes in cattle living in mountainous areas of Spain were studied. Fourteen calves grazed together with a flock of 120 cattle from May to October following the traditional system in mountainous areas (Permanent calves). Each month 2 helminth naive calves (Tracers) were added and grazed for four weeks throughout the grazing season. Each four weeks the tracers and two permanent calves were housed for two weeks before slaugther. Worm counts and species identification were performed. Faecal and blood samples were collected every two weeks from both groups of animals.

At slaughter calves the identified species were: Ostertagia ostertagi, O. lyrata, Teladorsagia circumcineta, Trichostrongylus axei, Cooperia oncophora, Trichostrongylus longispicularis, Capillaria bovis, Nematodirus helvetianus, Oesophagostomum radiatum, Chabertia ovina and Trichuris spp. Ostertagia ostertagi showed the highest abundance and prevalence, followed by Cooperia oncophora and Trichostrongylus axei. The highest intensity of infection was seen in May, June, September and November with an average of worm burden of 4,044, 3,755 (spring grazing in areas below 1,000 m), 2,780 (last month in areas higher than 1,000 m) and 2,821 (autumn grazing of areas below 1,000 m) respectively. The average worm burden during the grazing season was 5,741.4 worms/animal. The highest burden (7,999 worms/animal) was observed after two months of grazing. The highest percentages of larvae inhibition were in autumn, with maximum levels of 65,2% for Ostertagia spp. and 93.5% for Cooperia oncophora. Similar inhibition levels were observed in animals with and without previous exposure, suggesting that, as in other temperature countries in the Northern hemisphere, acquired immunity was not the primary cause of the hypobiosis.

# 219

Kinetics of Pasture Contamination by Gastrointestinal Nematode Larvae in the Pyrenees (Spain). SONIA ALMERIA\*, J. URIARTE, M. LLORENTE

The dynamics of contamination with gastrointestinal nematode larvae of mountain pastures were studied between March 1988 and December 1991 over 5 grazing areas at different altitudes (900 m to 2,100 m). Grass samples were taken every two weeks and larval differentiation performed. The areas were grazed by animals according to traditional systems of grazing in mountain areas. Stabling in wintertime (November-April), grazing in harvesting meadows (elevation = 900 m) in spring (May-June) and autumn (October-Nov.), and grazing areas between 1,200-2,100 m in the summer (July-September).

In prairies below 1,000 m infective larvae were found from the end of October until June of the next year. At higher altitudes a

bimodal pattern of pasture larvae contamination was observed. A first peak appeared between March and June with a second peak starting in September and continuing until November. Ostertagia spp., Cooperia spp., Trichostrongylus spp., Oesophagostomum spp., and Nematodirus spp were found, with Ostertagia spp. being the most prevalent, followed by Cooperia spp.. Nematodirus were found sporadically.

Analysis of this data indicates that initial infection of the animals was the result of ingestion of overwintered larvae on prairies below 1,000 m. This initial infection may be responsible for a high prairies contamination in summer that causes the second period of potential risk from September onwards. The contamination of the prairies below 1,000 m in autumn is may be a result of the movement of animals from the higher prairies, which in turn may be responsible for the animals infection prior to stabling. These results imply that the autumm pasture larvae population was the most important part of the epidemiology of nematode gastrointestinal larvae in the Pyrenees, because these larvaes remained in the pasture till next spring reinitiating the cycle of parasitism of the animals.

## 220

The Effect of Selection for Ivermectin (IVM) Resistance on Fitness of Helgimosomoides polygyrus (Nematoda). J.M. NJOROGE\* and M.E. SCOTT

life history traits variety of were characterized in five strains Heligmosomoides polygyrus: the stock "parent" strain (S), the 8th and 15th parasite generations after selection with ivermectin (G8 and G15), and the 8th and 15th generations of control passage strains (P8 and P15). Though the establishment of G8 ivmselected strain was significantly higher (p<0.05) than that of the parent strain (S), the egg production profile of S, G8 and P8 was similar between 7 and 28 days postinfection (pi). At generation 15, passage strain (P15) had a higher establishment (P<0.01) as well as a higher net egg output (P<0.001) between 7 and 28 days pi than the parent (S) or the ivm-(G15) strain. selected However establishment of the ivm-selected (G15) and passage strain (P15) were similar. To date, long-term survival are only available for S, G8 and P8 strains. Survival over 4 months was significantly lower (P<0.01) for the ivmselected (G8) and passage (P8) strains than for the parent strain (S). The net output of the three strains over 4 months differed significantly (p<0.01), but showed consistent trend. The per capita fecundity did not differ among the three These data suggest strains. that both ivermectin selection pressure and rapid passage result in fluctuating shifts attributes of Heligmosomoides polygyrus.

### 221

Effect of Albendazole Selection and/or Rapid Passage on Life History traits of Heliqmosomoides polygyrus (Nematoda) in Immunized Mice. A. CHEHRESA, M.E.SCOTT AND R.N. BEECH.

Albendazole (ABZ) selection and/or serial rapid passage of <u>H. polygyrus</u> was done for 10 generations in CDI mice, and every second generation was characterized for a spectrum of life history traits including traits The objective was immunized mice. determine whether a similar host response to challenge would occur against the different parasite strains. Groups of BALB/c mice were immunized with the stock strain (S) challenged with ABZ-selected strains (G2, G4, G6, G8, G10), the passage strains (P2, P4, P6, P8, P10), or with the stock strain (S). Simultaneous with the challenge infection, BALB/c mice were given a primary infection with each strain. Host response was measured by worm numbers and per capita fecundity, both which are known to be reduced immunized mice. One-month survival immunized mice were similar all among strains. However, percent reduction in worm burden in P10 and fecundity in P6 through P10 of passage (challenge infection compared to the primary infection) 1 month post-infection (pi) was significantly lower than in mice challenged with the stock strain (S). Also, PlO had a higher profile of reproduction over 1 month infection period compared to stock parasites (S). The values of these measures for G10 were intermediate between P10 and These results suggest that mice stock. immunized with the stock parasite were less able to mount an effective host response against the parasites exposed only to a rapid passage procedure, but were able to recognize and respond to parasites selected for albendazole resistance.

#### 222

Prevalence of Dirofilaria tenuis in Raccoons in Georgia. PAUL DAVIS\*, WILLIAM IRBY and OSCAR PUNG Dirofilaria tenuis is a subcutaneous filariid of the raccoon (Procyon lotor) which is transmitted by mosquitoes and occasionally infects humans. The parasite has not been reported to occur in raccoons from Georgia though a patient from the southeastern section of the state was recently treated for a D. tenuis-induced conjunctival nodule. To determine the prevalence of **D**, tenuis among raccoons in Georgia, animals were live-trapped, anesthetized and bled. Wet mounts of fresh raccoon blood and preparations of blood concentrated in 2% formalin were examined for the presence of D. tenuis microfilariae using a light microscope. We found that 48 of 90 raccoons (53%) trapped in the southeastern Georgia counties of Richmond, Bryan, Bulloch, Candler and Liberty were infected with the parasite. Numbers of microfilariae ranged from 100 to 14,750/ml of raccoon blood (mean  $\pm$  SD = 2,215  $\pm$  2,757). The mean length and width (μm) of microfilariae in 2% formalin was 292.7 ± 29.5 and  $5.0 \pm 1.3$  respectively. Microfilariae had button-

hooked tails and were unsheathed. Additionally, we have begun to examine mosquitoes trapped in southeast Georgia to determine which species serve as vectors of <u>D</u>. tenuis in this area and to determine the prevalence of the parasite in mosquitoes. To date, we have examined 290 mosquitoes (12 different species) but have found none infected with the parasite. To our knowledge, this is the first report of <u>D</u>. tenuis in raccoons from Georgia.

