92nd Annual Meeting of the American Society of Parasitologists and the 12th International Coccidiosis Conference

The Alamo Mission

The Hilton Palacio del Rio
San Antonio, Texas, June 27-July 1, 2017

Program & Abstracts

Thanks to Everyone Who Helped Make this Meeting Possible …

The American Society of Parasitologists gratefully acknowledges the following for their support, sponsorship, and hard work in putting together this year's annual meeting.

ASP Local Organizing Committee
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Mike Kemp - Honorary Committee Member
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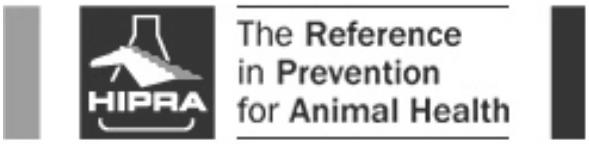
- Sierra Upton (sponsor of the Steve Upton Party for ASP Students; Sierra is the daughter of the late Dr. Steve J. Upton)
- Robert Grieve, ASP Past President (sponsor of the President's Reception)
- Tavis Anderson, Rebecca Cole, Susan Perkins, John Hawdon (Sponsors of "The Vortex")
- Debbie Contardi, Marriot and Renaissance, Caribbean and Mexico Resorts (sponsor of the Council Breakfast)
- Office of the Provost, Wake Forest University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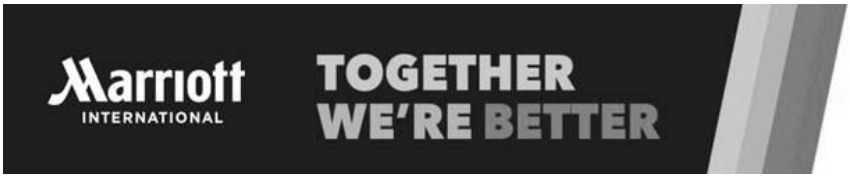
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Notes:

The American Society of Parasitologists
ESTABLISHED 1924

Welcome

We would like to welcome you to the 92nd annual meeting of the American Society of Parasitologists (ASP).

The ASP is a diverse group of over 800 scientists from industry, government, and academia who are interested in the study and teaching of parasitology. Founded in 1924, ASP members have contributed not only to the development of parasitology as a discipline, but also to primary research in systematics, medicine, molecular biology, immunology, physiology, ecology, biochemistry, behavior, and more.

Herman Eure and Kelli Sapp, Scientific Program Officers

Floor Plan, The Hilton Palcio del Rio
Conference Center (22nd Floor) and Mezzanine (2nd Floor)

Notes:

American Society of Parasitologist's Discrimination Policy

Statement of Policy: In accordance with the bylaws of the American Society of Parasitologists (ASP), the Society will afford an environment free from discrimination, harassment, and retaliation. The ASP will not tolerate actions, statements, or contacts that discourage the free expression and exchange of scientific ideas. This includes unequal treatment or harassment of any person based on their age, gender, gender identity or expression, marital status, sexual orientation, race, color, national or ethnic origin, religious identifications, beliefs or practices, disabilities, veteran status, or any other reasons or expressions that are unrelated to their scientific merit. Harassment, sexual or otherwise, shall be considered as a form of misconduct and violators will be subject to disciplinary actions, including expulsion from a society function or from the society itself.

Definition of Sexual Harassment: Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical conduct of a sexual nature. Sexual harassment does not refer to occasional compliments of a socially acceptable nature. It refers to behavior that is not welcome, is personally offensive, debilitates morale, and therefore, interferes with a collegial atmosphere. The following are examples of behavior that, when unwelcome, may constitute sexual harassment: sexual flirtations, advances, or propositions; verbal comments or physical actions of a sexual nature; sexually degrading words used to describe an individual; a display of sexually suggestive objects or pictures; sexually explicit jokes; unnecessary touching. What is perceived as acceptable to one person may be unwelcome by another. Those who have positions of authority or higher rank should be aware that others may be reluctant to outwardly express objections or discomfort regarding unwelcome behavior or language.

Other Types of Harassment: Remarks and behaviors based on other protected characteristics are also unacceptable to the Society. These include stereotyping, slurs, derogatory jokes or statements, and any hostile or intimidating acts.

Policy Scope: This policy applies to all attendees and participants at ASP meetings and functions, including social functions, tours, or off-site activities during the course of meetings and functions, and includes all members, guests, staff, contractors, and exhibitors.

Reporting an Incident: If any individual covered by this policy believes that they have experienced or witnessed harassment or bullying they should contact the society's designated individual **[Dr. Sara Brant, sbrant@unm.edu]**. No complainant will be required to discuss any incident with a respondent; no respondent will be required to discuss any incident with a complainant. All individuals (complainant or respondent) may bring an accompanying individual of their choice with them for support at any point when they discuss the matter with the society's designated individual, or during any course of an ensuing investigation.

Because allegations of discrimination, harassment and misconduct are sensitive matters with the potential to negatively impact the reputation of individuals, institutions, and/or our Society, confidentiality and discretion throughout the process is expected from all parties involved and is assured from the ASP's designated individual and all involved in the investigation.

Regardless, a complainant may speak in confidence with the society's designated individual without involving an official report, an investigation or a respondent. All complaints that are received will be treated seriously, and will be addressed promptly if that is the wish of a complainant. Any incidents of sexual assault should be immediately reported to the police. Note that many local and regional governments also consider a variety of behaviors to be reportable crimes regardless of the wishes of the complainant, respondent or of the society.

Investigation: Following the official report of an incident, the Society's designated individual, in consultation with ASP Council, will name an impartial investigator, usually an elected officer or Council member, and the respondent will be promptly notified. No one who has a conflict of interest with respect to the complainant or respondent will serve in this role. A complainant will be asked to

file a formal written complaint; the respondent will be notified immediately and prior to any discovery procedures. A respondent will be invited to respond to the complaint and allowed to bring evidence. The Council of ASP reserves the right to interview other individuals as witnesses at its own discretion. The investigator is allowed to seek counsel if they are in doubt as to how to proceed. When the investigation is complete, the findings will be communicated to the elected officers, as well as both to the complainant and respondent. Those officers without a conflict of interest will decide on appropriate disciplinary actions.

Retaliation: The Society will not tolerate any form of retaliation against individuals who report an incident, against those who are subject to a complaint, nor against those who participate in an investigation. Retaliation will be considered a form of discrimination in and of itself and offenders will be subject to disciplinary action, up to and including ejection from the society.

Disciplinary Action: If an individual harasses, retaliates, or knowingly makes a false claim, they will be subject to disciplinary action. These actions might range from a verbal warning to a request to leave the meeting or function without refund of fees and a reporting of the incident to the person's employer. Should repeated complaints, patterns of inappropriate behavior, or other events emerge, the society's by-laws permit its Council to exclude and eject members through a process that has no appeal.

Appeal & Questions: Should any person be dissatisfied with the result of an investigation or disciplinary action, they may appeal to the President of the Society, or to the highest ranking officer without a conflict of interest. Questions concerning the policy can be directed to an ASP officer or the ASP designated individual.

Day/Times	Activity/Function	Room/Space
June 27 (Tuesday)		
9:00-10:30 a.m.	ICC Session 1: Systematics, Taxonomy and Evolutionary Biology	Salon Del Rey A
10:30-11:00 a.m.	Coffee Break	Perfunction
11:00-12:30 p.m.	ICC Session 1: Systematics, Taxonomy and Evolutionary Biology	Salon Del Rey A
2:10-3:30 p.m.	ICC Session 2: Genetics and Comparative Genomics	Salon Del Rey A
3:30-4:00 p.m.	Coffee Break	Perfunction
4:00-5:30 p.m.	ICC Session 2: Genetics and Comparative Genomics	Salon Del Rey A
6:30-8:30 p.m.	ICC Welcome Reception	El Mirador C
June 28 (Wednesday)		
8:00 a.m.-Noon	ASP Council Meeting	El Mirador C
9:00-10:30 a.m.	ICC Session 3: Vaccines and Therapeutics	La Vista DEF
10:30-11:00 a.m.	Coffee Break	Foyer
11:00-12:30 p.m.	ICC Session 3: Vaccines and Therapeutics	La Vista DEF
2:00-5:00 p.m.	ASP: Host-Parasite Interactions I	La Vista AB
2:00-5:00 p.m.	ASP: Life Cycles and Epidemiology I	La Vista C
2:10-5:30 p.m.	ICC Session 4: Host-Pathogen Interactions	La Vista DEF
5:30-6:00 p.m.	ICC Planning Discussion	La Vista DEF
3:30-4:00 p.m.	Coffee Break	Foyer
7:00-10:00 p.m.	ASP/ICC Welcome Reception	El Mirador
June 29 (Thursday)		
8:30-10:30 a.m.	ASP President's Symposium	El Mirador
10:30-11:00 a.m.	Coffee Break	Foyer
11:00-Noon	ASP Student Business Meeting	La Vista AB
Noon-1:00 p.m.	Editorial Board Luncheon	La Reina
1:00-3:00 p.m.	Taxonomy, Systematics, Phylogeny I	La Vista AB
1:00-3:00 p.m.	Evolutionary Ecology I	La Vista DE
3:00-3:30 p.m.	Coffee Break	Foyer
3:30-5:30 p.m.	ASP Students' Symposium	La Vista AB
5:30-6:30 p.m.	ASP Student Social	La Vista Foyer
3:00-6:00 p.m.	Auction Set Up	El Mirador
6:00-7:00 p.m.	Auction Preview	El Mirador
7:00-9:00 p.m.	27th Annual ASP Student Auction	El Mirador

June 30 (Friday)

8:00-10:30 a.m.	Biochemistry/Physiology/ Chemotherapeutic & Drug Resistance/ Vector Biology	La Vista DE
8:30-10:30 a.m.	Taxonomy, Systematics, Phylogeny II	La Vista AB
8:30-10:15 a.m.	Host-Parasite Interactions II	La Vista C
10:30-11:00 a.m.	Coffee Break	Foyer
11:00-Noon	ASP President's Address	El Mirador C
2:00-5:15 p.m.	Evolutionary Ecology II	La Vista AB
2:00-3:30 p.m.	Life Cycles and Epidemiology II	La Vista DE
2:30-5:00 p.m.	Immunology	La Vista C
3:30-4:00 p.m.	Coffee Break	Foyer
6:00-7:00 p.m.	The Vortex*****	El Mirador A

July 1 (Saturday)

8:30-10:00 a.m.	Authors complete poster set up	La Vista DEF
8:00-10:45 a.m.	Taxonomy, Systematics, Phylogeny III	La Vista AB
8:00-11:15 a.m.	Genomics & Molecular Biology	La Vista C
9:15-9:45 a.m.	Coffee Break	Foyer
11:00-12:30 p.m.	Poster Session, coffee, snacks	La Vista DEF
1:00-1:50 p.m.	H.B. Ward Lecture	El Mirador
2:00-3:00 p.m.	ASP Awards and Business Meeting	El Mirador

***** Meet the Professional: a science "speed meet" for early career scientists. This gathering is an opportunity for "early in your career" scientists to meet an "established PI." Thinking about what's next after graduate school? Your next post doc? Need to practice your interview skills in a friendly environment? Want to network to build collaborations? Looking for a fun social activity with your colleagues on Friday night?

Tuesday Morning, 2017-06-27

9:00-12:30 pm ICC: Systematics, Taxonomy & Evolutionary Biology

Location: Salon Del Rey A

Presiding: **D. Duszynski**

Time (Abstract No.)

9:00 Opening Remarks & Welcome.

9:10 Introduction of Plenary Speakers.

9:20 (1) Plenary Speaker: **J Kvičerová**. EVOLUTIONARY PROCESSES, POPULATION STRUCTURE AND MORPHOLOGICAL FEATURES OF COCCIDIA PARASITIZING DIFFERENT GROUPS OF HOSTS.

10:00 (2) **L Hofmannova**, J Kvičerová, B Koudela, D Modrý. INTRANUCLEAR COCCIDIOSIS OF TESTUDINES THE LIFE CYCLE DISCOVERED.

10:15 (3) **JR Barta**, AN Leveille, MA Hafeez. MITOGENOMICS IN IDENTIFICATION AND EVOLUTION OF COCCIDIA AND OTHER APICOMPLEXAN PARASITES.

10:30-11:00 am **COFFEE BREAK**

11:00 (4) Plenary Speaker: **L Xiao**, Y Feng, Y Wang, DM Roellig. GENOME EVOLUTION AND HOST SPECIFICITY IN *CRYPTOSPORIDIUM*.

11:40 (5) **Y Feng**, L Xiao. GENETIC AND BIOLOGICAL SIMILARITY OF *CYCLOSPORA CAYETANENSIS* TO CECUM-INFECTING *EIMERIA* SPP. AS REVEALED BY COMPARATIVE GENOMIC ANALYSIS.

11:55 (6) **C Su**. GLOBAL GENETIC DIVERSITY AND POPULATION STRUCTURE OF *TOXOPLASMA GONDII*.

12:10 Panel Discussion.

Tuesday Afternoon, 2017-06-27

2:10-5:30 pm ICC: Genetics & Comparative Genomics

Location: Salon Del Rey A

Presiding: **M. Jenkins**

Time (Abstract No.)

2:10 Introduction of Plenary Speakers.

2:20 (7) Plenary Speaker: **B Striepen**. MOLECULAR GENETICS FOR CRYPTOSPORIDIOSIS.

3:00 (8) SL Sokol, A Primack, J Dubey, **JP Boyle**. USING COMPARATIVE AND FUNCTIONAL GENOMICS TO UNDERSTAND LIFE CYCLE EVOLUTION IN *T. GONDII*.

3:15 (9) **L Knoll**. DUAL TRANSCRIPTIONAL PROFILING OF MICE AND *TOXOPLASMA GONDII* DURING EARLY AND LATE CHRONIC INFECTION.

3:30-4:00 pm **COFFEE BREAK**

4:00 (10) Plenary Speaker: A Khan, A Kennard, B Gregg, J Zhang, K Shen, JP Dubey, J Parkinson, **ME Grigg**. BIOLOGICAL CONSEQUENCES OF GENETIC EXCHANGE AND INFLAMMASOME SENSORS ACTIVATED BY PROTOZOAN PARASITES.

4:40 (11) **DP Blake**, V Vrba, ID Jatau, MJ Nolan, G Underwood, FM Tomley. USING GENOME SEQUENCING TO DECIPHER CRYPTIC *EIMERIA*.

4:55 (12) F Guo, H Zhang, **G Zhu**. *CRYPTOSPORIDIUM PARVUM* LACTATE DEHYDROGENASE IS ASSOCIATED WITH THE PARASITOPHOROUS VACUOLE MEMBRANE AND IS A POTENTIAL TARGET FOR DEVELOPING THERAPEUTICS.

5:10 Panel Discussion.

Tuesday Evening, 2017-06-27

6:30 - 8:30 pm ICC WELCOME RECEPTION

Location: El Mirador C

Wednesday Morning, 2017-06-28

8:00 am – Noon ASP Council Meeting, El Mirador C

Presiding: **G.W. Esch**, Wake Forest University

9:00-12:30 pm ICC: Vaccines & Therapeutics

Location: La Vista DEF

9:00 Opening Remarks and Updates. **D. Duszynski**

Presiding: **G. Zhu**

Time (Abstract No.)

9:10 Introduction of Plenary Speakers.

9:20 (13) Plenary Speaker: **S Moreno**, Z Li. TARGETING A MAMMALIAN HOST METABOLIC PATHWAY FOR IMPROVED CHEMOTHERAPY AGAINST THE APICOMPLEXAN PARASITE *TOXOPLASMA GONDII*.

10:00 (14) **B Jordan**, L Tensa, G Albanese. APPLICATION AND CYCLING CHARACTERISTICS OF A COMMERCIAL COCCIDIA VACCINE APPLIED BY SPRAY AND GEL.

10:15 (15) **T Wang**, M Finklin, C Hofacre, G Mathis. EVALUATION OF THE EFFICACY OF DIFFERENT COCCIDIOSIS BIOSHUTTLE PROGRAMS AND POTENTIAL ALTERNATIVES TO AGPS ON BROILER PERFORMANCE.

10:30-11:00 am **COFFEE BREAK**

11:00 (16) Plenary Speaker: **X Suo**, X Liu, J Suo, X Tang, G Tao, C Duan, S Zhang, Q Liao. ON THE WAY TOWARDS ACTUALIZING RECOMBINANT EIMERIA AS A VACCINE VECTOR.

11:40 (17) **M Pagès Bosch**, R March Massos, E Del Cacho Malo. RELEVANCE OF MATERNAL IMMUNITY AGAINST COCCIDIOSIS AFTER IMMUNIZATION WITH SPORULATED OOCYSTS.

11:55 (18) **I Pastor-Fernandez**, V Marugãn-Hernãndez, F Tomley, D Blake. TARGETING TRANSGENIC ANTIGEN EXPRESSION TO OPTIMISE EIMERIA PARASITES AS VACCINE DELIVERY VECTORS.

12:10 Panel Discussion.

Wednesday Afternoon, 2017-06-28

2:00-5:00 pm *ASP: Host-Parasite Interactions I*

Location: La Vista AB

Presiding: **J. Camp**, Purdue University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

2:00 (25) **AR Pastor**, DA Smith, JR Barta. MOLECULAR CHARACTERIZATION OF FERRET ENTERIC COCCIDIA.

2:15 (26) **S Lee**, A Kim, D Lee, F Quan. PROTECTION INDUCED BY VIRUS-LIKE PARTICLES CONTAINING *TOXOPLASMA GONDII* MICRONEME PROTEIN 8 AGAINST HIGHLY VIRULENT RH STRAIN OF *TOXOPLASMA GONDII* INFECTION.

2:30 (27) **A Montoya**. A FIELD CASE OF *EIMERIA MIVATI* IN BROILERS.

2: 45 (28) † **C Li**, A Hu, JM Stout, JT Detwiler. FRESHWATER SNAILS EXHIBIT DENSITY-DEPENDENT IMMUNITY POTENTIALLY MEDIATED BY CHEMICAL COMMUNICATION.

3:00 (29) † **C Li**, JD Roth, JT Detwiler. STABLE ISOTOPE ANALYSIS INDICATES HOST-PARASITE ENCOUNTER RATES AND REVEALS UNEXPECTED ROUTES OF PARASITE TRANSMISSION.

3:15 (30) † **RW Koch**, RP Shannon, KD Gustafson, MG Bolek. MYSTERIOUS SNAIL HOSTS! DISTRIBUTION AND HOST USE OF ACANTHOCEPHALANS IN TWO SPECIES OF FRESHWATER SNAILS.

3:30-4:00 pm **COFFEE BREAK**

4:00 (31) † **SK Buddenborg**, L Bu, GM Mkoji, ES Loker. A GENOME-WIDE TRANSCRIPTOMIC ANALYSIS OF THE REPRODUCTIVE CONSEQUENCES AND DIVERSE IMMUNOLOGICAL RESPONSES IN FIELD-DERIVED EARLY AND PATENT *SCHISTOSOMA MANSONI* INFECTIONS OF *BIOMPHALARIA PFEIFFERI*.

4:15 (32) † **RP Snyder**, M Guerin, B Hargis, G Page, JR Barta. COCCIDIAL METAGENOMICS: HIGH THROUGHPUT DNA SEQUENCING OF *EIMERIA* SPECIES TO EVALUATE THE IMPACT AND CONTROL OF COCCIDIOSIS ON BROILER OPERATIONS.

4:30 (33) **FD Chevalier**, W Le Clec'h, B Gourbal, G Mitta, T Anderson. EXTREME QTL AND POOLED SEQUENCING FOR DETERMINING THE GENETIC BASIS OF HOST SPECIFICITY IN SCHISTOSOME PARASITES.

4:45 (34) **W Le Clec'h**, FD Chevalier, M Mcdew-White, V Menon, T Anderson. GENETIC ANALYSIS OF TRANSMISSION STAGE PRODUCTION IN SCHISTOSOME PARASITES.

2:00-5:00 pm ASP: Life Cycles and Epidemiology I

Location: La Vista C

Presiding: **M. Moser**, University of California Berkeley

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

2:00 (35) E Hürlimann, M Ouattara, **G Coulibaly**, D Kouassi, J Utzinger, G Raso, E Kouakou N'Goran. EPIDEMIOLOGY OF HELMINTHS AND INTESTINAL PROTOZOA IN THE CENTRAL OF COTE D'IVOIRE.

2:15 (36) † **J Vannatta**, R Moen. HELMINTH COINFECTION AND LANDSCAPE PATTERNS IN A SUBURBAN UNGULATE POPULATION.

2:30 (37) **A Mays**, V Faulkner, C Faulkner. PREVALENCE OF ZOONOTIC GASTROINTESTINAL PARASITES OF SHELTER DOGS AND CATS IN THE TRISTATE CUMBERLAND GAP AREA OF KENTUCKY, TENNESSEE AND VIRGINIA WITHIN THE APPALACHIAN REGION.

2:45 (38) **DS Lindsay**, SK Verma, D Scott, JP Dubey, AR Von Dohlen. MOLECULAR CHARACTERIZATION AND UNUSUAL DEVELOPMENT OF A *SARCOCYSTIS* SPECIES FROM A COOPERS HAWK (*ACCIPITER COOPERII*) IN CELL CULTURES.

3:00 (39) † **R Imai**, JR Barta. PREVALENCE OF POTENTIALLY ZOONOTIC AND NON-ZOONOTIC PARASITES IN DOMESTIC DOGS IN RURAL, URBAN AND FIRST NATIONS COMMUNITIES ACROSS ONTARIO, CANADA.

3:15 (40) † **E Rejman**, A McDonald, N Nemeth, JR Barta. SURVEY OF *EIMERIA* SPECIES IN ONTARIO WILD TURKEYS (*MELEAGRIS GALLOPAVO SILVESTRIS*).

3:30-4:00 pm **COFFEE BREAK**

4:00 (41) **TR Olariu**, C Petrescu, V Dumitrascu, MA Lupu. RISK FACTORS FOR *TOXOPLASMA GONDII* INFECTION IN ROMANIAN PREGNANT WOMEN.

4:15 (42) **J Adkins**, V Faulkner, D Spangler, C Faulkner. FIELD COLLECTION OF QUESTING AND HOSTED IXODID TICKS FROM THE CUMBERLAND GAP REGION OF TENNESSEE, VIRGINIA, AND KENTUCKY.

4:30 (43) **MA Lupu**, V Lazureanu, TR Olariu. TRICHINELLOSIS IN HOSPITALIZED PATIENTS IN WESTERN ROMANIA: A 4 YEARS RETROSPECTIVE STUDY.

4:45 (44) **AR Pastor**, JR Barta, S Hollamby, DA Smith. ENTERIC COCCIDIOSIS IN THE BLACK-FOOTED FERRET (*MUSTELA NIGRIPES*).

2:10-5:30 pm ICC: Host Pathogen Interactions

Location: La Vista DEF

Presiding: **H. Lillehoj**

Time (Abstract No.)

2:10 Introduction of Plenary Speakers.

2:20 (19) Plenary Speaker: **W Kim**, H Lillehoj. RECENT PROGRESS IN UNDERSTANDING HOST IMMUNE RESPONSE TO AVIAN COCCIDIOSIS: TH1 AND TH17 RESPONSES.

3:00 (20) G Tao, X Tang, **C Li**, X Liu, X Suo. CONSTRUCTION OF TRANSGENIC RABBIT COCCIDIA AND PROSPECT OF INDUCED IMMUNITY BY RECOMBINATION WITH VP60 FROM RHDV.

3:15 (21) **H Lillehoj**, W Kim. MODULATION OF PROTECTIVE IMMUNITY AGAINST COCCIDIOSIS BY A HOST-DERIVED DEFENSE PEPTIDE THAT SHOWS ANTI-PARASITIC ACTIVITY.

3:30-4:00 pm **COFFEE BREAK**

4:00 (22) Plenary Speaker: **M Pinard-Van Der Laan**. DECIPHERING HOST RESPONSES TO *EIMERIA* INFECTIONS FOR IMPLEMENTING INTEGRATED BREEDING STRATEGIES FOR IMPROVED RESISTANCE TO COCCIDIOSIS.

4:40 (23) **J Gigley**. NATURAL KILLER CELLS: FRIEND OR FOE IN CHRONIC TOXOPLASMOSIS.

4:55 (24) **MC Jenkins**. TRACKING *EIMERIA* OOCYST NUMBERS AND SPECIES COMPOSITION IN COMMERCIAL BROILER HOUSES.

5:10 Panel Discussion.

5:30 ICC Planning Discussion.

Wednesday Evening, 2017-06-28

7:00 - 10:00 pm ***ASP/ICC WELCOME RECEPTION***

Location: El Mirador

Thursday Morning, 2017-06-29

8:30-10:30 am ASP President's Symposium

Location: El Mirador

Presiding: **J. Hawdon**, The George Washington University

Theme: "Expanding Diversity in Parasite Ecology."

8:30 **J. Hawdon**. Introduction.

8: 40 (45) **CV Holland**. CHASING *ASCARIS* AGGREGATION: FROM FIELD TO LABORATORY.

9:10 (46) **KC Jacobson**. PARASITES, PATTERNS AND POLICY: PARASITE ECOLOGY FOR MARINE FISHERIES MANAGEMENT.

9:40 (47) **D Zelmer**. SCALING SYNCHRONY IN SUNFISH SYMBIONTS.

10:00-10:30 Questions, Closing Remarks.

10:30-11:00 am **COFFEE BREAK**

11:00 am-Noon ASP Student Business Meeting

Location: La Vista AB

Presiding: **K. Gallagher**, University of Connecticut

Thursday Afternoon, 2017-06-29

Noon – 1:00 pm Editorial Board Luncheon

Location: La Reina

1:00-3:00 pm Taxonomy, Systematics & Phylogeny I

Location: La Vista AB

Presiding: **J. Bernot**, The George Washington University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (48) **CK Blend**, NO Dronen, GR Racz, SL Gardner. TAXONOMIC MAIDS AT YOUR SERVICE: CLEANING UP *PSEUDOPECOELUS* (DIGENEA: OPECOELIDAE), A GENUS GROUPING SPECIES WITH SUSPICIOUSLY-WIDE HOST DIVERSITY.

1:15 (49) † **KS Herzog**, K Jensen. AN UNUSUALLY HIGH NUMBER OF NEW SPECIES OF *ANTHOCEPHALUM* (RHINEBOTHRIIDEA: ANTHOCEPHALIDAE) PARASITIZING A SINGLE SPECIES OF INDO-PACIFIC STINGRAY HOST.

1:30 (50) † **S Galen**, R Nunes, S Perkins. IS THE AVIAN MALARIA GENUS *LEUCOCYTOZOON* A GLOBAL PARASITE RADIATION? INSIGHTS FROM MOLECULES, MORPHOLOGY, AND ECOLOGY.

1:45 (51) † **RJ Swanteson-Franz**, A Schmidt-Rhaesa, MG Bolek, B Hanelt. WHEN PARASITES HIDE IN PLAIN SIGHT: DISCOVERY OF A HAIRWORM (NEMATOMPORPHA: GORDIIDAE) CRYPTIC SPECIES COMPLEX IN NORTH AMERICA.

2:00 (52) **JE Light**, OM Takano, CE Nessner, DR Gustafsson, PS Mitchell, G Voelker. HOST ASSOCIATIONS AND GENETIC DIVERSITY OF AVIAN CHEWING LICE (INSECTA: PHTHIRATPERA) FROM AFRICA.

2:15 (53) † **B Trevisan**, FP Marques. HETEROGENEITY IN CESTODE COMPOSITION THROUGHOUT HOST DISTRIBUTION AND ITS IMPLICATIONS ON SAMPLING STRATEGIES FOR CO-EVOLUTIONARY STUDIES.

2:30 (54) **TJ Achatz**, J Hildebrand, VV Tkach. PHYLOGENETIC INTERRELATIONSHIPS OF DICROCOELIID DIGENEANS PARASITIC IN BATS.

2:45 (55) † **M Doolin**, F Reyda, A Phillips, K Luth. MODERN METHODS AND OLD SPECIES: COMBINING MOLECULES AND MORPHOLOGY TO UNDERSTAND DIVERSITY IN *NEOECHINORHYNCHUS*.

3:00-3:30 pm **COFFEE BREAK**

1:00-3:00 pm Evolutionary Ecology I

Location: La Vista DE

Presiding: **K. Herrmann**, Tarleton State University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

1:00 (56) † **R Paseka**, R Grunberg. PARASITE STOICHIOMETRY FOLLOWS FUNCTIONAL AND PHYLOGENETIC PATTERNS.

1:15 (57) **SE Bush**, SM Villa, DH Clayton. RAPID EVOLUTION OF CRYPTIC COLOURATION IN ECTOPARASITES.

1:30 (58) † **MR Laidemitt**, SV Brant, MW Mutuku, GM Mkoji, ES Loker. EAST AFRICAN ECHINOSTOME DIVERSITY.

1:45 (59) **G Mouahid**, FD Chevalier, S Al Yafae, MA Idris, J Langand, V Menon, M Mcdew-White, T Anderson, H Moné. GENETIC MAPPING AND POPULATION GENETICS OF AN ADAPTIVE PARASITE TRAIT: LARVAL RELEASE TIME IN SCHISTOSOMES.

2:00 (60) † **L Eliuk**, J Detwiler. BEHAVIORAL EFFECTS OF TREMATODE PARASITISM ON FRESHWATER MOLLUSCS: DOES PARASITISM ALTER THE ATTRACTION BETWEEN FIRST AND SECOND INTERMEDIATE HOSTS?

2:15 (61) **AM Gleichsner**, D Minchella. OF MICE AND WORMS: DO UNRELATED PARASITE INFECTIONS DO MORE DAMAGE TO DEFINITIVE HOSTS?

2:30 (62) **FD Chevalier**, W Le Clec'h, PT Loverde, R Ramiro De Assis, G Oliveira, S Kinunghi, A Gouvras, B Webster, J Webster, A Emery, D Rollinson, T Anderson. POPULATION EXOMICS OF NATURAL SCHISTOSOME POPULATIONS USING SINGLE MIRACIDIA.

2:45 (63) † **ET Ebbs**, ES Loker, V Flores, SV Brant. COMPARATIVE POPULATION GENETICS OF TWO CONGENERIC DUCK SCHISTOSOMES, *TRICHOBILHARZIA QUERQUEDULAE* AND *T. PHYSELLAE*.

3:00-3:30 pm **COFFEE BREAK**

3:30-5:30 pm ASP Students' Symposium

Location: La Vista AB

Presiding: **K. Gallagher**, University of Connecticut

Theme: "Locating and Applying for Research Funding."

Time (Abstract No.)

3:30 Introduction.

3:40 (64) **JM Hawdon**. TRAINING GRANTS FROM THE NIH: WHAT ARE THEY AND HOW DO YOU GET ONE?

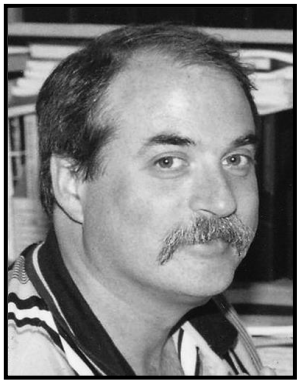
4:10 (65) **JL Cook**. THE NATIONAL SCIENCE FOUNDATION AND PERSPECTIVES ON GRANTS.

4:40 (66) **K Gallagher**. FINDING AND SUCCESSFULLY APPLYING TO SMALL GRANTS.

5:10-5:30 Questions and Closing Remarks.

5:30-6:30 pm ***The Steve Upton Party for ASP Students (sponsored by Sierra Upton)***

Location: La Vista Foyer



Steve J. Upton

Dr. Steve J. Upton (6/14/1953-7/29/2010) was an active member of the ASP. His research focused on numerous coccidia and *Cryptosporidium* species. He taught a variety of courses including human parasitology and medical parasitology during his tenure at Kansas State University as a Professor of Biology. Dr. Upton published 225 original research papers, 11 book chapters and 3 books. In 1996, he was the recipient of the H.B. Ward Medal.

Thursday Evening, 2017-06-29

6:00-7:00 pm **Auction Preview**

7:00-9:00 pm ***27th ANNUAL ASP STUDENT AUCTION***

Location: El Mirador

Friday Morning, 2017-06-30

8:00-10:30 am ***Biochemistry/Physiology/Chemotherapeutic & Drug Resistance/Vector Biology***

Location: La Vista DE

Presiding: **N. Negovetich**, Angelo State University
N. Cinar, Food & Drug Administration

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

8:00 (67) **D Calhoun**, G Bucciarelli, PT Johnson. CHEMICAL DEFENSES PROTECT LARVAE FROM PREDATORS AND DISEASE?

8:15 (68) **LM Brooks**, AR Badial, JG King. FUNCTIONAL AND PHYLOGENETIC ANALYSES OF RHS PROTEINS IN ARTHROPODS.

8:30 (69) **G Huang**, S Zhang, X Tang, X Liu, X Suo. *EIMERIA FALCIFORMIS* INFECTION PERTURBS THE METABOLIC PATHWAYS IN MICE.

8:45 (70) **SH Fitz-Coy**. CONTROLLING COCCIDIOSIS: A DAUNTING TASK.

9:00 (71) **W Le Clec'h**, T Anderson, FD Chevalier. A JOURNEY INTO THE BLOOD MICROBIOME OF *BIOMPHALARIA* SPP. SNAILS.

9:15 (72) † **E Moen**, J King. LAB STRAIN *ANOPHELES QUADRIMACULATUS* MIDGUT MICROBIAL COMMUNITY AND THE EFFECTS OF WILD LARVAL REARING ENVIRONMENTS ON MICROBIOME AND *PLASMODIUM BERGHEI* INFECTION.

9:30 (73) **A Rugel**, AB Taylor, X Cao, PJ Hart, SF Mchardy, R Ripley, F Chevalier, TJ Anderson, PT Loverde. WHY DOES OXAMNIQUINE KILL *SCHISTOSOMA MANSONI* BUT NOT *S. HAEMATOBIUM* OR *S. JAPONICUM*?

9:45 (74) **S Han**, C Leasure, S Kitchen, M Keaney, R Ratnappan, D O'Halloran, JM Hawdon. IDENTIFICATION AND CHARACTERIZATION OF A MULTI-DRUG RESISTANT STRAIN OF THE CANINE HOOKWORM *ANCYLOSTOMA CANINUM*.

10:00 (75) **S Nair**, I Cheeseman, V Menon, A Arya, F Nosten, T Anderson. WHAT MAKES A SUCCESSFUL DRUG RESISTANCE ALLELE?

10:15 (76) † **A Shrestha**, B Freudenschuss, R Jansen, B Hinney, B Ruttkowski, A Joachim. EXPERIMENTALLY CONFIRMED TOLTRAZURIL RESISTANCE IN A FIELD ISOLATE OF *CYSTOISOSPORA SUIS*.

10:30-11:00 am **COFFEE BREAK**

8:30-10:30 am Taxonomy, Systematics, Phylogeny II

Location: La Vista AB

Presiding: **O. Amin**, Institute of Parasitic Diseases
A. Phillips, Smithsonian's National Museum of Natural History

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

8:30 (77) **M Oneeb**, A Shaukat, H Naeem, MI Rashid, A Maqbool, MM Nazir. POLYMERASE CHAIN REACTION (PCR) BASED AMPLIFICATION OF MITOCHONDRIAL DNA TO DETECT *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* FROM LAHORE, PAKISTAN.

8:45 (78) **S Locke**, JJ Lopez-Cruz. MOLECULAR AND MORPHOLOGICAL DATA FROM *POSTHODIPLOSTOMUM*-LIKE, NEASCUS-FORMING DIPLOSTOMIDS IN BIRDS AND FISH.

9:00 (79) † **R Shannon**, M Bolek. THE HIDDEN DIVERSITY OF HEMOFLAGELLATE AND APICOMPLEXAN BLOOD PARASITES OF AMPHIBIAN AND REPTILE HOSTS FROM THE GREAT PLAINS REGION OF THE UNITED STATES.

9:15 (80) † **JP Bernot**, KA Crandall, GA Boxshall. EVOLUTION OF PARASITISM IN COPEPODS: A PHYLOGENETIC APPROACH USING THE OPEN TREE OF LIFE.

9:30 (81) **KC Bell**, BS Mclean, JM Allen, KP Johnson, JR Demboski, JA Cook. DOUBLE TAKE: COMPARATIVE PHYLOGENOMICS AND THE MOSAIC OF CHIPMUNK AND LOUSE DIVERSIFICATION.

9:45 (82) **M Bolek**, RA Shannon, KA Baum. THE NASTY RELATIONSHIP BETWEEN *OPHRYOCYSTIS ELEKTROSCIRRHA* AND THE MONARCH BUTTERFLY, *DANAUS PLEXIPPUS*! WHAT CAN BUYING INFECTED BUTTERFLY SPECIMENS ON THE INTERNET TELL US?

10:00 (83) **OM Amin**. ANATOMICAL VARIABILITY IN THE ACANTHOCEPHALA.

10:15 (84) **OM Amin**. STRUCTURAL-FUNCTIONAL RELATIONSHIPS AND CURIOSITIES IN THE ACANTHOCEPHALA.

10:30-11:00 am COFFEE BREAK

8:30-10:15 am Host Parasite Interactions II

Location: La Vista C

Presiding: **J.E. Light**, Texas A& M University
S. Greiman, Georgia Southern University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

8:30 (85) † **RL Grunberg**, MV Sukhdeo. PARASITIC STRATEGIES INFLUENCE HOST-PARASITE SCALING RELATIONSHIPS.

8:45 (86) **JN Caira**. LEARNING FROM THE COMPLEX SOCIETIES OF ARMY ANTS AND THEIR "GUESTS".

9:00 (87) **JE Light**, HA Folmar, AP Galán, RP Eckerlin, AP Dowling, T Campbell. A SURVEY OF SMALL MAMMAL ECTOPARASITES IN SOUTH TEXAS.

9:15 (88) † **N Wijewardena**. COMPARATIVE PHYLOGEOGRAPHY AND DIVERSITY OF FLEAS FROM AMERICAN PIKAS OF THE INTERMOUNTAIN WEST.

9:30 (89) **L Lu**, L Bu, S Zhang, ES Loker. A COMPARATIVE RNA-SEQ STUDY OF THE DETERMINANTS OF SUSCEPTIBILITY AND RESISTANCE OF THE MODEL SNAIL

BIOMPHALARIA GLABRATA TO THE HUMAN TREMATODE PARASITE *SCHISTOSOMA MANSONI*.

9:45 (90) **L Bassett**, K Herrmann, F Mitchell. SURVEY DETECTING VARIABILITY OF SMALL SUBUNIT RNA GENE OF *KNEALLHAZIA SOLENOPSAE* IN *SOLENOPSIS INVICTA*, IN SOUTH TEXAS.

10:00 (91) **MG Castillo**, N Dinguirard, TP Yoshino. EXPRESSION OF THIOESTER-CONTAINING PROTEINS IN RESISTANT AND SUSCEPTIBLE *BIOMPHALARIA GLABRATA* STRAINS AFTER IN VIVO EXPOSURE TO *SCHISTOSOMA MANSONI* MIRACIDIA.

10:30-11:00 am **COFFEE BREAK**

11:00-Noon *ASP President's Address*

Location: El Mirador C

Presiding: **N. Negovetich**, Angelo State University

11:00 Introduction of **Dr. Gerald W. Esch**

11:10 **G.W. Esch**, "Parasitism, and the discovery of veiled secrets."

Jerry Esch, President

Friday Afternoon, 2017-06-30

2:00-5:15 pm Evolutionary Ecology II

Location: La Vista AB

Presiding: **C.D. Criscione**, Texas A&M University

Time (Abstract No.)

2:00 (92) **MR Zimmermann**, CA Hollander, BN Griffith. PARASITISM DIFFERENCES BETWEEN MALE MORPHOTYPES OF BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*).

2:15 (93) **N Carpenter**, K Herrmann. VARIATION IN HELMINTH PARASITE COMPONENT COMMUNITIES OF GAMBUSIA AFFINIS AND THE EFFECTS ON HOST FITNESS.

2:30 (94) **VM Vidal Martinez**, OA Centeno Chalé, AL May Tec, L Aguirre Macedo. THE METAZOAN PARASITE COMMUNITIES OF FLATFISHES AS BIOINDICATORS OF THE ENVIRONMENTAL CONDITION OF THE CONTINENTAL SHELF OF THE PENINSULA OF YUCATAN, MEXICO.

2:45 (95) **IC Caballero**, CD Criscione. TESTING THE ROLE OF INBREEDING DEPRESSION IN THE INFECTION SUCCESS OF THE TAPEWORM, *OOCHORISTICA JAVAENSIS*.

3:00 (96) **SE Greiman**, D Menning, JA Cook, VV Tkach, EP Hoberg, AG Hope, SA Sonsthagen, SL Talbot. ONE GUT, TWO GUT, OLD GUT, NEW GUT: METAGENOMIC TOOLS FOR IDENTIFYING HELMINTH COMMUNITIES AND MICROBIOMES FROM MUSEUM ARCHIVED GASTROINTESTINAL TRACTS.

3:15 (97) **S Bromagen**, M Sukhdeo. DISTRIBUTION OF GILL MONOGENEANS (*HAPLOCLEIDUS* SP.) ON PUMPKINSEED (*LEPOMIS GIBBOSUS*) AND BLUEGILL (*LEPOMIS MACROCHIRUS*) SUNFISH.

3:30 – 4:00 pm **COFFEE BREAK**

4:00 (98) **P Robison**, A Mcgrew, P Schaffer, C Ghalambor. EFFECTS OF BRACKISH WATER TRANSFER ON METACERCARIAL LOAD IN THE TRINIDADIAN GUPPY (*POECILIA RETICULATA*).

4:15 (99) **JF Shea**. MATING BEHAVIOR OF THE HORSEHAIR WORM, *PARAGORDIUS VARIUS* (NEMATOMORPHA).

4:30 (100) **H Hollocher**, J Wilcox. UNPRECEDENTED EUKARYOTIC GUT MICROBIOME DIVERSITY WITHIN LONG-TAILED MACAQUES (*MACACA FASCICULARIS*) IN SOUTHEAST ASIA.

4:45 (101) **M Bolek**, CC Pierce, KD Gustafson. TADPOLE PARASITE COMMUNITY STRUCTURE: DO PARASITE LIFE CYCLES MATTER?

5:00 (102) **CD Criscione**, JT Detwiler. ELUCIDATING THE ROLE OF INBREEDING IN PARASITES: USING PEDIGREE RECONSTRUCTION DATA TO INFER TRANSMISSION, ASSESS INBREEDING DEPRESSION, AND PARTITION THE MATING SYSTEM.

2:00-3:30 pm Life Cycles and Epidemiology II

Location: La Vista DE

Presiding: **S. Buddenborg**, University of New Mexico

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

2:00 (103) **B Beechler**, A Jolles, S Budischak, M Smith, P Corstjens, V Ezenwa, R Spaan, G Van Dam, ML Steinauer. HIGHLY DYNAMIC SCHISTOSOME BURDENS IN FREE-RANGING AFRICAN BUFFALO: SPACE AND TIME DRIVE SCHISTOSOME ACQUISITION WHILE HOST IMMUNITY, NUTRITION, AND COINFECTION DRIVE WORM LOSS.

2:15 (104) **VR Flores**, SV Brant, G Viozzi, L Casalins, A Veleizán, ES Loker. A NEW SCHISTOSOME FROM NASAL TISSUE OF THE BLACK-NECKED SWANS, *CYGNUS MELANOCORYPHUS* (ANATIDAE).

2:30 (105) **C Anaya**, B Hanelt, MG Bolek. LAND HO! FIELD OBSERVATIONS AND EXPERIMENTATION ON A NEW *GORDIUS* SP. (NEMATOMORPHA: GORDIIDA) WITH THE FIRST DOCUMENTED TERRESTRIAL LIFE CYCLE FOR THE PHYLUM.

2:45 (106) **GJ Langford**, BE Ward. A PARASITE SURVEY OF LIZARDS ON ANDROS ISLAND, BAHAMAS: DO *ANOLIS* ECOMORPHS HOST DIFFERENT ASSEMBLAGES OF PARASITES?

3:00 (107) † **K Skinner**. PREVALENCE OF *TRYPANOSOMA CRUZI* WITHIN MAMMALIAN MUSEUM SPECIMENS IN WEST CENTRAL TEXAS.

3:15 (108) † **EA Zieman**, FA Jimenez, CK Nielsen. CHRONIC *CYTAUXZOON FELIS* INFECTIONS IN WILD CAUGHT BOBCATS (*LYNX RUFUS*).

3:30 – 4:00 pm **COFFEE BREAK**

2:30-5:00 pm Immunology

Location: La Vista C

Presiding: **J.F. Hillyer**, Vanderbilt University
J.G. King, Mississippi State University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

2:30 (109) † **ST Peper**, A Wilson-Fallon, SL Webb, J Gaskamp, SM Presley. ZOONOTIC AND ENZOOTIC PATHOGENS FROM WILD PIGS IN SOUTHERN OKLAHOMA.

2:45 (110) † **B Freudenschuss**, B Ruttkowski, A Abd-Elfattah, M Pagés, A Joachim. EFFECT OF *CYSTOISOSPORA SUIS* INFECTION ON ANTIBODY AND CYTOKINE RESPONSES IN IMMUNE COMPETENT WEANERS.

3:00 (111) **JF Hillyer**, GP League. COMPARATIVE ANALYSES OF IMMUNE RESPONSES IN LARVAE AND ADULTS OF THE MOSQUITO, *ANOPHELES GAMBIAE*.

3:15 (112) **C Li**, X Yan, H Lillehoj. INTESTINAL META-TRANSCRIPTOME COMPARISON REVEALS DISPARATE ANTIVIRAL TRANSCRIPTIONAL RESPONSE AND ITS ASSOCIATION WITH MITOCHONDRIA IN CHICKEN IMMUNITY DEVELOPMENT.

3:30 – 4:00 pm **COFFEE BREAK**

4:00 (113) **G Ahmad**. IN VITRO MORTALITY OF PROTOSCOLECES IN THE PRESENCE OF CELLS AND SERA FROM *ECHINOCOCCUS GRANULOSUS* INFECTED OR NON-INFECTED DOGS.

4:15 (114) **B Al-Adhami**, A Gajadhar. DEVELOPMENT AND EVALUATION OF SOME SEROLOGICAL TESTS FOR THE DETECTION OF *TOXOPLASMA* INFECTION IN MULTIPLE ANIMAL SPECIES.

4:30 (115) **MM Nazir**. PREVALENCE OF IGG ANTIBODIES TO *NEOSPORA* SPP. AND ASSOCIATED RISK FACTORS IN EQUIDS FROM SOUTHERN PUNJAB, PAKISTAN.

4:45 (116) **MA Muhammad**. COPROELISA DETECTION OF *FASCIOLA HEPATICA* IN GOATS.

Friday Evening, 2017-06-30

6:00 – 7:00 pm *El Mirador A*

***THE VORTEX**: Science Speed Meet for Early Career Scientists*

Saturday Morning, 2017-07-1

8:00-10:45 am Taxonomy, Systematics, Phylogeny III

Location: LaVista AB

Presiding: **T. Ruhnke**, West Virginia State University
S. Seville, University of Wyoming-Casper

Time (Abstract No.)

8:00 (117) **HA Pinto**, EA Murillo-Pulido, AL De Melo, SV Brant. EXPERIMENTAL AND MOLECULAR STUDY OF LARVAL TREMATODES IN PLANORBIDS FROM BRAZIL AND USA REVEAL A PUTATIVE NEW GENUS OF AVIAN SCHISTOSOME PRESENT IN THE AMERICAS, EUROPE AND AFRICA.

8:15 (118) **JS Portugal III**, GM Moraru, J King, SJ Mcinnis, CD Paddock, M Allerdice, T Becker, TC Smith, J Goddard. MOLECULAR PHYLOGENETIC ANALYSIS OF *DERMACENTOR PARUMAPERTUS* NEUMANN (ACARI: IXODIDAE) AND RELATED SPECIES.

8:30 (119) **T Ruhnke**. TOWARDS A RESOLUTION OF THE *INCERTAE SEDIS* SPECIES OF THE PHYLLOBOTHRIIDEA AND RHINEBOTHRIIDEA.

8:45 (120) **V Mantovani Bueno**, JN Caira. LOST (AND FOUND!) IN A SEA OF NOVELTY.

9:00 (121) **AJ Phillips**, R Salas-Monteil, S Kvist, A Oceguera-Figueroa. PHYLOGENETIC POSITION AND DESCRIPTION OF A NEW SPECIES OF MEDICINAL LEECH FROM NORTH AMERICA.

9:15 – 9:45 am **COFFEE BREAK**

9:45 (122) **CF Ruiz**, WB Driggers, CR Arias, SA Bullard. *NEOALBIONELLA* SP. FROM SKIN OF COMMON GULPER SHARKS (*CENTROPHORUS GRANULOSUS*) IN THE GULF OF MEXICO AND COMPARISON WITH *NEOALBIONELLA LONGICAUDATA*.

10:00 (123) **JR Roberts**, CR Arias, B Folt, JM Goessling, SA Bullard. TURTLE BLOOD FLUKES (DIGENEA: SCHISTOSOMATOIDEA: *HAPALORHYNCHUS* SPP.) INFECTING SOUTHEASTERN MUSK TURTLES (*TESTUDINES: KINOSTERNIDAE*).

10:15 (124) **MB Warren**, RP Koenigs, JR Roberts, CR Arias, SA Bullard. TAXONOMY OF NORTH AMERICAN SPECIES OF *ACIPENSERICOLA* (DIGENEA: APOROCOTYLIDAE), BLOOD PARASITES OF STURGEONS AND PADDLEFISH (ACIPENSERIFORMES).

10:30 (125) **SA Bullard**, CF Ruiz, CR Arias, J Rash, D Besler. *MYXOBOLUS CEREBRALIS* (ETIOLOGICAL AGENT OF WHIRLING DISEASE) INFECTS NORTH CAROLINA TROUTS (SALMONIDAE).

8:00-11:15 am Genomics & Molecular Biology

Location: La Vista C

Presiding: **G. Mayer**, Mahattan College
N. Carpenter, Tarleton State University

Time (Abstract No.)

† **denotes student presentation in the Best Student Presentation Competition**

8:00 (126) † **M Tessler**, S Kvist, ME Siddall. SALIVARY TRANSCRIPTOMICS AND ANTICOAGULANT DIVERSITY OF LEECHES.

8:15 (127) † **A Leveille**, J Barta. THIS IS GETTING RIDICULOUS! UNPRECEDENTED DIVERSITY FOUND IN MITOCHONDRIAL GENOMES OF A TURTLE PARASITE, *HAEMOGREGARINA BALLI*.

8:30 (128) † **KE O'Connor**, PA Conrad, AM Kjemtrup, RS Lane, M Yoshimizu, A Swei. A NEW PCR TOOL FOR DETECTING *BABESIA DUNCANI* IN NATURALLY INFECTED TICKS.

8:45 (129) † **P Kruth**, J Brisbin, K Moore-Dorsey, JR Barta. DEVELOPMENT OF MOLECULAR ASSAY FOR DETERMINING THE VIABILITY OF *EIMERIA* SPECIES OOCYSTS.

9:00 (130) **JG King**. DOES MOSQUITO SALIVA CONTAIN DNA THAT COULD BE RELEVANT TO SURVEILLANCE STRATEGIES?

9:15 – 9:45 am **COFFEE BREAK**

9:45 (131) **TC Van Warmerdam**, AL Drury, J Goddard, JG King. MOLECULAR ANALYSIS OF IMMUNOMODULATORY COMPONENTS IN THE BED BUG SALIVA.

10:00 (132) **K Gallagher**, J Caira, J Wegrzyn. DECIPHERING THE ORIGIN OF THE NOVEL MORPHOLOGY OF *LITOBOTHRIUM AENIGMATICUM* USING GENOMICS AND TRANSCRIPTOMICS.

10:15 (133) **HN Cinar**, J Lee, S Choi, C Lee, S Almeria, M Durigan, H Murphy, A Da Silva, G Gopinathrao. GENERATION ASSEMBLY AND ANNOTATION OF WHOLE GENOME SEQUENCES OF *CYCLOSPORA CAYETANENSIS* ISOLATES DIRECTLY FROM HUMAN STOOL SAMPLES.

10:30 (134) **K Boulton**, MJ Nolan, K Harman, A Psifidi, Z Wu, P Kaiser, S Bishop, FM Tomley, DA Hume, DP Blake. GENOMIC AND EIGEN ANALYSES PROVIDE VALUABLE INSIGHT INTO VARIATION IN HOST RESPONSES TO *EIMERIA TENELLA* INFECTION.

10:45 (135) **A Shrestha**, N Palmieri, A Abd-Elfattah, B Ruttkowski, M Pagés, A Joachim. GENE CSUI_005805 ENCODES FOR A *CYSTOISOSPORA SUIS* SPECIFIC TRANSMEMBRANE PROTEIN CRUCIAL FOR INTRACELLULAR DEVELOPMENT OF MEROZOITES.

11:00 (136) **X Tang**, J Suo, G Tao, D Hu, S Zhang, X Liu, X Suo. GENETIC MODIFICATION OF THE GLOBAL DISTRIBUTION PATHOGEN *EIMERIA TENELLA* USING CRISPR/CAS9 SYSTEM.

8:30-10:00 am Poster Display Boards delivered

Location: La Vista DEF

Authors may set up posters during this time.

11:00-12:30 pm Poster Session, coffee and snacks

Location: La Vista DEF

All authors must stand by your posters from 11:00-12:30.

CELL BIOLOGY

(137) **T Mikus**, J Volf. ANTIGEN DELIVERING SYSTEM FOR IMMUNIZATION SYSTEM AGAINST COCCIDIOSIS.

CHEMOTHERAPY AND DRUG RESISTANCE

(138) **MK Stuart**, ME Hammers. PROTEOMIC EFFECT OF TETRACYCLINE ON *TRICHOMONAS VAGINALIS.*

(139) **W Le Clec'h**, FD Chevalier, AC Alves De Mattos, PT Loverde, T Anderson. AN INTRIGUING RESISTANCE PHENOTYPE IN PRAZIQUANTEL-SELECTED *SCHISTOSOMA MANSONI*.

(140) **M Pereira**, FR Badoco, DC Tavares, G Kapadia, S Rao, LG Magalhães. ELUCIDATION OF THE IN VITRO AND IN VIVO ACTIVITIES OF CURCUMIN ANALOGS AGAINST *SCHISTOSOMA MANSONI.*

(141) **MA Guzman**. UNDERSTANDING THE GENETIC BASIS OF DRUG RESISTANCE IN *SCHISTOSOMA* SPP. AND THE DEVELOPMENT OF NOVEL THERAPEUTICS TO TREAT HUMAN SCHISTOSOMIASIS.

EVOLUTIONARY ECOLOGY

(142) **Z Faulkes**. MORE LARVAL TAPEWORMS IN SAND CRABS THAN MOLE CRABS.

(143) **CJ Brianik**, RL Grunberg, MV Sukhdeo. PARASITE AND HOST COMMUNITY STRUCTURE ALONG TWO RIVERINE ECOSYSTEMS.

(144) **AM Fedynich**, NJ Traub, A Bruno, D Rollins. SEX RATIOS OF THE COMMONLY OCCURRING NEMATODE *AULONOCEPHALUS PENNULA* IN NORTHERN BOBWHITES.

(145) **N Chodkowski**, RJ Bernot. USING STABLE ISOTOPES TO UNDERSTAND PARASITE EFFECTS ON HOST NUTRIENT ASSIMILATION.

(146) **EM Reinhart**, A Gleichsner, D Minchella. THE EFFECTS OF SECONDARY MICROPLASTICS ON THE RELATIONSHIP BETWEEN A TREMATODE PARASITE AND ITS INTERMEDIATE AND DEFINITIVE HOSTS.

(147) **DC Heins**, I Barber, CA Tilley. CASTRATORS AND THIEVES: EVIDENCE OF PARASITE STRATEGIES IN DIPHYLLOBOTHRIIDEAN CESTODES OF FISH.

GENOMICS AND MOLECULAR BIOLOGY

(148) **NS Gopal**, J Hayter, M Azaro, LM Brzustowicz. *PLASMODIUM* ALDOLASE QUANTIFICATION USING PORTABLE ISOTHERMAL LOOP MEDIATED AMPLIFICATION (LAMP) AND A SMARTPHONE.

(149) **L Bu**, S Buddenborg, S Zhang, ES Loker. COMPARATIVE STUDY OF DE NOVO TRANSCRIPTOME ASSEMBLY METHODS AND POTENTIAL IMPACTS ON DIFFERENTIAL GENE EXPRESSION.

(150) **M Mcdew-White**, S Nkhoma, V Menon, I Cheeseman, T Anderson. MICROSATELLITE AND TRANSCRIPTIONAL CHANGES IN A MALARIA MUTATION ACCUMULATION EXPERIMENT.

(151) **F Chevalier**, W Le Clec'H, M Mcdew-White, G Mouahid, H Moné, MA Idris, S Al Yafae, J Langand, N Holroyd, A Tracey, M Berriman, T Anderson. IMPROVING THE *SCHISTOSOMA MANSONI* GENOME ASSEMBLY USING GENETIC CROSSES AND LINKAGE ANALYSIS.

(152) **FD Chevalier**, W Le Clec'h, T Anderson. ROBUST EXOME SEQUENCING OF SINGLE SCHISTOSOME MIRACIDIA OPTIMIZING SNP CALLING AND ACCURACY ASSESSMENT.

(153) **J Limonta**, **N Dolce**, G Mayer. TEMPORAL AND SPATIAL PREVALENCE OF *GIARDIA LAMBLIA* IN ATLANTIC OYSTERS (*CRASSOSTREA VIRGINICA*) COLLECTED FROM ORCHARD BEACH, AND SOUNDVIEW PARK, NY, FROM 2014 TO 2016.

HOST-PARASITE INTERACTIONS

(154) **NJ Traub**, SA Shea, A Bruno, A Olsen, LA Brennan, D Rollins, AM Fedynich. ASSESSMENT OF PRECIPITATION ON THE NORTHERN BOBWHITE CECAL NEMATODE (*AULONOCEPHALUS PENNULA*) IN TWO TEXAS REGIONS.

(155) **N Chodkowski**. INVESTIGATING THE COMPLEXITY AND IMPORTANCE OF PARASITE TRANSMISSION THROUGH STUDENT-CENTERED TEACHING TECHNIQUES.

(156) **MJ Shepherd**, HR Yoder. PARASITE ASSEMBLAGES OF FRESHWATER FISHES FROM TWO BACKWATER HABITATS ALONG THE NECHES RIVER OF SOUTHEAST, TEXAS, USA.

(157) **SR Canas**, RM Lopez. HELMINTHS COMMUNITIES OF *PHILANDER OPOSSUM* (MAMMALIA: DIDELPHIDAE) IN AGUA FRIA, CHIAPAS.

(158) **MA Moran**, AM Barse. ECHINOSTOME TREMATODES IN THE MUD SNAIL (*ILYANASSA OBSOLETA*), THE RIBBED MUSSEL (*GEUKENSIA DEMISSA*) AND GULLS (*LARUS* SPP.) IN A DELAWARE SALT MARSH.

LIFE CYCLES AND EPIDEMIOLOGY

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Saturday Afternoon, 2017-07-1

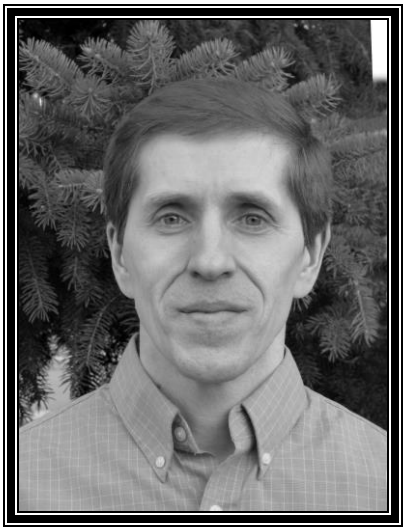
1:00 – 1:50 pm Henry Baldwin Ward Medal Lecture

Location: El Mirador

Presiding: **R. Cole,** USGS, National Wildlife Health Center
Chair, ASP Awards Committee

1:00 **R. Overstreet**, Introduction of the 2017 H. B. Ward Medal Recipient.

1:10 **V.V. Tkach**, "Parasites, people and continents: an unexpected journey."



Vasyl Tkach
Henry Baldwin Ward Medal

2:00 - 3:00 pm ASP Awards and Business Meeting

Location: El Mirador

ASP AWARDS

ASHTON CUCKLER NEW INVESTIGATOR AWARD

Presiding: **R.A. Cole,** US Geological Survey, Chair, ASP Awards Committee

The recipient of the 2017 New Investigator Award is **Heather Stigge**, College of Saint Mary.

Dr. Heather Stigge
Ashton Cuckler New Invesitgator Award

WILLIS A. REID JR. STUDENT RESEARCH GRANTS

Presiding: **G. Mayer**, Mahattan College

BEST STUDENT PRESENTATIONS AND MARC DRESDEN TRAVEL GRANT AWARDS

Presiding: **S. Seville,** University of Wyoming-Casper

ASP BUSINESS MEETING

Presiding: **G.W. Esch**, Wake Forest University

Thank you for attending this year's ASP meeting and have a safe trip home.
*See you **June 21-June 24, 2018** at our next meeting in **CANCUN, MEXICO.***

Abstract Listings

(1)

EVOLUTIONARY PROCESSES, POPULATION STRUCTURE AND MORPHOLOGICAL FEATURES OF COCCIDIA PARASITIZING DIFFERENT GROUPS OF HOSTS

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Evolutionary processes in parasites, associated with changes of host spectra, and adaptations to the new hosts, have been for a long time enigmatic and interesting biological phenomena. Based on the phylogenetic and population-genetic analyses, this research area posed many crucial questions, mainly concerning the conditions and mechanisms which underlie the evolutionary processes. The long-held view of host-parasite coevolution as a process determined mainly by cospeciation events has dramatically changed in recent years, especially due to the frequent incongruencies detected between host and parasite phylogenies. At the population level, even a geographically and taxonomically restricted model can be remarkably complex and flexible: when analyzed across several sites, individual genetic lineages may form mosaic of entirely different patterns, from strict coevolution (with host-mediated genetic structure of the parasite), to rapidly uncoupled genetic structures of both counterparts. This indicates that even under similar conditions (shared history between the host and parasites) the evolution and resulting genetic structure may be dominantly influenced either by the host or by other, environmental or inherent conditions. Eimerians parasitizing European *Apodemus* spp. and Arvicoline rodents represent such an example; during their evolution, colonizations and complete host switches have occurred multiple times. These events resulted in origin of several lineages with different degree of host specificity, ranging from a single host species to several host genera. Apart from these genetic features, the studied eimerians displayed a congruence between their morphological features and phylogenetic relationships inferred from the molecular data. This indicates that morphological traits of *Eimeria* are not organized randomly. In contrast to eimerians parasitizing rodent hosts, a different situation occurs in other group of coccidia, namely in genera *Haemogregarina* and *Hemolivia*. Despite the large number of samples collected from different localities and host species, neither any population structure nor host switching events were observed.

(2)

INTRANUCLEAR COCCIDIOSIS OF TESTUDINES – THE LIFE CYCLE DISCOVERED

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B. Koudela and **D. Modrý**, Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackého tř. 1946/1, 612 42 Brno, Czech Republic Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences, Pala

Intranuclear coccidiosis of testudines' (known as TINC) is an emerging disease in chelonians. An intranuclear coccidian parasite causing lethal systemic disease in chelonians was first identified in tissues

of the radiated tortoises (*Astrochelys* [*Geochelone*] *radiata*) histologically and by electron microscopy in 1990. Subsequently, the systemic intranuclear coccidiosis was confirmed in numerous tortoises, when the similar nonspecific symptoms (anorexia, lethargy, weakness, weight loss) were described. In 2006, the diagnostics by histological techniques and electron microscopy was supplemented by DNA-based diagnostics. Partial sequences of the 18S rDNA can be targeted by PCR assay for post- and antemortem diagnosis. Attempts to describe the TINC oocysts in faeces of tortoises have failed, however unsporulated oocysts were seen in tissues. Until our study, the identification of exogenous stages, life cycle and way of transmission of the intranuclear coccidium responsible for the TINC have not been discovered. We report herein the discovery of oocysts of an eimeriid coccidium in faeces of naturally infected leopard tortoises (*Stigmochelys* [*Geochelone*] *pardalis*) and their causal associations with TINC, which was proved by the partial 18S rDNA sequence analysis and experimental transmissions. Juvenile tortoises of *S. pardalis* were infected perorally with sporulated oocysts from the naturally infected individual. The tortoises started to shed unsporulated oocysts one month post infection. Symptoms of the infection developed few weeks later. Postmortally, the tissues were examined using histopathology, transmission electron microscopy and PCR. Histological findings showed numerous intracellular stages mainly in intestine, liver, lungs and kidney. Blood and all 16 tested organs were PCR positive. The obtained partial 18S rDNA sequences were homologous with GenBank sequences from previous TINC cases. The sequence of an eimeriid coccidium from *S. pardalis* determined in our study and the intranuclear coccidium isolated from *Indotestudo forsteni* (AY728896) formed a separate and well-supported lineage, which was basal to all other eimerians and was most closely related to *Goussia* and *Eimeria* infecting poikilothermic vertebrates. Chelonians represent one of the most threatened groups of vertebrates. Emerging pathogens not only hamper the captive breeding programs, but represent also enormous threat for the native populations that are to be reinforced by captive individuals. From this point of view, the knowledge on the epidemiology and transmission biology of TINC is an important step towards its prevention and treatment. This study was funded by the Grant Agency of University of Veterinary and Pharmaceutical Sciences Brno (project No. IGA 105/2017/FVL).

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(3)

MITOGENOMICS IN IDENTIFICATION AND EVOLUTION OF COCCIDIA AND OTHER APICOMPLEXAN PARASITES

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The use of comparatively short DNA sequences to aid in the identification and delimitation of coccidian species (i.e. DNA 'barcoding') has been used extensively since the advent of Sanger sequencing. Initially, partial nuclear small subunit rDNA (nu 18S rDNA) sequences were utiltized because of ubiquity and accessibility but nu 18S rDNA was too conserved for use at the species level with many coccidia. Over the last decade, DNA barcoding using short mitochondrial cytochrome *c* oxidase subunit I (mt COI) sequences has become used widely for species delimitation and, less commonly, for molecular phylogenetic studies. With improved and less costly sequencing methods, complete mitochondrial genomes are being generated for many apicomplexan parasites including coccidia. We have generated complete mitochondrial genome sequences from a wide variety of eucoccidian parasites belonging to various genera (e.g. *Eimeria*, *Isospora*, *Caryospora*, *Cyclospora*, *Choleoeimeria*, *Hepatozoon* and *Haemogregarina* among others). The mitochondrial genomes of eimeriid coccidia were structurally conserved with the order of the 3 CDS (COI, COIII and CytB) and multiple rDNA fragments essentially identical; however, *Choleoeimeria* had some modest rearrangements in mt genome structure. In contrast, sarcocystid coccidia had reduced mitochondrial genomes (with losses of both CDS and rDNA fragments) and the adeleid coccidia had wildly divergent mt genome structures (even within the same genus). Although phylogenies generated from mt genome sequences alone provided robust support for relationships among closely related coccidia, combining nu 18S rDNA and (even partial) mt COI sequences in a partitioned dataset analysed with suitable partition-specific substitution models proved the best method for inferring both recent and more ancient relationships among these parasites. A posteriori

analysis demonstrated substitution saturation of mt COI sequences, particularly at the 3rd codon position, for more distantly related taxa requiring accommodation in the alignment and Bayesian analyses.

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(4)

GENOME EVOLUTION AND HOST SPECIFICITY IN *CRYPTOSPORIDIUM*

L. Xiao, Centers for Disease Control and Prevention
Y. Feng and **Y. Wang**, East China University of Science and Technology
D.M. Roellig, Centers for Disease Control and Prevention

Cryptosporidium spp. are important apicomplexan pathogens of humans and various animals. Thus far, about 100 *Cryptosporidium* species and genotypes have been described based on differences in host specificity and DNA sequences at the 18S rRNA locus. Host adaptation is also known to occur within *C. parvum* and *C. ubiquitum*, with several gp60 subtype families preferentially infecting humans or different species of animals. Thus far, the genetic determinants for host specificity in *Cryptosporidium* spp. are poorly understood. Taking advantage of recent development in whole genome sequencing, we have sequenced the genomes of >200 isolates from 8 *Cryptosporidium* species. Comparative genomic analysis indicates that some *Cryptosporidium* species have very similar genomes despite difference in host range. They differ mostly in copy numbers of several multigene families in telomeric regions, with species of board host range having more copies of these *Cryptosporidium*-specific genes. *Cryptosporidium* spp. also differ significantly in sequences of genes encoding secreted proteins, especially those belonging to multigene families or located in subtelomeric regions. These differences in gene content and sequences also exist among host-adapted *C. parvum* or *C. ubiquitum* subtype families. Thus, telomeric gene duplications and losses and highly divergent secreted proteins could contribute to different host specificity among closely related intestinal *Cryptosporidium* species or *C. parvum* and *C. ubiquitum* subtype families. In contrast, gastric *Cryptosporidium* spp. appear to have very different genomic evolution and other mechanisms could be involved in host specificity determination.

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(5)

GENETIC AND BIOLOGICAL SIMILARITY OF *CYCLOSPORA CAYETANENSIS* TO CECUM-INFECTING *EIMERIA* SPP. AS REVEALED BY COMPARATIVE GENOMIC ANALYSIS

Y. Feng, South China Agricultural University
L. Xiao, Centers for Disease Control and Prevention

Cyclospora cayetanensis is an emerging foodborne parasite that has received high attentions because of the massive foodborne outbreaks they caused in recent years. The investigation of foodborne outbreaks of cyclosporiasis has been hampered by a lack of genetic data and poor understanding of pathogen biology. We sequenced the whole genome of *C. cayetanensis* from a patient in China using Illumina 100-bp paired-end technology. The apicoplast and mitochondrial genomes of *C. cayetanensis* are highly similar to those of cecum-infecting avian *Eimeria* spp. in both gene organization and sequences. A comparative analysis of the nuclear genomes has confirmed the similarities in genome organization, metabolic capabilities and potential invasion mechanism between *C. cayetanensis* and *Eimeria tenella*. Propanoyl-CoA degradation, GPI anchor biosynthesis, and N-glycosylation are some apparent metabolic differences between *C. cayetanensis* and *E. tenella*. The similar repertoire of host cell invasion-related proteins possessed by all coccidia suggests that *C. cayetanensis* has an invasion process similar to the one in *T. gondii* and *E. tenella*. However, the significant reduction in the number of identifiable rhoptry protein kinases, phosphatases and serine protease inhibitors indicates that monoxenous coccidia, especially *C. cayetanensis*, have limited capabilities or use a different system to regulate host cell nuclear activities. *C. cayetanensis* does not possess any cluster of genes encoding the TA4-type SAG surface antigens seen in *E. tenella*, and may use a different family of surface antigens in initial host cell interactions. Based on the

whole genome sequence data, a multilocus sequence typing tool was developed, with 2 to 10 geographically segregated sequence types at each of 5 selected loci. This new tool could be useful in case linkage and infection/contamination source tracking.

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(6)

GLOBAL GENETIC DIVERSITY AND POPULATION STRUCTURE OF *TOXOPLASMA GONDII*

C. Su, University of Tennessee

Elucidating *Toxoplasma gondii* population structure will enhance our understanding of the factors that have driven the proliferation of one of the most successful eukaryotic pathogens on earth. In the past decade, numerous studies have identified various genotypes of *T. gondii* in domestic and wild animals, providing significant information on genetic diversity of the parasite. Using the multilocus PCR-RFLP typing method, hundreds of genotypes were identified over 1,400 *T. gondii* samples worldwide. Overall, only a few genotypes dominate in Europe, Africa, Asia and North America. However, hundreds of genotypes coexist with none being notably dominant in South America. The Type II has been identified as the dominant lineage in Europe, North Africa and North America, but has a low frequency in South America. Our recent genetic analysis of the Type II strains suggested a recent expansion of this lineage following human migration.

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(7)

MOLECULAR GENETICS FOR CRYPTOSPORIDIOSIS

B. Striepen, University of Georgia

The protozoan parasite *Cryptosporidium* is a major cause of severe diarrhea in young children and an important contributor to early childhood mortality. *Cryptosporidium* is also an opportunistic pathogen in immunocompromised individuals. Currently, we lack effective drugs and vaccines to treat or prevent cryptosporidiosis. A main roadblock for their development has been the overall poor tractability of Cryptosporidium due to lack of continuous tissue culture systems, poor animal models, and lack of genetic tools. Recently we established a powerful molecular genetic model for this pathogen. This system allows us to tag, modulate or delete genes, and to use a variety of reporters to follow the parasite in vivo and in real time. We currently use these approaches to understand how the single host sexual lifecycle of *Cryptosporidium* unfolds, how the parasite interacts with its host at the cellular and whole animal level, and to enable the development of effective treatments with facile animal models and genetic target discovery and validation.

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(8)

USING COMPARATIVE AND FUNCTIONAL GENOMICS TO UNDERSTAND LIFE CYCLE EVOLUTION IN *T. GONDII*

S.L. Sokol and **A. Primack**, University of Pittsburgh
J. Dubey, United States Department of Agriculture
J.P. Boyle, University of Pittsburgh

Toxoplasma gondii has a vast host range and flexible multi-host life cycle while its nearest relatives are obligately heteroxenous and have more typically restricted host ranges. To identify the molecular determinants for these differences we conduct comparative studies between *T. gondii* and its nearest extant relative, *Hammondia hammondi*. Our published work shows that the *T. gondii* and *H. hammondi* genomes bear near perfect synteny, and that both species harbor similarly functional virulence effector gene repertoires. We are now investigating core features that distinguish these species. *In vitro*, *H. hammondi* sporozoites replicate significantly slower than *T. gondii* (0-1X per 24h compared to 1X per ~6h), suggesting key differences in the parasite cell cycle. *In vitro*, *H. hammondi* sporozoites spontaneously convert to terminally differentiated cyst-like stages, which cannot be subcultured *in vitro* and can only be propagated further after oral infection of felines. We have found that the inability to subculture *H. hammondi in vitro* is precisely timed, and linked this directly to spontaneous conversion to cyst-like stages *in vitro*. Interestingly the inability to subculture parasites *in vitro* correlates directly with the inability to infect rodent hosts, which provides a unique opportunity to investigate the terminal differentiation process *in vitro* and *in vivo*. We have now performed RNAseq analyses of *H. hammondi* and *T. gondii* during this stage transition *in vitro* and identified multiple classes of genes that are differentially regulated. Interestingly, in addition to known *T. gondii* cyst-expressed genes, *H. hammondi* zoites express a subset of transcripts that are only expressed during *T. gondii* infection of the feline gut epithelium. These data, while preliminary, suggest that one of the transcriptional switches that may underlie the life cycle flexibility that is unique to *T. gondii* is the suppressed expression of genes that are required for and/or expressed during the infection of cat cells.

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(9)

DUAL TRANSCRIPTIONAL PROFILING OF MICE AND *TOXOPLASMA GONDII* DURING EARLY AND LATE CHRONIC INFECTION

L. Knoll, University of Wisconsin Madison

The obligate intracellular parasite *Toxoplasma gondii* establishes a life-long chronic infection within any warm-blooded host. After ingestion of encysted parasites, *Toxoplasma* disseminates throughout the body as a rapidly replicating form during acute infection. Over time and after stimulation of the host immune response, *Toxoplasma* differentiates into a slow growing, cyst form that is the hallmark of chronic infection. Previously, we conducted a simultaneous RNA-seq analysis of *Toxoplasma* and their rodent hosts to better understand host and parasite responses during acute and chronic infection. Because *Toxoplasma* cysts are primarily localized to neurons, we sampled the forebrains of three infected and uninfected mice at each time point. We expected the host response to peak at acute infection because that is when the mice shows flu-like symptoms; however, only nine mouse genes were at least 20-fold more abundant during acute versus chronic infection. In contrast, almost 400 mouse genes were at least 20-fold more abundant during chronic compared to acute infection. We hypothesized that this increased host response we due to our sampling at relatively early chronic infection, 28 days post-infection, and that the host response would subside at a late infection time point, such at 6 months post-infection. To address this hypothesis, we extended our dual transcriptional profiling of mice and *Toxoplasma* to 21 and 28 days as well as 3 and 6 months post-infection. We also included three uninfected controls at each time point as well as male and female mice to address sex differences seen in multiple studies in the host response to *Toxoplasma*. Our initial analysis shows that several host genes that are more abundant at 28 days post-infection are still maintained at that abundance level at six months post-infection. These data suggest host and *Toxoplasma* interplay is still active during long-term chronic infection.

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(10)

BIOLOGICAL CONSEQUENCES OF GENETIC EXCHANGE AND INFLAMMASOME SENSORS ACTIVATED BY PROTOZOAN PARASITES

A. Khan, **A. Kennard**, **B. Gregg**, **J. Zhang**, **K. Shen**, **J.P. Dubey**, **J. Parkinson**, **M.E. Grigg**

How virulent strains emerge among protozoan populations is an important paradigm of eukaryotic pathogenesis. Using population genetic and WGS phylogenomic methods, our work has identified extant genetic exchange among circulating populations of natural isolates of *Toxoplasma gondii*, *Neospora caninum,* and *Sarcocystis* spp. To understand the biological consequences of such genetic admixture, we investigated the genetic factors that mediate acute virulence during murine *Toxoplasma* infection. Employing forward, reverse genetic, and genome-wide association (GWAS) techniques, we show that *Toxoplasma* acute virulence is highly dependent on the expression of a restricted set of polymorphic secreted pathogenesis determinants (SPDs) that are inherited in discrete haploblocks by genetic exchange. SPDs discharged from parasite secretory organelles target host immune signaling pathways and facilitate infection competency. We have identified novel SPDs activating inflammasome pathways, dysregulating immune homeostasis, and altering parasite pathogenesis. Utilizing GWAS on WGS data from 56 *T. gondii* strains, we identified four genomic regions (Chromosome VIIa, VIIb, VIII and IX) that encode novel SPDs associated with murine virulence. eQTL screening of progeny from a collection of *T. gondii* crosses has identified two parasite loci, in addition to GRA15, that upregulate IL-1β and modulate host inflammasome activation. Our data indicate that parasite effector proteins are released to alter parasite virulence, subvert host immune responses and maximize parasite transmissibility

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(11)

USING GENOME SEQUENCING TO DECIPHER CRYPTIC *EIMERIA*

D.P. Blake, Royal Veterinary College
V. Vrba, Bioproperties Pty Ltd
I.D. Jatau, Ahmadu Bello University
M.J. Nolan, Royal Veterinary College
G. Underwood, Bioproperties Pty Ltd
F.M. Tomley, Royal Veterinary College

Seven *Eimeria* species are recognised to infect chickens, all with a global enzootic distribution. Additionally, three cryptic Operational Taxonomic Units (OTUs x, y and z) have been described in populations of *Eimeria* recovered from chickens in Australia. More recently, all three OTUs have been detected in sub-Saharan Africa and/or South America, but their occurrence, pathology and the risk they pose is largely unknown. As the role for live vaccines in the control of coccidiosis increases it is important to determine the ability of current vaccine formulations to induce protection against challenge by OTU parasites. The development of genomic resources for each OTU is a key step towards understanding these parasites. Initially, oocyst morphology was determined for all three *Eimeria* OTUs using field isolates collected from Nigerian broiler chickens. Microscopic characterisation revealed oocyst length (L), width (W) and L/W ratios of 30.5 µm, 23.4 µm, 1.30 (OTUx); 26.8 µm, 22.9 µm, 1.17 (OTUy); and 17.6 µm, 15.3 µm, 1.15 (OTUz), respectively. Oocyst morphology was confirmed for OTUx and z by comparison with reference isolates collected in Australia. Subsequently, total genomic DNA extracted from Australian OTUx, y and z isolates were prepared for Illumina next generation sequencing employing a Nextera XT protocol. Automated sequence assembly for OTUx created 16,071 contigs, representing an assembly size of 42.9 Mb. For OTUy 40,800 contigs were produced, including 58.0 Mb DNA sequence. For OTUz 27,925 contigs were produced, including 50.6 Mb. Phylogenetic inference using a range of reference gene, mitochondrial and antigen coding sequences has revealed significant divergence from the seven recognised *Eimeria* species with OTUz found to be most distinct. The risk posed by these novel genotypes is unknown, but it is clear that an understanding of their pathogenic potential is now essential, with

appropriate diagnostic tools required to facilitate the assessment of escape from current anticoccidial vaccines and possible future vaccine development.