#### 223

Defining Seasonal Limits of <u>Dirofilaria immitis</u> Transmission in the USA. J. B. LOK\*, D. H. KNIGHT, M. O'Brien and G. SMITH

Assuming the presence of vector-competent mosquitoes and a pool of susceptible hosts and microfilaremic reservoirs, the transmission of the canine heartworm, Dirofilaria immitis is limited in part by the availability of sufficient environmental heat units in excess of a discrete developmental threshold of the parasite. We have compiled average monthly temperatures over a 30 year cycle from 200 weather stations across the United States via the National Climatic Data Center. These data have been incorporated into a published linear model for the accumulation of heat units permitting heartworm development (Heartworm Development Units, HDU's). The days of onset and cessation of heartworm transmission at the reporting stations have been calculated assuming a 30-day mosquito life span. For example, the model predicts that in an extreme year, transmission will last from Mar. 19 to Dec. 8 in New Orleans, from May 14 to Nov. 8 in St. Louis and from June 11 to Sept. 15 in International Falls, MN. The product of this analysis is an isoline map depicting the recommended dates of the first and last doses of macrolide chemoprophylaxis for regions across the USA. The model is being validated by studying effects of single 24 hr. cold shocks on day 2, 4, 6, 8, 10, 12 or 14 PI on development of larval D. immitis incubated at 27° C to infectivity in susceptible Aedes aegypti. Preliminary findings indicate no decrease in larval viability until the cold shocks fall below 14° C. (Supported by the American Heartworm Society).

## 224

An Ecological Study of the
Overwintering of Toxocara Canis
Eggs in Toronto, Ontario. REVA
BERMAN\*, R. HANSELL, and J. YANG
Infection with the eggs of Toxocara
spp. may result in Visceral Larva
Migrans, especially in young children
and small mammals. Past studies have
indicated indirectly that Toxocara
eggs may remain viable after being
exposed to winter conditions. If eggs
remain viable over the winter, then a

bioaccumulation would be expected in the springtime. To test the overwintering capabilities of Toxocara canis, flats of grass were seeded with embryonated and unembryonated eggs. The eggs were challenged with extremes of temperature and humidity by placing them outdoors during part of the winter season in Toronto. Maximum and minimum temperature changes within the microclimate of the experimental sites were recorded daily. The results of this ecological study are reported here.

#### 225

Peptide antigen common to Schistosoma mansoni and Biomphalaria glabrata found in snail cerebral ganglion. SHARON FILE\*, ANPING CHEN, ANTONIO FERNÁNDEZ and JOSÉ JIMÉNEZ

A number of vertebrate peptides and classical transmitters have been colocalized generally in platyhelminths and molluscs including Schistosoma mansoni and Biomphalaria glabrata. In order to pursue the possible participation of immunoactive peptides in the regulation of this host-parasite relationship, a series of small proteins have been isolated from B. glabrata extracts using immunoaffinity chromatography loaded with anti-schistosome IgG. After further purification by SDS-PAGE, the protein fractions were used to elicit polyclonal antibodies. Immunocytochemistry was then used to localize each fraction in the mollusc and various stages of the parasite. One fraction was localized in the tubercules on the dorsal surface of the adult male worm, in the vitelline material of developing eggs and in vesicles in some large cells of the cerebral ganglion of B. glabrata. The same antibody preparation recognizes a 43 kD protein (doublet) in extracts of adult schistosomes and B. glabrata cerebral ganglia on Western blot analysis. Studies to characterize these proteins have begun. Vasoactive intestinal peptide, cholecystokinin, met-enkephalin, vertebrate ACTH and beta-endorphin could not be colocalized with this protein using immunocytochemistry. Bioassays indicate that crude extracts inhibit phagocytosis by murine macrophages. Further testing with IEF-purified fractions is in progress. This study was supported in part by the University of Puerto Rico and Howard Hughes grant.

## 226

Intestinal Permeability Properties of the 7-14
Day Old Chicken. D.P.THOMPSON, N.F.HO,
J.S.DAY, B.A.OELSLAGER and T.G.GEARY
Primary screens for anticoccidial drugs identify
numerous agents that possess potent in vitro activity
against Eimeria tenella. The vast majority of these,
however, possess little or no in vivo activity when
administered orally to infected chickens. To test the
hypothesis that inadequate biovailability contributes
to this lack of in vivo activity, we examined the drug

transport properties of 7-14 day old Leghorn-Hubbard chickens using an in situ intestinal perfusion model. Permeants tested included salicylic acid, glycine. glucose and halofuginone. Rates of absorption were determined as a function of flow rate to delineate the separate contributions of the aqueous boundary layer (P<sub>nu</sub>) and the intestinal membrane (P<sub>m</sub>) to the effective permeability coefficient (P<sub>e</sub>). At flow rates that mimimize the effects of the aqueous boundary layer, P<sub>m</sub> values for glycine and glucose, which are actively transported across the intestinal epithelium, were 24.2 and 21.2 x 10<sup>5</sup> cm/sec, while that for halofuginone, which is passively absorbed, was 6.2 x 10<sup>-5</sup> cm/sec. The aqueous boundary layer was the rate-limiting barrier for absorption of salicylic acid, glycine and glucose, but it controlled only 32% of halofuginone absorption. These results indicate that the permeability properties of chick intestine are similar to those of mammalian intestine. Therefore, the lack of in vivo anticoccidial activity of most experimental compounds is not attributable to permeability properties of the chick intestine.

### 227

Efficacy of decoquinate against Neospora caninum in cell cultures. D.S. LINDSAY\*, J.M. BUTLER, M.A. TOIVIO-KINNUCAN, and B.L. BLAGBURN.

Neospora caninum is a protozoan parasite that is a significant cause of abortion in dairy cattle. Decoquinate, ethyl 6-(decyloxy)-7ethoxy-4-hydroxy-3-quinoline carboxylate, is an anticoccidial that is approved for use in cattle for the prevention of intestinal coccidiosis. The present study was done to determine the efficacy of decoquinate against N. caninum in cell cultures and to examine its mode of action against this parasite. Dose titration studies indicated that this agent was highly effective. Concentrations as low as  $0.0001 \mu g/ml (0.24 nM)$  allowed some development of the parasite but completely protected the host cell monolayer. Concentrations of 0.01 µg/ml (24 nM) and higher killed the parasite. Decoquinate was not effective against extracellular tachyzoites. Transmission electron microscopy revealed drug induced mitochondrial swelling and an increase in number and size of cytoplasmic vacuoles in treated tachyzoites. Drug resistance could be induced by selection for growth in permissive levels of decoquinate.