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(12)

CRYPTOSPORIDIUM PARVUM LACTATE DEHYDROGENASE IS ASSOCIATED WITH THE PARASITOPHOROUS VACUOLE MEMBRANE AND IS A POTENTIAL TARGET FOR DEVELOPING THERAPEUTICS

F. Guo, **H. Zhang** and **G. Zhu**
Texas A&M University, College of Veterinary Medicine & Biomedical Sciences

The apicomplexan *Cryptosporidium parvum* possesses a bacterial-type lactate dehydrogenase (CpLDH) that is considered as an essential enzyme, as this parasite lacks the Krebs cycle and cytochrome-based respiration, and mainly rely on glycolysis to produce ATP. Using immunofluorescence and immunoelectron microscopy, we found that CpLDH was localized in the cytosol in the extracellular parasites (e.g., sporozoites and merozoites). However, it becomes associated with the parasitophorous vacuole membrane (PVM) during the intracellular developmental stages, suggesting the involvement of PVM in the parasite energy metabolism. We also characterized the biochemical features of CpLDH and observed that, at lower micromolar levels, the LDH inhibitors gossypol and FX11 could inhibit both CpLDH activity (K_i = 14.8 μM and 55.6 μM, respectively), as well as parasite growth *in vitro* (IC_{50} = 11.8 μM and 39.5 μM, respectively). These observations not only reveal a new function for the poorly understood PVM structure in hosting the intracellular development of *C. parvum*, but also suggest LDH as a potential target for developing therapeutics against this opportunistic pathogen, for which fully effective treatments are not yet available.

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(13)

TARGETING A MAMMALIAN HOST METABOLIC PATHWAY FOR IMPROVED CHEMOTHERAPY AGAINST THE APICOMPLEXAN PARASITE *TOXOPLASMA GONDII*

S. Moreno and **Z. Li**, Center for Tropical and Emerging Global Diseases, University of Georgia

Toxoplasma gondii is an opportunistic pathogen that causes serious disease in immunocompromised patients. Treatment for these parasitic diseases is challenged by lack of effective drugs to eradicate chronic infection. New therapeutic agents or combinations of drugs to treat these infections are urgently needed. We are exploring the development of therapeutics that combine targeting host-encoded with parasite-encoded functions as a novel approach to develop highly effective treatments. We test this strategy by targeting the supply of isoprenoids for *Toxoplasma gondii* growth and survival. Synthesis of isoprenoid precursors in Apicomplexa occurs in the apicoplast and is essential. For longer isoprenoids, *T. gondii* expresses a farnesyl diphosphate synthase. Surprisingly, *null* mutants for this enzyme have a mild growth phenotype and an isoprenoid composition similar to wild type parasites because during intracellular growth parasites salvage FPP and/or GGPP from the host. However, the loss of the enzyme becomes phenotypically apparent during the extracellular phase of the parasite. Our hypothesis is that we should be able to inhibit intracellular growth with inhibitors of the host mevalonate pathway (statins). We proposed a synergistic interaction between statin treatment and pharmacological or genetic interference of the parasite isoprenoid pathway. A number of successful combinations were found to be effective both *in vitro* and *in vivo*. Mice were cured from a lethal infection with an hypervirulent strain of *Toxoplasma*

with combinations of host and parasite inhibitors. We propose a double-hit strategy combining inhibitors of host and parasite pathways as a novel therapeutic approach against Apicomplexan parasites.

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(14)

APPLICATION AND CYCLING CHARACTERISTICS OF A COMMERCIAL COCCIDIA VACCINE APPLIED BY SPRAY AND GEL

B. Jordan, The University of Georgia
L. Tensa, **G. Albanese**

Coccidiosis is an economically significant enteric disease of poultry caused by *Eimeria spp*. Coccidiosis can result in poor feed conversion, reduced weight gain, and can aid in the development of necrotic enteritis. Coccidiosis can be controlled using a vaccine containing live, sporulated oocysts delivered to day old chicks. The oocysts may or may not be attenuated, depending on the vaccine. Coccidia vaccines have typically been administered in a water diluent via spray cabinet, however a gel based delivery system has recently been introduced. The gel system is designed to elongate preening time and increase oocyst ingestion by creating more viscous gel droplets that remain intact on the chicks for a longer post-vaccination period. The purpose of this trial was to evaluate the application characteristics of a commercial coccidia vaccine applied by water spray or gel, and then analyze oocyst vaccine cycling patterns and protection from challenge in chickens vaccinated with these methods. The vaccine was mixed in each diluent and samples were taken to assess how well the vaccine remained in suspension. Vaccine application patterns for each diluent were then assessed by measuring the size and number of droplets applied onto a plexiglass sheet on a chick basket. Droplets of each obtainable size were collected and oocyst enumeration and speciation was performed. Vaccine, mixed in each diluent, was then applied to chicks to compare cycling patterns between the two application methods, as well as to determine protection from challenge. Results show that no settling occurred in either diluent after mixing per manufacturer's instructions. Application to the plexiglass revealed there were no differences in oocyst delivery from front to back for either delivery system. For the spray, the largest droplets containing the most oocysts were found along the outside edges of the spray pattern, indicating that chicks on the outside may receive more vaccine than chicks in the middle. As expected, the number of oocysts per droplet increased as droplet size of the spray administration increased, but stayed constant in the gel administration since the droplets were of uniform size. There was an even distribution of species in each drop that approximately mirrored the distribution in the vaccine bottle prior to application, except for *Eimeria acervulina*. Additionally, there were consistent decreases in oocysts per dose for both application methods from the working dilution to a sample collected directly from the spray nozzle or gel bar, with a further decrease in oocysts per drop for the spray application when looking at drops on the Plexiglass plate compared to no decrease for the gel application. There were differences present in the cycling pattern between application methods, with the timing of peak cycling being the most dramatic. Taken together, this data will aid poultry producers in deciding which vaccine delivery system will provide the best protection in their production system.

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(15)

EVALUATION OF THE EFFICACY OF DIFFERENT COCCIDIOSIS BIOSHUTTLE PROGRAMS AND POTENTIAL ALTERNATIVES TO AGPS ON BROILER PERFORMANCE

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The inclusion of ionophores in one or more phases of feeding is often used to ameliorate necrotic enteritis (NE) or subclinical enteritis in coccidiosis vaccination programs in the US (called bioshuttle programs); however, ionophores, considered as antibiotics in the US, are not accepted in antibiotic free (ABF) programs. Since the market trend of ABF has risen, more and more natural products have been applied in the field to improve performance. A total of 4800 one-day-old male broiler chicks were sprayed with a commercial coccidiosis vaccine and raised to 42 days (Trial 1) and 43 days (Trial 2) of age in randomly assigned pens on built up litter. Different probiotics, natural products, feed additive amprolium or water soluble amprolium were used in the trials. Feed conversion ratio and body weight were calculated on days 14, 35 and 42 or 43. Fecal samples from every treatmentgroup were collected, and oocyst counts were performed every 4 days from day 7 to the end of study. The results from Trial 1 demonstrated that adjusted FCR at day 42 ofBacillus licheniformis and Bacillus subtilis with or without amprolium 72.6 g/ton feed between days 15-35 groups were significantly lower than non-additive control group. In Trial 2, at 35 and 43days of age, the group with amprolium 113g/ton in feed between days 15-35 had the greatest weight gain and lowest adjusted feed conversion ratio.Short duration of water soluble amprolium 0.006% at days 14-16 was nearly as effective as continuous use of a low dose (72.6 g/ton) of amprolium from days 15-35. The capsicum-turmeric oleoresins combination product performed similarly to the amprolium and salinomycin treatments. The treatment group with amprolium (113g/ton) resulted in highest weight gain with the lowest feed conversion ratio.

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(16)

ON THE WAY TOWARDS ACTUALIZING RECOMBINANT *EIMERIA* AS A VACCINE VECTOR

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Eimeria-induced coccidiosis causes enormous economic losses to the industries of poultry, rabbits and other livestock. Anti-coccidial vaccines are now extensively used in poultry production to combat coccidiosis, an effective alternative to the drug-based control of coccidiosis that is being defeated by widely distributed anti-coccidial drug resistance and concerns over human health risks of drug residues in animal products. In fact, live oocyst-based anti-coccidial vaccines are among the most successful vaccines against infectious diseases in animals even when they are compared to anti-viral and anti-bacterial vaccines. Our group is endeavoring to develop recombinant/transgenic coccidial strains as a eukaryotic vaccine vector for the protection of farm animals against mucosal infections. In our previous studies presented at ICC-10 and -11 we had showed our potentiation of recombinant/transgenic *Eimeria* as a vaccine vector by: a) establishment of a stable transfection system in *Eimeria* parasites, b) successful manipulation of recombinant *Eimeria* to stimulate immune responses to exogenous model antigens expressed by recombinant parasites, and c) our ability to improve expressing levels of exogenous model antigens and to enhance immune responses to exogenous model antigens through co-expression of adjuvant molecules in the recombinant *Eimeria*. At ICC-12, I will present our recent results of: 1) targeting exogenous antigens to multiple locations in the recombinants and improved expressing levels of the antigens using the CRISPR/Cas9 genome editing system; 2) further polarized and enhanced stimulation of host immune responses by the recombinants; 3) achieving partial protection of immunized animals against challenges by targeted pathogens. We thank National Natural Science Foundation of China (31330076 and 31572507) for supporting this research and anticipate refinement of the novel vector system.

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(17)

RELEVANCE OF MATERNAL IMMUNITY AGAINST COCCIDIOSIS AFTER IMMUNIZATION WITH SPORULATED OOCYSTS

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In order to assess the relevance of maternal immunity when using *Eimeria* live vaccines, four different trials were performed. In the first trial, serologically positive and negative broiler breeders were obtained. To obtain a serologically positive group, broiler breeders were inoculated four times every two weeks with one dose of the vaccine HIPRACOX (composed of sporulated oocysts of *Eimeria acervulina*, *E.maxima*, *E.mitis*, *E.praecox* and *E.tenella*). The serologically negative group was obtained by avoiding any contact of the broiler breeders with Eimeria oocysts and a combination of anticoccidial treatments. Breeders were treated with a rotation program, which included Nicarbazin/Narasin, Diclazuril, Salynomicin, and Monensin. The offspring of these two groups of broiler breeders, broiler birds of one day of age, were used to perform three studies to confirm the effect of maternal immunity on the efficacy of vaccinal strains administered at one day of age. The vaccinal strains included in the studies were *E.acervulina* 003, *E.maxima* 013 and *E.tenella* 004. Before the beginning of any efficacy study, the broiler birds were tested to be serologically positive or negative by using an Indirect Immunofluorescence Test modified from Kouwenhoven (1976). The efficacy study protocol was done according to Williams (2000). The results obtained in the three studies performed confirm that, independently of the serological status of the vaccinated birds, the elimination of the vaccinal oocysts post-vaccination and the level of protection to challenge obtained in the different study groups can be considered the same. The results indicate lack of relevance of maternally derived antibodies to the protection against live coccidiosis vaccines when sporulated oocysts are used for the immunization.

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(18)

TARGETING TRANSGENIC ANTIGEN EXPRESSION TO OPTIMISE *EIMERIA* PARASITES AS VACCINE DELIVERY VECTORS

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Farmed poultry are under threat from multiple viral, bacterial, and parasitic infections. One of the most important threats, coccidiosis, has been ranked among the ten most economically significant enzootic livestock diseases with associated costs predicted to exceed £2.5 billion worldwide every year. Control of *Eimeria* species parasites, the cause of coccidiosis, relies on routine chemoprophylaxis and/or vaccination. Both are effective although drug resistance, and the relative cost and production capacity of the live parasite vaccines, can prove limiting. Recently, the availability of protocols supporting genetic complementation of *Eimeria* has raised the prospect of generating vaccinal parasite lines which also function as vaccine vectors, expressing and delivering heterologous proteins. Complementation with sequences encoding immunoprotective antigens from other *Eimeria* species offers an opportunity to reduce the complexity of species/strains in anticoccidial vaccines. Antigens which induce immune protection against other pathogens or zoonoses could also be included. Here, we describe the ongoing development of *Eimeria tenella*-vectored vaccines, highlighting the use of specific protein delivery signals to modify transprotein trafficking in an effort to improve antigen exposure to the host immune system. Specifically, fusion of the signal peptide (SP) from *E. tenella* microneme protein 2 (EtMIC2) to the mCherry reporter resulted in protein secretion. Further, addition of the glycophosphatidylinositol (GPI) anchor from *E. tenella* surface antigen 1 (EtSAG1) resulted in secretion and tethering to the sporozoite

surface. A sequence encoding the *Eimeria maxima* apical membrane antigen 1 (EmAMA1) ectodomain was added to each construct and used to create transgenic *E. tenella*. Vaccination of chickens using these transgenic parasites conferred protection against *E. maxima* challenge, with levels of efficacy comparable to those obtained using recombinant protein or DNA vaccines.

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(19)

RECENT PROGRESS IN UNDERSTANDING HOST IMMUNE RESPONSE TO AVIAN COCCIDIOSIS: TH1 AND TH17 RESPONSES

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Coccidiosis is one of the most economically important diseases of the chickens caused by *Eimeria* spp. since it destroys the intestinal epithelium resulting in nutrient malabsorption, body weight loss, and in severe cases, death. Since the life cycle of *Eimeria* parasites is complex and comprised of intracellular, extracellular, asexual, and sexual stages, host immune responses are quite diverse and complex. In the context of adaptive T cell immunity, it has been shown that IFN-γ-mediated Th1 response is dominant in *Eimeria* infection. However, since the discovery of Th17 type response which is distinct from the Th1 and Th2 responses, it has become evident that Th17 cells may play an important role in the host defense against various infections including parasitic infections. In order to determine if Th17 type response is also induced in chickens and its potential role in host response to coccidiosis, we investigated the expression levels of Th17 cells-related cytokines in *E. tenella*-infected chickens along with Th1 cells-related cytokines. Our recent studies showed that an increase of both $CD4^+IFN\text{-}\gamma^+$ and $CD4^+IL\text{-}17A^+$ cells in the *E. tenella*-infected cecum with increasing number of parasites in the feces. We also found that the mRNA levels of IL-17A and IFN-γ increased in the *Eimeria* antigen-stimulated splenic $CD4^+$ cells and these results suggest that the Th1- and Th17-associated cytokines are enhanced in *Eimeria*-infected tissues. Collectively, our study demonstrates that the Th17 as well as Th1 immune responses are implicated in host immune response to *E. tenella* infection in chickens.

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(20)

CONSTRUCTION OF TRANSGENIC RABBIT COCCIDIA AND PROSPECT OF INDUCED IMMUNITY BY RECOMBINATION WITH VP60 FROM RHDV

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Rabbit coccidiosis, caused by infection with *Eimeria* parasites, is a prevalent parasitic disease affecting worldwide rabbitries. Genetic manipulation of rabbit eimerian parasites was a far underdeveloped field compared with other coccidia. In our previous studies, transgenic lines of *E. intestinalis* and *E. magna* expressing reporter genes were constructed. The transgenic line of *E. magna* expressed enhanced yellow fluorescent protein (EYFP) and red fluorescent protein (RFP) targeted to different cellular compartments in the whole life cycle and elicited exogenous antigens-specific immunity in the rabbits' mesenteric lymph nodes. Thus, we aimed to evaluate the specific immunity of transgenic rabbit coccidia expressing heterologous immunodominant antigens. Capsid protein VP60 of rabbit haemorrhage disease virus (RHDV) is a highly immunogenic and ideal vaccine candidate against RHD. We constructed a single-cassette plasmid, *p*H-DEp2aVP60-A containing DHFR-Ts2m3m-EYFP and caspid protein VP60-P2 subunit (codon optimized) inserted between histone 4 promoter and 3' untranslated region of actin of *E. tenella*. Transfected sporozoites were injected into duodenum by surgery and oocysts were collected from

feces of rabbits 7~10 days after infection. The fluorescent oocysts, namely *EmagE-VP60* were propagated and selected by fluorescent activated cell sorting (FACS) combined with addition of 150 mg/kg pyrimethamine in the rabbit pellet. The transgenic rate was continuously increased from 0.07% to 50% after 7 rounds selection. Western blot of soluble antigen confimed the expression of VP60-P2 of the transgenic parasites. Immunofluorescent assay and immunohistochemistry showed that VP60-P2, in sporozoite and merogony stages, was expressed in the parasites' cytoplasm. With the emerging prospect of transgenic coccidia as live vaccine vehicles, evaluation of VP60-specific immunity induced by the transgenic parasites is of great value.

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(21)

MODULATION OF PROTECTIVE IMMUNITY AGAINST COCCIDIOSIS BY A HOST-DERIVED DEFENSE PEPTIDE THAT SHOWS ANTI-PARASITIC ACTIVITY

H. Lillehoj and **W. Kim**, Animal Bioscience and Biotechnology Laboratory, USDA-ARS

Host-pathogen interaction leading to protection against coccidiosis is complex, involving many aspects of innate and adaptive immunity to intracellular parasites. The etiologic agent of avian coccidiosis is *Eimeria*, a genus of eukaryotic obligate intracellular parasites belonging to the phylum Apicomplexa. Clinical manifestations of infection include damage to the intestinal epithelium, decreased nutrient absorption, inefficient feed utilization, and impaired growth rate, which, in severe cases, may lead to mortality. Finding antibiotic alternative solution to control coccidiosis is becoming increasing important globally due to the prevalence of drug resistant coccidia parasites in the field disease outbreaks. Our previous studies showed that intestinal $CD8^+$ cells are involved in sporozoite transport and host protection. Furthermore, selective depletion $CD8^+$ cells led to reduced disease resistance following infection with *E. tenella* or *E. acervulina*. Although the detailed immune mechanisms mediated by $CD8^+$ cells are not known, our recent study indicated that $CD8^+$ cells secrete anti-infective protein, NK lysin which is chicken homologue of human granulysin. We recently designed a cNK-2, a synthetic peptide derived from NK-lysin, and demonstrated its anti-parasitic activity against *Eimeria* spp. Besides anti-parasitic activity, we report in this presentation that chicken cationic peptide cNK-2 also interacts with host cells to modulate innate immune response in poultry through several MAPK signaling pathways. Based on these findings, we believe that cNK-2, an immunomodulatory agent is a good candidate as a novel antibiotic alternative to prevent or treat avian coccidiosis and other infections.

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(22)

DECIPHERING HOST RESPONSES TO *EIMERIA* INFECTIONS FOR IMPLEMENTING INTEGRATED BREEDING STRATEGIES FOR IMPROVED RESISTANCE TO COCCIDIOSIS

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In the context of intensification and specialization of poultry production, animal health issues are of increasing importance to the breeding sector because of huge related production losses caused for ex. by coccidiosis. Because of the multiple factors controlling host responses to *Eimeria* infections, most effective disease control strategies should be developed in an integrated animal health management approach, including prevention, cure, environment control and breeding for disease resistance. Yet, traits like resistance to coccidiosis are scarcely selected for because of a lack of easy measurable and relevant phenotypes and associated genetic markers which could be integrated in running breeding programs.

Therefore, our objectives are the identification of useable phenotypes and, using major advances in animal genomics, genes, gene products and regulatory networks involved in host pathogen interactions with a twofold goal: their use in selection and a better understanding of their functions and the underlying mechanisms. We performed an experimental infection with 2024 commercial broilers and measured phenotypes of animals' response (body weight gain (BWG), temperature, lesions and many blood parameters). Animals were genotyped using a 580K SNP panel, and the genome-wide association study evidenced genomic regions (22 SNPs) highly significantly associated to BWG, plasma colour and plasma β2-globuline concentration. Three SNPs associated with BWG explained 7.5% of the total variance for BWG in challenged birds. In parallel, transcriptomics analysis of liver and spleen was performed. The analysis of biological functions of genes showed the major role played by biological pathways of metabolism and some innate immune parameters. Interactions analysis showed very significant networks centred on tissue repair or cardiovascular functions, i.e. general robustness related functions. The genetic markers identified can be used now in selection programmes. Next step is to investigate how this can be applied to breeding in combination with novel vaccination.

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(23)

NATURAL KILLER CELLS: FRIEND OR FOE IN CHRONIC TOXOPLASMOSIS

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Natural killer (NK) cells are critical for early control of *Toxoplasma gondii* infection via their IFNγ production. Their role in immunity after acute infection has been cleared and during chronic infection with this opportunistic infection has not been explored. Previous studies demonstrate parasite latency and reactivation can be promoted by PD-1 dependent CD8 T cell exhaustion. NK cell exhaustion has been proposed to occur in tumors and chronic HCV infection. Therefore, we investigated whether NK cells become exhausted similar to CD8 T cells in chronic *T. gondii* infection. NK cells are not exhausted following a standard immune exhaustion profile. Their numbers do not decrease during late stage infection and they do not increase expression of PD-1 or LAG3. Unlike acute parasite infection where NK cells predominantly produce IFNγ and have little cytotoxic activity, NK cells in chronic parasite infection have reduced IFNγ and highly elevated cytotoxic activity. NK cells also become more highly mature KLRG1+ during late stage chronic infection and immune exhaustion compared to non-exhausted states. Importantly, we demonstrate after acute parasite infection is cleared in chronically infected animals, NK cell depletion is therapeutic and rescues mice from death caused by CD8 T cell exhaustion and parasite reactivation. This treatment reduced CD8 T cell apoptosis, increased parasite specific polyfunctional CD8 T cell responses and prevented parasite reactivation. We also demonstrate that unlike viral infection models where NK cells, very early during acute infection, negatively regulate adaptive immunity, NK cells positively regulate development of adaptive immunity during acute *T. gondii* infection. Therefore, we hypothesize NK cells, long after acute parasite infection is cleared, persist and negatively regulate late not early infection stage CD8 T cell responses to promote immune exhaustion and chronic Toxoplasmosis. Understanding how NK cells develop this response will improve therapies for individuals at risk for reactivation of chronic infections.

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(24)

TRACKING *EIMERIA* OOCYST NUMBERS AND SPECIES COMPOSITION IN COMMERCIAL BROILER HOUSES

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The purpose of this study was to compare *Eimeria* oocyst concentrations and species composition over time of growout in commercial broiler house litter during anticoccidial drug (ACD) or live *Eimeria* oocyst vaccine (VAC) control programs. Litter samples were collected over a 21 month period from a total of 15 different broiler farms encompassing a total of 45 individual houses during at least one complete growout cycle. Of these 15 broiler farms, 3 were followed for the entire 21 month period spanning 3 ACD and 4 VAC cycles. Samples were collected at 2, 4, and 7-8 weeks of growout corresponding to starter, grower, and withdraw periods of the ACD cycle. On a number of occasions, litter samples were obtained just prior to chick placement to identify possible sources of infection to newly hatched chicks. *Eimeria* oocysts were isolated from all samples, counted by microscopy and extracted for DNA to identify *Eimeria* species by ITS1 PCR using genus- or species-specific oligonucleotide primers. In general, *Eimeria* oocyst concentration in litter reached peak levels at 2-4 weeks of growout regardless of coccidiosis control measure being used. However, peak oocyst numbers were sometimes observed later in growout at 7-8 weeks possibly indicating some level of *Eimeria* drug resistance or incomplete vaccine coverage after hatch. *Eimeria maxima*, *E. acervulina*, *E. praecox*, and *E. tenella* were generally present in all samples, and no difference in the species composition was noted between houses on a particular farm. While *Eimeria* species composition was identical among houses, high *Eimeria* oocyst levels were randomly observed in one house compared to other houses in the same location. Of particular interest was the observed correlation between *E. maxima* oocyst numbers and chick mortality at different points of growout. This study showed that understanding the dynamics of *Eimeria* oocyst levels and species composition in litter during ACD or VAC programs may provide insight into the effectiveness of coccidiosis control measures in commercial broiler production.

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(25)

MOLECULAR CHARACTERIZATION OF FERRET ENTERIC COCCIDIA

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Black-footed ferrets (*Mustela nigripes*) are one of only three ferret species worldwide. Although formerly distributed throughout the North American prairies, black-footed ferrets (BFF) were listed as endangered by the International Union for Conservation of Nature in 1967, and declared extinct in the wild in the 1980s. Since 1986, a multi-institutional effort has been breeding BFFs in captivity with reintroductions into the wild in 27 locations. Three coccidial species, *Eimeria furonis*, *Eimeria ictidea* and *Cystoisospora (=Isospora) laidlawi*, have been described in the domestic ferret (*Mustela putorius furo*), a relative of the BFF. Coccidia morphologically consistent with the two *Eimeria* species have also been described in BFFs. The goal of this research was to use morphometric data and molecular diagnostic techniques to determine whether coccidia isolated from BFF are in fact the same species as those from domestic ferrets. Coccidial DNA was isolated from potassium dichromate preserved fecal samples from BFF bred at the Toronto Zoo, and from domestic ferret samples submitted to multiple veterinary diagnostic laboratories. DNA extraction from archived formalin fixed paraffin embedded (FFPE) intestinal samples (1999 to 2016) from both ferret species was also performed. A single coccidial species, *Eimeria* cf *ictidea*, was identified from BFF samples; only *E. furonis* and *C. laidlawi* were identified in domestic ferret samples. Sequencing of nuclear 18S and mitochondrial COI regions of *E. furonis*, *E.* cf *ictidea* and *C. laidlawi* were performed, and mitochondrial whole genome sequencing of both *E. furonis* and *E.* cf *ictidea* were achieved. Genotyping of the 3 species at nuclear and mitochondrial loci permitted unambiguous identification of these parasites from FFPE samples and fecal oocysts. Future sampling and genotyping of coccidia from BFF and domestic ferrets should permit determination of the host specificity, if any, of these coccidia among these hosts.

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(26)

PROTECTION INDUCED BY VIRUS-LIKE PARTICLES CONTAINING *TOXOPLASMA GONDII* MICRONEME PROTEIN 8 AGAINST HIGHLY VIRULENT RH STRAIN OF *TOXOPLASMA GONDII* INFECTION

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Toxoplasma gondii (*T. gondii*) microneme protein 8 (MIC8) represents a novel, functional distinct invasion factor. In this study, we generated virus-like particles (VLPs) targeting *Toxoplasma gondii* MIC8 for the first time, and investigated the protection against highly virulent RH strain of *T. gondii* in a mouse model. We found that VLP vaccination induced Toxoplasma gondii-specific IgG, IgG1 and IgG2a antibody responses in the sera. Upon challenge infection with RH strain of *T. gondii* tachyzoites, vaccinated mice showed a significant increase of both IgG antibodies in sera and IgA antibodies in feces compared to those before challenge, and a rapid expansion of both germinal center B cell (B220+, GL7+) and T cell (CD4+, CD8+) populations. Importantly, intranasally immunized mice showed higher neutralizing antibodies and displayed no proinflammatory cytokine IFN-γ in the spleen. Mice were completely protected from a lethal challenge infection with the highly virulent *T. gondii* (RH) showing no body weight loss (100% survival). Our study shows the effective protection against *T. gondii* infection provided by VLPs containing microneme protein 8 of *T. gondii*, thus indicating a potential *T. gondii* vaccine candidate.

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(27)

A FIELD CASE OF *EIMERIA MIVATI* IN BROILERS

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E. mivati, one of the nine species of *Eimeria* known to cause coccidiosis in chickens, has been a source of controversy among poultry pathologist. It was first described in 1959 by Edgar and Siebold as a parasite of the upper small intestine. They noted that the parasite moved down the intestine as the infection progressed. With the shift from in feed-antioccoidials to coccidisis vaccination in chickens for control of coccidiosis control, *Eimeria mivati* has to be considered. It has been reported that *Eimeria mivati* is pathogenic to chickens resulting in impared feed utilization, impared growth, and sometimes mortality depending of the level of challenge. A poultry company in the Southeast part of the USA was experimenting loss in performance and some mortality. During necropsy gross lesion appear to be similar to *Eimeria acervulina* but lesions extended to the midgut. Scrapings from the mid gut and observations under the microscope confirm the presence of *Eimeria mivati*. In addition, tissue samples were collected for histopathology. In the past, we did not have to deal with *Eimeria mivati* because anticoccidials were controlling it, but is safe to assume that *Eimeria mivati* resistance to anticoccidials may develop just as it has for other *Eimeria* species.

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FRESHWATER SNAILS EXHIBIT DENSITY-DEPENDENT IMMUNITY POTENTIALLY MEDIATED BY CHEMICAL COMMUNICATION

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Although animal aggregation can benefit individuals, grouping can also induce costs by creating an increased risk of disease transmission. In response, animals in groups can exhibit density-dependent prophylaxis (DDP), in which they invest more energy in their immune systems as the degree of crowding increases. Many invertebrates are required hosts for a variety of parasites, which could lead to selection for DDP. However, few invertebrates have been investigated for DDP, and thus the prevalence, mechanisms and evolution of this phenomenon is unclear. We used a freshwater snail-trematode system to test for evidence of DDP and employed lipid analysis to better understand the role of chemical communication in DDP. We predicted that snails would exhibit DDP because in nature they aggregate in large numbers and can become infected with many parasites including trematode larvae that emerge from first intermediate host snails to infect second intermediate host conspecific or heterospecific snails. To test for DDP, hemocyte counts were conducted on unexposed and trematode-exposed *Lymnaea elodes* snails (n = 528) which were raised at low, medium and high densities. To determine the role of chemical communication, hemocytes were counted from unexposed L. elodes (n = 60) that were individually raised in snail-conditioned water (SCW) or control water and lipid analysis was conducted on each water type. We found that snails raised in medium and high densities produced more hemocytes than those raised in low density and that parasite-exposed snails had more hemocytes than unexposed snails. In addition, snails individually raised in SCW produced more hemocytes than in control water. Several fatty acids including arachidonic acid and eicosapentaenoic acid were only found in SCW. These compounds are biosynthetic precursors of eicosanoids, which are known to play a role in host-parasite interactions. Our study demonstrates that snails exhibit DDP which broadens the extent of this phenomenon among invertebrate taxa, and also suggests that chemical communication underlies DDP.

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(29)

STABLE ISOTOPE ANALYSIS INDICATES HOST-PARASITE ENCOUNTER RATES AND REVEALS UNEXPECTED ROUTES OF PARASITE TRANSMISSION

A. Li, **J.D. Roth** and **J.T. Detwiler**, University of Manitoba

Many models of parasite transmission include an “encounter” parameter. For trophically-transmitted parasites, encounter is influenced by wildlife diet, yet host diet is often unknown or not quantified. Therefore, it can be difficult to relate encounter rates from theoretical models to natural systems. One way to quantify diet is to integrate stable isotope analysis (SIA) with Bayesian mixing models. These models estimate the relative proportion of prey and other food items in host diet. We hypothesized that if encounter rates are related to diet, then diet estimates for prey and food items involved in parasite life cycles will be positively related to parasite infection patterns. We used a muskrat-trematode system to test this hypothesis because muskrats are omnivores and hosts to several trematode species. Muskrats from three wetlands were necropsied for trematode parasites and a stable isotope sample was prepared from the muscle tissue of each individual (n = 67). Stable isotope samples from prey and plants were analyzed from the same sites (n = 63). A diversity of trematodes were recovered including one plant-encysting and three animal-encysting species. The most prevalent trematode was the plant-encysting species, *Quinqueserialis quinqueserialis*, which was also suggested by SIA because plants were the main food item (>50%) at all sites. There was a positive relationship between percent of plant in the diet and number of *Q. quinqueserialis* in individual muskrats from two of the three sites. At the third site, a negative relationship was found between these variables, and there was a significantly positive relationship between *Q. quinqueserialis* infection intensity and the proportion of snails in the diet. In laboratory, we confirmed that these parasites encysted on snail shells, thus suggesting a new route of transmission for plant-encysting parasites. This study shows that combining SIA and parasite infection data could help calibrate parasite transmission models and reveal unexpected routes of transmission.

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MYSTERIOUS SNAIL HOSTS! DISTRIBUTION AND HOST USE OF ACANTHOCEPHALANS IN TWO SPECIES OF FRESHWATER SNAILS

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In many acanthocephalan life cycles, a vertebrate paratenic host is used to bridge the ecological gap between the intermediate and definitive hosts. However, there have been few reports of freshwater snails serving as paratenic hosts for acanthocephalans. To assess how commonly freshwater snails serve as hosts for acanthocephalans, two species of freshwater snails, *Helisoma trivolvis* and *Physa acuta*, were collected from various wetlands throughout Payne Co., Oklahoma. Additionally, snails were sampled on a monthly basis for a year from a single location to further investigate seasonal variation of infection. Snails were dissected for juvenile acanthocephalans by examining the entire body and then flattening snail tissue between two slides. Acanthocephalans were identified to *Neoechinorhynchus* spp., which most likely infect turtle definitive hosts and ostracod intermediate hosts in nature. Among all sites sampled, 7 of 27 (26%) contained snails infected with acanthocephalans, with *H. trivolvis* being more commonly infected than *P. acuta*. Depending on the site, prevalence and mean intensity ranged from 4–79% and 1–3.6, respectively. Throughout the year, prevalence and mean intensity peaked at 73% during the summer and decreased to 0% during the winter. Among all acanthocephalans recovered from snails, 88% were found encysted in the head foot of snails; whereas 12% were attached with their proboscis to the mantle collar underneath the shell. Lastly, acanthocephalans were twice as large in snails as reported from ostracods. These results suggest that 1) freshwater snails may be important hosts for the transmission of *Neoechinorhynchus* spp.; 2) location and season have a strong effect on the variation of acanthocephalan infections in snails; 3) acanthocephalans are using two different microhabitats within snail hosts; and 4) acanthocephalans appear to be growing and developing within snail hosts, which has important implications on establishment in the definitive host.

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A GENOME-WIDE TRANSCRIPTOMIC ANALYSIS OF THE REPRODUCTIVE CONSEQUENCES AND DIVERSE IMMUNOLOGICAL RESPONSES IN FIELD-DERIVED EARLY AND PATENT *SCHISTOSOMA MANSONI* INFECTIONS OF *BIOMPHALARIA PFEIFFERI*

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G.M. Mkoji, Kenya Medical Research Institute
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Biomphalaria pfeifferi exhibits a high degree of compatibility with *Schistosoma mansoni* and likely transmits more cases of this parasite to people than any other snail species. Ironically, we know relatively little at the molecular level regarding the interactions of *B. pfeifferi* and *S. mansoni* from early-stage sporocyst transformation to the development of cercariae. To redress this shortcoming, using field-derived west Kenyan representative of schistosomes and snails, we have undertaken dual RNAseq of three intramolluscan developmental stages (1- and 3-days post exposure and patent, cercariae-producing infections). A high-quality, well-annotated *de novo B. pfeifferi* transcriptome was assembled from over a half billion non-*S. mansoni* paired-end reads. Transcripts involved in pathogen recognition and binding (hemocytin, C1q, fibrinogen-related, and c-type lectins) were abundantly up-regulated at all time points. Reactive oxygen species are seen throughout infection with Cu-Zn SODs up-regulated during early infection (1- and 3-dpe) and thioredoxins up-regulated in patent snails. Several transcripts that promote snail reproduction were up-regulated at 1- and 3-dpe suggesting that host modifications occur early in

infection. Although we see an increase in transcripts inhibitory to reproduction in patent snails, there is also an increase in yolk ferritin, serotonin, and dopamine indicating that these snails may be retaining some reproductive function and are not permanently or irrevocably castrated. We have also characterized core snail transcripts required for basic biological functions that are present in all groups, like soma ferritins for mineral absorption and the oxygen-transporting transcript hemoglobin. Individual snails within each group exhibited unique transcript activity, suggesting that *B. pfeifferi* response to *S. mansoni* infection is not homogeneous. These field-derived snails harbored notable symbionts including several microsporidian species, a yeast with antimicrobial properties, and a known molluscan-infecting protist. Our results provide unique insights into schistosome-snail interactions taking place in a natural transmission focus, potentially including candidate molecules amenable to manipulation to facilitate new control approaches targeting the ability of larval schistosomes to succeed in their snail hosts.

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COCCIDIAL METAGENOMICS: HIGH THROUGHPUT DNA SEQUENCING OF *EIMERIA* SPECIES TO EVALUATE THE IMPACT AND CONTROL OF COCCIDIOSIS ON BROILER OPERATIONS

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Coccidiosis is an intestinal disease costing the global poultry industry US$700 million annually to control. Several species of the genus *Eimeria* cause the disease. Understanding the *Eimeria* infection patterns within broiler flocks managed using current control strategies (i.e. anticoccidial medication or live coccidiosis vaccination) will become critical to the Canadian market as it embraces Raised Without Antibiotic (RWA) production. Quantifying oocysts in fresh fecal material (i.e. oocysts per gram, OPG) is a reliable method for determining the current infection level. Identification of *Eimeria* species relies on *in vivo* challenge in naïve birds or molecular typing using PCR, both of which have limitations. Commercial broiler farms in southern Ontario were sampled during summer 2016 and winter 2017. Half of the flocks sampled used preventive anticoccidial medication programs and the other half used live coccidiosis vaccination. Samples consisted of pooled, fresh fecal droppings collected throughout the chicken house using a standardized methodology. Sampling of each flock was conducted on day 7, 14, 21, 28 and 35 following chick placement. OPG was calculated for all samples and oocysts from positive samples were purified for later molecular identification. Chicken performance data regarding growth, feed consumption and mortality were collected. Oocysts were identified by extracting DNA and then using the DNA as template in a 2-step, nested PCR targeting the mtCOI locus. The primary amplicon using genus-specific primers was gel-extracted for later species-specific PCR and next-generation sequencing (NGS). This is the first comparison of two coccidiosis control strategies following oocyst shedding on a weekly basis. Vaccinated flocks had peak OPG of 25,000 to 218,000. Three medicated flocks were found with peak OPG counts above 745,000; such high fecal OPG counts provide compelling evidence of anticoccidial resistance. The observation of *Eimeria* species in nearly all flocks highlights the challenges commercial producers face as they begin RWA broiler production.

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EXTREME QTL AND POOLED SEQUENCING FOR DETERMINING THE GENETIC BASIS OF HOST SPECIFICITY IN SCHISTOSOME PARASITES

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Interactions between parasitic trematodes and their aquatic snail hosts provide a classical example of gene-for-gene co-evolution. Trematode infections typically sterilize snails, leading to selection of costly defense mechanisms, while parasites evolve to circumvent these defenses. We used a genetic approach to identify the parasite genes involved in overcoming snail defenses in the *Biomphalaria glabrata* (snail) - *Schistosoma mansoni* (parasite) system. We performed genetic crosses between two schistosome populations (SmBRE and SmLE) with distinctive patterns of host specificity: while both parasite populations infect BgBRE snails, only one (SmLE) can infect a second snail population (BgBS90). The F1 parasite progeny from our crosses were unable to infect BgBS90, while ability to infect BgBS90 snails was recovered in some F2 progeny. To identify the genome regions involved in snail specificity we used the extreme QTL (X-QTL) approach, developed by yeast and malaria researchers. In this method, pooled F2 progeny are selected for the trait of interest (i.e. ability to infect different snail populations), and then pools of selected or unselected progeny are quantitatively genotyped to measure allele frequencies genome-wide. We compared exome sequences of F2 parasite pools before and after infection of the two snail lines. Two genome regions (on chromosome 2 and 3) showed dramatic allele frequency distortion in parasites infecting BgBS90, and clearly underlie host specificity. We will now identify the specific genes involved in host specificity using RNAi approaches. Our long-term aim is to identify interacting genes in both parasite and snail to understand host-parasite evolution at the molecular level.

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(34)

GENETIC ANALYSIS OF TRANSMISSION STAGE PRODUCTION IN SCHISTOSOME PARASITES

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Parasite traits associated with transmission success, such as the number of infective stages released from the host, are expected to be optimized by natural selection. However, in the trematode parasite *Schistosoma mansoni*, a key transmission trait – the number of cercariae larvae shed from infected *Biomphalaria spp*. snails – varies within and between different parasite populations and selection experiments demonstrate that this variation has a strong genetic basis. We used genetic crosses to determine the genetic architecture of this critical transmission related life-history trait. A *S. mansoni* isolate from Brazil (SmBRE) sheds very low numbers of cercariae, and causes minimal mortality to snails, while another new world parasite (SmLE) sheds 8 fold more cercariae (mean (±se) cercariae per shedding: 284±19 vs 2352±113) and causes high mortality to snails. We conducted two independent three generation genetic crosses between these two parasite lines (SmBRE and SmLE), and determined shedding profiles of parent parasites, F1 and F2 progeny from inbred *B. glabrata* snails. We sequenced the ~15 Mb exomes from parents, F1 progenitors and 188 F2 progeny for each crosses, revealing 9,140 and 9,465 SNPs fixed for alternative alleles in the two crosses, and conducted a classical QTL (*i.e.* Quantitative Trait Locus) analysis. The QTL analysis revealed potential QTLs on chromosome 1. We are now in position to identify candidate gene(s) involved in a key life-history trait that is critical for transmission in an important human pathogen.