## 228

Efficacy of Triclabendazole against Fasciola hepatica in Experimentally Infected Goats. SANTIAGO HERNANDEZ\*, VICTORIA JIMENEZ, JAVIER MARTINEZ and ALVARO MARTINEZ.

Efficacy of Triclabendazole (Fasinex®), a fasciolicide of benzimidazole group, was tested at a single oral dose of 10 mgr/kg b.w. against goats experimentally infected with Fasciola hepatica. Sixteen animals of Spanish "serrana" breed were used in the experiment. Age of the goats at the moment of the infection was six months, and they were divided in four groups of five animals each: Group 1, infected with 200 metacercariae and treated 4 weeks post-infection; Group 2, infected with 200 metacercariae and treated 8 weeks post-infection; Group 3, infected with 200 metacercariae and treated 16 weeks post-infection, and Group 4, infested with 200 metacercariae, untreated and used as control group. Animals were killed after treatment, and livers were examined to recover the flukes not affected by triclabendazole. The mean number of flukes recovered in the control group is 27,7. Efficacy of Triclabendazol is 90,7% when applied at 4 weeks postinfection, 99,1% at 8 weeks and 100% when is used at 16 weeks post-infection. These results showed that triclabendazole is more effective against mature flukes. Size of the flukes varies between 8 mm and 28 mm in control group, and there are not significative differences in the size between groups.

(Supported by CICYT project AGF92-0985)

# 229

Prepatent Period, Mean Fluke Burdens and Percent Take in Sheep Experimentally Infected with Metacercaria of the Liver Fluke Fasciola hepatica. DANIEL E. SNYDER\*.

During the development of an animal model to evaluate compounds with flukicidal activity, sheep were experimentally infected with metacercaria of the liver Nineteen sheep (Mean fluke Fasciola hepatica. weight=37.3 kg) were each orally infected with 150 metacercaria. Individual rectal fecal samples were collected starting at week 6 post-infection (PI) and collected weekly thereafter out to week 12 PI when each animal was necropsied. Fecal samples (2 g) were processed using a sedimentation technique and scored as positive or negative. At necropsy the entire liver, gallbladder and adjacent duodenum were dissected and examined and the number of flukes found recorded. At weeks 6, 7, and 8 PI, fluke eggs were not detected in the fecal samples of any of the sheep. At weeks 9, 10, 11, and 12 Pl, 32%, 74%, 95% and 100% of the sheep,

respectively, had positive fecal egg counts. Animals that became positive remained positive at subsequent fecal collection dates out to necropsy. At necropsy, the mean number of flukes recovered was 60 (range 31-94; S.E.=4.18) and the calculated mean percent take of administered metacercaria was 40%. Moderate fluke associated damage to the livers was noted at necropsy. An inoculum of 150 F. hepatica metacercaria given orally to sheep produced patent infections in all 19 sheep at 12 weeks PI. The administration of experimental compounds to evaluate efficacy against adult fluke infections in sheep could be given at 12 weeks PI using this experimental model.

#### 230

Does RM340 depress relaxation less than thiacetarsamide in pulnionary artery from heartworm infected dogs studied in vitro?

DS MAKSIMOWICH\*, JF WILLIAMS, L KAISER

Acute pulmonary complications associated with adulticide treatment in heartworm infected dogs have been attributed to embolization of dead worms. However, adverse reactions are often seen prior to worm death, suggesting that other mechanisms may be involved. In addition, RM340, an adulticide awaiting approval in the US, reportedly causes higher worm mortality than thiacetarsamide without greater thromboembolism, further suggesting that embolization of dead adult parasites is not solely responsible for complications seen after adulticide treatment. An alternate explanation for acute reactions after adulticidal therapy could be depressed vascular relaxation induced by the arsenicals. Experiments were designed to test the hypothesis that adulticides depress vascular relaxation. Isolated rings of pulmonary artery from heartworm infected dogs were suspended in tissue baths & dose-response relationships to the vasoconstrictor norepinephrine, the endothelium-independent vasodilator nitroglycerin, and the endothelium-dependent vasodilator methacholine were done +/- thiacetarsamide or RM340 at concentrations calculated to be similar to peak therapeutic concentration. Comparisons were made between untreated control rings, and rings treated with either thiacetarsamide or RM340. Thiacetarsamide significantly depressed relaxation to nitroglycerin, but RM340 did not. Relaxation to methacholine was significantly depressed by both thiacetarsamide and RM340. Norepinephrine constriction was not different. Whereas, thiacetarsamide alters both endothelium-independent and endothelium-dependent relaxation, RM340 selectively depresses endotheliumdependent relaxation. RM340 has no apparent direct influence on vascular smooth muscle, which could account for the fewer side effects seen after treatment with RM340. (NIH AI #35757 & AI #01082)

# 231

Correlation Between <u>In Vitro</u> and <u>In Vivo</u> Potency and Onset Kinetics for Selected Anthelmintics. EILEEN M. THOMAS', SANDRA S. JOHNSON, TOM J. VIDMAR, GEORGE A. CONDER, NORMAN F. HO and DAVID P. THOMPSON.

The activities of selected anthelmintics on the gastrointestinal nematode, Haemonchus contortus, were compared using in vitro and in vivo assays. In the in vitro assay, the concentration- and timedependent effects of the compounds on adult H. contortus were measured using an automated motility recording system. The concentration required to reduce motility by  $\geq 50\%$  (EC<sub>50</sub>) and time to reduce motility by  $\geq 50\%$  (t<sub>50</sub>) at the rapeutically relevant concentrations were determined. In the in vivo assay, compounds were evaluated by determining the percentage reduction of H. contortus in experimentally infected jirds at various concentrations and times after oral dosing to determine EC90 and t90 values. The comparative potencies and onset kinetics between the in vitro and in vivo assays correlate highly for compounds within a chemical class. However, between chemical classes, high in vitro potency and rapid onset kinetics does not always extend to the in vivo model. Except for the benzimidazoles, compounds that are active in vivo against H. contortus at ≤1 mg/jird are also active in vitro at ≤3 µM. Benzimidazoles are effective in the in vivo assay at 28-30 h but are completely inactive in the in vitro assay. Results of these studies suggest the potential utility of an in vitro assay using a target nematode to examine intrinsic potency and onset kinetics of anthelmintic compounds identified in mechanism based screens.

## 232

Efficacy of Azadirachtin and Pyriproxyfen Against Developing Stages of the Cat Flea, Ctenocephalides felis. A.A. MARCHIONDO\*, S.W. FOGT, D.L. HEIMBICHNER, and S.L. STROH

Azadiractin is a natural insect growth regulator (IGR) extracted from seeds of the tropical neem tree. This limonoid IGR is similar in structure to the insect Pyriproxyfen molting hormone. ecdysone. (2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine) is a new synthetic IGR considered to be a juvenile hormone mimic. Both IGRs were evaluated in vitro at concentrations of 0, 0.25, 0.5, 1.0 and 2.0 mg/ft<sup>2</sup> for activity against developing stages of the cat flea. Treated paper discs from each of the five concentrations were seeded on test day (TD) 0 with 100 newly collected flea ova (less than 18 hrs old), covered with rearing medium, and incubated at 26-28° C and 80-85% relative humidity. Direct counts of larvae (ovicidal activity), pupae (larvicidal activity) and adult fleas (inhibition of adult emergence) were made on TD 7, 13 and 28, respectively. No detectable ovicidal activity was observed for either IGR regardless of the level of concentration tested. Pyriproxyfen provided

100% larvicidal activity and inhibition of adult emergence. In contrast, azadirachtin provided only 14.6% and 10.9% larvicidal activity at levels of 0.5 and 2.0 mg/ft², respectively. Azadirachtin provided 16% inhibition of adult flea emergence at 0.25 mg/ft², 28% at 0.5 mg/ft², no detectable inhibition at 1.0 mg/ft², and only 10% at 2.0 mg/ft². Further in vitro evaluations were conducted with pyriproxyfen at 0, 0.125, 0.25, 0.5, 0.75, 1.0 and 2.0 mg/ft² on carpet swatches. Ovicidal activity of >90% was detectable at all treatment concentrations at TD 7. At TD 114, larvicidal activity ranged from 42.3-76.9%. However, inhibition of adult flea emergence was 87.2% at 0.125 mg/ft², 94.9% at 0.25 mg/ft², and 100% at all higher concentrations. Microscopy of developing larvae from coccons revealed structural abnormalities.

### 233

Development and Utilization of a DNA Probe Assay for Quantitation of <u>Eimeria</u> Parasite Load in <u>In Vivo</u> Studies. HELEN PROFOUS-JUCHELKA\*, M. HOZZA, J. ONDROF, M. KREIDER, and D. SCHMATZ

Presently, there is no quantitative method for evaluating the effects of a drug or vaccine on the specific stages of Eimeria parasite development in vivo. To this end, an assay utilizing a repetitive parasite-specific DNA probe has been developed. The assay has been validated with known coccidiostats using E. tenella as the infectious agent. The coccidiostats which were tested include salinomycin, robenidine, nicarb, amprol, and nitrophenide. These coccidiostats are known to effect different stages of the parasite's life cycle and the results obtained confirm their stage specificity. The DNA probe assay was also used in conjunction with a visual immunoassay to determine the stages of parasite growth which are effected by the chicken's immune system after the animals had been immunized by a trickle infection of sporulated oocysts. Results indicate that both of the asexual stages as well as the early sexual stages are targeted by the host's immune response. Using this DNA probe assay one can now quantitate total parasite load in infected gut tissue and relate this to various other parameters such as protein levels, enzyme activities and metabolic products. This assay can also be used with other species of chicken coccidia in vivo and is sensitive enough to monitor E. tenella growth in culture.

## 234

Pathological Changes in the Jejunum of Calves Naturally Infected with Giardia and Cryptosporidium. N. RUEST\*, C. GIRARD, Y. COUTURE and G.M. FAUBERT

Giardiasis and cryptosporidiasis are frequently diagnosed in calves at our large animal clinic here at the Veterinary School. There have been few studies reported in he literature concerning the pathology caused by these two intestinal parasites. The aims of this study were to follow the histologic changes in the villi and crypts located in the jejunum of naturally infected calves. We have also followed the changes in the number of intraepithelial lymphocytes during the acute phase of the infection. For this purpose, 29 calves aged between 7 to 10 days old were bought at a local auction. The animals were housed in a barn in individual stalls to avoid cross-contamination. They were fed twice daily with milk. Stool specimens were collected three times per week over a period of 45 days. The first specimen was collected at day 35 after birth, and they were analyzed for the presence of Giardia cysts and Cryptosporidium oocysts. Six calves did not pass any cysts or oocysts and were used as controls. Fifteen of them passed cysts of Giardia, five of them passed both cysts and oocysts, and three of them passed oocysts only. The villus to crypt ratio index in the control group was 1.76; for the Giardia-infected group, it was 1.08. In the calves infected with Cryptosporidium, the ratio was 1.18, which is not significantly different when compared to the control group. To our surprise, the calves infected with both parasites had an index of 1.37, which is comparable to the control group. The number of intraepithelial lymphocytes per square mm of jejunum tissue was 21. This number was doubled in the calves infected with Giardia, but was slightly lower in the other infected groups. All the infected calves had intermittent diarrhea and the presence of mucus was seen in many stool specimens. (Supported by NSERC Grant #A-9374.)

# 235

Plasmodium falciparum Malaria Parasites Imaged by Soft X-Ray Microscopy

CATHLEEN MAGOWAN\*, MARIO MORONNE, and WERNER MEYER-ILSE

Intracrythrocytic malaria parasites develop from the ring stage, to a trophozoite, to a multinucleated schizont during a 48 hour cycle. Development is accompanied by the elaboration of new intracrythrocytic membranes, the formation of numerous structures and organelles within the parasite, and hemoglobin metabolism resulting in the formation of hemozoin (malaria pigment).

We are using the technique of soft x-ray imaging microscopy to study the intraerythrocytic stages of P. falciparum. Soft x-ray microscopy has been made practical by the advancing technologies of x-ray sources, x-ray optical components (lenses and mirrors), and x-ray detectors. It does not compete with electron microscopy and scanning probe microscopies for resolution, but offers the ability to take images of thick samples (up to about 10um) in an aqueous environment at 5 times better resolution than visible light. Because of the high demand on the light source required, most advanced x-ray microscopes use synchrotron radiation sources such as the High Resolution Zone-Plate Microscope at the Advanced Light Source in Berkeley.

The accompanying images demonstrate the resolution we have achieved examining intraerythrocytic malaria parasites with the x-ray microscope. A membrane system (the parasitophorous vacuolar membrane) can be seen within the red cell, as well as structures such as the parasite's food vacuole. We encourage everyone to view these images and consider possible applications for x-ray microscopy in your research.

## 236

Growth and Developmental Surface Ultrastructure of Plagiorchis muris from Rat

HO-CHOON WOO AND SUNG-JONG HONG\*

Metacercariae of Plagiorchis muris were collected from dragonfly, Sympetrum eroticum, and fed to albino rats. Recovery rate of P. muris from rats was remained over 47.0% until 14 days post-infection (PI) but dropped to 4.0% at 28 days Pt. Excysted metacercaria (EMC), 0.255 mm long and 0.147 mm wide, grew rapidly from 5 days PI and reached to 1.989 mm in length and 0.687 mm in width at 7 days Pl. Ovary and vitelline glands appeared in 2-day-old worms, and testes and cirrus sac in 3-day-old ones. Intrauterine eggs were formed in several worms at 4 days Pl and in all worms at 5 days Pl, then increased in number by 14 days Pi but decreased remarkably thereafter. Cytoplasmic processes of tegument were of low differentiation on EMC, velvety on juvenile worms, and more fine on adults. Whole surface of EMC was beset with simple peg-like tegumental spines and sparse posteriorly. Tegumental spines became spade-shape of single point with corrugations on juvenile and on adult worms without changing in distribution pattern. Dome-shape sensory receptors without cilium were arranged on rim of oral and ventral suckers and dense around oral sucker in EMC. This receptor was increased in number around ventral sucker of 1- and 3-day-old juvenile worms. Apple-shape sensory receptors with a short cilium at apex were concentrated around oral sucker of all developmental stages. Two types of sensory receptors were occurred bilaterally symmetrical dorsally and ventrally.