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(35)

EPIDEMIOLOGY OF HELMINTHS AND INTESTINAL PROTOZOA IN THE CENTRAL OF CôTE D'IVOIRE

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Intestinal parasitic infections due to protozoa and helminths are a major public health problem in developing countries. Epidemiological data of these parasites are relevant for the development of an effective preventive and integrated control strategy. From August to September 2014, a cross-sectional epidemiological survey was conducted in communities of three (03) sub-districts in central Côte d'Ivoire composed of 56 villages. Stool samples and urine were examined respectively with three parasitological methods: Kato-Katz, formalin-ether concentration for stool and urine filtration method for urine. A questionnaire was administered to households to collect data on sanitation and hygiene practices. Hookworm was the helminth mostly observed in these three sub-districts of central Côte d'Ivoire. The prevalence of hookworm was respectively 35.3%, 34.2% and 10.9% in Djékanou, Kpouèbo and Taabo. The other species of helminths were found with prevalence lower than 10%. *Entamoeb*a *histolytica/dispar* and *Giardia lamblia* were similarly prevalent in the three sub-districts. Boys aged 5-15 years were statistically the most infected with hookworms. Logistic regression analysis showed that hookworm infection was positively correlated with open defecation (OR = 1.27; p = 0.01). Garbage deposit near the household is positively associated with *G. lamblia* (OR = 1.32; p = 0.007). Data from this study will serve as a baseline to monitor the effect of integrated interventions on re-infection by helminths and intestinal protozoa.

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(36)

HELMINTH COINFECTION AND LANDSCAPE PATTERNS IN A SUBURBAN UNGULATE POPULATION

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Fascioloides magna (giant liver fluke) and *Taenia hydatigena* (thin-necked bladderworm) are common helminth parasites of white-tailed deer (*Odocoileus virginianus*) in North America. The known effect of infection by each of these parasites on host fitness is variable, yet the impacts and drivers of coinfection in deer are unknown. Landscape patterns influence the transmission dynamics of these helminths and may help predict infection risk. In Fall of 2014 - 2016, 165 deer livers were collected in the city of Duluth, Minnesota and examined for helminth infections. Prevalence of giant liver fluke was 44% and prevalence of thin-necked bladderworm was 19%. The probability of infection was positively related to liver mass for both species. This was likely due to host age as both liver mass and infection probability increase with age. Coinfection with both parasites was also common (13%) and a statistically significant association between infections was found. This association may have been related to abiotic factors, deer behavior, and/or immunology. Fluke prevalence was correlated with the proportion of wet cover types in deer hunting areas, which was likely related to intermediate host habitat and deer foraging behavior. Bladderworm

infection metrics were not strongly correlated with any landscape variable because this parasite has two highly mobile hosts.

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PREVALENCE OF ZOONOTIC GASTROINTESTINAL PARASITES OF SHELTER DOGS AND CATS IN THE TRISTATE CUMBERLAND GAP AREA OF KENTUCKY, TENNESSEE AND VIRGINIA WITHIN THE APPALACHIAN REGION

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Healthy dogs and cats re-homed from shelters are screened for behavioral suitability, vaccinated, and spayed or neutered. However, due to limited resources, not all are screened for parasitic infections or receive anthelminthic treatment prior to adoption. This poses a significant health risk to anyone in contact with these animals, especially new owners who may not be informed about potential zoonotic parasite transmission to their families. The purpose of this study was to determine the prevalence of zoonotic parasites in shelter animals available for adoption. Fecal samples from the kennel floor of 68 dogs and the litterboxes of 30 cats (98 total) were collected from August through October 2016. Animal health was assessed using the 1-9 Purina Body Condition Score system (BCS), age was estimated by dental examination, and animal relinquishment noted as either stray or owner surrendered. Fecal samples were analyzed on day of collection using centrifugation flotation in Fecasol (sodium nitrate) solution. Parasite prevalence in dogs was 86.7% and in cats was 46.7%, with an overall prevalence of 74.5 % in both. In dogs, infections with the zoonotic parasite, *Ancylostoma caninum* was the most prevalent at 73.5% and 13.2% were positive for *Toxocara canis*. In cats, infections with *Toxocara cati* were the most prevalent at 23.3%. Kittens (< 6 months) were more likely to be positive (p=0.02), however there was no association seen in puppies (<6 months). For all animals assessed, there were no statistical associations between positivity and BCS or relinquishment. Although previous studies reported high prevalence of parasitic infections from shelter animals, the results of this study specifically show that 74.5% of healthy adoptable animals (based on BCS) harbor zoonotic parasites. The One Health concept of beneficial human-animal bond is compromised if pet ownership results in risk to human health. Therefore, the results of this ongoing study show the need for community-aimed recommendations to seek veterinary care of re-homed animals.

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(38)

MOLECULAR CHARACTERIZATION AND UNUSUAL DEVELOPMENT OF A *SARCOCYSTIS* SPECIES FROM A COOPER'S HAWK (*ACCIPITER COOPERII*) IN CELL CULTURES

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Few studies have been reported examining the asexual development of *Sarcocystis* in cell cultures. Most of these studies have demonstrated that sporozoites enter host cells and develop by schizogony (endopolyogeny) producing merozoites that egress and enter new host cells and repeat schizogony. This asexual development leads to destruction of the monolayer and lesions (areas devoid of intact host cells).

The lesions are produced when the infected host cell ruptures due to multiplication of schizonts producing merozoites. The present report describes the unusual development of schizonts of a *Sarcocystis* species isolated from the intestines of a Cooper's hawk, *Accipiter cooperii*. The bird was submitted to the Carolina Raptor Center, Huntersville, NC, exhibiting neurologic signs (seizures, tremors) and was euthanized because of poor prognosis 2 days later. Sporozoites from excysted sporocysts were inoculated onto monkey kidney (CV-1) cells and these stages were used to establish a continuous culture of the parasite. The isolate was characterized by PCR amplification of merozoite DNA and sequencing at 3 regions of nuclear ribosomal DNA units; *18SrRNA*, *28S rRNA*, and *ITS-1*, and the mitochondrial cytochrome c oxidase subunit 1 locus. Analysis of *ITS-1* sequences confirmed its membership in the genus *Sarcocystis* and indicated an especially close relationship to other parasites in this genus that employ birds as their hosts. Molecular characterization and development observed *in vitro* for this isolate were different from an isolate reported previously as *Sarcocystis* sp. ex *Accipiter cooperii*. Merozoites were seen 12 days post inoculation (PI) and extracellular merozoites were motile. As developmental time in culture continued an unusual developmental process was observed. The schizonts were sausage shaped (longer than wide and usually slightly curved) and the schizonts remained in what appeared to be the original host cell as they underwent development. This created large areas of accumulations of schizonts (AcSch) in areas adjacent to uninfected host cells. Stages that were the size of schizonts were seen in the media 30 days along with floating remnants of host cells (floaters). Examination of cell culture media using differential contrast microscopy and Giemsa stained preparations demonstrated that there was a mixture of extracellular merozoites, extracellular schizonts (ESch) and floaters. When the cell culture media was removed and replaced with fresh media and examined the following day this media would contain extracellular merozoites, ESch and floaters. As the length of time in culture increased the size of AcSch increased with little or no visible damage to the adjacent uninfected CV-1 cells. We have observed merozoites, ESch, and floaters in media for over 60 days. We have not observed active egress of schizonts, cell penetration by ESch or motility of ESch. We do not know if the ESch are viable and can produce merozoites. It is possible that they are nonviable and have been excreted somehow by the host cell. The presence of floaters in the media may be the result of ESch release. We are attempting to determine how these schizonts are released or egress from host cells. Supported by NSF grant # 1505407 to ARVD and an IRC grant to DSL.

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(39)

PREVALENCE OF POTENTIALLY ZOONOTIC AND NON-ZOONOTIC PARASITES IN DOMESTIC DOGS IN RURAL, URBAN AND FIRST NATIONS COMMUNITIES ACROSS ONTARIO, CANADA

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A wide range of parasitic infections are prevalent in Canadian domestic dogs. However, no study to date has examined the prevalence of potentially zoonotic parasites in dogs in varied communities across Ontario. Access to permanent veterinary services is limited in some areas of Ontario and veterinary clinics are not usually found on First Nations reserves. Human exposure to zoonotic parasites may be elevated where free-roaming dogs have access to contaminated water and infected raw meat through scavenging. Wild or domestic dogs may serve as bridges for such zoonoses. There is also concern regarding transmission of parasitic infections from infected to healthy dogs within these communities. Our goal was to determine the prevalence of potentially zoonotic and non-zoonotic parasitic infections in domestic dogs that may impact the health of humans and domestic dogs in these Ontario communities. Fecal samples (n=127) from dogs in rural, urban or First Nations communities were collected to determine the prevalence of parasites identified in each community; parasites with potential zoonotic risk to humans were of particular interest. Samples were processed using the Cornell-Wisconsin centrifugal flotation method employing sucrose flotation medium (SG of 1.27-1.33). Preliminary results showed that the overall prevalence (at least one parasite identified) of intestinal parasites in the canine fecal samples was 20.5% (n=26); only 2 dogs were infected with more than one parasite. Protistan parasites detected included *Giardia* sp. and *Cystoisospora* sp. and the helminths detected included hookworms (*Ancylostoma*/*Uncinaria* sp.), ascarids (*Toxocara*/*Toxascaris* sp.) and whipworms (*Trichuris* sp.). Of the

28 parasite infections identified, 18% (5/28) were potentially zoonotic and 82% (23/28) were non-zoonotic. This study serves as a preliminary assessment of the prevalence of dog parasites in select Ontario communities; potentially zoonotic parasitic infections were found that may warrant more active surveillance and, potentially, more active control.

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SURVEY OF *EIMERIA* SPECIES IN ONTARIO WILD TURKEYS (*MELEAGRIS GALLOPAVO SILVESTRIS*)

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The native eastern wild turkey (*Meleagris gallopavo silvestris*) population in Ontario has grown significantly since reintroduction of this extirpated species in 1984. By 2007, the estimated population grew to more than 70 000 birds providing increased hunting opportunities and diversification of Ontario's natural ecosystem. Rapidly growing wild turkey populations have the potential to be effective natural reservoirs for, or be affected by, diseases of domestic turkeys. Domestic and wild turkeys belong to the same species and therefore are susceptible to coccidiosis caused by the same *Eimeria* species. Ecological differences, such as population density, habitat and nutrition, may affect the prevalence of these parasites and intensity of infections. In 2015, as part of a larger study on surveying disease pathogens present in Ontario wild turkeys, 109 adult wild turkey carcasses were obtained during the 2015 spring hunting season (April 25th to May 31st). Intestinal contents from proximal duodenum to cloaca were collected to determine the prevalence of *Eimeria* species. Detection and enumeration of *Eimeria* oocysts were performed using the McMaster counting method with saturated sodium chloride floatation medium to determine the total number of oocysts per gram (OPG). From the wild birds sampled, 84/126 (77.1%) tested positive for *Eimeria* oocysts (<175 to >5000 OPG); morphological speciation was not attempted because of the overlap in morphometrics among *Eimeria* spp. in turkeys. Samples with higher OPG counts had DNA extracted from oocysts purified by centrifugal salt flotation followed by surface bleaching. A nested PCR method targeting the mtCOI locus with genus-specific followed by species-specific primers was used to identify *Eimeria* species in each sample. Samples were found to contain one or more of the following *Eimeria* species: *E. dispersa*, *E. meleagridis*, *E. adenoeides* and *E. innocua*. More than half of the samples contained multiple *Eimeria* species. High prevalence and considerable diversity of *Eimeria* species was detected in these adult Ontario wild turkeys.

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RISK FACTORS FOR *TOXOPLASMA GONDII* INFECTION IN ROMANIAN PREGNANT WOMEN

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Serologic screening and exposure factors for *Toxoplasma gondii* infection were determined among 208 consecutive pregnant women residing in Western Romania. The seroprevalence of *Toxoplasma gondii* was assessed by demonstration of serum *T. gondii* IgG and IgM antibodies with Vitros anti-*Toxoplasma* IgG and IgM assays, designed for the Vitros ECiQ immunodiagnostic system (Ortho-Clinical Diagnostics, NJ). A short questionnaire interview was carried out to obtain information regarding the risk factors associated with *T. gondii* infection, including age, residential area, history of eating raw or undercooked meat or unwashed fruits/ vegetables, contact with soil and keeping pets (cats and dogs). Additional demographic data were acquired regarding their gestational age, number of previous spontaneous

abortions, occupation and educational level. *T. gondii* IgG and/ or IgM antibodies were demonstrated in 55.8% (116/ 208) of pregnant women and their presence increased with age from 51.8% (12-20 years), to 52.8% (21-30 years) and to 64.3% (31-41 years). In general, no significant difference in the seroprevalence of *T. gondii* infection was found between the pregnant women with and without exposure to the risk factors studied. However, *T. gondii* seroprevalence decreased with increasing level of education from 100% in those with elementary school only, to 58.8% in those with middle school and 49.6% in those with high school or College, respectively. Occupation appeared to be a risk factor: housewives and women working with meat (in restaurants, food stores) had a higher *T. gondii* seroprevalence (71.1%) compared to those with different jobs (53.8%) ($p=0.046$). Women with ≥4 live births had a higher risk for *T. gondii* infection compared to those without any previous births ($p<0.002$). Of women with demonstrated *T. gondii* antibodies, 24.7% (24/ 97) had spontaneous abortions compared to 12.3% (10/ 81) of those in whom specific antibodies were not detected ($p=0.036$). Our results indicate a high prevalence of *T. gondii* antibodies in Romanian pregnant women. Risk factors for *T. gondii* past infection were being in the older age group, working with meat, having a low level of education, higher gravidity and histories of spontaneous abortions.

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FIELD COLLECTION OF QUESTING AND HOSTED IXODID TICKS FROM THE CUMBERLAND GAP REGION OF TENNESSEE, VIRGINIA, AND KENTUCKY

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Adult and nymphal ixodid ticks (n=82) were collected from field sites and animal hosts in the Cumberland Gap region where the states of Tennessee, Virginia, and Kentucky are contiguous, and 2 animal shelters in adjacent Kentucky counties. Three genera of ticks were identified in order of decreasing frequency, *Dermacentor varriabillis* (55%), *Ixodes* sp. (32%), and *Amblyomma americanum* (13%). Questing ticks were collected with a flannel drag on 46 sampling occasions between May and July 2016. Ticks acquired from this sampling effort were exclusively *D. varriabillis*, predominately the adult stage (88%), and male (63%). Higher tick recovery rates were associated with sampling shaded trails compared to open pastures. *Ixodes* sp. (86%) was the predominate tick associated with animal hosts throughout the fall and winter months. The occurrence of *D. varriabillis* with shelter acquired dogs between May and July is consistent with our observation of peak questing activity evidenced by the flannel drag sampling effort. All tick species recovered from the animal hosts appeared to demonstrate substantial residence time and 86% of female *Ixodes* sp. ticks were engorged or showed evidence of feeding. Prolonged feeding time is a well-established risk factor associated with increased transmission efficiency of tick borne infectious diseases. These results are interesting and warrant further investigation in light of anecdotal reports of Lyme borreliosis based on antibody detection in whole blood samples from pet dogs residing in local communities within the region.

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TRICHINELLOSIS IN HOSPITALIZED PATIENTS IN WESTERN ROMANIA: A 4 YEARS RETROSPECTIVE STUDY

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Trichinellosis is a zoonosis caused by *Trichinella* species, a nematode that infects a broad range of domestic and wild animals. Human trichinellosis occurs through consumption of raw or undercooked meat infected with *Trichinella* larvae and the symptoms are related to the number of larvae ingested. The present study assesses the prevalence and risk factors of human trichinellosis in Western Romania over a period of 4 years.In this retrospective study were included 65 consecutive patients diagnosed and hospitalized with trichinellosis in three counties from western Romania (Timis, Arad, Hunedoara) between January 1st 2012 and December 31st 2016. Epidemiological, clinical, laboratory and therapeutic data were collected from these patients' medical records. Patients were aged between 2-68 years (mean age = 36.15). Twenty nine (44.6%) patients were females and 42 (64.4%) came from rural area. Pork meat was the source of infection in 61 (93.8%) patients and wild boar meat in 4 (6.2%). Myalgia (81.3%), fever (56.9%), eyelid edema (52.3%) and asthenia (47.7%) were the most frequent symptoms. Thirty-four (52.3%) patients had high levels of white blood cells and 61 (93.8%) eosinophilia. In 17 (26.1%) of the patients, the hospitalization period ranged between 10 and 17 days. Albendazole was used in 59 (90.8%) cases. Trichinellosis is still an important public health problem in Western Romania. Implementation of sanitary education programmes, for both swine breeders and consumers, and strict hygienic measures are imperative.

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(44)

ENTERIC COCCIDIOSIS IN THE BLACK-FOOTED FERRET (*MUSTELA NIGRIPES*)

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The black-footed ferret (BFF) (*Mustela nigripes*), the only North American ferret species, was declared extinct in the wild in 1987. Since 1986, a multi-institutional consortium has been breeding BFFs in captivity with reintroductions into their former geographic range. Coccidial enteritis is a major cause of death in young, captive BFFs but can affect all age classes. This disease can reduce the number of captive-bred ferrets available for release to the wild; consequently, the prevention and control of coccidial outbreaks is an important part of the BFF recovery program. Despite reports of coccidial enteritis in BFF since the 1970s, the disease has been poorly characterized in this species, with emphasis placed on disease treatment and prophylaxis. Furthermore, the endangered status of the BFF has limited the ability to conduct in vivo studies of the disease. The goal of this research was to improve the health of captive and wild BFF through better recognition of the etiologic agents and understanding of the natural history of enteric coccidiosis in this species, and through development of a model for experimental investigations of the disease. Data on morbidity, mortality, clinical signs and shedding of coccidial oocysts from ferrets at the Toronto Zoo were collected from 2014-2016. Coccidia isolated from BFF fecal samples and identified in a retrospective examination of BFF necropsy tissue were characterized using morphometric data and/or molecular diagnostics, and compared to novel and published data from domestic ferrets (*Mustela putorius furo*). Only one coccidial species, *Eimeria* cf *ictidea*, was identified from enteritis and mortality events in captive BFF from 1999-2016; mitochondrial whole genome sequencing of this pathogen was achieved. A pilot study performed using the domestic ferret showed that patent infection can be induced with *E. cf ictidea* originating from BFF, opening the way for future investigations into the control and treatment of enteric coccidiosis in a mustelid model.

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(45)

CHASING *ASCARIS* AGGREGATION: FROM FIELD TO LABORATORY

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Ascaris lumbricoides is a remarkably infectious and persistent parasite that infects in the order of 800 million people. Among the 17 neglected tropical diseases (NTDs), fundamental aspects of *Ascaris* biology and epidemiology remain unknown. The associated chronic morbidity, including growth retardation and effects on cognitive development, is linked to intensity of infection. However not all hosts are infected equally, a phenomenon known as aggregation. Aggregation has been described as a universal law in parasite ecology, but the mechanisms that contribute to such a pattern have proved elusive. Part of the explanation for this lies in the dynamic nature of this epidemiological pattern coupled, with the difficulty in exploring complex contributors in host-parasite systems. I will describe how our field-based studies on *Ascaris* aggregation and predisposition among Nigerian children have progressed to the development of a mouse model of susceptibility and resistance, with a particular focus on the role of the liver during larval migration. Recent preliminary work on the proteomics of the murine liver revealed a higher abundance of mitochondrial proteins, particularly those associated with the oxidative phosphorylation pathway and reactive oxygen species (ROS) production in the relatively resistant mice. However, our understanding of the role of the liver in *Ascaris* infections, particularly at the molecular level, remains deficient. Furthermore, from a wider perspective, large-scale deworming programmes are being rolled out worldwide and there is an urgent need to understand how such control may alter patterns and processes in human geohelminth infection. In addition, the growing perception that infection with *Ascaris* and other geohelminths have an effect on the host immune response, with consequences for concurrent infectious diseases, such as malaria, greatly enhance the public heath significance of such parasites. To conclude, *Ascaris* as a case study of ascarids of human importance can highlight a range of fascinating and enigmatic aspects of the host-parasite relationship including the phenomenon of aggregation and its consequences. It is hoped that such insights will encourage further investigation and greater investment in what undoubtedly remains a classic neglected disease.

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PARASITES, PATTERNS AND POLICY: PARASITE ECOLOGY FOR MARINE FISHERIES MANAGEMENT

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Marine fisheries management is a delicate balance between exploitation and conservation in a dynamic ecosystem. Management policy is supported by the best available science. One example of how parasite ecology contributes information to marine fisheries management is the use of parasites as biological tags for commercially important marine fishes. Although ecosystem management is an ultimate goal, current management rests primarily at the level of fish stocks. Parasites have helped define stocks of marine fishes and their distributions since the first study almost 80 years ago. The usefulness of this tool to fishery managers depends upon the knowledge of the ecology of individual parasites, or communities, chosen as markers and specific characteristics of their life histories. Another example of how parasite ecology can better inform management is through an improved understanding of marine food webs and habitats. Biological patterns and processes in the Northeast Pacific Ocean are greatly influenced by high spatial and temporal dynamics. These dynamics, including a recent increased variability in the physics and biology of the Northeast Pacific, provide challenging opportunities for both scientists and fisheries managers. For marine parasite ecology, the effects of climate change on this, and other, marine ecosystems provides unique opportunities and deserves greater attention in the fields of both theoretical and applied ecology.

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SCALING SYNCHRONY IN SUNFISH SYMBIONTS

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Persistence of populations is fundamentally linked to all aspects of community ecology, and is of particular importance in the evaluation of disease dynamics. Asynchronous dynamics within a metapopulation can increase the probability of persistence by facilitating “rescue effects”, where local extinction events are rescued by dispersal of individuals from more abundant demes. Because the factors that influence population growth tend to be spatially correlated, one would expect the degree of synchrony among populations or demes to decline with distance, as distant individuals experience different stochastic influences (the Moran effect). The appropriate scale for examining declining synchrony will depend on the dispersal capabilities of the organisms themselves, as individuals will essentially experience the average environment within their home range. Parasitic organisms tend to be dependent on their hosts for dispersal, and so one would expect the appropriate scale at which asynchrony can be detected to be much larger for parasites inhabiting vagile hosts. Young-of-the-year sunfish (Centrarchidae) were collected monthly from 4 localities on Lake Strom Thurmond, separated by an average linear distance of 5.75 km, with the goal of determining whether asynchrony could be detected among the component populations of their parasites, and whether the scale at which asynchrony (if present) occurred varied with host use. The specific hypothesis being tested was that asynchrony among component populations of autogenic parasite species should be more likely to occur, and occur at a smaller scale than for allogenic parasite species.

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TAXONOMIC MAIDS AT YOUR SERVICE: CLEANING UP *PSEUDOPECOELUS* (DIGENEA: OPECOELIDAE), A GENUS GROUPING SPECIES WITH SUSPICIOUSLY-WIDE HOST DIVERSITY

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Pseudopecoelus is a large genus comprised of 39 species parasitizing marine fish from shallow to deep waters. Its members possess a typical opecoeline combination of characters including an unspecialized and sessile, protuberant or pedunculate ventral sucker, blindly-ending caecae, a cirrus pouch that is either small or absent, and a genital pore located on the sinistral side of the forebody. This genus also has remarkably low host specificity, infecting at least 17 orders of fish in general and 20 families of deep-sea fish in particular. A morphological study of the digeneans parasitizing deep-sea fish collected in the 1960’s and 1970’s in the eastern Pacific Ocean west of Oregon and Vancouver Island revealed a new species of *Pseudopecoelus* within the bigfin eelpout, *Lycodes cortezianus* (Perciformes: Zoarcidae); however, we came to realize that a new morpho-taxonomic scheme was sorely needed to identify member species of this genus and an updated key was produced. We also began to question the true validity of *Pseudopecoelus*. Given the large number of fish hosts, their broad phylogenetic diversity, and the varied (or even unknown!) diets of the fish reported as hosts for species of *Pseudopecoelus*, it is evident that species assigned to this genus span hosts with a very wide array of life histories. This presentation will present our new combination of species level diagnostic characteristics as well as observations of parasite and host diversity within *Pseudopecoelus* – touching upon the strikingly wide range in numbers of host species infected (i.e. some *Pseudopecoelus* species infect many fish [12–15 spp.] while others have much narrower [1 sp.] ranges). A brief discussion of species allocated to this genus in the deep sea will also be offered. Whether this variability is either an artifact of sampling or might reflect unrecognized taxonomic distinctions within *Pseudopecoelus* is hard to confirm at this point; species herein have been studied thus

far only morphometrically. As of February 2017, NCBI/GenBank contains no DNA sequence information for any species of *Pseudopecoelus*. In order to ascertain the true taxonomic legitimacy of *Pseudopecoelus*, we feel that this genus would be an ideal candidate for future studies combining molecular, morphological and life history data.

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AN UNUSUALLY HIGH NUMBER OF NEW SPECIES OF *ANTHOCEPHALUM* (RHINEBOTHRIIDEA: ANTHOCEPHALIDAE) PARASITIZING A SINGLE SPECIES OF INDO-PACIFIC STINGRAY HOST

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Over the past decade, the elasmobranch-hosted tapeworm genus *Anthocephalum* has received increased taxonomic and systematic attention. Since 2009, 11 new species of *Anthocephalum* have been described from ten batoid host species, and two additional species have been transferred to the genus. As a result, the number of valid species of *Anthocephalum* is now 18. While species in this genus are known from a number of genera in the batoid families Dasyatidae, Urotrygonidae, and Torpedinidae, congeners of *Anthocephalum* have been reported from only three host species: two *Anthocephalum* species each parasitize the dasyatids *Hypanus americanus*, *H. longus*, *and Himantura leoparda* (one of these six *Anthocephalum* species still awaits formal description). This study represents the first examination of the dasyatid *Urogymnus granulatus* for species of *Anthocephalum*. Six new species were discovered. They could be differentiated from one another and from the 18 valid species in the genus using a combination of standard morphological methods, including light and scanning electron microscopy and histological sectioning. Morphological species boundaries for all six species were confirmed using molecular sequence data. Moreover, the sequenced replicates of one of these six species were each recognized as belonging to one of two molecularly distinct sister clades; however, the members of these respective clades are as of yet morphologically indistinguishable from one another. The collective morphology of all new species of *Anthocephalum* from *U. granulatus* suggests that characters such as the anterior extent of the uterus, vitteline follicle distribution, and scolex microthrix pattern may be more diagnostic than previously understood. *Urogymnus granulatus* is now recognized as host to the single greatest number of species of *Anthocephlaum* of any batoid examined to date. Interestingly, these congeners may not necessarily form a monophyletic group.

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IS THE AVIAN MALARIA GENUS *LEUCOCYTOZOON* A GLOBAL PARASITE RADIATION? INSIGHTS FROM MOLECULES, MORPHOLOGY, AND ECOLOGY

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The avian malaria parasite genus *Leucocytozoon* was described in 1904 and within two decades the majority of currently recognized species had been described based on morphology and host associations. Unfortunately, our understanding of species diversity within this globally abundant malaria genus has progressed little in the intervening century. There are currently 33 described species of *Leucocytozoon*, many of which are considered to have wide geographic distributions and generalist host use strategies. However, recent surveys of avian haemosporidian parasites using DNA barcoding techniques have suggested that *Leucocytozoon* may be more speciose than previously recognized, which raises the question: is *Leucocytozoon* a species poor genus of widely distributed host generalists, or alternatively a

global species radiation characterized by restricted geographic distributions and cryptic host specificity? Here we integrate multi-locus sequence data and coalescent species delimitation models with microscopic study of parasite morphology and ecological data on host specificity to estimate species level diversity within this genus. We sampled *Leucocytozoon* parasites from across geographically disparate localities and from a broad diversity of host species, encompassing three abundant morphospecies: *Leucocytozoon majoris*, *L. fringillinarum*, and *L. dubreuili*. We found that morphospecies are not monophyletic groups, but rather each morphotype has evolved multiple times independently. Furthermore, within morphotypes we found evidence for cryptic host specificity to avian families. Coalescent species delimitation methods differed in their estimates of species diversity across our sampled lineages, though there was strong and consistent support for species-level divergence in association with transitions to different avian host families. However, there was also evidence for *Leucocytozoon* speciation within single avian families, indicating that speciation in this system can occur without host-switching. Our integrative analysis suggests that *Leucocytozoon* species diversity may be underestimated by a factor of ten or more, illustrating the need for continued study of the complex history of diversification in this group.

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WHEN PARASITES HIDE IN PLAIN SIGHT: DISCOVERY OF A HAIRWORM (NEMATOMPORPHA: GORDIIDAE) CRYPTIC SPECIES COMPLEX IN NORTH AMERICA

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The recognition of parasite cryptic species not only has implications for the study of biodiversity but also for the study of epidemiology, disease ecology, and host-parasite relationships. Hairworms of the genus *Gordius* are well known for their lack of reliable, species-level morphological characters and thus have become the target for cryptic species studies. *Gordius difficilis* Montgomery 1898 was characterized by a bifurcating male posterior end with a v-shaped postcloacal crescent and a semicircular row of bristles extending around the cloacal opening. Since its description, any worm fitting these 3 characters, and found in the US, has been identified as *G. difficilis*. Recently, worms fitting the description of *G. difficilis* were found in vastly different habitats (desert, alpine, plains, prairie), thus raising the possibility of crypsis. Worms were collected from the Midwest and Southwest, and analyzed using morphology (SEM) and molecular (CO1) data. Our data suggests that these specimens represent 3 new species: 2 were collected in the Southwest (SW in New Mexico and Arizona; AZ in Arizona), 1 in the Midwest (MW in Nebraska, Wisconsin, Iowa). MW and AZ have a textured, concave, polygonal, superficial cuticle pattern extending up the lateral sides of the male bifurcation. Males have a postcloacal crescent with a boomerang shape and serrated edge. SW has smooth, superficial, concave, polygons not extending up the bifurcating lobes. Males have a boomerang shaped postcloacal crescent, with a smooth edge, and unique short, tapered, and rounded bristles. MW have a medially curving bifurcation and some individuals have bristle-like projections between lobes. Genetic divergence of the CO1 gene support the validity of these 3 new species (>10% divergence). This cryptic species complex is the second discovered within North America. *Gordius* cf. *robustus* was found to be a complex containing 8 species. With the addition of the 3 new species discovered here, the diversity of recognized *Gordius* species within North America has gone from 3, to 13 in just 4 years.

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HOST ASSOCIATIONS AND GENETIC DIVERSITY OF AVIAN CHEWING LICE (INSECTA: PHTHIRATPERA) FROM AFRICA

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Parasitic chewing lice (Insecta: Phthiraptera) of birds are found everywhere their avian hosts are distributed, and their host relationships and taxonomy have been well studied in many regions. Compared with Europe and the Americas, however, the ectoparasite fauna of African birds is poorly understood despite the avian fauna being relatively well-known. Recent field expeditions exploring the avian diversity in South Africa, Benin, and the Democratic Republic of the Congo, and the fact that these specimens are stored in natural history museums, allowed an opportunity to examine louse specimens from across Africa. The goal of this study was to investigate avian louse host associations and genetic diversity to increase our understanding of African parasite biodiversity. Over 1600 avian specimens were examined for lice, and approximately 125 new louse-host associations were observed. Portions of the mitochondrial COI and nuclear EF-1α genes were amplified and phylogenetically analyzed, revealing multiple new genetic lineages of lice. Our work reveals possibly of as many as 60 new chewing louse species. Examining biogeographic patterns in parasitic lice across the entire region of Sub-Saharan Africa indicated that lice tend to follow host distributions rather than grouping by geographic region. Given the lack of current data on chewing louse species distributions in Africa, this study adds to the knowledge of host associations, geographic distribution, and genetic variability of avian chewing louse species in Africa.

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(53)

HETEROGENEITY IN CESTODE COMPOSITION THROUGHOUT HOST DISTRIBUTION AND ITS IMPLICATIONS ON SAMPLING STRATEGIES FOR CO-EVOLUTIONARY STUDIES

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We surveyed the cestode fauna of the stingray *Styracura schmardae* (Werner) from 4 localities throughout its geographic range in the Caribbean Sea. *Styracura* forms a sister clade to Neotropical freshwater stingrays (Potamotrygonidae). For this reason, we might expect that cestode lineages of these marine stingrays are closely related to the ones of freshwater potamotrygonids. We found that the parasite diversity of species of *Anindobothrium* Marques, Brooks & Lasso, 2001 and *Rhinebothrium* Linton, 1890 is heterogenous throughout the geographic range of this host. Of the two species of *Anindobothrium*, one is restricted to the coasts of Colombia and Trinidad & Tobago, while the other is restricted to Belize and Panama. A similar pattern was observed for species of *Rhinebothrium*, with one species restricted to Colombia, while the second occurs off the coast of Panama. The distribution observed for these cestode lineages is congruent with the present understanding of the areas of endemism in the Caribbean Sea. Since co-evolutionary studies require accurate taxonomic and phylogenetic information to unambiguously establish associations within the lineages involved, our results suggest that the role of α- and β-richness on the composition of the cestode fauna within target hosts must be taken into account. In general, it is assumed that individual hosts are the unitary measure of sampling effort. However, if β-richness plays an important role in shaping the cestode composition of target hosts, as our study suggests, additional efforts have to be invested to recognize the species diversity associated with host lineages. We predict that the patterns of cestode distribution observed for *S. schmardae* are likely to be replicated for other batoids as more data from other host groups are compiled. Within this framework, we highlight the importance of increasing the sample size across the distribution of host species in co-evolutionary studies in order to optimize the recognition of species and track historical host-parasite associations.

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PHYLOGENETIC INTERRELATIONSHIPS OF DICROCOELIID DIGENEANS PARASITIC IN BATS

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The Dicrocoeliidae Looss, 1899 is a large, globally distributed and very diverse family of digeneans parasitizing reptiles, birds and mammals. The vast majority of dicrocoeliids parasitize the gall bladder and bile ducts in the liver of their definitive hosts, while relatively few taxa are found in intestines and, exceptionally, other organs. Despite the great number of described species and genera within the Dicrocoeliidae, the systematics and phylogenetic interrelationships among dicrocoeliids remain unclear, mainly due to high level morphological homogeneity among many taxa. Among mammals, dicrocoeliids are most common and diverse in rodents; in contrast, bats and insectivores have a very limited dicrocoeliid diversity with only a few species reported from different parts of the world. In this study, we used partial sequences of the nuclear ribosomal 28S gene to examine phylogenetic relationships of several dicrocoeliid species from shrews, bats and rodents from North and South America, Europe, Africa and Southeast Asia. Even the limited number of currently available sequences have demonstrated multiple host switching events within most clades including dicrocoeliids parasitic in both birds and mammals. Combination of morphological and molecular analyses revealed two new species and a new genus of dicrocoeliids in South American bats and European shrews. In addition, molecular phylogeny has allowed reassessment of systematic value of some morphological and biological characteristics and helped to reveal mistakes in morphological descriptions of known taxa. Dicrocoeliids from small mammals did not form clear patterns associated with either hosts or geographic regions. This study was funded in part by the National Science Foundation project numbers DEB 1120734.

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MODERN METHODS AND OLD SPECIES: COMBINING MOLECULES AND MORPHOLOGY TO UNDERSTAND DIVERSITY IN *NEOECHINORHYNCHUS*

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K. Luth

Neoechinorhynchus is an acanthocephalan genus with 115 valid species, 33 of which parasitize North American freshwater fishes. This presentation addresses preliminary findings of the first modern systematic investigation (i.e. combined study of genetic sequence and morphological data) and presents the first phylogeny of *Neoechinorhynchus* species that parasitize U.S. fishes. The goals of this project are to illuminate the previously unknown species boundaries for a subset of species in this large, complex genus by combining molecular data from a 3-gene (28S, ITS, and COI) phylogeny with detailed morphological study of type and fresh specimens, and to redescribe the species *N. prolixoides* Bullock 1963. Essential to this work is the collection of fresh material from type localities for morphological and molecular analyses in conjunction with study of type specimens. Host specificity seems to be greatest in the 13 species that are described from hosts of the fish family Catostomidae (suckers), so the authors are directing special attention to these species. Authors Doolin and Reyda have, to date, collected multiple species of the genus in the northeastern USA, including specimens of *N. prolixoides* from its type host *Erimyzon oblongus* (creek chubsuckers) at its type locality in New Hampshire. Efforts are currently underway to collect specimens of three other species of *Neoechinorhynchus* from their respective type

localities in Oklahoma and Mississippi. To this point, molecular work has been conducted on 84 specimens collected from five fish families across 22 states, and to date, results include a 28S-based phylogeny consisting of five clades based on host family, with varying degrees of host specificity. Morphological studies corroborate these preliminary molecular findings, indicating the presence of 5 species in the freshly mounted material. This work constitutes a portion of the presenting author's Master's degree.

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PARASITE STOICHIOMETRY FOLLOWS FUNCTIONAL AND PHYLOGENETIC PATTERNS

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Parasites interact with the cycling of elements such as N and P by responding to environmental nutrient availability or altering host nutrient recycling function. Data on these topics are available for only a few host-parasite species pairs, and they reveal species-specific variation in the interactions between parasites and nutrients. Ecological stoichiometry provides a framework for quantifying the exchange of multiple elements in host-parasite interactions. Measuring the ratios of elements composing parasite tissues and assessing the factors that drive variation among species will be useful for understanding interspecific differences in parasite nutritional demand, the magnitude of resource extraction from hosts, and the functional importance of parasitism to nutrient recycling. Despite growing interest in the ecological stoichiometry of parasitism, few studies have measured the elemental content of parasite tissues, and none have done so for multiple parasite species. In this study, we measured the elemental content (%C, N, and P) and molar ratios (C:N, C:P, N:P) of a diverse assemblage of parasitic helminths, then asked what ecological and evolutionary variables were linked to stoichiometric variation among species. We sampled 27 macroparasite taxa, spanning 4 phyla, from 16 host species inhabiting freshwater ecosystems in New Jersey. Macroparasites varied widely in their elemental content, exhibiting four-fold variation in %N and five-fold variation in %P. Across all species, parasite body size had a strong negative relationship with %P and positive relationships with C:P and N:P. This pattern supports the growth rate hypothesis, which predicts that smaller taxa require more P to support their relatively higher growth rates. Parasite phylum was also an important determinant of %P, C:P, and N:P. Life cycle stage and infection location were related to variation in %N and %P, respectively. Parasite functional feeding group and trophic level were not related to any stoichiometric variables. Phylogenetic correction for all statistical analyses will be necessary to determine the independence of these patterns from parasite phylogeny. This project is the first to document variation in the organismal stoichiometry of parasites, which will be important to understanding relationships between parasitism and nutrient cycling. Our results provide support for the growth rate hypothesis and identify several phylogenetic and life history variables linked to variation in parasite stoichiometry.

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RAPID EVOLUTION OF CRYPTIC COLOURATION IN ECTOPARASITES

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Cryptic colouration is one of the most compelling examples of evolution by natural selection. Darwin and Wallace predicted that selection by visual predators would lead to the evolution of crypsis through

background matching by prey. Yet, there are few compelling, experimental demonstrations of the selective advantage of cryptic colouration using live organisms in their natural environment. To date, nearly all documented cases of crypsis have involved predator-prey interactions. However, cryptic colouration may also evolve in parasites, which are one of the most abundant groups of organisms on Earth. For example, background matching may facilitate the ability of external parasites to escape from the behavioural defenses of their hosts, such as grooming by mammals, preening by birds, or cleaning symbioses in fish and other aquatic organisms. Here we demonstrate that preening does, in fact, select for background matching by feather lice on birds, and that cryptic coloration rapidly evolves in response to preening-mediated selection. Our results are the first demonstration of the evolution of crypsis by parasites. More broadly, we show how experimental tests of selection, coupled with experimental evolution, can shed light on macroevolutionary patterns.