# 237

Metacestode of Canine Origin In Mice. EDWARD G. PLATZER\*, W.J. HERNANDEZ, and W. BOYCE

Metacestodes recovered from a dog with peritoneal cestodiasis were injected intraperitoneally into a female Swiss-Webster mouse. After ten weeks no apparent growth or multiplication of the metacestodes had occurred. The surviving metacestodes were sub-inoculated into five SW mice. The metacestodes grew and multiplied significantly during the subsequent 15 week interval. The metacestodes were acephalic and cystic. In further growth studies, we found that he metacestodes grew and multiplied faster in female BALB/c mice. Adaptation of this canine metacestode to a murine host provides a model system for investigation of the biology and chemotherapy of peritoneal cestodiasis.

### 238

Development of the Tapeworm Diphyllobothrium alascense from the Kuskokwim region of Alaska. A.M. ADAMS\* and R.L. RAUSCH

Diphyllobothrium alascense was described from sled dogs from Chevak, Alaska, a village situated ca. 5 km from the Bering Sea on the Yukon-Kuskokwim delta. In early December 1969, a series of burbots, Lota lota L., was collected at the village of Tuluksak, situated on the Kuskokwim River about 150 km above its mouth in Kuskokwim Bay. Examination of these fishes revealed a minute plerocercoid in the mucus covering the gastric mucosa. The strobilar stage of D. alascense was identified from subsequent infections of dogs. Dogs were autopsied at intervals of 2, 2.8, 3, 5, 10, 14, 21 and 32 days. All tapeworms recovered were processed using standard methods. The purpose of this study is to describe the development of the strobilar stage of D. alascense from the experimental infections conducted earlier. Genital and uterine pores were present by the 14th day of development; genital Anlagen were present by the 10th day. As the period of development increased, the genital Anlagen appeared to occur earlier in the strobilae. Segments in some strobilae were gravid by the 23rd day. In general, length of strobilae increased with time, although a lag phase was present in the first week. However, some plerocercoids scarcely developed during the period of rapid growth for other tapeworms within the same dog. The scolices of these tapeworms increased in length even though the no other development occurred. Within the same individual host, some strobilae of D. alascense may rapidly mature while others exhibit delayed development.

## 239

Comparison of Sodium Azide and Refrigeration for Preservation of Hookworm Eggs for Fecal Thick Smear Examination. GEORGE J. GREER and NANCY A. NIX

Kato-Katz fecal thick smear is frequently used to quantify eggs per gram of feces for various species of intestinal helminths and schistosomes. This technique has the advantages of being relatively simple, inexpensive, and fast. A major drawback is that feces cannot be preserved in substances that alter the consistency of the feces, such as formalin. A small quantity of sodium azide, 2 to 3 mg per gram of feces, effectively preserves the feces without noticeably altering its consistency. There is no significant change in eggs counts for Trichuris trichiura and Ascaris lumbricoides for up to 6 months after preservation with sodium azide. The objective of our study was to determine if sodium azide and/or refrigeration are suitable methods for preservation of specimens to be examined quantitatively for hookworm eggs using the fecal thick smear. Changes in the number of eggs per gram of feces were measured over a 2 month period in samples maintained under 4 conditions. The conditions included (1) no preservative, ambient temperature; (2) sodium azide, ambient temperature; (3) no preservative, refrigeration (4°C); (4) sodium azide, refrigeration. Our results indicate that refrigeration and refrigeration plus sodium azide more effectively preserve hookworm eggs in fecal samples than does sodium azide at ambient temperatures.

# 240

Surface ultrastructure and encystment of <u>Paragordius</u> <u>varius</u> larvae (Nematomorpha, Gordioidea). PATRICK J. MIKLOS, PAUL D.LEWIS, JR.\*, and DOUGLAS F. BRAY

Thirteen of 15 crickets Gryllus pennsylvanicus (Orthoptera) from Lethbridge, Alberta, were infected with a total of 17 Paragordius varius (11 females, 6 males) which emerged spontaneously when the crickets were placed in tap water. Paired male and female worms mated, and females deposited egg strings within 24 h of emergence. Males and females died very shortly thereafter. Egg strings were maintained in tap water at 22C; larvae began to hatch after 14 d and hatching continued for 5-7 d. Larvae move feebly for up to 2-3 wk. Larvae and egg masses were attached to poly-L-lysine coated cover-slips, and some larvae were placed on blades of grass and allowed to dry before processing and examination by SEM. The larval preseptum contains 3 sets of 6 spines each, of which the ventral spine in the outermost set is bifid. There is a central evaginable proboscis which bears a pore at its tip and is armed with 3 longitudinal rows (1 ventral and 2 lateral) of paired teeth.

The postseptum bears 2 pairs of ventrolateral spines near the posterior end, and a ventral pore which is located near the anteriormost pair of spines. Larvae on grass blades assumed a characteristic J-shaped posture and were coated with a (secreted?) material which blanketed them and attached them to the grass. Larvae may encyst on emergent vegetation when temporary ponds dry and thus become available for ingestion by their orthopteran hosts.

#### 241

Helminths of *Rattus* spp. in southern and central Puerto Rico. CHRISTINA MALDONADO\* and WIESLAW J. KOZEK

Previous study to define the profile of helminthic fauna of Puerto Rican murines, and to identify the zoonotic species, was continued by trapping and examining rats captured in Adjuntas, Maricao, Trujillo Alto and Cayo Santiago. The 49 rats ((39 Rattus rattus and 9 R. norvegicus) examined to date harbored a total of 15 species of helminths: Strongyloides venezuelensis, S. ratti, Nippostrongylus braziliensis, strobilocercus of Taenia taeniaformis, Capillaria hepatica, Angiostrongylus cantonensis, Trichosomoides craussicauda, Ganguleterakis spumosa, Ecoleus gastricus, Gongylonema neoplasticum, Mastophorus sp., Hymenolepis diminuta, Moniliformis moniliformis, and and unidentified trichostrongylid and a trematode. Four of the recovered helminths: C. hepatica, A. cantonensis, H. diminuta and M. moniliformis have zoonotic potential. Some of the helminth species appear to have a broad distribution throughout the Island; distribution of others, e.g. A. cantonensis, M. moniliformis, H. diminuta, T. craussicauda, E. gastricus and E. neoplasticum appear to be more limited. The results obtained confirmed our previous observations of high prevalence of helminthiases in local rats and identified zoonotic species which present a potential hazard to human health. This study is being continued to characterize more completely the helminthic fauna of local Rattus spp. as well as those from the neighboring islands.

# 242

Chicken Line Differences in *Eimeria tenella*-induced Changes in Splenic T-lymphocyte Subpopulations. K. ZYAN\* and H. LILLEHOJ.

E. tenella infection induces a significant change in splenic T-lymphocyte subpopulations. In order to investigate genetic differences in host response to E. tenella infection, changes in splenic T-lymphocytes were assessed following primary and secondary

infections with E. tenella. Spleen lymphocytes were stained with monoclonal antibodies detecting various T-lymphocyte subpopulations and staining was analyzed using a flow cytometer. Significant increases in CD3<sup>+</sup> and CD8<sup>+</sup> lymphocytes were seen in SC chickens at 6 days post primary infection (ppi), whereas TK and Saxsal chickens showed a significant increase in these cells at 9 days ppi. CD4<sup>+</sup> T-lymphocytes were significantly increased at 6 days ppi in SC and TK chickens, but not in Saxsals. Following secondary infection, CD3<sup>+</sup> and CD8<sup>+</sup> lymphocytes were increased in all chickens, whereas no changes were seen in CD4<sup>+</sup> cells. These results suggest that kinetic differences in the changes in T-lymphocyte subpopulations reflect genetic differences in induction of protective immunity to coccidian parasites. (Supported by the Binational Agricultural Research and Development Grant US2047-91R.)

### 243

Cytokine and Antibody Isotype Profile of Immune Responses Induced by Iscoms Containing *Toxoplasma gondii* antigen. ANNA LUNDÉN\* and ANDERS SJŎLANDER

Previous studies have shown that immunization of mice with T. gondii antigens incorporated into immunostimulating complexes (iscoms) results in partial protection against a lethal challenge infection. The aim of this study was to characterize the antibody and T cell response induced by such iscoms. Balb/c mice were immunized subcutaneously, and 5 and 42 days later local lymph node (LN) and spleen cells were prepared. In vitro stimulation with T. gondii antigen of LN and spleen cells taken 5 days after immunization resulted in a strong proliferative response. Activated cells also produced high levels of IL-2 and IFN-γ and low levels of IL-4. Forty-two days after immunization, the response of LN cells was less pronounced, while that of spleen cells had increased. Serum from mice immunized twice contained high levels of T. gondii specific IgG1, IgG2a and IgG2b. The results suggest that iscoms containing T. gondii antigen efficiently recruit Th1 cells and, to some extent, also activate Th2 cells. The high capacity of the induced cells to produce IFN-y is likely to be of importance, since this cytokine has been identified as a major mediator of immunity to T. gondii.

## 244

Cell Transfer of Resistance to Repeat <u>Trypanosoma</u> <u>cruzi</u> Infection In Mice. AIMEE SLEEPER and <u>E.C. ROWLAND\*</u>

We have investigated a <u>Trypanosoma cruzi</u> infection model system using BALB/c inbred mice which show high acute phase parasitemia, survival of the acute phase and evidence of chronic phase myocarditis. Considering the idea that constant exposure to this parasite is experienced by those in endemic areas, we initiated a study of repeat infections using this mouse strain. Mice infected for a second time 85 days after the first infection, which was about 40 days after the acute phase had subsided, were found to have no parasites in their blood. Control of this second dose of the parasites was further indicated by a lack of a boosting effect on the antiparasite ELISA titers. For example, the isotype titers

for mice infected once for 130 days was similar to those for mice twice infected for 130 and 45 days. To establish the immune basis of this resistance phenomenon, BALB/c mice infected for 90 days were used as a source of immune sera and immune spleen cells for transfer into naive mice prior to infection. Although multiple doses of 0.1 nil immune sera/mouse were found to partially decrease the parasitemia of the recipient mice, the adoptive transfer of 100x106 spleen cells/mouse was shown to provide protection similar to that seen upon repeat infection. Similarly, adoptive transfer of 100x106 spleen cells from 90 day infected mice before infection was able to provide protection to SCID mice. These results confirm other recent reports of a cell mediated mechanism responsible for resistance to T. cruzi infection in mice.

## 245

Isolation and Immunogenicity of Phylogenetically Conserved Intestinal Antigens from *Haemonchus placei*. CARLA SIEFKER\*, LORA RICKARD BALLWEBER, and CODY P. COYNE

Intestinal proteins isolated from some parasites have been found to be capable of inducing protective immunity in vaccinated hosts. Intestinal tissue from Haemonchus placei is being examined as a potential source of host protective antigens. Soluble proteins were extracted from the intestine of a laboratory strain of adult H. placei and used to produce monoclonal antibodies (mAbs). Immunohistochemical studies with the mAbs verified the intestinal location of the epitope(s) in the laboratory strain and a recent field isolate of H. placei. Epitope(s) were conserved among species within the Trichostrongyloidea and Strongyloidea but were absent in the Ancylostomatoidea. Calves were subsequently challenged with H. placei intestinal homogenate and the humoral immune response was profiled.

# 246

Partial characterization of stage related proteins of <u>Capillaria hepatica</u> and their immune recognition in experimentally infected muskrats. JOANNA <u>BORUCINSKA\*</u>, A. GARMENDIA

Capillaria hepatica is the cause of granulomatous hepatitis in animals and humans throughout the world. The prevalence of this disease is highly underestimated because a specific diagnostic procedure is lacking. Immune responses against soluble egg proteins have been studied in mice, but there is no detailed knowledge about parasitic antigens and their immunogenicity. We studied hepatic capillariasis in muskrats which appear to be the natural host for C. hepatica in the Northeastern United States. Muskrats were infected intragastrically with 8,000 embryonated eggs obtained from naturally infected muskrats. Serum was

collected every 24 hours until day 28 post infection when all animals were euthanised. Histologic examination revealed severe granulomatous parasitic hepatitis in all infected animals. Parasitic antigens were prepared separately from unembryonated eggs, eggs containing first stage larvae and adult worms all obtained from livers of experimentally infected mice. Antigens were separated under reducing conditions in a sodium dodecyl sulfate polyacrylamide gel (SDS-Page), transferred to nitrocellulose and probed with sera from infected muskrats; preinfection sera were used as negative controls. Distinct banding patterns were found for each of the three parasitic stages. Western blots from sera obtained 24 days post infection suggest immune recognition of distinct stage specific and common antigens.

## 247

Effects of a phosphorylcholine-containing filarial excretory-secretory product on lymphocyte signalling pathways. WILLIAM HARNETT\*, MAUREEN D. DEEHAN and MARGARET M. HARNETT.

A characteristic of infection with filarial nematodes is the induction of lymphocyte hyporesponsiveness. Although the cause of this is uncertain, increasing evidence suggests a role for products excreted-secreted (ES) by the parasite, particular those containing a and in phosphorylcholine (PC) moiety. We have recently demonstrated that the PC-ES, ES-62, can inhibit anti-Ig induced murine B cell proliferation, and that this can be mimicked by PC-BSA or PC alone. In an attempt to understand the biochemical mechanisms underlying this result, we have investigated the effect of ES-62/PC on B cell signal transduction pathways. We have found no evidence to support a negative effect on the generation of inositol phosphates, but have witnessed (i) an inhibition of protein tyrosine kinase activity, and (ii) a downregulation of protein kinase C, both at the level of expression and activity. Preliminary experiments on T cells indicate similar effects.