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EAST AFRICAN ECHINOSTOME DIVERSITY

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Echinostomes have long been used to address basic questions regarding host-parasite interactions, systematics, and biodiversity. We are interested in echinostomes because they are commonly transmitted at our field sites in West Kenya, and because they produce rediae that may prey upon other trematode species - including sporocysts of *Schistosoma mansoni* - within their snail intermediate hosts. We have pieced together a dominance hierarchy among the trematode species infecting *Biomphalaria* from West Kenya, and a previously-undescribed echinostome is the most dominant species there. These studies would be greatly facilitated by having a more comprehensive understanding of the species composition and host usage patterns of tropical African echinostomes. Consequently, we have sequenced (28S and ND1) over 60 different samples of echinostomes from three different countries in East Africa. There are at least six species from four different genera in the family Echinostomatidae that are transmitted by African *Biomphalaria* alone. At least two of these species can also use planorbid snails from other genera as their first intermediate hosts. Interestingly, the cercariae of some of these echinostomes have peculiar refractile structures lying just posterior to their oral suckers. Only a few previous studies have reported similar features, in echinostomes transmitted by Neotropical *Biomphalaria* and *Bulinus* from South Africa. Other anatomical similarities have been noted between echinostomes transmitted by South American and African *Biomphalaria* species, and additional studies will allow us to determine if representatives from the different continents are close relatives or represent parallel acquisitions of peculiar structural features. In the future, we will continue to pin down the interactions between echinostomes and other trematodes and further elucidate their diversity in East Africa. This study was supported by NIH grant R01 AI101438.

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GENETIC MAPPING AND POPULATION GENETICS OF AN ADAPTIVE PARASITE TRAIT: LARVAL RELEASE TIME IN SCHISTOSOMES

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Parasites show exquisite adaptations to ensure transmission to new hosts, but the genetic bases of these adaptations are poorly understood. The timing of cercarial shedding from schistosome infected snails is critical for successful transmission to the vertebrate host. In Oman, *Schistosoma mansoni* parasites collected from rats shed cercariae nocturnally (~8pm) while parasites collected from humans shed cercariae during the day (11am). This project was designed to understand the genetic basis for nocturnal shedding. We conducted reciprocal genetic crosses between nocturnal and diurnally shedding Omani schistosomes, determined cercarial shedding profiles in parents, individual F1 and F2 progeny. We then sequenced exomes of parasites from each cross and used linkage mapping approaches to determine the genome regions underlying this trait. We found a strong quantitative trait locus on chr. 1 (LOD = 6.1) and a secondary peak on chr. 6. The chr. 1 peak contains a compelling candidate locus (Hes-1) that encodes a transcription repressor known to influence cell proliferation, embryogenesis and developmental timing in *Drosophila*. We are currently investigating the population genomics of Omani schistosome populations by exome sequencing field collected parasites showing nocturnal or diurnal shedding, and in future work, we will exploit the growing functional genetics toolbox for schistosomes to determine the loci underlying this trait and the mechanisms underlying timing of cercarial release. Our central goal is to understand, at the molecular level, a key parasite trait critical for transmission and host specificity.

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BEHAVIORAL EFFECTS OF TREMATODE PARASITISM ON FRESHWATER MOLLUSCS: DOES PARASITISM ALTER THE ATTRACTION BETWEEN FIRST AND SECOND INTERMEDIATE HOSTS?

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Many parasites cause host behavioral changes which increase the likelihood of transmission to the next host. Few studies have examined the step from the first intermediate host to the second intermediate host in trematodes**,** where transmission of cercarial larvae relies on proximity to the next host. One study found that potential second host heterospecific snails aggregated more with echinostome-parasitized first host snails compared to non-parasitized first hosts. To further examine this result, we performed a series of behavioral experiments with *Echinoparyphium* sp. to determine if potential second host snails (*Helisoma trivolvis*) were more attracted to parasitized first host snails (*Lymnaea elodes*). In a Y-maze tank a choosing snail (*H. trivolvis*) was placed in the base and a stimulus snail (*L. elodes*) was restrained in either arm. Four treatments (30 replicates each) were each digitally filmed for 30 minutes: T1) control/no snail, T2) 1 non-parasitized stimulus, T3) 1 parasitized stimulus, T4) 1 parasitized and 1 non-parasitized stimulus. In T2 and T3, snails spent more time with the stimulus and entered the snail arm more times than the non-snail arm. Non-parasitized snails in T2 were not chosen first, but parasitized snails in T3 were. When both parasitized and non-parasitized stimuli were present (T4), responding snails spent significantly more time in proximity to parasitized hosts. These results indicate that potential second host snails were more attracted to parasitized first host snails over non-parasitized snails of the same species. To investigate a possible mechanism for this behavioural change, high-performance liquid chromatography was used to test whether parasites were affecting the chemical communication of the stimulus snail. We will discuss whether there were differences in the amount and type of fatty acids in parasitized vs non-parasitized *L. elodes* conditioned water. This study demonstrates that parasites alter the behavior of second intermediate host snails and explores the role of chemical communication in parasite-altered behavior.

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OF MICE AND WORMS: DO UNRELATED PARASITE INFECTIONS DO MORE DAMAGE TO DEFINITIVE HOSTS?

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Intraspecific competition between co-infecting parasites can influence the amount of virulence, or damage, they do to their host. Kin selection theory dictates that infections between related parasite individuals should have lower virulence than infections between unrelated individuals, because they benefit from inclusive fitness and increased host longevity. These predictions have been tested in a variety of microparasite systems, and in larval stage macroparasites within intermediate hosts, but the influence of adult macroparasite relatedness on virulence has not been investigated in definitive hosts. This study used the human parasite *Schistosoma mansoni* to determine whether definitive hosts infected with related parasites have lower virulence than hosts infected with unrelated parasites, and to compare the results from intermediate host studies in this system. The presence of unrelated parasites in an infection decreased parasite infectivity and total worm establishment in hosts; impacting the less virulent parasite strain more severely. Unrelated parasite co-infections had similar virulence to the more virulent of the two parasite strains. We combine these findings to complementary studies of the intermediate snail host and describe trade-offs in virulence and selection within the life cycle. Our results in this host-parasite system suggest that unrelated infections may select for higher virulence in definitive hosts while selecting for lower virulence in intermediate hosts.

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POPULATION EXOMICS OF NATURAL SCHISTOSOME POPULATIONS USING SINGLE MIRACIDIA

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Population genomic analyses of parasites such as malaria, trypanosomes and leishmania have provided insights into the selection pressures imposed by both human and invertebrate hosts, but comparable research on schistosomes has lagged behind because adult parasites live in the blood vessels and only microscopic miracidia larvae are available for analysis. We developed a robust, inexpensive approach for capturing and sequencing of the ~15 Mb *Schistosoma mansoni* exome that can be used for single miracidia hatched from eggs isolated from feces. Here we describe population genomic analysis of exome sequence data from 137 miracidia (one from each patient sampled) from Brazil, East Africa (Tanzania) and West Africa (Senegal and Niger). All the African samples come from the Schistosome Collection at the Natural History Museum (SCAN collection). We scored 155,057 SNPs and detail patterns of genomic variation in autosomal and mitochondrial DNA, characterize SNP variation at candidate vaccine and drug resistance loci, and examine geographic differentiation in allele frequencies to identify genome regions under strong directional and balancing selection. Such scans for selection in natural populations can

provide a valuable complement to targeted analyses of adaptive biomedical traits (e.g. host specificity or drug resistance) conducted in the laboratory.

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COMPARATIVE POPULATION GENETICS OF TWO CONGENERIC DUCK SCHISTOSOMES, *TRICHOBILHARZIA QUERQUEDULAE* AND *T. PHYSELLAE*

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Host-parasite systems exist across complex and ecologically heterogeneous landscapes; they evolve at the population level and are shaped by many co-occurring factors (immunology, evolutionary constraints, ecology). Host ecology (dispersal, distribution, life history) is thought to be important in shaping parasite microevolution, however identifying relevant ecological factors is challenging as it is unknown to what extent evolutionary history has determined contemporary microevolutionary patterns. In an effort to control for this, we assessed the population genetics of two congeneric trematodes (*T. querquedulae* and *T. physellae*), which are assumed to have evolved under similar evolutionary constraints. These worms infect ducks and freshwater snails, both *T. querquedulae* and *T. physellae* infect *Physa* spp. snails, which are common snails throughout North America and one species (*Physa acuta*) is globally invasive. These worms are associated with different duck groups in distinct lineages and with different habitat preferences and distribution. *Trichobilharzia querquedulae* is found globally while *T. physellae* is restricted to North America. In comparing *T. querquedulae* (n=100) and *T. physellae* (n=60) from 20 different localities we see strong discordance of population genetic patterns and intraspecific variation, based on CO1 & ND4 genetic markers. *Trichobilharzia querquedulae* maintains greater genetic diversity and effective population sizes than *T. physellae*. Within host diversity of *T. querquedulae* is equal to between host diversity, suggesting a well-mixed and genetically diverse metapopulation. Geographic structuring within *T. physellae* is minimal and genetic diversity is low, relative to *T. querquedulae*. These data represent populations' sampled across the range of two widely distributed congeneric trematodes and reveal strikingly different microevolutionary stories, suggesting the importance of evolutionary and ecological forces in shaping their contemporary populations and consequently the evolutionary potential of *Trichobilharzia* populations.

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TRAINING GRANTS FROM THE NIH: WHAT ARE THEY AND HOW DO YOU GET ONE?

J.M. Hawdon, The George Washington University School of Medicine & Health Sciences

So you want to get your graduate degree working on a medically important parasite? Or you want to join a medical parasitology lab for your post-doctoral research? Medical parasitology labs are usually supported by grants from the National Institutes of Health, and often, insufficiently. Bringing your own funding to the lab, as a graduate student or post-doc, not only benefits you and your career, it will free up money in your lab for additional people, supplies or equipment, thereby scoring points with your PI. The NIH has a mechanism to obtain grants to fund your training called the Ruth L. Kirschstein National Research

Service Award. I will discuss the specific grants available based on your career stage and future plans, how to write the application, and what reviewers are looking for in a proposal. This will allow you to increase your odds of obtaining pre- or post-doctoral funding.

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THE NATIONAL SCIENCE FOUNDATION AND PERSPECTIVES ON GRANTS

J.L. Cook, Sam Houston State University

Grants to support scientific research are a part of the culture of our discipline due to the inherent nature of our work and the need for adequate funding to support that work. However, grant funding has become more than just a mechanism to allow scientists to conduct their research. Applying for and receiving grants has become a way for employers and peers to measure productivity and in most cases, getting grants is required to receive tenure and promotions in Academia. This has increased the importance of getting a grant but has also increased the competitiveness of these grants. Thus, there is more than one perspective on grants from the viewpoint of researchers, administrators, and scientific program directors. This presentation will discuss my viewpoint of this situation as I have experienced it in my roles as researcher, Chief Research Officer for my University, and Program Director at the National Science Foundation. Information will also be given on National Science Foundation Programs, especially those pertinent to students such as the Doctoral Dissertation Improvement Grant (DDIG) and the Graduate Research Fellowship Program (GRFP).

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FINDING AND SUCCESSFULLY APPLYING TO SMALL GRANTS

K. Gallagher, University of Connecticut

Small grants may not fund a student's entire research project, but they are valuable ways to fund the generation of preliminary data so as to improve chances of obtaining larger grants in the future, to supplement an already funded project in order to improve or expand it, or even to fund small side projects. No matter how the funds are used, small grants demonstrate the ability to apply for and successfully obtain funding. Fortunately, a great variety of small grant programs are available to both undergraduate and graduate students throughout their academic careers. However, these programs are often not widely publicized and are thus difficult to find. The goal of this talk is to help familiarize students with the process of searching for and applying for small grants. Some of the forums that are available for searching for funding opportunities, such as PIVOT and grants.gov, will be discussed. An overview of some of the different small grant programs that are available, for example through agencies such as Sigma Xi and scientific societies, such as the Society of Integrative and Comparative Biology, will be presented. The requirements for the various applications will be reviewed. Advice from students who have previously applied for and received the various small grants will also be shared. The goal of the talk is to provide an overview of some of the grants available to students at the different stages of their academic careers and to help students improve their applications.

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CHEMICAL DEFENSES PROTECT LARVAE FROM PREDATORS AND DISEASE?

D. Calhoun, University of Colorado Boulder
G. Bucciarelli, University of California
P.T. Johnson, University of Colorado Boulder

All amphibians have dermal glands, many of which secret toxins or noxious substances. Newts in particular possess the potent neurotoxin tetrodoxtin (TTX), for which the highest concentrations are found in species within the genus *Taricha*. Adult *Taricha* are thought to use TTX primarily as a chemical defense against vertebrate predators such as garter snakes. However, less is known about how TTX functions to defend larval newts against natural enemies, including both aquatic macroinvertebrate predators and trematode parasites. Here we experimentally investigated the effects of TTX on cercariae of five species of trematode parasites known to infect larval amphibians and seven orders of aquatic macroinvertebrates commonly found with newt larvae. Specifically, we used dose-response curves to investigate the sensitivity of trematode cercariae to varying concentrations of TTX and how this differed among parasite species. We further compared the sensitivity of parasites to common macroinvertebrates during 24 hr trials. Using experimental bioassays to test the effects of waterborne TTX, we found that TTX significantly reduced the survival of trematode cercariae, but that the magnitude of such effects varied among species. More specifically, *Ribeiroia ondatrae* – which causes mortality and limb malformations in amphibians – was the least sensitive to TTX whereas the kidney-encysting *Echinostoma trivolvis* was most sensitive. Tested macroinvertebrate taxa showed no significant response to exogenous TTX exposure, even at concentrations 16x higher than used for trematode cercariae. Our results suggest that TTX in larval newts may provide some level of protection against trematode infections and highlight the importance of future work assessing both its effects on in vitro host infections and the palatability of larval newts to invertebrate predators.

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FUNCTIONAL AND PHYLOGENETIC ANALYSES OF RHS PROTEINS IN ARTHROPODS

L.M. Brooks, **A.R. Badial** and **J.G. King**, Mississippi State University

The RHS protein family is comprised of large transmembrane proteins that are present in diverse bacterial groups and in the genomes of several interesting arthropods, including mosquitoes and potentially *Cimex*. Previous studies have focused on the nature of horizontal gene transfer of *RHS* genes between *Wolbachia* and mosquitoes. Our prior research has also shown that mosquito RHS proteins form a major immunogenic component of the saliva in both major mosquito lineages. Several recent studies have suggested a function of RHS proteins as contact-dependent growth inhibition systems in bacteria. Therefore, we hypothesize that arthropod RHS proteins might act to manipulate the microbiome of certain insect tissues. This research focuses on the function of arthropod RHS proteins by using in vitro expression and bacterial bioassays to analyze the inferred functional domains of RHS proteins from *Aedes albopictus*, *Cimex lectularius*, and *Wolbachia*. Up-to-date phylogenetic analyses of known RHS genes, including codon-bias analyses, will be presented, as well as ongoing wet-lab experiments confirming the presence of *RHS* genes in the genome of *Cimex lectularius* through tissue-specific quantitative PCR and long range PCR coupled with sanger sequencing.

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EIMERIA FALCIFORMIS INFECTION PERTURBS THE METABOLIC PATHWAYS IN MICE

G. Huang, **S. Zhang**, **X. Tang**, **X. Liu** and **X. Suo**, China Agricultural University

Eimeria infection results in host tissue damage and metabolic dysfunction. Better understanding of interference of host metabolic pathways by protozoan infection can help to reveal useful targets for diagnosis of protozoan diseases. To determine the global host metabolic responses associated with protozoan infection, we established an *Eimeria falciformis* infection model in BALB/c mice and subsequently monitored the metabolite changes in the serum samples using gas chromatography/mass spectrometry (GC/MS)-based metabolomics technique. Metabolic profiling revealed that *E. falciformis* infection dramatically changed numerous metabolite pathways, including citrate (TCA) cycle, ATP-binding cassette (ABC) transporters, phenylalanine metabolism and biosynthesis of unsaturated fatty acids, with the majority of metabolites being down-regulated on day 7 post infection (pi) and being recovered on day 14 pi. In particular, level of phenylacetic acid, which involves in the catabolism of many aromatic compounds, dropped more than 10-fold and significantly affected the TCA cycle (p=0.0054). Importantly, a 2-fold down-regulation of phenylacetic acid still occurred near the end of the oocyst-shedding period (day 14 pi). Our study reveals phenylacetic acid may be a potential diagnostic biomarker of *Eimeria* infection. We thank National Natural Science Foundation of China (31330076 and 31572507) for supporting this research.

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CONTROLLING COCCIDIOSIS: A DAUNTING TASK

S.H. Fitz-Coy, Merck AH

Many improvements have occurred in the commercial poultry industry; re-using litter for several flocks of chickens, managing feed intake, light intensity and duration during each grow-out. Some other changes are bird genetics, age at which the birds are marketed and densities at which the birds are grown. Also better environmental conditions inside the houses due to evaporative cooling and air quality are other major improvements in the commercial industry and many more production improvements. For more than 50 years, the control of coccidiosis has mainly been with the use of in-feed anticoccidials and with some use of live coccidia vaccines. Shuttle programs and product rotation are quite common and considered good management practices. But over use of an anticoccidial drug in the presence of a moderate coccidia challenge may lead to the development of drug tolerant or even drug resistant strains of coccidia. Vaccinating chickens with drug sensitive strains of coccidia has shown great promise; this practice enables the addition of drug sensitive strains of coccidia to the population within the house environ. Mathis *et al,* 2003, and Peek and Landman, 2006, demonstrated that there are benefits for using Coccivac-B® or Paracox® in a rotational program with anticoccidials. The data showed sensitivity was restored to Clinacox™ (diclazuril) or diclazuril and monensin. Recently, some commercial poultry companies have used an anticoccidial (ionophore or chemical) following the administration of a live coccidia vaccine. However, these drugs are not fed until 13 to 21 days or 24 to 28 days of age. The verdict on this practice is not yet out. The review of data from 1960's until 2013 has indicated we might not have been good stewards of managing and maintaining the efficacy of available anticoccidials. For example, the efficacy of the most commonly used anticoccidials (nicarbazin, salinomycin or monensin) at launch the range of drug efficacy was 85-90%. Recent data has shown drug efficacy has significantly declined. Anticoccidial sensitivity tests (AST) for several field isolates of *E. maxima* from several broiler production areas in the US to salinomycin shown considerable variability in coccidia susceptibility; 45% of the isolates resistant, 36% partial resistant and 19% demonstrated some susceptibility. To maintain the efficacy of the anticoccidial drugs, a more judicious use of these products is required. This should include using vaccines and anticoccidials and regular monitoring drug sensitivity and or regular necropsy sessions.

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A JOURNEY INTO THE BLOOD MICROBIOME OF *BIOMPHALARIA* SPP. SNAILS

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Texas Biomedical Research Institute, San Antonio, Texas

The microbiome – the microorganism community found on or within an animal's body – is increasingly recognized shape many aspects of biology and is a key determinant of health and disease. The central aim of this study is to investigate the microbiome of aquatic snails (genus *Biomphalaria* sp.) that are the intermediate host for the human blood fluke parasite (*Schistosoma* sp.), causative agent of a parasitic disease that infects ~67 million people in sub-Saharan Africa and South America. In this work, we characterized the diversity and abundance of microorganisms within the *Biomphalaria* hemolymph (*i.e.* blood) using next generation sequencing (Illumina MiSeq) of the V4 variable region of the bacterial 16S ribosomal DNA. We characterized the microbiome composition of hemolymph from 5 snails from each of 7 different populations of *B. glabrata*, and from one population of *B. alexandrina*. We observed (i) 2,329-6,976 operational taxonomic units (OTUs) per snail (total 76,097), where OTUs are defined as sequences differing by >3% and (ii) significant differences in microbiome composition at the level of individual snails, snail population and snail species. Moreover, we hypothesize that the microbiome may represent a critical, but unexplored intermediary in the snail-schistosome interaction. Indeed hemolymph is in very close contact with the parasite at each step of its development. To investigate this aspect of the host-parasite interactions, we will characterize the microbiome of *S. mansoni* infected snails across the parasite life-cycle.

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LAB STRAIN *ANOPHELES QUADRIMACULATUS* MIDGUT MICROBIAL COMMUNITY AND THE EFFECTS OF WILD LARVAL REARING ENVIRONMENTS ON MICROBIOME AND *PLASMODIUM BERGHEI* INFECTION

E. Moen and **J. King**, Mississippi State University

Vector competence of mosquitoes has been linked to the conditions in which the larvae mature to adults. The microbial community obtained from their rearing environment is suspected to be one key factor in this interplay. Prior to becoming infective, the zygotes of *Plasmodium* parasites must make their way to the midgut lining and spend a significant amount of time developing as oocytes before releasing sporozoites, which then make their way to the salivary glands. While the parasite is inside the midgut, the microbial flora inside the gut could affect the development of *Plasmodium*. A better understanding of the effect of rearing environment on microbiome and *Anopheles-Plasmodium* interactions could be useful for understanding observed lab vs. field differences in *Plasmodium* biology and could help drive future control efforts. This explorative study focused on the effects of larval rearing on microbiome establishment in a North American malaria vector. We used lab-strain *Anopheles quadrimaculatus* Say reared in 1) standard laboratory settings, and 2) in water from two different native habitats in Mississippi. As a control we included 3) an analysis of the gut microbiome from wild adult *An. quadrimaculatus*. Illumina-based 16S amplicon sequencing was used to determine the eubacterial content of the midguts from the adults. Wild mosquitoes showed more variation in diversity at the bacterial phylum level than either lab strain treatments. Each mosquito microbiome was dominated by Proteobacteria. *Asaia spp.* bacteria were observed in very high quantities in the lab strain mosquitoes reared in water from the refuge, comprising 89-96% of total content. Likewise 40% of the total content in the lab strain mosquito reared

under clean laboratory conditions was *Asaia spp*. However, in the wild mosquitoes, both had less than 2% of their total content represented by *Asaia spp*. We conclude from these initial results that there are substantial differences between the gut microbiomes of lab strain mosquitoes reared under varying conditions and wild mosquito populations. Ongoing studies investigating the microbiome of lab-strain *An. quadrimaculatus* reared in the wild and their competency for harboring *Plasmodium berghei* infections will also be discussed.

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WHY DOES OXAMNIQUINE KILL *SCHISTOSOMA MANSONI* BUT NOT *S. HAEMATOBIUM* OR *S. JAPONICUM*?

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S.F. McHardy, University of Texas at San Antonio
R. Ripley, University of Texas at San Antonio
F. Chevalier and **T.J. Anderson**, Texas Biomedical Research Institute
P.T. LoVerde, University of Texas Health Science Center

The major species of *Schistosoma* affecting humans are *S. mansoni, S. haematobium,* and *S. japonicum*. There is currently only one method of treatment (monotherapy), the drug Praziquantel. Constant selection pressure through mass chemotherapy - this year will see the administration of over 250 million doses - has yielded evidence of resistance to PZQ. Previous treatment of *S. mansoni* included the use of oxamniquine (OXA), a prodrug that is enzymatically activated in *S. mansoni* but is ineffective against *S. haematobium* and *S. japonicum*. The OXA activating enzyme was identified by our laboratories as being a sulfotransferase (SmSULT). One focus of this research is to understand why OXA does not kill S. haematobium or S. japonicum and with this information reengineer OXA to be effective against *S. haematobium* and *S. japonicum*. An alignment of the sulfotransferases (SULT) shows that SmSULT, ShSULT and SjSULT share considerable sequence identity (71% Sm/Sh; 58% Sm/Sj, and 58% Sh/Sj) and predicted structural similarity. We sought to understand how differences in the amino-acid composition of Sm-, Sh-, and SjSULTs gave rise to species-specific drug action. Using site-directed mutagenesis, we demonstrated that SmSULT modified to look like ShSULT and vice versa each could activate OXA in an in vitro assay ie the SULTs were functional. We next evaluated the transcriptional differences between the SULTs by qPCR and Digital PCR. SmSULT transcription was 100X ShSULT and 1000X SjSULT. The differences in transcription account in part for the inability of OXA to be cidal in Sh and Sj. Next we employed an iterative process which lead to the identification an OXA derivative (CIDD790) that is effective against *S. mansoni* (100% killing), *S. haematobium* (80% killing) and *S. japonicum* (80% killing). These results demonstrate that understanding the mechanism of action of a drug and its structure function relationship can lead to novel cidal drugs.

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IDENTIFICATION AND CHARACTERIZATION OF A MULTI-DRUG RESISTANT STRAIN OF THE CANINE HOOKWORM *ANCYLOSTOMA CANINUM*

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D. O'Halloran, The George Washington University
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Hookworm disease remains one of the most significant infectious diseases. Control is limited to administration of single dose of a benzimidazole (BZ) drug through mass drug administration (MDA) programs, but re-infection in endemic regions necessitates frequent treatment. The rapidity with which BZ resistance developed in populations of livestock parasitic nematodes suggests that expanded MDA programs will generate resistant hookworms also. Therefore, molecular tests to monitor the frequency of resistance alleles are required to avert the development of BZ resistance. While several mutations in the β-tubulin gene have been implicated in BZ resistance in trichostrongyles, phenotypically resistant hookworms lack any of the known resistance mutations. A verified BZ resistant strain would be valuable for determining the mechanism of hookworm BZ resistance, and would allow correlation between in vitro tests and drug efficacy data. We report the identification and characterization of a multi-drug resistant strain of the canine hookworm *Ancylostoma caninum*. Feces from a dog that remained egg positive after 3 treatments with fenbendazole were cultured and infective larvae (iL3) used to infect a naïve dog. A larval development assay (LDA) was used to determine the EC50 and EC95 concentrations of thiabendazole (TBZ) for our sensitive lab strain WMD. Neither concentration had any effect on development of the resistant KGR strain worms. Sequencing revealed the presence of a known mutation associated with BZ resistance, and qPCR indicated that the frequency of the mutant allele was >99% in 2 generations of KGR strain passaged in our lab. To confirm that the mutation is associated with resistance, we are determining if the hookworm *tub-1*^{KGR} can impart resistance on wildtype *C. elegans* when expressed *in trans*. Additionally, KGR worms are resistant to pyrantel pamoate and partially resistant to ivermectin. This multi-drug resistant strain will prove invaluable for investigations into the mechanism of AR in hookworms.

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WHAT MAKES A SUCCESSFUL DRUG RESISTANCE ALLELE?

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More than 125 different *kelch13* alleles, each containing different single amino acid substitutions, have arisen in SE Asian malaria parasite (*Plasmodium falciparum*) populations under artemisinin selection over the past 15 years in a dramatic example of a soft selective event. However, just one of these alleles (C580Y) is now outcompeting other alleles in multiple different countries and is spreading towards fixation. Here we examine the transcriptional and fitness consequences of C580Y, relative to another less successful kelch13 mutation (R561H), to try to determine what is special about C580Y. Specifically, we test the hypothesis that C580Y causes less transcriptional disruption than R561H, and carries lower fitness costs. We used CRISPR/Cas9 to edit a wildtype kelch13 parasite genotype to carry C580Y, R561H, or control edits with only synonymous changes. We are now measuring transcript abundance across the parasite lifecycle, and determining relative fitness in head-to-head competition experiments, to provide direct comparisons of these two artemisinin resistance mutations on the same genetic background. Our overall goal is to better understand the dynamics of an ongoing selective event by careful functional examination of the phenotypic properties of different competing resistance alleles.

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EXPERIMENTALLY CONFIRMED TOLTRAZURIL RESISTANCE IN A FIELD ISOLATE OF *CYSTOISOSPORA SUIS*

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Cystoisospora suis is the causative agent of porcine neonatal coccidiosis, characterized by transient, pasty to watery diarrhea in suckling piglets in with the consequence of weight loss, ill thrift and occasionally secondary bacterial diseases with high morbidity. The only effective drug currently registered in Europe for metaphylactic treatment is toltrazuril which is administered as an oral suspension once in the first week of life. Constant treatment regimens in intensive production systems are applied for ca. 20 years, and the possibility of resistance development has not been addressed so far despite limited availability of treatment alternatives. In 2016 a pig farm in The Netherlands complained about pasty feces in suckling piglets between days 10 and 15 of life despite toltrazuril application in the absence of bacterial or viral pathogens. Evaluation of the administered amount of toltrazuril revealed no underdosing, and resistance was suspected. Piglets experimentally infected with the field isolate in question (4th day of life, d.o.l.) were treated with 0, 20 or 30 mg/kg of body weight (BW) of toltrazuril on the 6th d.o.l. (n=8 piglets/group) and fecal samples were taken daily individually from the 8th to the 22nd d.o.l. to evaluate oocyst excretion and fecal consistency. A separate litter infected with a toltrazuril-susceptible strain of *C. suis* and treated with 0 or 20 mg/kg BW (n=5 piglets/group) was infected and sampled identically for comparison. Toltrazuril completely suppressed oocyst excretion and diarrhea in the susceptible strain, while neither the recommended (20 mg/kg BW) nor the increased dose had an effect on oocyst shedding of the Dutch field isolate, indicating a complete loss of efficacy of toltrazuril as already indicated by the farm history. As no effective and economically sustainable alternative treatment is available in such cases, veterinarians and farmers should be aware of the possibility of resistance development and be instructed to undertake measures to retain the efficacy of toltrazuril in intensive piglet production systems.

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POLYMERASE CHAIN REACTION (PCR) BASED AMPLIFICATION OF MITOCHONDRIAL DNA TO DETECT *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* FROM LAHORE, PAKISTAN

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Introduction: Prompt detection of *Plasmodium* infection following rational treatment is a paramount to attain a reduction of disease burden in endemic areas like Pakistan. Malarial diagnosis using light microscopy often does not give diagnosis at species identification of *Plasmodium*. High throughput sensitive molecular techniques are prerequisite in malaria elimination settings. Methods: In the present study the mitochondrial DNA of *Plasmodium* species was targeted by using PCR assay to get more accurate results. The total 130 suspected blood samples were analyzed using cytochrome c oxidase genes of *Plasmodium falciparum* (cox III) and *Plasmodium vivax* (cox I) using species specific primers. Results: A total 81/130 samples were slide positive for *Plasmodium* (78 for *P. vivax* and 3 for *P. falciparum*). While no mixed infection was found. On PCR out of 130 blood samples, 83 were malaria positive. Among which 75 (90.36%) were *P. vivax*, 7 (8.44%) were *P. falciparum* positive and one patient was found with mixed infection. Conclusion: Compared to microscopic results, the sensitivity and specificity of the PCR was higher. The development of a simple and specific PCR is needed to avoid irrational use of antimalarials. It would be used in clinical or epidemiological studies with applications in malaria control program.

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MOLECULAR AND MORPHOLOGICAL DATA FROM *POSTHODIPLOSTOMUM*-LIKE, NEASCUS-FORMING DIPLOSTOMIDS IN BIRDS AND FISH

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Metacercariae in several genera in the Diplostomidae Poirier, 1886 form a neascus, which is characterized by a flat forebody and well-developed hindbody inside a spacious, thin-walled cyst. Distinguishing genera and especially species in neascus-forming metacercariae is difficult. We obtained partial sequences (500-600 base pairs) of cytochrome *c* oxidase 1 (CO1) from neascus-type metacercariae resembling *Posthodiplostomum* from fish from North America and the Caribbean, and in some cases from adult forms in birds. Divergence in CO1 indicates at least four species are present in fish on the island of Puerto Rico: two in centrarchids, one in poecilids and one in a mugilid. Preliminary data suggest metacercariae of these species can be distinguished morphologically, but at present it is difficult to identify to genus those without links to adult forms. One example of this difficulty is "*Posthodiplostomum*" from *Fundulus*. A neascus from *Fundulus diaphanus* in Quebec we previously identified as *Posthodiplostomum* was linked by CO1 to adult *Mesoophorodiplostomum pricei* (Krull 1934) collected from gulls. Since Krull described *M. pricei* from adults from a gull fed wild *F. diaphanus* and *F. heteroclitus* harboring neascus-type metacercariae, in Washington, DC, there have been no further mentions in fish. However, metacercariae of *Posthodiplostomum* are often reported from *Fundulus* in this region. This suggests that metacercariae of *M. pricei* may be more common than indicated by their absence from the literature and, possibly, are sometimes misidentified as *Posthodiplostomum*.

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THE HIDDEN DIVERSITY OF HEMOFLAGELLATE AND APICOMPLEXAN BLOOD PARASITES OF AMPHIBIAN AND REPTILE HOSTS FROM THE GREAT PLAINS REGION OF THE UNITED STATES

R. Shannon and **M. Bolek**, Oklahoma State University

Compared to blood parasites of mammalian and avian hosts, little information is available on host specificity, prevalence and distribution of blood parasites of amphibians and non-avian reptiles. The few available surveys suggest that amphibians and reptiles are commonly infected with a diverse group of blood hemoflagellates and apicomplexans. However, currently no information is available on these parasites in amphibian and reptile hosts from the Great Plains region of the United States. To investigate this, 7 locations in north central and southeastern Oklahoma were surveyed for amphibians and non-avian reptiles for blood protozoans. A total of 295 amphibians and reptiles from 9 families and 22 species were examined and found to be infected with 15 species/morphotypes of blood parasites. Eleven species/morphotypes of *Trypanosoma* infected amphibian hosts, 1 species of *Haemogregarina* infected reptilian hosts, and 3 species/morphotypes of *Hepatozoon* infected amphibian and reptile hosts. In terms of host parasite relationships, *Hepatozoon* and *Haemogregarina* species infected aquatic hosts; whereas *Trypanosoma* species/morphotypes infected aquatic and arboreal hosts. However, because blood parasites are difficult to identify based on morphology alone, we are in the process of sequencing the 18s rRNA and gGAPDH genes of the 11 *Trypanosoma* morphotypes and the ITS and CO3 genes of the three *Hepatozoon* and *Haemogregarina* morphotypes. Thus far, we have sequenced the 18s rRNA gene of 5 *Trypanosoma* morphotypes and found them to be genetically distinct. Additionally, we have sequenced the ITS and CO3 genes of *Hepatozoon* morphotypes from 8 anuran individuals. Phylogenetic analyses of these *Hepatozoon* sequences indicate that at least two species of *Hepatozoon* infect anuran hosts in Oklahoma. Our work characterizing the diversity of blood parasites infecting amphibians and reptiles in

the Great Plains will elucidate their species relationships and create the foundation allowing for future studies on their host specificity and life cycles.

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EVOLUTION OF PARASITISM IN COPEPODS: A PHYLOGENETIC APPROACH USING THE OPEN TREE OF LIFE

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Across the tree of life, our understanding of evolutionary relationships is in a state of flux as additional taxa and characters are included in phylogenetic analyses. As a result, there has been a shift in systematics from considering phylogenetic trees as static results to treating them as dynamic hypotheses to be explored in further analyses. The Open Tree of Life project (opentreeoflife.org) provides access to a continually updated tree of life by hosting a comprehensive, dynamic, and digitally-available tree that synthesizes published phylogenies with taxonomic data for all 1.8 million known species. In this study, we capitalize on this synthesis approach to estimate a phylogeny for copepods. The evolutionary relationship of copepods, and parasitic copepods in particular, is contentious because many taxa have reduced segmentation and lost appendages, making it difficult to identify homologous structures on which copepod classification typically relies. In order to better understand copepod relationships, we conducted a review of published phylogenies on copepods. By combining these published phylogenies with a new phylogeny we generated based on copepod sequences from GenBank and the underlying taxonomic framework from WoRMS that includes all 12,000 described copepod species, we generated a synthetic phylogeny for the Copepoda. With this synthesis tree, we survey transitions to parasitism in the Copepoda and examine morphological evolution across the group. This study identifies regions of the copepod tree that are under-studied with respect to phylogeny and serves as a framework for future studies on copepod relationships.

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DOUBLE TAKE: COMPARATIVE PHYLOGENOMICS AND THE MOSAIC OF CHIPMUNK AND LOUSE DIVERSIFICATION

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Comparative phylogenomic investigations can elucidate processes shaping host and parasite evolution. We used over 800 genomic loci to investigate the evolutionary history of two sucking lice (Anoplura), *Hoplopleura arboricola* and *Neohaematopinus pacificus*, infesting western North American chipmunks (Sciuridae: *Tamias*). Parasite variation was placed within the framework of a newly generated chipmunk phylogeny based on over 3000 ultraconserved element (UCE) loci. Given the high diversity of the hosts (23 species) and the dynamic history of climatic cycling across western North America, we predicted that lice would contain divergent lineages reflecting both host associations and the dynamic geologic and climatic history of this region. For both louse species, we recovered lineages largely correspondent with a

set of related host lineages (i.e., single host species or host species complexes). However, relationships among host-associated lineages vary between louse species, reflecting different durations of host association as well as unique histories of host switching. These outcomes suggest that diversification of different parasite species inhabiting similar niches is impacted by a temporal and geographic mosaic of processes extending beyond simple host diversification.

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(82)

THE NASTY RELATIONSHIP BETWEEN *OPHRYOCYSTIS ELEKTROSCIRRHA* AND THE MONARCH BUTTERFLY, *DANAUS PLEXIPPUS*! WHAT CAN BUYING INFECTED BUTTERFLY SPECIMENS ON THE INTERNET TELL US?

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The pathogenic neogregarine *Ophryocystis elektroscirrha* infects the hypodermal tissues of monarchs (*Danaus plexippus*) and queen butterflies (*D. gilippus*). However, the transmission and distribution of these parasites in other butterfly species is not well understood. Currently, 2 routes of transmission have been proposed for *O. elektroscirrha* and include horizontal transmission, when butterflies deposit protozoan oocysts (spores) on milkweed leaves and more commonly through maternal transmission, when females deposit oocysts on eggs during oviposition. Caterpillars become infected when they ingest oocysts from milkweed leaves or egg cases after hatching. However, the mechanism of oocyst transfer from infected female butterflies to their eggs is unknown. To investigate this, we examined the abdomen region of infected female monarchs using scanning electron microscopy. Our results indicate that all infected female monarchs contained *O. elektroscirrha* oocysts in their ovipore. This observation supports the maternal transmission route of *O. elektroscirrha*. More importantly, because caterpillars of many butterfly species ingest their egg cases after hatching, our observations suggest that the genus *Ophryocystis* should be maintained in butterfly lineages and potentially infect other species of butterflies. To test this hypothesis, we sampled 23 species of milkweed butterflies from 8 genera including all 11 *Danaus* spp. for *Ophryocystis* infections by buying dry butterflies sold for the butterfly collector trade. Based on oocyst morphology and 18S rDNA sequences, at least 5 species of milkweed butterflies from 2 genera collected from 4 continents were infected with *Ophryocystis* spp. However, oocyst morphology and host pathology, defined as embedded oocysts in the cuticle of butterflies, was conserved within clades but distinct among clades of milkweed butterflies. The implications of our findings are discussed in terms of conservation of migratory monarch butterfly populations and the geographic distribution and co-occurrence of *Ophryocystis* infected butterfly species.

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(83)

ANATOMICAL VARIABILITY IN THE ACANTHOCEPHALA

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Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, the Middle East, and North and East Africa, are described. The presentation is in 2 parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, male and female reproductive organs, and lemnisci. Newly described structures including (a) Para-receptacle

structure (PRS) and hoods in certain species as well as a new order of Acanthocephala from Vietnamese birds are also featured.

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(84)

STRUCTURAL-FUNCTIONAL RELATIONSHIPS AND CURIOSITIES IN THE ACANTHOCEPHALA

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This treatment of variability in the Acanthocephala is in 3 parts (1) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala and worm reproductive cycle. (2) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth. (3) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here.

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(85)

PARASITIC STRATEGIES INFLUENCE HOST-PARASITE SCALING RELATIONSHIPS

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The metabolic theory of ecology (MTE) is a framework used to describe global patterns in abundance, distribution and diversity of all species on Earth. A growing appreciation of the ubiquity of parasites in nature has led to an increase in efforts to include parasites in ecological theory and more recently in MTE. The aim of this study is to describe differences in general patterns in MTE based on variations in parasitic strategies. Specifically, we evaluate the applicability of Damuth's rule of density-mass allometry (DMA), which postulates that an organism's mean population density and body size will scale with an exponent of -0.75 across taxa. We collected field data from 35 distinct host-parasite interactions across 3 lake and 2 riverine ecosystems, and quantified parasite body size, density and host body size. Parasites were then assigned to distinct groups based on their vagility (autogenic, allogenic), consumer strategy (grazer, absorber) and ontogenic stage (adult, larval) to describe differences in parasitic strategies. We constructed log-linear models to evaluate the relationship between body size and population density of the parasites. Our analysis indicates a scaling exponent -0.25 describes the DMA relationship when using parasite body size as a predictor variable (R^2= 0.23, p=0.01), and this significantly departs from the theoretical value of -0.75 based on free-living species (p=0.03). When we used host body size as the predictor variable the value of the DMA scaling exponent was -0.99 (R^2=0.71, p<0.0001) and again this deviated from theory (p=0.004). Our results indicate that host body size is a better predictor variable when compared to parasite body size, but neither lines up with MTE. Furthermore, our analyses indicate that parasite consumer strategy and ontogenic stage can significantly influence this relationship when using parasite body size as the predictor variable (ontogenic stage slopes: larval= -0.70, adult= -1.55; p=0.02; consumer strategy: absorber= -0.86, grazer= -1.87; p=0.003). Parasite vagility was the only significant strategy when using host body size as a predictor variable (vagility slopes: allogenic= -1.31, autogenic= -1.07; p=0.03). In analyses by other investigators, parasites are generally treated as a homogeneous group of consumers. However, our data suggest the variable strategies represented amongst parasitic taxa can influence the general findings in MTE.

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(86)

LEARNING FROM THE COMPLEX SOCIETIES OF ARMY ANTS AND THEIR "GUESTS"

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Donation of the Carl W. and Marian E. Rettenmeyer army ant guest collection to the University of Connecticut Biodiversity Research Collections has led to renewed interest in this collection and the intriguing relationships among army ants and the hundreds of species associated with these hymenopterans. These associated taxa include a diversity of arthropods such as beetles, flies, springtails, millipedes, butterflies, and a wide array of mites. The term "guest" has been used to refer to these myrmecophiles (i.e., species that associate with ants by choice) because in so many cases the exact nature of the interspecific association is not clearly understood. Across taxa, mutualism, commensalism, phoresis, and of course, parasitism, have all been invoked. There are guests that run in army ant swarm raids and guests that live in the refuse deposits of the colony. The intimacy of some of these associations is attested to by myrmecoid staphylinid beetles that look and behave like their army ant "hosts" and by mites of the genus *Larvamima*, which bear an uncanny resemblance to, and in fact are treated by the ants as, ant larvae. By far the most intriguing guests however, are the mites that are associated with the individual ants themselves. These taxa occupy different sites on the ants and exhibit bizarre body forms depending on their specific site of attachment—the intimacy of these associations is undeniable. For example, mites of the genus *Macrocheles* live with their mouthparts embedded between the tarsal claws at the tips of the legs of the ants, and ants have been observed using the hind legs of the mites as substitutes for their own tarsal claws! A three year project funded by NSF's Collections in Support of Biological Research (CSBR) program aims to stabilize and database the millions of specimens in the Rettenmeyer collection. One of the primary goals of the project is to expand interest in this poorly known, but exceedingly complex and potentially highly informative system.

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(87)

A SURVEY OF SMALL MAMMAL ECTOPARASITES IN SOUTH TEXAS

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Texas is a geographically variable state containing a large diversity of plants and animals. Due to widespread private land ownership, organismal biodiversity (especially invertebrate and parasitic taxa) is poorly known across the southern portion of the state. We investigated the diversity of small mammal ectoparasites on private properties owned by the East Foundation in an effort to document and better understand invertebrate biodiversity across South Texas. Small mammals (primarily rodents) were captured and carefully inspected for ectoparasites. Each specimen was vigorously brushed over white paper and then scanned by eye using forceps to move fur around. All brushings and specimens obtained by eye were examined and sorted under a dissecting microscope. In total, 337 individual mammals representing 18 species were retained and examined for ectoparasites. Of these, 16 species and 273 individuals (81%) were parasitized by a total of 2,225 individuals of mites (1,295 specimens), lice (653), ticks (194), and/or fleas (83). Mites parasitized 16 mammal species and were found on 55.5% of the host individuals examined. Lice were collected from 9 of the mammal species examined, occurring on 24.6% of the specimens examined. Ticks also were present on 9 of the species examined, with 22.3% of the mammals examined serving as hosts. Lastly, fleas represented the least abundant class of ectoparasite found, with 7 mammal species and 15.4% of the host individuals examined parasitized. Specific host associations will be described, including a new association of a hoplopleurid louse parasitizing the

northern pygmy mouse (*Baiomys taylori*). Our work will aid in broadening the knowledge of general biodiversity in this area, including possibly discovering species and new associations for South Texas.

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(88)

COMPARATIVE PHYLOGEOGRAPHY AND DIVERSITY OF FLEAS FROM AMERICAN PIKAS OF THE INTERMOUNTAIN WEST

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American pikas (*Ochotona princeps*) are small mammals that are widely distributed across North America's Intermountain West. Previous investigations revealed five geographically distinct mitochondrial lineages within *O. princeps*. These lineages represent genetically distinct pika populations that have been evolving independently in association with different mountain systems of the Intermountain West. In contrast, diversity of endoparasitic helminths are not structured geographically in the same way. Instead, there are two primary parasite assemblages, one distributed across southwestern pika populations and one found across the northeastern part of the host range. These contrasting patterns suggest that the shared history of pikas and their parasites had different consequences for the evolutionary trajectories of these organisms. Here we investigate whether or not patterns of ectoparasite diversity suggest a history that is more similar to that of the host or that of the endoparasites. We characterized the flea diversity within American pikas based on a sample of 823 flea specimens collected from 37 localities in the Intermountain West. We identified 13 flea species, two of which are common and known to be specific to pikas. The population genetic structure of the most common flea species, *Ctenophyllus armatus*, was examined to test for phylogeographic concordance between host and parasite diversity. We generated DNA sequences from the mitochondrial COII gene for 72 fleas representing 22 different localities, and show general congruence between the phylogeographic structure of the fleas and that of the endoparasites. This pattern is consistent with post-glacial population movement from Southern Rocky Mountains and Utah to the Cascade Range and Rocky Mountains. Strong phylogeographic congruence between host and parasites is not supported by our analysis.

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A COMPARATIVE RNA-SEQ STUDY OF THE DETERMINANTS OF SUSCEPTIBILITY AND RESISTANCE OF THE MODEL SNAIL *BIOMPHALARIA GLABRATA* TO THE HUMAN TREMATODE PARASITE *SCHISTOSOMA MANSONI*

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RNA-Seq is a powerful technique for ascertaining the transcriptional responses of both parasites and hosts as they encounter one another. This technique is facilitated when the genomes of the organisms involved are known and can be referenced. This is now the case for both *Schistosoma mansoni* and its most important snail hosts in the Neotropics, *Biomphalaria glabrata*. By exposing susceptible (M line) and resistant (Salvador or BS-90) strains of *B. glabrata* to *S. mansoni*, and by then employing RNA-Seq,

we can gain a comprehensive overview of how the two strains of *B. glabrata* vary in their responses to the same parasite and how the transcriptional responses of *S. mansoni* vary in conducive vs. hostile molluscan environments. We have initiated an RNA-Seq project to investigate differences in gene expression between the two strains of *B. glabrata* during various stages of *S. mansoni* exposure. Individual juvenile M-line snails and BS-90 snails were exposed individually to 10 miracidia of PR1 *S. mansoni* and four snails of each strain were harvested at 2 and 8 days post-exposure (dpe). Additionally, four exposed snails of each strain were harvested at 40 dpe (shedding *S. mansoni* cercariae in the case of M line snails). Non-exposed M-line and BS-90 served as controls. A PCR-based assay was used to confirm the presence of *S. mansoni* in exposed snails. RNA from three individual snails from each strain and time point were used for Illumina NextSeq500 library preparation and sequencing. All samples yielded relatively consistent raw read counts (mean 8.2 M reads, standard deviation 1.2 M reads). A preliminary analysis showed that the total number of differentially expressed genes in M-line snails is much higher than in BS-90 snails. We expect to identify specific host factors associated with resistance or that support successful schistosome development, as well as prominent *S. mansoni* transcripts that could be targeted to suppress the parasite's successful development. Supported by NIH grant R01 AI 101438 and P30GM110907.

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(90)

SURVEY DETECTING VARIABILITY OF SMALL SUBUNIT RNA GENE OF *KNEALLHAZIA SOLENOPSAE* IN *SOLENOPSIS INVICTA*, IN SOUTH TEXAS

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The Red Imported Fire Ant, *Solenopsis invicta,* is an invasive species in the southeastern United States, causing negative impacts on the agriculture industry, environment, and economy. A common pathogen is the microsporidium, *Kneallhazia solenopsae*. There are several different *K. solenopsae* SSU gene variants (SSUGV): North America (NA), Widely Distributed (WD), Mexico (MEX), and USA. The SSUGV are not randomly distributed and appear to be dispersed by geographical location**.** It is unclear what would cause SSUGV localization, whether that be ant genetics or environmental factors. The objectives in this study were to determine the prevalence and geographic distribution of different *K. solenopsae* SSUGV in south Texas. Fifty-eight colonies were sampled from 32 counties in south Texas. After DNA extraction and examination by PCR, the samples were prepared for Next Generation Sequencing (NGS). The NGS data were analyzed with DNAStar Lasergene. Five counties had NA (5 samples) as the major SSUGV, 12 counties (24 samples) were determined to have WD, and another 12 (15 samples) did not have a significant number of *K. solenopsae* SSU gene variants (Negative). The final three counties were as follows: one county had WD (2 samples) and Negative (2 samples), and 2 counties had NA (8 samples) and Negative (2 samples). The prevalence for the total number of NA sequences was 34.1%, WD was 42.4%, MEX was 11.6%, USA was 0.3% and 11.7% were negative. The NA SSUGV appears to be more geographically localized than the other variants. It may be possible to use bait containing NA *K. solenopsae* spores from south Texas to infect colonies in other parts of Texas. If those ant colonies become infected and it is determined that the NA SSUGV is present, then the bait will be determined as successful.

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(91)

EXPRESSION OF THIOESTER-CONTAINING PROTEINS IN RESISTANT AND SUSCEPTIBLE *BIOMPHALARIA GLABRATA* STRAINS AFTER IN VIVO EXPOSURE TO *SCHISTOSOMA MANSONI* MIRACIDIA

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The thioester-containing proteins (TEPs) are a group of molecules that have functions associated with innate immunity. They form a family of ancient molecules present in all metazoan organisms with diverse functions including protease inhibitors and opsonins. We previously identified nine different TEP sequences in the genome of the freshwater snail *B. glabrata*. Eight of these proteins have not been described before, and their potential role in the immune defense *of B. glabrata* snails is still unknown. To begin their functional characterization, in the present study we measured the changes in TEP transcript expression over time in resistant (BS-90) and susceptible (NMRI) strains of *B. glabrata* following exposure to *S. mansoni* miracidia. The transcripts tested included four newly described TEPs (A2M, CD109, C3, and TEP2), in addition to a previously reported TEP, identified in this study as TEP1. Preliminary results of replicate experiments demonstrate differences in constitutive steady-state transcript levels between resistant and susceptible *B. glabrata* strains, with higher levels of CD109 and C3 transcripts in resistant BS-90 snails compared to the susceptible NMRI strain, but lower TEP2 transcript levels in BS-90 vs. NMRI snail strains. Unexpectedly, exposure of snails to *S. mansoni* miracidia had little effect on TEP gene expression, with the exception of TEP2, which exhibited a significant decrease at 48h in parasite-exposed BS-90 snails compared to unexposed BS90 controls. In summary, none of the TEP transcripts tested demonstrated increased expression within the first 48 h after *S. mansoni* exposure. However, the two snail strains exhibited differences in the steady-state constitutive levels of some of these molecules supporting their possible role in influencing innate susceptibility/resistance to infection.

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PARASITISM DIFFERENCES BETWEEN MALE MORPHOTYPES OF BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*)

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Bluegill sunfish (*Lepomis macrochirus)* are an important North American sport fish with a cosmopolitan distribution across the United States and Canada. These fish are sexually dimorphic with males being larger and more brightly colored than females. Additionally, there are two male morphotypes, dominant, brightly colored α-males, and β-males, which resemble females in both appearance and behavior. The two male morphotypes differ significantly in terms of mating behavior, territoriality, and diet. These behavioral and feeding differences may result in α-males harboring greater parasite diversity and parasite load than β-males. This was tested by collecting, necropsying, and identifying parasites from 636 *L. macrochirus* sampled from 11 ponds in Northwest Virginia, and comparing parasite diversity and parasite load in the male morphotypes. When compared to β-males, α-males consistently had greater parasite species richness as well as greater prevalence and intensity for both trophically and non-trophically transmitted parasite species sampled in this study. Furthermore, α–males had a more diverse and complex diet than β-males, which may have contributed to the differences in parasite species richness and parasite load. The disparities in parasitism may be contributing to the maintenance of multiple mating strategies in an evolutionarily stable system established by the 2 male *L. macrochirus* morphotypes by potentially affecting overall fitness and survival of the heavily infected α–male hosts, which is an avenue for future studies.

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VARIATION IN HELMINTH PARASITE COMPONENT COMMUNITIES OF *GAMBUSIA AFFINIS* AND THE EFFECTS ON HOST FITNESS

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In North Central Texas streams, seasonal changes in ambient temperature and rainfall result in dynamic variation in microhabitat structure. Since the complex life cycles of parasites require the presence of multiple hosts, the seasonal variations in microhabitats could influence parasite component communities within these ecosystems. The presence of parasites, particularly intestinal helminths, reduces host foraging ability, body condition, and potentially resources available for developing offspring thereby reducing host fitness. Western mosquitofish (*Gambusia affinis*) are facultative matrotrophs, exhibiting adjustments in post-fertilization provisioning to some offspring within a brood using recently acquired resources. Since larger offspring are more likely to survive, maternal contributions are expected to increase fitness. The objectives of this study were to 1) investigate spatial and temporal variation in parasite component communities and 2) examine the effect of helminth component communities on host fitness. Mosquitofish were collected from three sites on the Paluxy River, monthly from June 2015 through August 2016. All helminth parasites were collected during necropsy and subsequently identified. For the second objective, ten embryos of each developmental stage from each fish (n=98) collected from April through August 2016 were randomly selected and individually weighed. From 495 mosquitofish, 5,269 helminths were collected with an overall helminth prevalence of 90.0%. We found Shannon diversity was significantly varied by collection site and with river discharge. Additionally, we found embryo weight to be significantly affected by maternal weight, brood size, embryo stage, as well as intestinal cestodes and nematodes and parasitic diversity. These results demonstrate that helminth component communities infecting mosquitofish in the Paluxy River vary through space and in response to environmental variation. The results further suggest intestinal helminths affect nutrients available for maternal provisioning to developing embryos.

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THE METAZOAN PARASITE COMMUNITIES OF FLATFISHES AS BIOINDICATORS OF THE ENVIRONMENTAL CONDITION OF THE CONTINENTAL SHELF OF THE PENINSULA OF YUCATáN, MéXICO

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The Peña-Nieto administration is opening again oil exploration started in the 70´s along the continental shelf of the Peninsula of Yucatán (PY). As a part of a larger research project to establish the baseline environmental conditions of this region prior oil exploration, we studied the metazoan parasite community metrics of flatfishes as indicators of aquatic environmental health. Our aim was to describe the metazoan parasite communities of flatfish species at the component and infracommunity levels along the continental shelf of the PY. The study area included 18 sampling sites, where we obtained data on species richness, relative abundance of the metazoan parasite communities, as well as contaminants (e.g. hydrocarbons, heavy metals) from water, sediments and organisms, nutrients and physicochemical variables. We used redundancy analysis (RDA) to examine the potential statistical relationships between the contaminants and physicochemical variables from the water, sediments, and fishes, and the parasite community metrics. Overall, 161 flatfish individuals from eight species were examined for parasites, recovering 63 metazoan parasite species. Unidentified larval cestodes, the digeneans *Lecithochirium* sp. and *Stephanostomum* sp. were numerically dominant at the component and infracommunity levels. The pool of parasite species of the flatfishes from this region was twice as rich in species number as that reported for the southern Gulf of Mexico. The parasite community metrics had significant negative

statistical associations with fluoranthene, 2-methilnaphthalene and anthracene detected in the flatfishes. No other variable in water, sediments or flatfishes exceeded the range considered safe for marine organisms. The most likely explanation for the large pool of parasites found in flatfishes along the continental shelf of the Peninsula of Yucatán is the good environmental condition of the region, which allows the completion of parasite life cycles.

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(95)

TESTING THE ROLE OF INBREEDING DEPRESSION IN THE INFECTION SUCCESS OF THE TAPEWORM, *OOCHORISTICA JAVAENSIS*

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We know little about the occurrence and effects of inbreeding in wild parasite populations and there remain gaps in our understanding of how flatworm parasite mating systems impact host-parasite interactions. In hermaphroditic parasites that self-mate, inbreeding could be costly if infection success depended on heterozygosity (either via overdominance or masking of deleterious alleles). For example, inbreeding depression, the reduction in fitness of inbred offspring compared to outbred offspring, could manifest upon infection of a host if the parasite does not have the appropriate genetic variation to deal with host defenses. Two previous experimental studies measuring inbreeding depression in flatworms have produced differing results. In one study, a progenetic trematode, *Coitocaecum parvum,* showed no evidence of inbreeding depression based on several fitness parameters measured in offspring. In contrast, studies on the tapeworm *Schistocephalus solidus* showed evidence of reduced growth, lower infection and transmission rates, and limited intraspecific competitive ability among inbred individuals. With only two systems examined, additional data are needed to assess the role of parasite mating systems in impacting host-parasite interactions. Our goal was to test if the mating system of *O. javaensis* impacted the infection success of parasite offspring in its beetle intermediate host, *Tribolium castaneum*. If heterozygosity is important to infection then selfed offspring may experience inbreeding depression. However, our prior work shows that *O. javaensis* is highly inbred and populations with a history of inbreeding may be less affected by inbreeding depression because of the purging of deleterious recessive alleles. In addition, prior studies on hermaphroditic animals have measured 'apparent inbreeding depression' because paired animals cannot be forced to outcross. We will present a novel direct way of testing inbreeding depression by using parent-offspring genotype data.

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ONE GUT, TWO GUT, OLD GUT, NEW GUT: METAGENOMIC TOOLS FOR IDENTIFYING HELMINTH COMMUNITIES AND MICROBIOMES FROM MUSEUM ARCHIVED GASTROINTESTINAL TRACTS

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Natural history collections spanning multiple decades provide critical historical baselines to measure and understand changing relationships among Earth's biodiversity. With the advancement of molecular technologies, like next generation sequencing, museum samples now can be utilized in novel ways to

address significant questions about environmental change and species interactions, including community dynamics. Shrews occupy diverse habitats, host a speciose and abundant parasite fauna (97 helminth species in North America), and are wide spread across the US. The goal of this project was to apply a new technique (metagenomic sequencing of all organisms in the gut) to identify the helminth parasites and microbiomes (bacteria) of small insectivorous mammals (shrews), including in museum specimens prepared in varying ways. Through comparison of guts preserved in ethanol to freshly collected samples, we examined how parasites and other symbionts (bacteria) are changing both spatially (throughout the US) and temporally (over time). We successfully sequenced the parasite communities (using the genes 12S, 16S, 28S) and bacterial microbiota (16S) of 46 museum archived whole gastrointestinal tracts (GI) of shrews. Guts were obtained from the Museum of Southwestern Biology and from recent field collections and varied both in timespan preserved (ranging from 16 years to 4 months old) and preservation method (70% ethanol stored at room temperature, 95% ethanol at room temperature, and flash frozen in liquid nitrogen and stored at -80°C). No differences were observed in alpha- or beta-diversity among museum-archived samples and newly collected samples. This newly developed technique allows critical examination of the distributions and interactions among multiple groups of organisms through time.

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DISTRIBUTION OF GILL MONOGENEANS (*HAPLOCLEIDUS* SP.) ON PUMPKINSEED (*LEPOMIS GIBBOSUS*) AND BLUEGILL (*LEPOMIS MACROCHIRUS*) SUNFISH

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Most parasite infrapopulations are aggregated within their host populations, typically infecting few hosts heavily while having low intensity on most hosts. In our lake ecosystem, the same monogenean parasite species (*Haplocleidus sp.)* infects two Sunfish species differentially. These species, when infecting Pumpkinseed Sunfish follow a typical aggregated distribution in their host populations (k=1.44). However, the same monogenean species infect Bluegill Sunfish in the same systems; the infrapopulations are not aggregated, but are normally distributed in hosts according to a Shapiro Wilk normality test ($p > 0.05$). In addition, there is a positive relationship between host body weight and parasite abundance in Pumpkinseed Sunfish ($r^2 = 0.7405$, $p < 0.05$) but not in Bluegill Sunfish ($r^2 = -0.009243$, $p > 0.05$). These data indicate very different host-parasite interactions occur between these two cohabitating sunfish species. We are currently measuring several parameters related to the fitness of the monogeneans in both Bluegill and Pumpkinseed Sunfish. These will include per capita egg production in live infections, spatial distribution on the gills (macro and micro using histological techniques), and rates of establishment in hosts given standard infections of oncomiracidia in lab conditions. Preliminary data suggests that the Pumpkinseed is the more typical host.

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EFFECTS OF BRACKISH WATER TRANSFER ON METACERCARIAL LOAD IN THE TRINIDADIAN GUPPY (*POECILIA RETICULATA*)

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All species have a geographic range, but identifying the factors that limit the range boundaries are poorly known for most species. Interactions with parasites may influence range limits if resistance or tolerance declines beyond the range boundary. However, few studies have investigated how parasite loads change within and outside the host geographic range. On the island of Trinidad, the tropical fish *P. reticulata* inhabits mountainous and lowland freshwater streams but avoids brackish waters. A close relative, *P.*

picta inhabits both lowland freshwater and brackish water streams. In lab studies, *P. reticulata* has been shown to be physiologically tolerant of brackish water, suggesting some environmental factor in brackish waters may limit dispersal and range expansion. Here we test if digenean trematode parasites play a role in limiting the distribution of *P. reticulata*, but not *P. picta*, by measuring the metacercarial parasite loads under freshwater and brackish water conditions. We assessed the total mean metacercarial load in three freshwater populations of *P. reticulata* and found substantial variation between them (loads = 10.8, 16.2, and 0). The total mean metacercarial load of *P. picta*, was found to increase between fresh (7.1) and brackish water (19.2) sites. To mimic the effects of dispersal we exposed one upstream population of *P. reticulata* to brackish water thought to contain live cercaria for a period of nine days. There was a non-significant trend for *P. reticulata* exposed to brackish water for increased internal metacercarial load and decreased mean external metacercarial load. However, there was a significant correlation between the weight of the fish transferred to brackish water and their total metacercarial load, with fish between 0.07 and 0.12 grams having the highest parasite load. Our results suggest that movement of intermediate sized *P. reticulata* into novel brackish environments may be inhibited via the increased visceral metacercarial load, however, further investigations are warranted to better understand the relationship between parasite load, body size, and salinity.

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MATING BEHAVIOR OF THE HORSEHAIR WORM, *PARAGORDIUS VARIUS* (NEMATOMORPHA)

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Few studies have examined the mating behavior of parasites. Yet understanding how parasites find each other could lead to treatments based on disrupting their mating behavior. We examined the mating behavior of adult freshwater horsehair worms, *Paragordius varius*, under laboratory conditions. Horsehair worms infect various terrestrial insects, which they manipulate into entering water where the adults emerge, mate and lay eggs. We asked if hairworm adults locate each other randomly or non-randomly. To test this, we established male-male, female-female or female-male pairings using virgin adult hairworms. We placed the individuals from these pairs in the opposite corners of a 10-gallon aquarium and timed how long it took for the adults to locate each other under red light conditions in 15-minute trials. We hypothesized that if adults locate each other non-randomly, then individuals from female-male pairs would locate each other sooner than same sex pairs. Although not statistically significant, the results support our hypothesis with the average time to first contact between female-male pairs (n = 25) taking 217 seconds versus 266 seconds for same sex pairs (n = 24). All individuals from male-male pairs made first contact while only 64% of the individuals from female-male pairs (n = 39) and 64% of the female-female pairs (n = 29) made first contact. After making contact with another adult, males wrap their posterior end around the other adult to initiate copulation. 64% of the males did this with females in an average of 305 seconds compared to 47% of males that initiated copulatory behavior with another male in 367 seconds. Choice experiments will determine if adult hairworms use pheromones to locate each other. Results could lead to the development of drugs that interrupt the mating behavior of closely related human nematode parasites.

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(100)

UNPRECEDENTED EUKARYOTIC GUT MICROBIOME DIVERSITY WITHIN LONG-TAILED MACAQUES (*MACACA FASCICULARIS*) IN SOUTHEAST ASIA

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The majority of eukaryotes have been suggested to live on or in other hosts, but the diversity and ecology of these symbiotic eukaryotes remains consummately uncharacterized, despite unprecedented

contemporary interest in prokaryotic microbiomes. Key ecological roles played by eukaryotes in free-living systems and the ubiquity of parasitism, commensalism, and mutualism in eukaryotes suggest that symbiotic eukaryotes may make important contributions to host-associated communities. While previous studies on the host-associated eukaryotic communities of vertebrates have reported low levels of diversity relative to both sympatric prokaryotic and free-living eukaryotic communities, these findings may be more indicative of differences in the methodologies used to characterize these communities than they are of actual ecological differences between these biological systems. To assess the potential for such hidden diversity within guts of non-human primates, we utilize a novel Illumina sequencing approach to characterize eukaryotic diversity within the feces of wild long-tailed macaques (*Macaca fascicularis*) on two islands in Southeast Asia: Singapore and Bali, Indonesia. We report substantially higher levels of eukaryotic diversity than previously reported from the feces of primates. All five super-groups of eukaryotic life were represented, and several taxonomic groups were found to be common across all samples, suggesting the existence of a core eukaryotic community with the capacity to perform consistent ecological functions within these macaque hosts. Despite these commonalities, differences in eukaryotic gut assemblages were also detected that could be attributed to differences in host geography and diet. Our results are discussed within the context of how ecological guilds operating in the gut of macaques can drive community assemblage of symbiotic eukaryotes.

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TADPOLE PARASITE COMMUNITY STRUCTURE: DO PARASITE LIFE CYCLES MATTER?

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Currently, little information is available on parasite community structure in larval amphibians, specifically tadpoles of anurans. We examined the parasite community structure in tadpoles of five anuran species from an ephemeral wetland in northcentral Oklahoma. Specifically, we were interested in how species-specific factors, such as size, feeding strategies, and habitat partitioning among larval anurans affect parasite community structure. Additionally, we assessed whether parasite life cycle strategies affected tadpole parasite community composition. During May–August 2015 and April–June 2016, we collected tadpoles of southern leopard frogs, *Rana sphenocephala*, Blanchard's cricket frogs, *Acris blanchardi*, Cope's gray treefrogs, *Hyla chrysoscelis*, spotted chorus frogs, *Pseudacris clarkii*, and Great Plains narrow-mouthed toads, *Gastrophryne olivacea*. The compound parasite community was dominated by larval trematode stages (mesocercariae and metacercariae), with only two gravid adult helminth species present, the trematode *Megalodiscus temperatus*, and nematode *Gyrinicola batrachiensis*. The parasite component communities were depauperate, with a maximum of six parasite species/types per component community. Although parasite host specificity cannot be ruled out, our results indicate that tadpole size was the primary factor determining parasite abundances and intensities. However, after controlling for species-specific differences in tadpole size, parasite life cycle strategy and host species were the major factors affecting tadpole parasite community structure.

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ELUCIDATING THE ROLE OF INBREEDING IN PARASITES: USING PEDIGREE RECONSTRUCTION DATA TO INFER TRANSMISSION, ASSESS INBREEDING DEPRESSION, AND PARTITION THE MATING SYSTEM

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Inbreeding is a critical evolutionary mechanism that not only alters genotype frequencies, but also magnifies the effect of drift, affects selection efficiency, and can have immediate fitness impacts on inbred offspring, i.e., inbreeding depression. In hermaphroditic species, rates of selfing and kin-mating impact inbreeding. Even though parasitic flatworms are one of the most species rich groups of hermaphroditic organisms, we know virtually nothing of their mating systems in nature. Hence, we lack an understanding of the role of inbreeding in parasite evolution. The natural mating systems of parasitic flatworms have remained elusive due to the inherent difficulty in generating progeny-array data in many parasite systems. New developments in pedigree reconstruction analyses allow direct inference of realized selfing rates in nature by simply using a sample of genotyped individuals. We built upon this advancement by utilizing the closed mating systems, i.e., individual hosts, of endoparasites. In particular, we created a novel means to use pedigree reconstruction data to estimate potential kin-mating rates in hermaphroditic parasites. With data from natural populations of a tapeworm, we show how our newly developed methods can be used to test for co-sibling transmission and inbreeding depression. We then show how independent estimates of the two mating system components, selfing and kin-mating rates, account for the observed levels of inbreeding in the populations. Thus, our results suggest that these natural parasite populations are in inbreeding equilibrium. Pedigree reconstruction analyses along with the new companion methods we developed will be broadly applicable across a myriad of hermaphroditic parasite species. As such, we foresee that a new frontier will emerge wherein the diverse life histories of flatworm parasites could be utilized in comparative evolutionary studies to broadly address ecological factors or life history traits that drive mating systems and hence, inbreeding in natural populations.

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HIGHLY DYNAMIC SCHISTOSOME BURDENS IN FREE-RANGING AFRICAN BUFFALO: SPACE AND TIME DRIVE SCHISTOSOME ACQUISITION WHILE HOST IMMUNITY, NUTRITION, AND COINFECTION DRIVE WORM LOSS

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Schistosomes are trematode parasites of global importance, causing infections in millions of people, livestock, and wildlife. Most studies on schistosomiasis, involve human subjects; as such, there is a paucity of longitudinal studies investigating parasite dynamics in the absence of intervention. As a consequence, despite decades of research on schistosomiasis, our understanding of its ecology in natural host populations is centered around how environmental exposure and acquired immunity influence acquisition of parasites, while very little is known about the influence of host physiology, coinfection and clearance in the absence of drug treatment. We used a 4-year recapture study in free-ranging African buffalo to investigate natural schistosome dynamics. We asked (i) what are the spatial and temporal patterns of schistosome infections; (ii) how do parasite burdens vary over time within individual hosts; and (iii) what host factors (immunological, physiological, co-infection) and environmental factors (season, location) explain patterns of schistosome acquisition and loss in buffalo? We found that microgeographic structure explained some variation in parasite burdens among hosts, indicating spatial transmission hotspots. Also, abundance was highly seasonal suggesting not only seasonal transmission, but also seasonal loss of worms. Gains in schistosome abundance in the dry season were partially offset by natural losses in the wet season, but overall, parasite burdens ratcheted up over time, suggesting a lack of acquired immunity as animals age. None of the host or co-infection parameters we measured affected

schistosome acquisition in individual buffalo. By contrast, variation in schistosome loss was associated with immunologic and nutritional factors, as well as co-infection by the gastro-intestinal helminth *Cooperia fuelleborni*. Our results demonstrate that schistosome infections are surprisingly dynamic in a free-living mammalian host population, and point to a role for host factors in driving variation in parasite clearance, but not parasite acquisition which is driven by seasonal changes and spatial habitat utilization. Our study illustrates the power of longitudinal studies in natural host populations for discovering mechanisms underlying parasite dynamics in individual animals and populations.

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A NEW SCHISTOSOME FROM NASAL TISSUE OF THE BLACK-NECKED SWANS, *CYGNUS MELANOCORYPHUS* (ANATIDAE)

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In the family Schistosomatidae, 9 schistosome species have been reported from nasal tissues of their hosts, 2 from mammals and 7 from birds, with all the avian representatives thus far placed in the genus *Trichobilharzia*. All previous reports of avian nasal schistosomes have been reported from the Eastern Hemisphere, 4 species from Rwanda (Africa), 2 from Australia (Oceania) and 1 from Europe/Middle East. A recent parasitological survey of *Cygnus melancoryphus*, an anatid endemic to South America, revealed the first adult nasal schistosome worms from the Western Hemisphere. To characterize this new species, worms were described both morphologically and genetically. Some worms were fixed in in hot formaldehyde 4% for morphological studies and others were stored in 96% ethanol for genetic analysis of the nuclear 28S and ITS as well as the mitochondrial *cox*1 gene regions. The combination of diagnostic morphological features does not match with any described avian schistosome genus. These worms have a filiform body with spiny tegument, a rounded posterior end, two muscular suckers, a robust but short gynaecophoric canal with thickened cross bands, and more than 100 testes. The longest worm fragment was 9.6 mm. The genetic results confirm their distinctiveness and place them at the base of the large avian schistosome clade, and not with the more commonly found genera, *Allobilharzia*, *Anserobilharzia*, *Trichobilharzia*, *Dendritobilharzia* or *Gigantobilharzia*. In fact, these worms grouped with a previously undescribed clade, designated “lineage 1” of larval schistosomes from the Patagonian freshwater snail *Chilina gibbosa*. Chilinid snails comprise a distinctive family endemic to southern South America. This first reported nasal schistosome from the western Hemisphere represents a new genus, with both its definitive and intermediate hosts being species endemic to South America.

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LAND HO! FIELD OBSERVATIONS AND EXPERIMENTATION ON A NEW *GORDIUS* SP. (NEMATOMORPHA: GORDIIDA) WITH THE FIRST DOCUMENTED TERRESTRIAL LIFE CYCLE FOR THE PHYLUM

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All gordiids have complex life cycles but are considered aquatic in their free-living phase. However, recently we discovered a new *Gordius* sp. in Oklahoma which occurs in terrestrial habitats. To investigate the transmission of this *Gordius* sp., during 2014-2017 a total of 1,455 adult free-living worms were collected from lawns, open fields, and road gutters from 20 sites in Payne Co., OK. Additionally, field observations based on the presence of sperm drops on free-living female worms indicated that most worms mated within 24 hours of appearing on lawns, but disappear within days of being observed in these habitats. Finally, surveys of earthworms and land snails from locations where adult free-living worms were observed indicated they were commonly infected with *Gordius* type cysts suggesting gordiid larvae are present in the soil. To test our field observations, we performed comparative laboratory assays on egg laying behavior of the new *Gordius* sp. collected from terrestrial environments and the aquatic gordiid, *Paragordius varius*. As expected, both gordiid species deposited egg strings when female worms were placed in water. However, when worms of both species were placed on soil, all individuals of the aquatic *P. varius* died and dried up; whereas 80% of the *Gordius* sp. individuals collected from terrestrial habitats burrowed within minutes into the soil. More importantly, some female *Gordius* sp. began depositing egg strings within days of burrowing in the soil. Examination of the eggs of the new *Gordius* sp. indicate they are unlike the eggs of any other hairworm species and contain double membranes suggesting these eggs may be resistant to desiccation. Taken together, our observations on the common occurrence of free-living worms in terrestrial habitats where worms mate, the ability of female hairworms to burrow in the soil where they deposit double membraned eggs, and *Gordius* type cysts being present in terrestrial invertebrates, strongly suggest that this species represents the first documented hairworm species with a terrestrial life cycle.

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A PARASITE SURVEY OF LIZARDS ON ANDROS ISLAND, BAHAMAS: DO *ANOLIS* ECOMORPHS HOST DIFFERENT ASSEMBLAGES OF PARASITES?

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Lizards are one of the most abundant terrestrial vertebrates on Bahamian islands, yet few studies have surveyed these hosts for parasites. The 4 species of *Anolis* on the island occupy different ecological niches and have different body types, and thus are considered different ecological morphotypes (ecomorphs). In addition to completing a baseline survey, we hope to determine if *Anolis* ecomorphs host different assemblages of parasites or if parasite species are shared among ecomorphs. In March 2015 and 2017, we conducted a parasite survey to ascertain the prevalence, abundance, and distribution of parasites within 7 species of lizards commonly found on Andros Island, Bahamas: *Ameiva auberi, Anolis angusticeps, Anolis carolinensis, Anolis distichus, Anolis sagrei, Hemidactylus frenatus, and Leiocephalis carinatus*. We collected lizards from three regions of the northern section of Andros Island, then dissected and examined hosts for parasites at ForFar Field Station. We also made blood smears at ForFar. We identified parasites at Florida Southern College, and we calculated prevalence, mean abundance and intensity for all parasite species. Our preliminary results suggest that the nematodes *Cyrtosomum* sp. and *Spinicauda spinicauda* were the most common parasites in lizards, whereas no blood protozoans have been located to date. We will discuss differences between parasite parameters among locations, host species, and years. Overall, our study suggests that *Anolis* ecomorphs have different parasite assemblages, although some parasites are shared among most anoles.

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PREVALENCE OF *TRYPANOSOMA CRUZI* WITHIN MAMMALIAN MUSEUM SPECIMENS IN WEST CENTRAL TEXAS

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Trypanosoma cruzi is a protozoan parasite and the causative agent of Chagas Disease. The life cycle of *T. cruzi* involves a dynamic relationship among Triatomine vectors (Hemiptera: Reduviidae), reservoir hosts, and humans. The presence of reservoir hosts is one of the many risk factors that can influence human risk of Chagas. While there have been a wealth of studies on the subject of *T. cruzi*, including descriptions of vector distribution and natural reservoir hosts, the West central region of Texas is sorely lacking survey results. Thus, the overall objective of this study is to assess the prevalence of infection with *T. cruzi* in reservoir hosts by utilizing museum mammalian host tissue collected from 8 counties of west central Texas. DNA was extracted from frozen liver tissue deposited in the Angelo State Natural History Collection and analyzed using *T. cruzi*-specific primers (TCZ1, TCZ2, TCZ3, and TCZ4) in nested PCR. Temporal and geographical correlations between *T. cruzi* prevalence and the various species of mammalian reservoirs will be discussed.

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CHRONIC *CYTAUXZOON FELIS* INFECTIONS IN WILD CAUGHT BOBCATS (*LYNX RUFUS*)

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Cytauxzoon felis is an intraerythrocytic apicomplexan of felids native to the United States. Infection in domestic cats (*Felis catus*) can result in the highly fatal disease cytauxzoonosis. The lone star tick (*Amblyomma americanum*) and the American dog tick (*Dermacentor variabilis*) are competent vectors of *C. felis*. Bobcats (*Lynx rufus*) are the natural wild animal reservoir of *C. felis*. Domestic cats and bobcats that become infected with *C. felis* and survive initial infection are thought to remain subclinically infected for the remainder of their lives. There is, however, no conclusive evidence that this occurs in wild bobcats, as this would require capture of live bobcats and subsequent recapture of the same individuals. In this study we live-trapped bobcats over a period of 3 years (2015, 2016, and 2017). During this study we recaptured 4 bobcats for 2 consecutive years and 1 bobcat for 3 consecutive years. This represents a unique, multi-year collection of samples from wild caught bobcats. These bobcats were all infected with *C. felis* at the initial capture and at the subsequent recapture(s). Theses bobcats were both polymerase chain reaction (PCR) positive and had positive identification of piroplasms on blood films. This represents the first evidence of multi-year infection of *C. felis* in wild bobcats. These data show that bobcats can sustain *C. felis* infection for years with important implications for the epizootiology of this emerging feline disease.

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ZOONOTIC AND ENZOOTIC PATHOGENS FROM WILD PIGS IN SOUTHERN OKLAHOMA

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Wild pig (*Sus scrofa*) populations in the United States are a concern because of their increasing numbers and expanding range. Wild pigs pose a threat to the environment, economy, and human health and safety. One specific area of concern is the potential of wild pigs to harbor infectious pathogens that can be spread to humans and livestock. As part of an ongoing abatement project, wild pig serum samples from Oklahoma, USA are screened for antibodies against zoonotic pathogens such as brucellosis (*Brucella* spp.), Chagas disease (*Trypanosoma cruzi*), and tularemia (*Francisella tularensis*), as well as two enzootic pathogens, porcine reproductive and respiratory syndrome virus (PRRSV) and pseudorabies virus (PSRV). Enzyme-linked immunosorbent assays (ELISA) are used to detect antibodies for *T. cruzi*, PRRSV, and PSRV. Rose Bengal card tests are used to detect the presence of antibodies for *Brucella* spp. and slide agglutination testing are performed to detect the presence of antibodies for *F. tularensis* and confirmed by tube agglutination. Overall, *Brucella* spp. antibodies were detected in 4.9% (24/489), *T. cruzi* in 31.8% (54/170), *F. tularensis* in 9.4% (16/170), PRRSV in 0.4% (2/481), and PSRV in 23.2% (105/452) of our samples. Identifying infectious pathogens in wild pig populations is necessary for local, state, and federal governments to establish appropriate legislative regulations addressing transport and sale. Modeling the dissemination of pathogens to other animals, as well as through abiotic medium, may expand the knowledge base for invasive species, and help reduce the public health concerns associated with wild pigs.