# 248

Immune Response in Mice Infected with a Tasmanian Devil Isolate of *Trichinella pseudospiralis*. K. ALFORD\*, D.L. OBENDORF, T.M. FREDEKING, E. HAEHLING and G.L. STEWART

Enteritis in mice at 9 days following infection with *Trichinella spiralis* was twice that seen in mice infected with the original isolate of *Trichinella pseudospiralis* (OI),

and nine times that seen in mice infected with T. pseudospiralis recently isolated from a Tasmanian devil (DI). Enteritis was lower during the remainder of the study in mice from the latter group of animals compared to those infected with either of the other two parasites. Inflammation in the diaphragm muscles of mice infected with T. spiralis was dramatically greater than that seen in mice infected with either isolate of T. pseudospiralis. In concurrent infections with either T. spiralis and T. pseudospiralis (DI) or T. spiralis and T. pseudospiralis (OI), muscle inflammation was significantly less than that for mice infected with T. spiralis alone. In mice concurrently infected with T. spiralis and T. pseudospiralis (DI), the number of T. pseudospiralis muscle larvae was similar to that seen in infections with this parasite alone, while the number of T. spiralis larvae recovered was half that seen in animals infected with T. spiralis alone. Larvae recovered from diaphragm muscles of mice infected concurrently with T. spiralis and T. pseudospiralis (OI) were similar (T. pseudospiralis) or two times (T. spiralis) that seen in mice infected with either species alone. Inflammatory reaction around subq implanted cotton string was profoundly suppressed in mice infected with either isolate of T. pseudospiralis compared to that seen in T. spiralis-infected or uninfected animals.

# 249

Analyses of Immune Responses of SLA<sup>a</sup> Minipigs that React against encysted <u>Trichinella spiralis</u> Muscle Larvae. JOAN K. LUNNEY\*, JANE BRYANT, AND SARAH HYATT

Certain genetically defined NIH minipigs when challenged with Trichinella spiralis exhibit a unique reactivity against the previously encysted muscle larvae remaining from the primary inoculation. Genetic studies have revealed that a minimum of two genes control this response, one gene within the major histocompatibility complex or swine leukocyte antigen (SLA) complex, and a second, as yet unknown gene. Extended studies demonstrated that 47% of minipigs that express a least one copy of the  $SLA^{a}(ax)$ haplotype are "Responders" against encysted Trichinella spiralis muscle larvae. Comparative analyses of cells from peripheral blood and local (mesenteric and submandibular) lymph nodes of Responder versus Non-Responder pigs during the early time post challenge have revealed no differences in important immune cell subsets (CD2, CD4, CD8, B cells, monocytes) or in parasite specific blastogenic responses or interleukin-2 (IL-2) production. Therefore, we have extended our studies to assess additional cytokines. Cells and tissues, collected at different times during the

post-challenge period, were frozen directly or restimulated in culture with concanavalin A, and cell pellets and supernatants harvested at 3 and 24 hours. Analyses for IL-2, IL-4, IL-10, tumor necrosis factor and interferon-gamma mRNA are underway. These studies should help reveal the effector mechanisms employed by Responder pigs as they react against the encysted <u>Trichinella spiralis</u> muscle larvae.

### 250

Contradicting Primitive Assumptions: Evidence for a More Advanced Placement of the Acoelomate Phytum Platyhelminthes. JASON A. CURTIS\*, K. LEE, D. J. MINCHELLA, and P. T. LOVERDE

Because organisms in the Phylum Platyhelminthes lack a coelom, they have traditionally been regarded as the most primitive of the animal groups with bilateral symmetry. Another possibility is that the acoelomate condition in parasitic flatworms, such as Schistosoma mansoni, is derived from a coetomate protostome lineage. This study utilizes the complete large subunit (28S) rRNA sequence from S. mansoni and a data set of eight other animal sequences to test these evolutionary assumptions. The gene was cloned and sequenced, and this sequence was then manually aligned with the previously existing alignment of the 28S rRNA genes (representing Arthropoda, Nematoda, and Chordata). Unrooted phylogenies were generated under Maximum Parsimony and the distance-based Fitch-Margoliash algorithm. Under all analyses, the least favored branching pattern was that which paired the two coelomate phyla, Arthropoda and the Chordata. This finding suggests that alternate hypotheses of coelomate evolution, including the possibility that the split between protostome and deuterostome lineages represents the earliest divergence of the bilaterat animals, warrant renewed consideration. These results also require that the traditional assumption, which places the Platyhelminthes as the most primitive bilateral phylum, needs to be reevaluated.

# 251

Molecular Phylogeny of Some Free-Living and Parasitic Nematodes Based on the 28S Ribosomal RNA Gene LEO X. LIU\*

Molecular phylogenetic studies of nematodes to date have largely concentrated on relatively closely related species. Systematic analysis of distantly related species may provide more insight into the evolutionary relationships of a broad range of nematodes, including nematode parasites of plants and invertebrates as well as free-living species and parasites of vertebrate animals. Ribosomal RNA gene sequences are powerful targets for molecular phylogenetic and taxonomic studies. Sense and antisense oligonucleotide primers for PCR were designed based on conserved sequences of the 5' end of the 28S rRNA gene of *Caenorhabditis elegans* (Ellis,1986) and several parasitic nematode species (Qu,1986). Using these

primers and template genomic DNA extracted from single or bulk worms, ~300 bp PCR products from the nematode 28S rRNA target region have been reliably amplified from approximately 20 nematode species studied thus far, including representatives of Enoplid, Rhabditid, Tylenchid, Strongylid, Ascarid, Spirurid, and Oxyurid orders. PCR products were subcloned into pBluescript or the pCR TA cloning vector for automated DNA sequencing. The multiple nematode 28S rRNA sequences were manually aligned after initial alignment using the Clustal and Jotun-Hein algorithms. Maximum parsimony and neighbor-joining distance methods were used for phylogenetic tree construction. Phylogenetic trees based on this 28S rRNA sequence are in excellent agreement with traditional published phylogenies: species within each major order constitute separate clades, tylenchid species are most closely related to rhabditid species, and ascarid and spirurid species are more closely related to each other than to the other clades. These results indicate that analysis of the 28S rRNA gene is a useful molecular tool for phylogenetic studies of distantly related nematode species.

### 252

Molecular Karyotyping of Anuran Trypanosomes. ZHAO-RONG LUN\* and SHERWIN S. DESSER

The chromosomes of 12 species and isolates of anuran trypanosomes from different geographical regions were investigated by pulsed field gradient gel electrophoresis (PFGE) to determine their number and size, and also to provide data for differentiation among isolates and species. Twelve to 16 chromosomes ranging from 0.45 to 2.2 megabase (Mb) pairs were found in each of the trypanosomes. Ten to 12 of these chromosomes were large ranging from 0.7 to 2.2 Mb. Two to 4 intermediate sized chromosomes ranged from 0.4 to 0.7 Mb. Minichromosomes were not observed in any of the isolates examined. Results from PFGE indicated that different species of anuran trypanosomes displayed distinct karyotype patterns, while isolates from the same region were similar. These data agree with those of results from isoenzyme comparison and random amplified polymorphic DNA analyses.