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EFFECT OF *CYSTOISOSPORA SUIS* INFECTION ON ANTIBODY AND CYTOKINE RESPONSES IN IMMUNE COMPETENT WEANERS

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Cystoisospora suis, a coccidian parasite causing diarrhea specifically in neonates, induces marked economic losses in piglet production. It is thought that the cellular immune system plays a role in the control of coccidiosis but the transfer of *C. suis*-specific colostral antibodies from sows to their offspring was shown to provide partial protection against infections. This study aimed to analyze the host's immunological control measures against *C. suis* and to identify markers for immune protection. It moreover aimed to optimize the antigen delivery to pregnant sows to improve passive protection of piglets. Sixty immune competent weaners were orally infected at different frequencies with different doses of oocysts or merozoites and compared to non-infected pigs. Until slaughter, blood was drawn weekly and parasitological parameters were evaluated daily. IgG and IgA titers in blood serum and intestinal mucus against two stages of *C. suis* were determined by IFAT. Relative-quantitative real-time PCR was used to measure the mRNA expression of cytokines (TNF-α, IFN-γ, TGF-β, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-27) in white blood cells, spleens and mesenteric lymph nodes. Infections significantly increased serum titers and resulted in a seroconversion within the first two weeks post infection for most groups, with IgG against merozoites displaying the most pronounced increase. Concentrations of specific mucus antibodies were insignificant. Cytokine profiles varied highly between individuals and differed only slightly between groups, not indicating a clear trend. Thus, the local immune system, primarily the cellular components, might play a more relevant role than systemic responses. From the current perspective we conclude that serum antibodies could serve as markers and that the infection mode does probably not severely influence the immune reaction. To further study cellular immune responses, cytokines in serum samples and supernatants of peripheral blood mononuclear cells, splenocytes and lymphocytes stimulated with merozoite antigen will be analyzed.

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COMPARATIVE ANALYSES OF IMMUNE RESPONSES IN LARVAE AND ADULTS OF THE MOSQUITO, *ANOPHELES GAMBIAE*

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Mosquitoes are holometabolous insects that encounter pathogens during all stages of their life cycle. Mosquito larvae exist under the constant threat of infection from the microbes that inhabit their aquatic environment. By contrast, mosquito adults likely encounter pathogens less frequently during interactions in their terrestrial environment, including blood feedings. Because of differences in the ecology of these two life stages, and because the reproductive potential of larvae rests entirely ahead of them, we hypothesized that larvae are better equipped to survive a microbial infection than adults. To test this hypothesis, we infected larvae and adults with bacteria and measured the bacterial load in the hemocoel 24 hours later. We found that fourth instar larvae are better equipped at killing bacteria than adults. To uncover the mechanisms behind this phenotype, we measured various immune parameters, including the number of circulating and sessile hemocytes, the phagocytic ability of hemocytes, the antimicrobial and melanization activity of hemolymph, and the infection-induced transcription of immune-related genes. Overall, we found higher cellular and humoral immune proficiency in larvae when compared to adults.

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INTESTINAL META-TRANSCRIPTOME COMPARISON REVEALS DISPARATE ANTIVIRAL TRANSCRIPTIONAL RESPONSE AND ITS ASSOCIATION WITH MITOCHONDRIA IN CHICKEN IMMUNITY DEVELOPMENT

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Background: Availability of a large number of data sets in public repositories and the advances in integrating multi-omics methods have greatly advanced our understanding of biological organisms and microbial associates, as well as large subcellular organelles, such as mitochondria. Mitochondrial organelles have an important role for understanding antibiotic-host interactions and cellular immune response development. Here, we collected publicly available data sets of intestinal microbial community transcriptomic profiling of healthy broiler chickens from hatch to market age as well as from their hens in order to investigate several important antiviral/antibacterial genes from the mitochondria and host change. Results and conclusion: Breeder source influenced the mitochondrial gene expression only at hatch, with no statistically significant effect on the gene expression at 2 weeks. After 4 weeks (4w), there was a significant variation in mitochondrial gene expression between the monitored and control flocks. At hatch, cytochrome c oxidase subunit (*cox*-1 and *cox*-3), the main subunit of cytochrome c oxidase complexes, was expressed at significantly higher rates than at 2 weeks (2w), but NADH dehydrogenase subunit 6 (*ND6*) was lower. The significant change of *cox*-1 gene expression among samples of 4w-c1, 4w-c3, 4w-c4, 6w-c1, and 6w-c4 (for individual chickens in the control groups at 4-weeks or 6-week age) may contribute to the structural changes of *cox*-1 gene with age. For hens, the most up-regulated immune response transcripts included: linker protein for immunoglobulin and μ polypeptides precursor (*IGJ*, NP_989594), polymeric immunoglobulin receptor precursor (*PIGR*, NP_001038109), and immunoglobulin-like receptor (*CHIR-IG1-5*, NM_001031331). While the most down-regulated immune-response transcripts detected for hens were:leucine rich repeat and Ig domain containing 1 (*LINGO1*, UniProtKB/Swiss-Prot: Q50L44) and *CDC42* effector protein (*CDC42EP1*,HGNC:17014). Whereas for chicks at hatch, toll-like receptor 21 (*TLR21*) and late endosomal/lysosomal adaptor MAPK and MTOR activator 2 (*LAMTOR2*) were the most up-regulated immune-response genes. In this study, several important antimicrobial genes in chicken mitochondria were identified in correlation with immune-response gene transcripts, such as *MavS*, a mitochondrial antiviral-signaling protein. Our analysis also identified that the antiviral response is present in chickens during their early development as we detected

induction of host immune response genes at hatching in the meta-transcriptome samples. These data provide new knowledge to support an age-based strategy for viral attenuation and vaccine development in poultry.

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IN VITRO MORTALITY OF PROTOSCOLECES IN THE PRESENCE OF CELLS AND SERA FROM *ECHINOCOCCUS GRANULOSUS* INFECTED OR NON-INFECTED DOGS

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It is well known that cell mediated immune response plays an important role in regulating the helminth infection but in relation to *Echinococcosis* the bulk of the investigations concerns humans and other intermediate host. Not much information is available on the CMI response of the definitive hosts against *Echinococcus*. Here we report some data using the microtiter plate direct assays of killing protoscoleces of *Echinococcus granulosus,* which provided an excellent system for investigation of larvicidal events. Incubation of protoscoleces in the medium containing infected or fresh normal serum or cells or a combination of these and/or cells alone collected from non-infected animals resulted in the production of a very interesting results. A sharp increase in the percent killing of the protoscoleces was noticed by the cells collected from infected animals. The percent mortality of the protoscoleces was more than 71% in the presence of the immune cells from infected animal. A further increase in the level of killing of protoscoleces was brought about by the mixture of cells and sera from infected dogs. When the protoscoleces were incubated with normal dog cells, only 9.16% of the larvae were killed. Increasing the number of cells per protoscolex did not appreciably affect the percent killing ($p=0.9$). Incubation of the protoscoleces in normal serum was not much different from that of the normal cells. Based on the results of this preliminary study on the *in vitro* effect of the cells and sera, it can be concluded that the killing of the protoscoleces, gradually increased with the concentration of the cells and serum and the effect was more pronounced in the infected animals than that of the control animals indicating a predominant role for cell mediated immunity during the infection of dog *with E. granolosus*. It can be summarized that the cells from infected animals in the presence of the serum killed significant number of protoscoleces indicating the involvement of specific antibody or complement dependent cell mediated cytotoxicity. The details of these results will be presented during the talk.

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DEVELOPMENT AND EVALUATION OF SOME SEROLOGICAL TESTS FOR THE DETECTION OF *TOXOPLASM*A INFECTION IN MULTIPLE ANIMAL SPECIES

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Toxoplasma gondii is a zoonotic protozoan parasite which can cause significant disease in most warm blooded animals including humans. Surveillance testing of human and animal populations is essentail to estimate disease prevalence, assess food safety risks and establish control programs. Serological tests are the most practical means to detect the prevalence of the disease in a broad range of hosts. The modified agglutination test (MAT) is a sensitive serological method to detect *Toxoplasma* IgG antibodies in livestock and wildlife. An in-house MAT was developed using tachyzoites cultivated in tissue culture rather than in mouse conventional preitoneumand evaluated on samples of serum and meat juice from experimentally infected pigs. Samples were also tested by an in-house IFA and ELISA-A/G, as well as by

commercial ELISA-IgG and MAT. Comparative analysis of test results from these samples showed excellent agreement between all tests. Serum and/or blood samples from a variety of other domestic and wildlife host species, naturally or experimentally infected, were also tested by in-house and commercial MAT. High correlation between test results for most of these hosts was obtained. Therefore, the in-house MAT was subsequently used as a screening test to detect antibodies to *Toxoplasma* in meat juice samples collected from pigs from various provinces across Canada.

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PREVALENCE OF IGG ANTIBODIES TO *NEOSPORA* SPP. AND ASSOCIATED RISK FACTORS IN EQUIDS FROM SOUTHERN PUNJAB, PAKISTAN

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Neospora caninum is recognized as one of the most prevalent causes of abortion and neonatal mortalities in cattle. *Neospora hughesi*, the only other member of the genus, causes reproductive problems in horses. The antigens of these parasites are similar and cross reactive in most serological assays and the results are best reported as *Neospora* species when equine samples are used. There is limited data available on the prevalence of *Neospora* antibodies in equids worldwide. The purpose of current study was to describe seroprevalence of *Neospora* infection in equids from Pakistan. In this cross sectional study sera from 631 equids (324 horses, 218 donkeys and 89 mules) were examined from southern region of Punjab province, Pakistan to determine the prevalence of antibodies against *Neospora spp*. Antibodies to *Neospora* spp. were detected using a commercially available competitive ELISA (VMRD, Inc., Pullman, USA). IgG antibodies to *Neospora* spp. were found in 147 (23.3%) of 631 animals. The prevalence by host was 16.0%, 32.6% and 26.9%, for horses, donkeys and mules, respectively. Statistically, significant *(P<0.05)* differences in prevalence were observed among animal hosts with donkeys having the highest prevalence and the horses with the lowest. Highest prevalence was seen among animals raised in contact with ruminants (26.2%) and pets especially dogs (29.5%), indicating a significant difference *(P<0.05)* of prevalence between two categories (with and without presence of domestic ruminants and dogs). Seroprevalence was significantly *(P<0.05)* higher in castrated animals (33.6%) than female (23.83%) and male (18.9%) equids. The prevalence ranged in different breeds of horses from 31.4% (other breeds) to 3.7% (Morna) and in different purpose of use from 26.8% (draughting) to 12.4% (breeding). The prevalence rate was significantly *(P<0.05)* higher in female animals with history of early pregnancy loss 44.2% (19/43), and late pregnancy loss 30.6% (23/75) than that of female equids have had no exposure of pregnancy loss, 9.4% (9/96). Statistical analysis indicating a significant association between feeding style and protozoan infection; rearing shelter was also a significant risk factor for *Neospora* infection. No significant difference was noted among age groups and risk of *Neospora* infection. This study is the first report on serological survey of *Neospora* infection in equines from Pakistan.

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COPROELISA DETECTION OF *FASCIOLA HEPATICA* IN GOATS

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Fascioliasis is an economically important parasitic disease of small ruminants mainly caused by a trematods of the genus *Fasciola*. The most important species responsible for fascioliasis are *Fasciola hepatica* and *Fasciola gigantica*. The study was conducted during January 2016 to June 2016 for detection of fascioliasis in small ruminants in Multan. A total of 100 samples were collected randomly for examination of GIT parasites especially for prevalence of fascioliasis. The animals under study were selected randomly without any discrimination of sex, age and breed. During Coprological parasitological examination of goats of district Multan, the total number of positive cases of *Fasciola hepatica* was 33/100 (33%) in goats by sedimentation technique(s) and by direct examination of the slides 19/100 (19%) while through floatation method 25/100 (25%) cases were diagnosed respectively. During the study of commercial ELISA kits, for Copro-antigen, the positive cases of *Fasciola hepatica* was 41% and ELISA antibodies was 39% respectively. It is concluded that BioX ELISA of Copro-antigen and sero-ELISA have detected higher percentage of Fasciolasois as compared to fecal examination. Both ELISA kits had high sensitivity and specificity as well. The application of commercial ELISA can provide a reliable method for the early diagnosis of fascioliasis in small ruminants.

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EXPERIMENTAL AND MOLECULAR STUDY OF LARVAL TREMATODES IN PLANORBIDS FROM BRAZIL AND USA REVEAL A PUTATIVE NEW GENUS OF AVIAN SCHISTOSOME PRESENT IN THE AMERICAS, EUROPE AND AFRICA

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Despite the worldwide importance of avian schistosomes, the etiological agents of human cercarial dermatitis, global species diversity, life cycles and distribution of these trematodes are not well known. Studies related to the molecular phylogeny of schistosomes have advanced in the last decades, and have revealed several unnamed genera and species. In the present study, larval trematodes found in planorbids from Brazil (*Biomphalaria glabrata)* and USA (*Gyraulus parvus*) were used for morphological and molecular studies (28S, ITS and COI). The larvae obtained from *B. glabrata* were used in experimental infections of ducks (*Cairina moschata*), which 24 dpi resulted in finding schistosome eggs with a long polar filament in the faeces. No adult worms could be found. Eggs with this distinctive morphology were previously reported from naturally infected anatids from Brazil (*Schistosoma pirajai*) and South Africa (*Trichobilharzia* sp. 4), and in ducks experimentally infected with cercariae from *Anisus vortex* in Czech Republic (perhaps *Gigantobilhariza vittensis*). In North America, in addition to the larvae from *G. parvus*, an adult fragment was found in a wild anatid, *Aix sponsa,* but no eggs were found for comparison. The molecular phylogenetic analyses showed that the Brazilian and North American schistosomes are sister taxa, but these two clades do not group with any other clade or named genus. Interestingly, the available ITS sequences of the European sample show that it also belongs to this clade of American specimens, consistent with the egg morphology. The clade found here, plus the distinctive egg morphology suggest that these worms can be found globally. Although obtaining adult parasites are need to the formal description, some biological characteristics can be predicted for this new genus of avian schistosome: anatids and planorbids respectively as intermediate and definitive hosts, adults long and thin, eggs with a long polar filament, cercariae with relatively long furcae. The involvement of these parasites in cases of cercarial dermatitis cannot be ruled out.

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MOLECULAR PHYLOGENETIC ANALYSIS OF *DERMACENTOR PARUMAPERTUS* NEUMANN (ACARI: IXODIDAE) AND RELATED SPECIES

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Dermacentor parumapertus Neumann is an under-studied tick found on black-tailed jackrabbits (*Lepus californicus*) throughout arid regions of western North America. A unique strain of the human pathogen *Rickettsia parkeri* (causative agent of American boutonneuse fever) was recently isolated from this species. Geographically-associated variations in ornamentation were noted in a previous study, which encouraged us to further investigate geographically distinct groups within this species using molecular techniques. We extracted DNA from 45 *D. parumapertus* (12 Texas, 18 Utah, 6 Arizona, 1 Nevada), 6 *D. andersoni,* and 2 *D. occidentalis*. We amplified an ~615 bp fragment of COI, an ~350 bp fragment of 12S, and an ~560 bp fragment of COII (mtDNA), as well as an ~360 and ~370 bp fragment of ITS2 (nDNA). Both nDNA and mtDNA sequences were concatenated respectively. Trees were developed using Maximum Parsimony, Maximum Likelihood, and Bayesian analysis (BA). Concatenated nDNA BA preliminary results give strong support to a "Southern" clade (100%) with ticks collected from Texas, and a "Northern" clade (100%) containing non-Texas ticks separated into a Utah group (93%) and an "intermediate" group (92%) containing ticks from Arizona, Utah, and Nevada. Some *D. andersoni* and both *D. occidentalis* were grouped together with a posterior probability of 95%. Preliminary mtDNA BA results are inconclusive, supporting a weak basal Utah group, with another clade (83%) consisting of Texas, Arizona, Nevada, some Utah, and all *D. andersoni*. Further analysis is underway to elucidate these relationships, and investigate the possibility of relatively recent introgression, or incomplete lineage sorting

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TOWARDS A RESOLUTION OF THE INCERTAE SEDIS SPECIES OF THE PHYLLOBOTHRIIDEA AND RHINEBOTHRIIDEA

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Phyllobothriidea and Rhinebothriidea are two of the newest cestode orders, having been established in 2009 and 2014, respectively. Recent work has focused on resolving the status of the genera that belong to these orders and determining valid species for these genera. Species that are poorly known or inadequately described have been accorded *incertae sedis*, *species inquirendum*, *nomen dubium*, or *nomen nudum* status. Ruhnke et al. (in review) listed 29 *incertae sedis* species associated with the Phyllobothriidea, housed for now in one of the following genera: *Anthobothrium*, *Crossobothrium*, *Marsupiobothrium*, *Monorygma*, *Orygmatobothrium*, *Phyllobothrium*, and *Pithophorus*. Ruhnke et al. (in press) listed 36 *incertae sedis* species within the Rhinebothriidea, housed for now in one of the following genera: *Anthobothrium*, *Echeneibothrium*, *Phyllobothrium*, *Pillersium* and *Rhinebothrium*. Given our current understanding of these genera, most of these *incertae sedis* species are inappropriately placed. However, examination of the original descriptions of these species reveals that for many, proper generic placements can be made now that generic boundaries in the Phyllobothriidea are coming into focus. The generic placements of others are more problematic, and it appears that the establishment of new genera may be needed for some species. With respect to the 29 *incertae sedis* species of Phyllobothriidea, four are consistent with *Paraorygmatobothrium*, and two with *Orectolobicestus*. Seven species are likely members of the clade that includes *Alexandercestus, Hemipristicola, Nandocestus,*

Orectolobicestus, Paraorygmatobothrium, Ruhnkecestus, Scyphophyllidium and *Thysanocephalum*. Two species are consistent with *Monorygma* and one appears to be related to *Monorygma*. Eight species primarily from skates will be placed in one of two new genera. Three species may each need novel generic assignments. Two species are possibly rhinebothriideans. For the 32 *incertae sedis* species of Rhinebothriidea, 11 are consistent with *Anthocephalum*, eight with *Rhinebothrium*, four with *Stillabothrium*, two with *Rhodobothrium*, and one each with *Echeneibothrium*, *Paraorygmatobothrium* and *Spongiobothrium*. One *incertae sedis* rhinebothriidean is likely a proteocephalidean, and three are truly of uncertain placement. A combination of morphological and molecular investigation of present collections has provided a starting point for the resolution of these *incertae sedis* species. However, new collections from the type hosts will be needed for many in order to fully address their status.

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LOST (AND FOUND!) IN A SEA OF NOVELTY

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Historically, tapeworm species parasitizing skates have commonly been reported from multiple host species, resulting in an apparent pattern of low species diversity, low host specificity, and broad geographic distribution of these tapeworms. For example, *Echeneibothrium variabile*, has been reported from 8 skate species in 13 distinct localities across Europe and the USA. Furthermore, although skates represent a highly diverse group of elasmobranchs, their superficial morphological homogeneity has led to approximately half of the several hundred species being assigned to the single genus *Raja*. This may have reinforced the notion that skate tapeworms exhibit low species diversity and are able to parasitize several species of hosts, because their hosts were considered to be closely related. Advances in the systematics and taxonomy of skates over the past 25 years have included the generation of a robust elasmobranch phylogeny based on molecular data, which has greatly contributed to the revision and stability of the current status of skate taxonomy. The majority of the species once placed in *Raja* has now been assigned to one of the 35 other skate genera currently recognized. This revised systematic framework has led to questions about the apparent low species diversity, and therefore low host specificity, observed in their tapeworms. Necropsy of 24 skate species—19 for the first time—allowed us to examine questions of low diversity and host specificity in tapeworms, by focusing on, but not limited to, species of *Echeneibothrium*. In total, 15 of these species of skates hosted *Echeneibothrium* species. Contrary to previous work, 43 distinct morphotypes of *Echeneibothrium*, 34 of which are potentially new to science, were found. Sequencing of a subset of the morphotypes revealed that at least some *Echeneibothrium* species seem to track host phylogeny and are, in fact, highly host specific and restricted in distribution. A more accurate understanding of the taxonomy and systematics of skates and their tapeworms has allowed us to revise the patterns of host associations in this system, directing our efforts for more meaningful future research questions.

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PHYLOGENETIC POSITION AND DESCRIPTION OF A NEW SPECIES OF MEDICINAL LEECH FROM NORTH AMERICA

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A new species of medicinal leech is described using novel specimens collected in Maryland, South

Carolina, and Georgia (USA) and museum specimens from the collections of the National Museum of Natural History, Smithsonian Institution. Morphology of the specimens was examined with a stereomicroscope and internal morphology through dissection. *Macrobdella* species are distinguished and easily identifiable by the number and arrangement of copulatory pores on the ventral side of the body. Superficially, the new species is most similar to Macrobdella decora as both species possess 4 copulatory pores (2 rows with 2 pores each), yet the new species has 4 annuli between the female gonopore and the first pair of copulatory pores rather than 5 annuli as in *M. decora*. Scanning electron microscopy of the jaws revealed between 60 – 68 teeth on each of the lateral jaws similar to M. decora with approximately 65 teeth on each lateral jaw. Phylogenetic analyses (parsimony and maximum likelihood) based on mitochondrial cytochrome c oxidase 1 and nuclear 18S rDNA and 28S rDNA sequence data derived from the novel specimens supports the monophyly of the genus and recovered the new species as the sister taxon to *Macrobdella decora*. The geographic distribution of this species spans different biogeographic regions in eastern North America and overlaps the distribution of *Macrobdella ditetra*. This is the first description of a new macrobdellid species since the description of *Macrobdella diplotertia* in 1975 and suggests that cryptic diversity remains to be described from geographic regions historically under-sampled for *Macrobdella*.

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NEOALBIONELLA SP. FROM SKIN OF COMMON GULPER SHARKS (*CENTROPHORUS GRANULOSUS*) IN THE GULF OF MEXICO AND COMPARISON WITH *NEOALBIONELLA LONGICAUDATA*

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Little is known about the biodiversity of parasite component communities infecting the geographically widespread deepwater gulper sharks (Centrophoridae: *Centrophorus*). *Neoalbionella* (Copepoda: Siphonostomatoida: Lernaeopodidae) comprises 7 nominal species that infect skin and gill of sharks (Elasmobranchii) representing 2 orders (Squaliformes; Carcharhiniformes), 4 families (Centrophoridae; Etmopteridae; Squalidae; Scyliorhinidae), 6 genera (*Centroscyllium*; *Centrophorus*; *Etmopterus*; *Apristurus*; *Squalus*; *Scyliorhinus*), and 8 species. Each species of *Neoalbionella* is reportedly highly host specific (infecting a single host). An exception to this is *Neoalbionella longicaudata*, which infects the leafscale gulper shark (*Centrophorus squamosus*; type host; North Atlantic Ocean off Iceland); common gulper shark (*Centrophorus granulosus*) and dwarf gulper shark (*Centrophorus atromarginatus*) (both Pacific Ocean off Japan). In March 2015, we collected 44 female and 18 male lernaeopodids that resemble *N. longicaudata* from the skin of 10 of 11 (90.1% prevalence; 6.2 mean intensity) common gulper sharks captured using longline gear in the northeastern Gulf of Mexico off Florida. Although the original description of *N. longicaudata* was incomplete and a redescription based on its type materials is needed, several features of the newly-collected lernaeopodids were distinctive or absent from the description of *N. longicaudata*. Females of *Neoalbionella* sp. had a first antenna with 6 setae, a second antenna with a smooth exopod tip, and the mandibular formula P1, S1, P1, S1, P1, S1, B5. Males had a conspicuous mediative process. This is the first report of a species of *Neoalbionella* from the Gulf of Mexico and contributes to our knowledge of parasites that infect seldom-examined deepwater gulper sharks.

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TURTLE BLOOD FLUKES (DIGENEA: SCHISTOSOMATOIDEA: *HAPALORHYNCHUS* SPP.) INFECTING SOUTHEASTERN MUSK TURTLES (TESTUDINES: KINOSTERNIDAE)

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As part of an ongoing survey of freshwater turtle blood flukes (TBFs), we sampled four musk turtle (Testudines: Kinosternidae) species from rivers in Alabama and Florida: the loggerhead musk turtle (*Sternotherus minor*), the Eastern musk turtle (*Sternotherus odoratus*), the stripe-necked musk turtle (*Sternotherus peltifer*), and an innominate loggerhead musk turtle (*Sternotherus* cf. *minor*). This survey resulted in the discovery of infections by *Hapalorhynchus reelfooti* (ex. *S. minor*, *S. peltifer*, *S.* cf. *minor* [all comprising new host and locality records], *S. odoratus* [new locality records]), *Hapalorhynchus* cf. *stunkardi* (ex. *S. minor*, *S. odoratus*), and a new species of *Hapalorhynchus* (ex. *S.* cf. *minor*). *Hapalorhynchus* cf. *stunkardi* differs from *Hapalorhynchus stunkardi* by having a longer forebody, shorter ceca, and smaller testes. The new species closely resembles *H. stunkardi* but can be differentiated by having a smaller ventral sucker, shorter ceca, nearly same-sized testes, a cirrus sac not abutting the dextral cecum, and a uterus dorsal to the ovary and anterior testis. Comprising donated material and examinations of 19 turtle species (11 never-before-reported TBF hosts) from rivers in Alabama (no previous TBF records), Florida, Mississippi, Malaysia, Vietnam, and Peru (first adult freshwater TBF records from South America), the ongoing TBF survey has resulted in the identification and collection of 28 TBF species (14 new species) assigned to *Spirorchis*, *Hapalorhynchus*, *Vasotrema*, *Coeuritrema*, and *Baracktrema*.

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TAXONOMY OF NORTH AMERICAN SPECIES OF *ACIPENSERICOLA* (DIGENEA: APOROCOTYLIDAE), BLOOD PARASITES OF STURGEONS AND PADDLEFISH (ACIPENSERIFORMES)

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The monophyletic fish blood flukes (Aporocotylidae) comprise ~148 species of 36 genera infecting freshwater, marine, and estuarine fishes. Freshwater fish blood flukes are little studied compared to marine species, but those of North America's inland fishes have essentially been ignored aside from a few papers published in the 1950's and 1980's. Collectively, 8 species of *Sanguinicola* infect basses (Centrarchidae), minnows (Cyprinidae), trouts (Salmonidae), and perches (Percidae). *Acipensericola petersoni* infects the heart of American paddlefish, *Polyodon spathula*, and is the only named aporocotylid to infect a paddlefish or sturgeon (Acipenseriformes) anywhere. We opportunistically sampled the heart of >150 lake sturgeon (*Acipenser fulvescens*) harvested during the annual recreational ice spearfishery in the Lake Winnebago System during Feb 2001, 2008, 2014, and 2017. A few adults and numerous juveniles of a blood fluke were collected and assigned to *Acipensericola* based on the presence of spike-like tegumental body spines arranged in ventrolateral rows, a large bowl-shaped anterior sucker, inverse U-shaped ceca, a column of intercecal testes, and an intertesticular ovary. Light and scanning electron microscopy of these specimens plus sequence data from the large subunit ribosomal DNA (28S) were pending at abstract submission. However, specimens of *Acipensericola* sp. are strikingly similar to those of *A. petersoni*. Given their hosts' geographic (Mississippi River vs. Great Lakes) and phylogenetic (Polyodontidae vs. Acipenseridae) separation, the level of morphological and molecular divergence

between their blood flukes is of interest regarding natural history, biogeography, and biodiversity of North American freshwater fish parasites.

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MYXOBOLUS CEREBRALIS (ETIOLOGICAL AGENT OF "WHIRLING DISEASE") INFECTS NORTH CAROLINA TROUTS (SALMONIDAE)

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The myxozoan parasite *Myxobolus cerebralis* was introduced into North America in the mid-twentieth century with infected trout imported from Europe and is now recorded in 24 states and 26 countries. This parasite is the causative agent of whirling disease; an economically and ecologically devastating disease of salmonids. Heavily-infected (diseased) fishes exhibit "whirling" behavior (tail chasing, disequilibrium, erratic swimming) plus skeletal and pigment abnormalities that are obvious to and can alarm anglers. Microscopy and molecular biology were herein used to provide the first documentation of infections of *M. cerebralis* in trouts from North Carolina river basins. A total of 1,085 rainbow trout (*Oncorhynchus mykiss*), 696 brown trout (*Salmo trutta*), and 319 brook trout (*Salvelinus fontinalis*) from 43 localities across 9 river basins were screened. Myxospores were observed microscopically in pepsin-trypsin digested heads of rainbow trout and brown trout from the Watauga River Basin. Those infections were confirmed using the prescribed nested polymerase chain reaction (PCR, 18S rDNA), which detected infections also in rainbow trout, brown trout, and brook trout from the French Broad River Basin and the Yadkin Pee-Dee River Basin. Myxospores were 9.0–10.0 μm (mean=9.6 ± SD=0.4; N=119) long, 8.0–10.0 (8.8 ± 0.6; 104) wide, and 6.0–7.5 (6.9 ± 0.5; 15) thick and had polar capsules 4.0–6.0 (5.0 ± 0.5; 104) long, 2.5–3.5 (3.1 ± 0.3; 104) wide, and with 5 or 6 polar filament coils. Myxospores from these hosts and rivers were morphologically indistinguishable, indicating conspecificity, and the resulting 18S rDNA sequences derived from these myxospores were 99.5–100% similar to published GenBank sequences ascribed to *M. cerebralis*. This report comprises the first taxonomic circumscription and molecular confirmation of *M. cerebralis* in the southeastern United States south of Virginia. Surveillance for infections by *M. cerebralis* in North Carolina trouts is ongoing.

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SALIVARY TRANSCRIPTOMICS AND ANTICOAGULANT DIVERSITY OF LEECHES

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Bloodfeeding leeches require potent anticoagulants to prevent blood from clotting while feeding and during their long digestion periods. Accordingly, leeches have evolved a diverse and powerful suite of anticoagulants and bioactive salivary proteins. These powerful anticoagulants have made leeches particularly useful for plastic and reconstructive surgeries; however, few leech species have been surveyed for these important proteins. We sequenced salivary transcriptomes using next generation sequencing to determine the repertoires of transcribed anticoagulants in several families of leech that vary in their relatedness and prey choice. We then assessed the selection pressures and phylogenetic relationships of these proteins. Leeches were found to transcribe a large variety of anticoagulants, which exhibited substantial differences even between closely related species: indeed many species transcribe proteins not expressed by other leeches in the same family or even genus. A number of these anticoagulants were not

previously known from leeches, but instead from venomous snakes such as the king cobra (e.g., ohanin). It is even clear that non-bloodfeeding leeches transcribe anticoagulants in their salivary tissue, despite their switch to predatory lifestyles. Common leech anticoagulants were often under positive selection for a number of species (e.g., *Ozobranchus*, which feeds on sea turtles). Furthermore, several species had phylogenetically divergent protein sequences (e.g., leeches that prey on sharks).

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THIS IS GETTING RIDICULOUS! UNPRECEDENTED DIVERSITY FOUND IN MITOCHONDRIAL GENOMES OF A TURTLE PARASITE, *HAEMOGREGARINA BALLI*

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Haemogregarina is a genus of the suborder Adeleorina (Apicomplexa). These are heteroxenous blood parasites that infect glossiphoniid leeches as definitive hosts and amphibians, fish and aquatic reptiles as intermediate hosts. In 2001, Siddall and Desser observed that the various *Haemogregarina* species infecting North American freshwater turtles were morphologically indistinguishable and consequently synonymized these to a single species, *Haemogregarina balli*. Molecular characterization could be a useful for distinguishing cryptic species among these morphologically indistinguishable parasites. *Haemogregarina balli* obtained from various turtle host species were characterized genetically using nuclear (nu) and mitochondrial (mt) DNA sequences. Initially, DNA was isolated from the blood of a common snapping turtle (*Chelydra serpentina*) from Ontario infected with *H. balli*. A single-species adeleorinid parasite infection was assumed to be present based on morphological features of intraerythrocytic gamonts. Polymerase chain reaction and Sanger sequencing were used to generate a partial nu ribosomal 18S sequence (1091 bp) that contained 11 single nucleotide polymorphisms (SNPs). Surprisingly, from the same DNA sample, 3 complete parasite mt genomes (~6100 bp) with remarkably high pairwise differences of 20-30% were obtained. Further parasite collection was performed in Ontario (Canada), Pennsylvania and Texas (U.S.A.) generating DNA from *H. balli*-positive blood samples collected from *C. serpentina* (n=11), *Macrochelys temminckii* (n=1), *Chrysemys picta* (n=16), *Trachemys scripta* (n=2), *Sternotherus odoratus* (n=3) and *Glyptemys insculpta* (n=2). Preliminary analysis revealed partial nu 18S sequences (~500 bp) containing SNPs and overall pairwise differences of 4-6% between isolates. Partial mt sequences (~500bp) have identified 3 additional unique mt genotypes with pairwise differences of 20-30% among the 6 mt genotypes found thus far. Many of the isolates contained 2 or 3 genotypes concurrently with no apparent association of mt genotypes to particular turtle host species.

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A NEW PCR TOOL FOR DETECTING *BABESIA DUNCANI* IN NATURALLY INFECTED TICKS

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Human babesiosis is an important emerging tick-borne disease in North America. Most documented cases are caused by the protozoan parasite, *Babesia microti*, in the Eastern and Midwestern United States. In the Pacific Coastal region, rare cases of human babesiosis have been ascribed to *Babesia duncani*, first

identified in 1991, as well as to closely related *Babesia* spp. infecting wildlife. While the tick vector and mammalian reservoir hosts for *B. microti* are well characterized, the vector and reservoir hosts for *B. duncani* are unknown despite previous investigations of several human-biting ticks and wild rodents. Identification of potential hosts and vector species has been hampered by the lack of a specific and sensitive molecular diagnostic tool that can identify piroplasm species and does not co-amplify other eukaryotic DNA. We developed a PCR assay targeting the beta-tubulin gene, a highly variable locus in other related piroplasms (Cacciò et al. 2000). Using this assay, we spiked tick DNA extracts with a *B. duncani* isolate derived from a human patient (WA-1) and with related *Babesia* spp. from Californian wildlife. This assay was highly specific, with a sensitivity of $2.27x10^{-12}$ pg/ul template DNA. This level of detection was sensitive enough to detect *B. duncani* in larval ticks, and it offers researchers a new tool for elucidating the natural transmission cycle of *B. duncani*.

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DEVELOPMENT OF MOLECULAR ASSAY FOR DETERMINING THE VIABILITY OF *EIMERIA* SPECIES OOCYSTS

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J. Brisbin and **K. Moore-Dorsey**, Ceva Animal Health
J.R. Barta, University of Guelph

Eimeria spp. (phylum Apicomplexa) are the causative agents of coccidiosis, a disease of particular importance to the poultry industry. Disease severity is a function of host immunity and quantity of viable oocysts ingested. Infection by *Eimeria* spp. is self-limiting and highly immunogenic, providing species-specific protection against future infection. Establishment of subclinical yet immunogenic infection through administration of live oocysts is therefore an efficient and widely-used strategy for the control of coccidiosis in commercial operations. Administration of appropriate numbers of oocysts, for which viability must be known, is critical to live vaccines efficacy. Oocyst viability cannot be determined morphologically, and current gold-standards for the determination of viability require time-consuming and costly *in vivo* infection trials. We have developed molecular assay that shows promise for the determination of *Eimeria* spp. oocyst viability based on the transcript abundance of α-actin assessed using a 2-step reverse transcriptase and qPCR (RT-qPCR) process. A strong correlation between α-actin transcript abundance and oocyst viability was observed in preliminary trials; absolute quantification data measured α-actin transcript abundance in non-viable oocysts at less than 5% of viable (stored <2 months) oocyst controls. Optimization of the assay is continuing using samples of oocysts from a single *Eimeria* species. However, the qPCR primers have been designed to amplify the same target from all *Eimeria* spp. that infect chickens; consequently, the current assay may require little modification to work with all these parasites of chickens. Future research will explore the application of next-generation sequencing (NGS) technologies to permit quantification of transcript abundance of individual species within a mixed species sample; combining RT-qPCR with NGS may ultimately permit simultaneous enumeration and viability of individual *Eimeria* spp. within a sample containing multiple parasite species.

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DOES MOSQUITO SALIVA CONTAIN DNA THAT COULD BE RELEVANT TO SURVEILLANCE STRATEGIES?

J.G. King, Mississippi State University

Emerging mosquito-vectored diseases, such as Zika virus, remain a worldwide threat. Novel tools that facilitate the tracking of such diseases could help us understand their spread and possibly help save lives. One such tool that has been recently pioneered is the use of sugar-bated FTA cards, coupled with real-time RT-PCR (qPCR), for the detection of viral particles that are secreted in the saliva of wild *Aedes* mosquitoes. We hypothesize that if apocrine secretion occurs at a sufficient rate in the mosquito salivary glands, as reported in *Drosophila*, that we will be able to detect mitochondrial DNA in the saliva via sensitive molecular techniques such as PCR. We used artificially-induced salivation and "bite-blot" assays on FTA cards coupled with real-time PCR to test for the secretion of mitochondrial DNA in a viral vector mosquito, *Aedes albopictus*. For quantitative PCR, we targeted a small (<150 base pair) region of COI to increase sensitivity to degraded DNA and several methods of DNA collection and concentration were compared for efficiency. Both melt curve and Standard curve analyses suggested that the primer pairs designed as part of this work are specific and effective. Results suggest that detectable amount of mitochondrial DNA are normally released into saliva. Ongoing experiments will also be discussed that aim to determine whether nuclear DNA can be detected in the saliva and if antibody-based assays targeting nuclear proteins show that nuclear components are released into the mosquito saliva.

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MOLECULAR ANALYSIS OF IMMUNOMODULATORY COMPONENTS IN THE BED BUG SALIVA

T.C. van Warmerdam, **A.L. Drury**, **J. Goddard** and **J.G. King**, Mississippi State University

The resurgence of the bed bug, *Cimex lectularius,* in the United States exemplifies its role as a persistent parasite. Their potential for infestation and the cutaneous and systemic reactions resulting from their bites warrants further investigation into the mechanisms underlying this essential component of their lifecycle. Several previous studies have addressed the *Cimex* salivary transcriptome and proteome and have identified a salivary Nitrophorin protein as a putative factor mediating host inflammation responses. However, no massively parallel sequencing based project has been published for the salivary transcriptome, and with the recent completion of the bed bug genome, the time is right for such a study to be conducted. Through Illumina-based RNA-seq analyses using the new Harlan strain *Cimex* genome as a reference, we are currently collecting and analyzing interstage salivary transcriptome data on *C. lectularius*. Our primary aim is to define the genes critical for the bedbugs' unique ability to serve as a persistent nuisance species. Data from a parallel project will also be presented, in which we are working to silence or knock-out a major component of the bed bug sialome, Nitrophorin. Our ultimate goal in silencing Nitrophorin is to use human-based bioassays to test for this protein's involvement in inflammation. Collectively, this data will offer new insights into the evolutionary origin and functionality of the bed bug salivary transcriptome.

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DECIPHERING THE ORIGIN OF THE NOVEL MORPHOLOGY OF *LITOBOTHRIUM AENIGMATICUM* USING GENOMICS AND TRANSCRIPTOMICS

K. Gallagher, **J. Caira** and **J. Wegrzyn**, University of Connecticut

This study begins to examine the evolution of *Litobothrium aenigmaticum*, a bizarre tapeworm that parasitizes pelagic thresher sharks in Taiwan and Mexico. Although phylogenetically nesting robustly within the genus *Litobothrium*, this species looks nothing like its congeners. This study will use comparative genomics and transcriptomics to investigate gene sequence, expression, or regulation differences that might account for this novel morphology. Living specimens of *L. aenigmaticum*,

Litobothrium daileyi, and *Litobothrium amplifica* were collected from 2 pelagic thresher sharks in Taiwan. A subset of each was preserved in 95% ethanol and a subset in RNAlater. Small insert (i.e. 350 and 550 bp in length) libraries were generated for genomic sequencing of all 3 species. Mate-pair libraries, 2 and 7 kb in length, were also prepared for *L. aenigmaticum*. Rough estimates of genome sizes, obtained using the program *Jellyfish*, were 350 Mb for *L. aenigmaticum*, 360 for *L. daileyi*, and 390 Mb for *L. amplifica*. Following removal of low quality and/or short reads, using *Sickle* for the small insert libraries and *Trimmomatic* the mate-pair libraries, reads were assembled using *SOAPdenovo* and *ABYSS2*. Assembly quality was evaluated using *QUAST*. Genome scaffolding was done using *SSPACE* and quality again evaluated using *QUAST*. These refined assemblies yielded genome size estimates of 330–360 Mb for *L. aenigmaticum*, 345–349 Mb for *L. daileyi*, and 345–350 Mb for *L. amplifica*. Our results indicate that *Litobothrium* genomes are more than twice the size of all published cyclophyllidean genomes, which range from 115–141, but are only one-third the size of the current 1.2 Gb estimate for a diphyllobothriidean species. RNA extraction and library preparation are currently underway. Preliminary RNA yields indicate that transcriptome libraries can be generated from a single specimen of *L. aenigmaticum*, but specimens of *L. daileyi* and *L. amplifica* may need to be pooled. Once sequenced the RNAseq libraries will be mapped back to the assembled genomes for differential gene expression analyses.