## 253

Expression of Piroplasm Proteins of *Theileria* sergenti (Korean Isolate) and Its Immunogenecity in laboratory animals. S.W. KANG\*, C.H. KWON, E.J. CHOI and Y.D. YOON

The DNA fragments encoding piroplasm surface protein (p33) of *Theileria sergenti* was cloned and expressed in Baculoviruses. The expressed p33 was characterized by indirect fluorescent antibody (IFA) and Immunoblotting analysis.

The antigenecity of expressed polypeptide was further tested through the inocultation to guinea pig. The immunized guinea pig antisera by p33 expressed cell lysate showed similar FA patterns and reacted with the same molecular weight protein of *Theileria sergenti*, in immunoblotting analysis, thus suggesting this proteins can be the possible candidate for subunit vaccine.

### 254

Effect of Brefeldin-A(BFA) on the Golgi Complex of *Tritrichomonas foetus*: A Laser Scanning Confocal and Electron Microscopic Study. J.C.A. ALMEIDA\*, M., BENCHIMOL and W. DE SOUZA

Brefeldin-A is a fungal metabolite that has been used as a powerful tool to study the membrane traffic in different cell types. Its more prominent effect is disturbing the traffic within Golgi structure and the disassembly and migration of Golgi elements to the endoplasmic reticulum. The purpose of this work is to investigate the action of BFA in Tritrichomonas foetus, a parasitic protozoan from the urogenital tract of cattle. For observation in Confocal Laser Scanning Microscopy (CLSM), the cells were incubated 10 min in a solution of NBD-Ceramide 1.4 $\mu$ M, a fluorescent lipid which has affinity for the Golgi Complex. For transmission electron microscopy the parasites were treated from 2 sec to 60 min, fixed and processed routinely for TEM. The action of the drug have occurred very quickly, but surprisingly, after about 15 minutes even in presence of the drug, the Golgi Complex becames spontaneously reestructured. The appearing of an extensive tubular network was observed at ultrastructural level. The results suggest that T. foetus have an overcoming mechanism in presence of the drug, differently of other cell types. (Supported by CNPq, FINEP, CAPES and FENORTE.)

# 255

Cloning of Two Putative Calcium Channel cDNAs from the triclad flatworm *Bdelloura candida*. ROBERT M. GREENBERG and PETER A. V. ANDERSON\*

Voltage-gated calcium ( $Ca^{2+}$ ) channels are integral membrane proteins that regulate the influx of  $Ca^{2+}$  required for excitation-contraction and excitation-secretion coupling in muscle and nerve, respectively. Structurally, they consist of an  $\alpha_1$ -subunit, which is capable of forming a functional channel, together with various regulatory or auxiliary subunits ( $\alpha_2/\delta, \beta, \gamma$ ). In vertebrates, there are several subtypes of  $Ca^{2+}$  channels, each with distinctive physiological, pharmacological and structural properties. Voltage clamp recordings (Blair and Anderson, 1993, 1994) from neurons and muscles dissociated from *Bdelloura* reveal the presence

of several distinct classes of voltage-activated current, including at least one slowly-inactivating Ca<sup>2+</sup> current. Given the importance of these proteins for the normal neurornuscular activity of these organisms, and the relatively large amount of information known about the physiological and pharmacological properties of calcium currents in this species, we sought to determine the structure of Bdelloura Ca2+ channels. cDNA fragments encoding two Ca2+ channel α, subunit-like sequences were amplified from Bdelloura RNA by RT-PCR. The degenerate primers used were designed against conserved amino acid sequences in the pore-forming region of domain I and the S5-S6 loop of domain II of other cloned Ca2+ channel α, subunits. The two amplified sequences differ from one another, and are encoded by two different mRNAs. Preliminary sequencing data suggest that the one of the two Bdelloura cDNAs is most closely related to classical, L-type, Ca2+ channels, while the other is most similar to the mammalian N-type Ca2+ channels.

### 256

Differentiation Between the Human Hookworms Ancylostoma duodenale and Necator americanus Using PCR-RFLP, J.M. HAWDON

Although traditionally considered the same for treatment purposes, the two major hookworms infecting humans, Ancylostoma duodenale and Necator americanus, differ significantly in their life histories. These differences must be considered when designing control strategies. However, identification of the species infecting a particular host population or individual has been problematic, as the eggs of the two species cannot be reliably differentiated. With this in mind, a PCR-based technique for the differentiation of hookworm species that infect humans was developed. A 474 bp fragment of the 3' untranslated region of the A. caninum cAMP-dependent protein kinase catalytic subunit gene (PKA) was amplified from A. duodenale and N. americanus genomic DNA. Digestion of the amplified DNA with the restriction enzymes HpaII, MboI, TaqI and ThaI generated specific restriction fragment length polymorphism (RFLP) patterns unique to each species. The technique can distinguish between pure and mixed hookworm infections, and can amplify DNA from a single egg. The primers also amplify the fragment from the DNA of several other species of hookworms that infect humans and animals. The technique is fast, simple, and hookworm-specific, and represents a considerable savings in time over current methods used for distinguishing between human hookworm infections.

# 257

Cloning and Sequencing of a Kunitz-type Protease Inhibitor from the Hookworm Ancylostoma caninum. J.M. HAWDON, B.F. JONES\*, M. CAPPELLO and P.J.HOTEZ

Using degenerate primers derived from conserved sequences of Kunitz-type protease inhibitors, an 89 bp fragment was amplified from adult stage Ancylostoma caninum cDNA library DNA by PCR. The fragment was used as a probe to isolate a 2.3 kb clone from the library. Nested deletions were constructed by exonuclease digestion, and the overlapping clones sequenced using vector specific primers. The 5' end, containing the methionine start codon ATG, was absent from the original clone, and was isolated from adult cDNA by 5' RACE. The full length cDNA, termed Ancylostoma Kunitz-type protease inhibitor (AKPI), contained an 11 bp 5' untranslated region (UTR), a 759 amino acid open

reading frame (ORF), and an 80 bp 3' UTR containing a polyadenylation signal (AATAAA) 20 bases upstream of the poly(A) tail. The ORF encoded a peptide with a calculated molecular weight of 84,885 and a pI of 8.46. The protein contains 11 Kunitz-type domains, as determined by the spacing between cysteine residues: C-8x-C-15x-C-7x-C-12x-C-3x-C. Comparison of AKPI to protein databases revealed significant homology with the tissue factor pathway inhibitors (TFPI; 35-39%), inter- $\alpha$ -trypsin inhibitors (39-42%), and  $\alpha$ -1-microglobulins (41-43%) of several species. Experiments are underway to express AKPI in bacterial cells to produce protein for antibody production.