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GENERATION ASSEMBLY AND ANNOTATION OF WHOLE GENOME SEQUENCES OF *CYCLOSPORA CAYETANENSIS* ISOLATES DIRECTLY FROM HUMAN STOOL SAMPLES

H.N. Cinar, Food and Drug Administration
J. Lee, **S. Choi**, **C. Lee**, **S. Almeria**, **M. Durigan**, **H. Murphy**, **A. da Silva**, **G. Gopinathrao**

The increasing globalization of the food supply has contributed to the spread of *Cyclospora cayetanensis* worldwide. This is a human specific coccidian parasite associated with food and waterborne outbreaks in developing and developed nations. Whole Genome Sequencing (WGS) of *C.* cayetanensis presents challenges due to the absence of culture methods for the organism. Genomic DNA must be extracted from naturally infected individuals' stool samples that also contain intestinal bacterial flora and other contaminants. Furthermore, the only accessible biological stage of the organism is the oocyst, which is very resistant to physical and chemical disruption. Here we present a laboratory workflow for human fecal samples containing *C. cayetanensis* oocysts. The workflow involves sieving, density gradient centrifugations, surface sterilization treatments with bleach and detergents for oocyst purification, followed by genomic DNA extraction using physical sharing with glass beads. Genomic libraries are constructed using the "Ovation Ultralow System (NuGen)" to allow genome sequencing via Next Generation Sequencing (NGS) on Illumina MiSeq platforms. CLC workbench was used for de-novo genome assembly and mapping reads to a draft reference genome. Using this workflow, we were able to obtain assemblies of whole genomes of five *C. cayetanensis* isolates originating from Nepal (3 isolates) and Indonesia (2 isolates). Our workflow seamlessly combines both conventional laboratory techniques and NGS approaches. A subset of predicted genes from the assembly was confirmed in our sample collection using PCR. This will facilitate molecular method development for detection and differentiation of *C. cayetanensis* isolates in clinical, food, and environmental samples. The objective of this project is to fill a critical knowledge gap by increasing genome sequence information for C. cayetanensis, which is essential for the development of molecular methods for outbreak investigation.

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GENOMIC AND EIGEN ANALYSES PROVIDE VALUABLE INSIGHT INTO VARIATION IN HOST RESPONSES TO *EIMERIA TENELLA* INFECTION

K. Boulton, **M.J. Nolan**, **K. Harman**, **A. Psifidi**, **Z. Wu**, **P. Kaiser**, **S. Bishop**, **F.M. Tomley**, **D.A. Hume**
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Eimeria species parasites can cause coccidiosis, most notably in poultry where control relies on routine chemoprophylaxis and/or live parasite vaccination. Both approaches can be highly effective, although legislative pressure on drug use, increasing drug resistance and the limited production capacity of many vaccines pose significant problems. In response there is now considerable incentive to breed chickens with greater resistance to *Eimeria*. Here, 1,200 Cobb500 broilers were subject to *Eimeria tenella* or mock challenge for use in a genome-wide association study (GWAS). Phenotypes percentage body weight gain (%BWG) and caecal lesion score (CLS) were assessed as measures of productivity and pathogenicity, supplemented by serum interleukin-10 (IL-10) as a biomarker of intestinal inflammation. A 62K SNP array was used to genotype all individuals. Significant phenotypic variation was recorded in response to infection in all traits. %BWG exhibited a negative correlation with CLS and IL-10, with a positive correlation between CLS and IL-10. Eigenanalysis revealed three phenotypically distinct groups. The first Eigenvector classified traits recognisable as susceptibility to *E. tenella* infection. Specifically, %BWG was reduced, while CLS and IL-10 both increased. The second and third Eigenvectors revealed that resistance is not simply the inverse of susceptibility, identifying subpopulations with increased %BWG and decreased CLS (true resistance, IL-10 also increased) or increased %BWG despite increased CLS (tolerance, IL-10 decreased). GWAS identified suggestive genome-wide significant SNP markers associated with CLS and IL-10, and a chromosome-wide significant SNP marker for %BWG. Pathway analysis identified candidate genes putatively responsible for %BWG under stress, the extent of caecal lesion damage and innate response in this population of infected birds. Visualization of Eigenvectors provides a valuable insight into variation in the immune responses of animals to diseases and, in conjunction with GWAS, may aid identification of potential biomarkers.

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GENE CSUI_005805 ENCODES FOR A *CYSTOISOSPORA SUIS* SPECIFIC TRANSMEMBRANE PROTEIN CRUCIAL FOR INTRACELLULAR DEVELOPMENT OF MEROZOITES

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Integrated proteomics and reverse vaccinology approaches suggest that a 42 kDa uncharacterized merozoite protein of *Cystoisospora suis* encoded by gene CSUI_005805, might be a relevant vaccine candidate due to its high immunogenic score, high expression level and species-specificity. The entire coding sequence of the CSUI_005805 gene was amplified and cloned into pQE-31expression vector. Recombinant protein was expressed in *Escherichia coli* as a 42 kDa fusion protein and used for immunization of SPF chickens. The specificity of the expressed recombinant protein was evaluated in an immunoblot, and relative levels of expression in different developmental stages and subcellular localization were determined by quantitative real-time PCR and indirect immunofluorescence assay, respectively. The recombinant protein was recognized by antiserum from chickens immunized with recombinant CSUI_005805 (rCSUI_005805) protein as well as by porcine anti-*C. suis* serum in an immunoblot indicating that despite prokaryotic expression, the recombinant CSUI_005805 protein maintained antigenic determinants recognized by antibodies against naïve protein and could elicit an immune response in the host. Immunofluorescence labeling and confocal microscopy revealed localization primarily at the surface of both merozoites and sporozoites. Incubation of sporozoites with chicken anti-rCSUI_005805 sera resulted in the inhibition of invasion by 50.1% compared to pre-immune sera *in vitro*, as assessed by qPCR. The mRNA expression profiles revealed differential gene expression during the early development of *C. suis in vitro*, with higher transcript levels in merozoites compared to sporozoites. Interestingly, once the sporozoites had invaded the enterocytes, the transcription level

steadily increased during merogony, indicating that this protein might be important for the survival and establishment of merozoites. Owing to its specificity, localization and expression pattern, CSUI_005805 could be exploited as an attractive candidate for alternative control strategies against *C. suis* such as vaccines.

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GENETIC MODIFICATION OF THE GLOBAL DISTRIBUTION PATHOGEN *EIMERIA TENELLA* USING CRISPR/CAS9 SYSTEM

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Eimeria parasites are widespread pathogens that infect a wide variety of livestock and birds and are also potential vaccine delivery vehicle carrying and releasing antigens to mucosal immune system. Our previous studies showed that immunization with transgenic *E. tenella* with *Toxoplasma gondii* SAG1 gene randomly inserted into the genome partially protected the host from subsequent *T. gondii* challenge. Fusing genes of pathogens' antigens with immunodominant antigens of *E. tenella* or inserting them into the regions of the parasites genome targeted by immunity will promote the progress of recombinant *Eimeria* parasites as vaccine delivery vehicles. However, current methods for editing the parasite genes have been inefficient. The CRISPR/Cas9 system is a powerful technique for genome editing and has been widely employed in a wide variety of organisms on account of its high efficiency and accuracy. In this study, we established a genomic editing platform of *E. tenella* using the CRISPR/Cas9 system. The regulatory elements of CRISPR/Cas9 system, including 5' and 3' UTRs, nuclear localization sequence of Cas9 cassette and the promoter driven gRNA transcription, were optimized from *E. tenella*. We conducted a Cas9-dependent DNA repair experiment *in vitro* to test the system. We introduced a stop codon into the enhanced yellow fluorescent protein (EYFP) reporter that ablated fluorescent activity (DeadEYFP). We co-transfected *E. tenella* sporozoites with Cas9-gRNA-DeadEYFP plasmid and a repair DNA and observed EYFP expression at 60 hours after transfection. In another independent experiment, we transfected EtER sporozoites (a transgenic line of *E. tenella* stably expressing EYFP) with Cas9-gRNA plasmid and found EYFP negative offspring that confirming Cas9 has efficiently disrupted EYFP. Our results, for the first time, presented the CRISPR/Cas9-mediated genetic modification in *E. tenella*, which would favor thorough studies of transfected *Eimeria* as vaccine delivery vehicle, the biology of apicomplexan parasites and beyond.

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ANTIGEN DELIVERING SYSTEM FOR IMMUNIZATION SYSTEM AGAINST COCCIDIOSIS

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The functional delivering system of the individual antigens or package of antigens to the host antigen presenting cells (APC) is one of the key prerequisite of the developing immunization system against coccidiosis. It have been constructed the DNA vectors with the set of gene modifications, that regulate their transcription depending on the type of cell, whether it is a transport prokaryotic cell or *vice versa* the eukaryotic target cell of the host organism. First using the marker gene green fluorescent protein (GFP) it was verified the high gene expression in eukaryotic cells and loss of the gene expression in

bacterial transport cells. Consequently the bacterial cells *E. coli* with the formerly tested DNA constructs have been cultivated together with the phagocyting chicken macrophage HD11 cells and with the chicken fibroblast cells DF1. The positive HD11 (GFP expressing) cells have been detected and that functionality of DNA constructs confirmed. This result will allow to test the new set of modifications of antigens from the *E. tenella*. The ultimate goal of this antigen delivering concept is to use it for the immunization system against coccidiosis infection.

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PROTEOMIC EFFECT OF TETRACYCLINE ON *TRICHOMONAS VAGINALIS*

Chemotherapy & Drug Resistance POSTER

M.K. Stuart and **M.E. Hammers**, A.T. Still University of Health Sciences

Trichomonas vaginalis is the most common non-viral sexually transmitted pathogen worldwide. Trichomoniasis is currently treated with the 5-nitroimidazoles metronidazole and tinidazole, but up to 10% of the parasites have developed resistance to these drugs. The need for alternative therapies has led to evaluation of tetracycline (TET) as a potential treatment for trichomoniasis. Transcriptome analysis by next-generation sequencing, performed by other investigators, identified *T. vaginalis* genes that were up- or down-regulated by exposure to TET. The goal of the study presented here was to determine whether TET-induced changes at the RNA level correlate with changes occurring at the protein level. Parasites were cultured for 4 h in TYM medium containing 0 and 500 µg/mL TET. Lysates of treated and untreated parasites were subjected to electrophoresis through 20-cm polyacrylamide denaturing gels, and the proteins were stained with colloidal Coomassie Blue G-250. Proteins displaying prominent up- or down-regulation were identified by MALDI-ToF/MS analysis. In the presence of TET, synthesis of enolase and glycogen phosphorylase was up-regulated, demonstrating a positive correlation with TET-induced transcription and suggesting a role for TET in alteration of carbohydrate metabolism. Production of inositol-1-phosphate synthase was down-regulated by TET, in direct opposition to results of transcriptome analysis, which showed that TET increased the RPKM (reads per kilobase per million mapped reads) value of the gene 485-fold. TET increased synthesis of alpha-actinin, but the effect of TET on alpha-actinin gene transcription has not been reported. The results from these experiments will help ascertain how transcriptomic alterations caused by TET translate into proteomic expression, and may aid in identifying the antibiotic's mode of action against *T. vaginalis*.

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AN INTRIGUING RESISTANCE PHENOTYPE IN PRAZIQUANTEL-SELECTED *SCHISTOSOMA MANSONI*

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Praziquantel (PZQ) monotherapy is used to treat schistosomiasis. Current mass treatment campaigns (2016-2021) aim to distribute 250 million PZQ tablets per year, constituting a 10-fold increase in tablet distribution, and intensifying selection for PZQ resistance in schistosome populations. PZQ-resistant parasites have been reported from the field, and can be selected in the laboratory by treating either rodent or snail hosts. Typically, 3-5 fold difference in IC_{50} is reported between sensitive and resistant parasite lines. We conducted careful *in vitro* measurement of dose-response in a PZQ resistant parasite line

(SmLE-PZQ-R) generated by PZQ treatment of SmLE-infected snails. To conduct dose response experiments, we placed adult male worms in 24-well plates (10 parasites per well) at doses that ranged from 0-233 μM PZQ. We observed an IC_{50} of 40.81 μM (+ SE 7.81 uM) for male worms, compared to 2.77 μM (+ SE 0.22 uM) for SmLE. This is a 14-fold difference, slightly greater than previously observed. Of greater interest, we repeatedly observed that 30-35% of male worms were still alive after exposure to PZQ concentrations up to 233 μM. This trait was observed in all trials conducted, suggesting that that this laboratory selected parasite population is polymorphic for drug response, and contains individuals that are impervious to high dose PZQ treatment. In future work, we will sequence the exome from pools of SmLE-PZQ-R worms that survive or die following PZQ-treatment. We will then compare allele frequencies across the genome to identify the genome region(s) that underlie this intriguing PZQ-resistance phenotype.

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ELUCIDATION OF THE IN VITRO AND IN VIVO ACTIVITIES OF CURCUMIN ANALOGS AGAINST *SCHISTOSOMA MANSONI*

M. Pereira, **F.R. Badoco**, **D.C. Tavares**, **G. Kapadia**, **S. Rao**, **L.G. Magalhães**

Schistosomiasis control programs are based mainly on chemotherapy, which is almost limited to praziquantel (PZQ). However, due to the widespread and intensive use of PZQ, there is an increasing concern about the development of drug-less sensive strains. In order to provide new hit/lead structures, which can be used in drug development to control schistosomiasis, the *in vitro* and *in vivo* activities of six curcumin analogs were studied against *Schistosoma mansoni*. Schistosomicidal activity and morphological changes were determined against adult worms *S. mansoni* LE (Luiz Evangelista strain) *in vitro*. Schistosomicidal analogs were evaluated for cytotoxicity and genotoxicity *in vitro* and *in vivo* followed by studies during experimental model of chronic schistosomiasis. The analogs 1 and 2 caused the highest *in vitro* activities against adult worms which showed a lethal concentration for 50% of parasites (LC_{50}) of 11.7 and 10.2 μM at 24 h, respectively. Additionally, analogs 1 and 2 reduced the total number of *S. mansoni* eggs produced by adult worms and induced morphological changes in tegument and organelles. The results a showed that analogs 1 and 2 were 4.8 and 2.0 times more toxic to adult worms *S. mansoni* than to fibroblasts cells, respectively. Genotoxic and cytotoxic studies *in vivo* indicated that analogs 1 and 2 were tolerated at the oral doses of 200 and 400 mg/kg. The *in vivo* antiparasitic activity of analogs 1 and 2 were conducted in female BALB/c mice infected with *S. mansoni* and significant reductions in total worm burden were observed at single oral doses of 400 mg/kg (57.2 and 66.3%, respectively). Additionally, analogs 1 significantly reduced the number of eggs in the liver of infected mice by 42.5 and 71.0%, respectively. Similarly, histological analysis of the livers showed a significant reduction in the diameter of the granulomas. The above antiparasitic results which compare reasonably well with PZQ, make a compelling case for considering curcumin analogs as promising compounds for future investigations of the action mechanism and on *in vivo* efficacy.

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UNDERSTANDING THE GENETIC BASIS OF DRUG RESISTANCE IN *SCHISTOSOMA* SPP. AND THE DEVELOPMENT OF NOVEL THERAPEUTICS TO TREAT HUMAN SCHISTOSOMIASIS

M.A. Guzman, UT Health San Antonio

Human schistosomiasis is a debilitating, life-threatening disease affecting more than 250 million people in as many as 78 countries. Currently, schistosomiasis is labeled a neglected tropical disease and is second only to malaria as “the most devastating parasitic disease”. There is only one drug of choice effective

against all three major species of *Schistosoma*, Praziquantel. However, as with many monotherapies, resistance is emerging. Previously used therapies include Hycanthone and Oxamniquine, however shortcomings such as carcinogenicity and affordability, respectively, resulted in their discontinuation. While the mechanism of action of Praziquantel is unknown, the need to understand the gene(s) underlying resistance is imperative. Through a collaborative effort, we have identified the possible chromosome containing the gene(s) underlying resistance. Using RNA-interference and molecular techniques, we aim to determine the mode of resistance, as well as provide insight into the mechanism of action of Praziquantel. In addition, our collaborations with medicinal and structural chemists have enabled us to develop and test novel, affordable drug derivatives to be used in conjunction with Praziquantel to combat the ever-growing threat of resistance. One such successful derivative is CIDD-0066790. Using RNAi, we have successfully silenced the resistance-associated gene to Oxamniquine, the parent drug, providing the opportunity to determine the derivative's mechanism of action. The need to not only understand the genetic basis underlying drug resistance to Praziquantel, but also to design, screen, and develop future therapeutics to treat this disease is imperative and has the potential to save millions of lives.

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MORE LARVAL TAPEWORMS IN SAND CRABS THAN MOLE CRABS

Z. Faulkes, The University of Texas Rio Grande Valley

Larval tapeworms (*Polypocephalus* sp.) embed themselves in the nervous systems of crustaceans. Previous work showed that these tapeworms infect white shrimp (*Litopenaeus setiferus*) and manipulate the behavior of this host species. This study shows that *Polypocephalus* sp. also infect sympatric sand crabs (*Lepidopa bendicti*) and mole crabs (*Emerita benedicti*). Both species were collected from the beaches of South Padre Island, Texas. The nervous systems were dissected from individuals, dehydrated in a progressive alhohol series, and cleared in methyl salicylate. The patterns of infection differ: 100% of *L. benedicti* examined to date were infected, compared to 25% of *E. benedicti* were infected. The number of parasites in *L. benedicti* ranged from 7 to 54, while only a single tapeworm has been found in any *E. benedicti* individual. Both species are collected in the same time and place, but the feeding behaviour of these two species differs: *L. benedicti* is a sediment feeder, while *E. benedicti* is a filter feeder. This suggests the hypothesis that *Polypocephalus* sp. are found in sand grains and infect *L. benedicti* via ingestion, while *E. benedicti* avoid infection by filter feeding.

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PARASITE AND HOST COMMUNITY STRUCTURE ALONG TWO RIVERINE ECOSYSTEMS

C.J. Brianik, Rutgers University, School of Environmental and Biological Sciences
R.L. Grunberg, **M.V. Sukhdeo**,

This survey describes changes in parasite and host community composition along two rivers, the Passaic (PR) and Raritan River (RR) in New Jersey. Our sampling methods and theoretical framework reflect those from the river continuum concept (RCC), which describes predictable shifts in host communities (i.e. macroinvertebrates and fish) along a stream gradient. We sampled 3, 100-m transects along the RR and PR in Fall 2016. The 100-m transects were divided into 5, 20-m transects, and 5 fish samples were taken at each site along this transect. At each sampling site we collected field data on the fish, macroinvertebrate and parasites communities. We also measured several abiotic characters of the stream, such as mean stream width (RR: Site 1 = 9.46m, Site 2 = 13.70m, Site 3 = 21.32m; PR: Site 1 = 7.12m, Site

2 = 13.00m, Site 3 = 19.68m), pH (RR: Site 1 = 8.35, Site 2 = 8.58, Site 3 = 8.79; PR: Site 1 = 7.51, Site 2 = 7.29, Site 3 = 7.58), and salinity (RR: Site 1 = 222.60, Site 2 = 222.20, Site 3 = 137.40; PR: Site 1 = 177.20, Site 2 = 341.80, Site 3 = 406.40). In the RR we recovered 2 fish and 8 parasite species, and 11 fish and 14 parasite species in site 1 and 2, respectively. In the PR we recovered 6 fish and 4 parasite species, and 7 fish and 15 parasite species in site 1 and 2, respectively. Trematode species dominated the parasite communities in site 1 and 2 in both the RR and PR (RR: site 1=92.6%, site 2=95.8%; PR: site 1=97.1%, site 2=87.8% trematodes) .Our preliminary analysis suggests that both host and parasite species richness increase along these two riverine ecosystems and the parasite communities are dominated by larval trematodes.

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SEX RATIOS OF THE COMMONLY OCCURRING NEMATODE *AULONOCEPHALUS PENNULA* IN NORTHERN BOBWHITES

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Sex ratios of nematodes at the component population level are predicted to be female biased at low prevalence and mean intensity and approach 1:1 when prevalence and mean intensity increase. Sex ratios of *Aulonocephalus pennula* in northern bobwhites (*Colinus virginianus*) were examined to learn more about sex ratios at the infrapopulation and component population level of this commonly occurring cecal nematode. We used a database containing 128 *A. pennula* infected bobwhites (prevalence 90%, intensity 140 ± 1.5, range 1–1,162) collected from the Rolling Plains ecoregion of Texas and sorted bobwhites into 6 groups based on infection levels of 10–20, 40–60, 80–100, 120–140, 160–180, and 200–250 worms. Infrapopulation and component population sex ratios of *A. pennula* were determined from 5 bobwhites randomly selected from each group. In this presentation, we report our results and discuss our findings on productivity and persistence of *A. pennula* within this quail host population.

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USING STABLE ISOTOPES TO UNDERSTAND PARASITE EFFECTS ON HOST NUTRIENT ASSIMILATION

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Parasites are infectious agents in all organisms, communities, and ecosystems. Despite parasitism being a common consumer strategy, the effects of parasites on host nutrient recycling is largely unexplored. Stable isotopes are used to identify dietary signatures of consumers, trophic position, and species interactions in ecosystems. Our goal was to use stable isotopes to examine variation in nutrient assimilation with parasite load. Trematode parasites grow and develop in a mollusk intermediate host and feed on host tissues. Given that parasitized hosts maintain a higher required metabolism, nutrient assimilation may differ between parasitized and non-parasitized individuals. We fed ^{15}N-enriched algae to parasitized and non-parasitized freshwater *Physa acuta* snails collected from the White River in east-central Indiana to trace the assimilation rate of nitrogen through host and parasite tissues over 10 days. Snails ranged in size from 8.0 to 12.5 mm and were dissected to separate snail foot and gonadal tissue from trematode sporocyst, rediae, and cercariae. The samples were dried at 60˚C and analyzed at the Marine Biological Laboratory. We hypothesized that parasitized snails will assimilate nitrogen at a faster rate relative to the uninfected treatment. Despite a lot of variation, parasitized individuals have greater ^{15}N signatures than non-parasitized individuals for all tissue types. These differences in assimilation rates

may have important consequences that could help explain the mechanisms of changes in ecosystem nutrient dynamics.

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THE EFFECTS OF SECONDARY MICROPLASTICS ON THE RELATIONSHIP BETWEEN A TREMATODE PARASITE AND ITS INTERMEDIATE AND DEFINITIVE HOSTS

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The influx of plastic materials into the environment has had many negative impacts. One issue of ecological concern is the accumulation of plastics in water. When plastics breakdown, added chemicals leach into the surrounding waters. One common chemical, Bisphenol A (BPA), can imitate estrogen, binding to activation sites, and disrupting the endocrine system. BPA has been shown to induce precocious puberty and other reproductive disorders, however its impact on other organismal interactions, including host-parasite disease dynamics, is largely unknown. To elucidate the impact of BPA on disease transmission and intensity, we exposed the aquatic snail *Biomphalaria glabrata*, infected with a human trematode parasite, *Schistosoma mansoni*, to three concentrations of BPA: low (well water), medium (0.05 µg/L) and high (5.0 µg/L). The number of parasites released from each snail, and the period in which parasites remained active were measured to record transmission. Additionally, we infected mice to determine the impact of BPA within the definitive host. Results showed that the parasite abundance in the control and high snail treatments were similar, but medium exposed snails had a significantly higher parasite output than either control or high exposure treatments. Historic interpretation of parasite output would suggest that the medium treatment would therefore result in higher transmission rates, however we did not find evidence of this when we looked at parasite infection success for this treatment in the definitive host infections. We discuss the accuracy of previous transmission measurements and the implications that BPA could have on parasite transmission and future patterns of disease.

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CASTRATORS AND THIEVES: EVIDENCE OF PARASITE STRATEGIES IN DIPHYLLOBOTHRIIDEAN CESTODES OF FISH

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Parasites manifest two different strategies relative to the effects on host energy budgets, direct manipulation of host energy allocation by “castrators” and simple nutrient theft by “consumers”, which are expected to cause demonstrably different effects on host reproductive function and thus allow insight into the parasite’s strategy. This poster will present a review of the two strategies and investigations into the host-parasite relationships of three model systems revealing these strategies: roach – *Ligula intestinalis*, threespine stickleback – *Schistocephalus solidus*, and ninespine stickleback – *Schistocephalus pungitii*.

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PLASMODIUM ALDOLASE QUANTIFICATION USING PORTABLE ISOTHERMAL LOOP MEDIATED AMPLIFICATION (LAMP) AND A SMARTPHONE

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PROBLEM: Malaria control efforts are limited in rural areas due to lack of simple quantitative monitoring methods for infected patients. A strong need exists to detect *P falciparum* and *P vivax* infections in the sporozite stage before reaching the human liver. No current methods exist to detect such low levels of parasite except expensive laboratory grade equipment (polymerase chain reaction). A low-cost system to monitor response without the use of expensive equipment and electricity is needed. *Plasmodium* aldolase and histidine rich protein 2 (HRP2) gene products are potential biomarkers. METHODS: A 3 part electricity free system to measure plasmodium DNA using isothermal loop mediated amplification (LAMP) was created: 1) heating chamber using off-the-shelf camping equipment, 2) microfluidic chip, and 3) smartphone app to detect color change. A 3 layer microfluidic chip was fabricated using optically clear acrylic and a CO2 laser. Lamination was performed with 3M 501FL adhesive. The chip contained silica membranes to bind DNA and serve as a reaction chamber. Microchannels in the chip directed fluid in the proper direction to allow for reagent flow. Color change was measured via camera on a smartphone using an application programmed in Java. The reverse complement strands of primers were designed in LAMP Designer. BsT DNA polymerase, Qiagen reagents and 0.5 mM crystal violet were used for the LAMP amplification. Extracted *P falciparum* DNA was ordered from ATCC and stock dilutions were prepared. The color reading performance at 60 minutes incubation of stock dilutions of DNA in twenty (20) separate microfluidic LAMPs were compared. RESULTS: A standard curve was generated which compared the relationship between color intensity measured on the smartphone vs DNA concentration. A total of 60 samples from 20 chips were used for the linear regression. After log transformation the correlation was high (R2 = 0.90) indicating good reproducibility of results. CONCLUSION: A low cost microfluidic system could help identify asymptomatic carriers of the malaria parasite. The cost of the microfluidic system is approximately $5 per sample. Further testing is ongoing to compare how well the microfluidic LAMP compares to quantitative PCR.

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COMPARATIVE STUDY OF DE NOVO TRANSCRIPTOME ASSEMBLY METHODS AND POTENTIAL IMPACTS ON DIFFERENTIAL GENE EXPRESSION

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Whole transcriptome sequencing via RNA-Seq technology provides a specific temporal and spatial perspective in molecular biology activities. Increasingly RNA-Seq studies for non-model organisms have been emerging, facilitated by the ever-decreasing costs of next generation sequencing (NGS). The quality of de novo transcriptome assembly is critical for the downstream analyses, including differential gene expression. In Africa, the freshwater snail *Biomphalaria pfeifferi* serves as the major intermediate host of the human parasite *Schistosoma mansoni*. However, its relative *B. glabrata* is the only representative of the genus for which a sequenced draft genome is thus far available to serve as a reference. In practice, there is a risk of losing unique *B. pfeifferi* genes if the *B. glabrata* genome is used as a reference to analyze differential gene expression analysis in B. *pfeifferi*. In this project, we devised and executed multiple

workflows for de novo transcriptome assembly, using raw reads from both Illumina RNA-Seq and 454 sequencing of 18 field collected *B. pfeifferi* individuals, some of which were exposed to *S. mansoni*. For the initial transcriptome assembly, we used Trinity software (genome-guided and de novo options) and Newbler. These initially assembled sequences were then merged into a final assembly using software CAP3, CD-HIT or EvidentialGene. The quality for the final transcriptome from different workflows was assessed with Trinity evaluation scripts and BUSCO software. These assemblies were also used to perform differential gene expression analysis using the workflow contains reads counting tool RSEM and R/Bioconductor statistical test packages (edgeR, DESeq, and EBSeq). The preliminary results indicate that the workflow with EvidentialGene will yield a *B. pfeifferi* transcriptome with genes homologous to most of the reference *B. glabrata*, in addition to a good number of novel genes. From this, we expect to build an efficient workflow for accurate de novo transcriptome assembly. Supported by NIH grant R01 AI 101438 and P30GM110907.

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MICROSATELLITE AND TRANSCRIPTIONAL CHANGES IN A MALARIA MUTATION ACCUMULATION EXPERIMENT

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Microsatellite sequences are widely assumed to evolve neutrally, but can also play an important role in bacterial pathogenesis and human disease and have been proposed as a means for fine-tuning transcription, so the neutral assumption maybe questionable. The malaria parasite *Plasmodium falciparum* is absurdly AT-rich and contains microsatellites on average every ~1 kb across the 23 Mb genome. This project was designed to determine the microsatellite mutation rate in malaria parasites, and to investigate whether microsatellites are key determinants of transcriptional change in his pathogen. We maintained 40 parasite lines derived from a single parasite cell for 83 to 261 days, with frequent bottlenecking to a single cell to minimize effective population size, allowing us to measure mutations accumulated over >15000 mitoses. We illumina sequenced genomes of both progenitor and end-point mutation accumulation (MA) parasite lines in duplicate and called microsatellites using a validated LOBSTR pipeline. We scored 21,654 microsatellite loci, with 13937 scored in >50% of MA lines. Calls were 99.47% concordant in duplicate sequence runs from independent sequence libraries. We observed 1353 microsatellite mutations, giving rates of $1.04 \times 10^{-5} – 3 \times 10^{-7}$ /cell division for different motif lengths: hence in a single infection (10^{11} parasites) we expect to see 10^4 - 10^6 independent mutations at any single microsatellite locus. Furthermore, many of these microsatellites are found either within or close to gene sequences. We are currently examining transcript variation across the parasite lifecycle in a subset of these MA parasite lines to determine how microsatellite length changes influence transcript abundance.

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IMPROVING THE *SCHISTOSOMA MANSONI* GENOME ASSEMBLY USING GENETIC CROSSES AND LINKAGE ANALYSIS

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The number of eukaryotic genomes sequenced is constantly increasing but assembly quality of these genomes is often poor, limiting their use for population genomic and QTL mapping studies. We describe use of laboratory genetic crosses and linkage information to assess assembly accuracy and to determine the location of unassigned scaffolds in the human blood fluke *Schistosoma mansoni*, which causes intestinal schistosomiasis in ~67 million people in Sub-Saharan Africa, Middle East and South America. The parasite genome (~363 Mb) is composed of 7 autosome and ZW sex chromosomes. The genome (v1) was released in 2009 and contained 19,022 scaffolds, while version 5 (released in 2011) contained 884 scaffolds, and v7 (unreleased), which incorporates PacBio long read sequencing and optical mapping, contains 379 scaffolds. We performed 5 experimental genetic crosses, and sequenced the ~15 Mb exome from a total of 458 F2 progeny allowing us to determine scaffold positions and identify assembly errors using segregation at ~20,000 Mendelian inherited SNPs. The maps drawn using v5 allowed us to relocate 288 unassigned scaffolds comprising 94 Mb (25% of the genome), within or between chromosomes. The maps drawn using the v7 showed a huge improvement with only 73 relocations, comprising 9.7 Mb or just 3% of the genome. Our analysis demonstrates that (i) the v7 *S. mansoni* genome assembly is in an excellent state and close to completion, (ii) PacBio and optical mapping approaches vastly improve assembly, and (iii) combining linkage with bioinformatics is a powerful approach for generating high quality genome assemblies

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ROBUST EXOME SEQUENCING OF SINGLE SCHISTOSOME MIRACIDIA – OPTIMIZING SNP CALLING AND ACCURACY ASSESSMENT

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Small parasites and larval stages pose a problem for population genomic analyzes because limited amounts of DNA template are available, while the large size of many parasite genomes makes sequencing complete genomes prohibitively expensive. For example, schistosome adults live in human blood vessels and only microscopic larval miracidia (~150 μm long) are available for analysis. We evaluate the accuracy of exome sequencing of single miracidia following whole genome amplification and exome capture using a custom Agilent SureSelect array designed to capture 92% of the ~15 Mb exome. We used a "truth" set of 11,923 SNPs to optimize calling parameters using VQSLOD variant recalibration. We then used a test set of 16 F1 miracidia to evaluate call accuracy. Because the test set F1 miracidia were obtained from a cross between single genotype male and female worms, we could predict the SNP alleles in F1s and quantify the error rate. We scored 24,341 SNPs with an accuracy of 97.74%. We were also able to evaluate capture bias of alleles from genome regions showing high levels of polymorphism compared with the reference genome from which the capture array was designed. Extensive multiplexing of samples prior to genome capture reduced costs to ~$250/exome while maintaining accuracy. We conclude that scoring of genome-wide exomic SNPs from miracidia is feasible, economical and extremely accurate. This approach will allow research on schistosomes (and other parasite species or small invertebrates) to progress from population genetics using small numbers of markers, to population genomics utilizing genome-wide marker information.

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TEMPORAL AND SPATIAL PREVALENCE OF *GIARDIA LAMBLIA* IN ATLANTIC OYSTERS (*CRASSOSTREA VIRGINICA*) COLLECTED FROM ORCHARD BEACH, AND SOUNDVIEW PARK, NY, FROM 2014 TO 2016

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Giardia lamblia, a flagellated protozoan parasite, if ingested, infects the lumen of the small intestines in humans. This parasite is found in various animals, such as dogs, cats, and birds. *G. lamblia* is most commonly transmitted to humans via ingestion of contaminated food and water. Its presence in aquatic environment is relatively unorthodox and its presence in public waterways is a public health concern. The goal of this study is to determine the prevalence of *G. lamblia* in Atlantic oysters *(Crassostrea virginica*) collected from Orchard Beach (16 specimens) and Soundview Park (26 specimens), Bronx, NY. The oysters were collected on September 15, 2016 at low tide. Tissues were dissected followed by DNA extraction and PCR analysis. Thus far, 13 of the 26 samples from Soundview Park were tested for the presence of *G. lamblia* DNA. We found a prevalence of 46.15% (6/13), indicating presence of *G. lamblia* in Soundview Park. Additional experiments will be conducted to determine whether there is a difference in the prevalence and genotype of *G. lamblia* between the two sites. Furthermore, we will be able to assess the temporal variation in *G. lamblia* prevalence at Orchard beach from 2014-2016. In conclusion, Atlantic Oysters can be used as a biological sentinel to detect *G. lamblia* in public waterways and reservoirs.

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ASSESSMENT OF PRECIPITATION ON THE NORTHERN BOBWHITE CECAL NEMATODE (*AULONOCEPHALUS PENNULA*) IN TWO TEXAS REGIONS

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Northern bobwhites (*Colinus virginianus*) are known to host upwards of thirty species of indirect life cycle parasites; however, the cecal nematode *Aulonocephalus pennula* dominates the infracommunity in Texas and little is known about factors influencing its life history. Bobwhite populations have been shown to naturally follow a boom and bust cycle that corresponds with annual precipitation. Precipitation is also a driving force for vegetation growth and insect intermediate host productivity. Thus, it is likely that the population dynamics of indirect life cycle helminths are also influenced by precipitation. We hypothesized that during years with above-average rainfall, prevalence and intensity of *A. pennula* would "boom" and during years of below-average rainfall prevalence and intensity would "bust" in two independent regions of Texas, Rolling Plains and South Texas. *Aulonocephalus pennula* prevalence and intensity from 4 hunting seasons (2012–2016) in South Texas and 3 trapping seasons (2011–2013) in the Rolling Plains were compared to the Palmer Drought Severity Index precipitation metric. We discuss how our findings relate to assessing the role of precipitation on the common cecal nematode of northern bobwhites in two Texas regions.

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INVESTIGATING THE COMPLEXITY AND IMPORTANCE OF PARASITE TRANSMISSION THROUGH STUDENT-CENTERED TEACHING TECHNIQUES

N. Chodkowski, Ball State University

Scientific teaching allows instructors to assess the effectiveness of their teaching strategies. Many STEM instructors are implementing new teaching pedagogies using student-centered teaching techniques and active-learning strategies to promote critical thinking and problem solving skills. As part of the Scientific Teaching Fellowship Program at Ball State University, I used the backwards design to create a module on parasitic relationships to animal physiology in an introductory biology course. I used the explore-expand-explain pedagogy to evaluate students' abilities to connect the environment with biotic interactions and appreciate the complexity of organisms related to human health and wildlife issues. Students were asked to explore features of parasites before coming to lecture. In class, I used a bookends teaching approach, which incorporated a series of mini-lectures and activities for students to expand their understanding of parasite biology. Students then worked collaboratively to solve case studies concerning parasite life cycles and transmission strategies. The case studies required a mixture of quantitative reasoning and applications to biological systems. After the module, students gained a better understanding and appreciation of parasites life cycles. Of the students who participated, 90% (18 of 20) students were able to correctly define the word parasite. The case studies improved retention of parasitic organism examples and showed evidence for improvement in problem solving skills. Students were also successfully able to apply this information to a new case study and many indicated a preference for active learning over having a traditional lecture. This study will be conducted again in a larger lecture Spring 2017. Overall, these results provide support for the use of active learning strategies in the classroom.

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PARASITE ASSEMBLAGES OF FRESHWATER FISHES FROM TWO BACKWATER HABITATS ALONG THE NECHES RIVER OF SOUTHEAST, TEXAS, USA

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A total of 32 fish representing 7 species from family Centrarchidae (*Lepomis macrochirus, L. megalotis, L. microlophus, L. gulosus, Pomoxis annularus, Micropterus salmoides, M.punctulatus*) 1 species from family Lepisosteidae (L*episosteus oculatus*) and 2 species from family Catostomidae (*Minytrema melanops*, *Carpiodes carpio*) were collected from two locations on the Neches River on three sampling dates (July, 25 2016, August, 2 2016, and August, 10 2016). Hosts were caught, put on ice and transported back to the lab, necropsied, and examined for endo and ectoparasites. Thirty fish were (94%) were infected with one or more parasite. A total of 935 parasite individuals were found representing 4 phyla. These included Arthropoda (Copepoda), Platyhelminthes (Trematoda, and Monogenea) Nematoda, and Mollusca (Glochidia). At the time of this abstract, the project is ongoing and parasites are being identified to species or lowest possible taxonomic unit. Centrarchid hosts, collectively made up the largest numbers of fish hosts sampled and revealed the most speciose parasite communities. Centrarchid parasite infracommunities were dominated by the metacercariae of the trematode, *Posthodiplostomum minimum*. Both the Gar (Lepisosteidae) and Suckers (Catostomidae) were collected in smaller numbers and revealed depauperate parasite communities.

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HELMINTHS COMMUNITIES OF *PHILANDER OPOSSUM* (MAMMALIA: DIDELPHIDAE) IN AGUA FRIA, CHIAPAS

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Although some studies about helminths have been made in Mexico, our knowledge of their ecological dynamics is limited yet. Specifically, the factors that explain the assemblage of helminth communities of *Philander opossum* are still unknown. In the present study, the helmintofauna of *P. opossum* is recorded and analyzed at infracommunity and community component level, in order to infer the possible factors that could explain the assembly of helminth communities. Forty nine opossum's were collected at the beginning of March and for three consecutive years, using conventional catch techniques for medium-sized mammals. The hosts were subjected to a general parasitological examination. The helminths obtained were identified taxonomically. The ecological analysis was made according to the definitions and formulas proposed by Bush et al. (1997). Nonparametric statistical tests were performed to analyze the variation in descriptors of different years and between males and females. We recorded 12 taxa of helminths. Most of the species are considered specialized at the subfamily level, which suggests that the phylogenetic factor is important for the composition of the helminth community. The ecological analysis shows that the species of helminths at intracommunity level have an abundance that varies significantly between the years, and it is associated with *C. tentaculata* in 2013 and with *R. coronatus* in 2014 and 2015. However, a similar analysis showed that there is not significant variation between the abundances of helminths in male and female hosts. According to this, males and females have similar habits and diets. Phylogenetic factors, the omnivorous diet of females and males in this opossum species, and environmental factors (like local climatic events such as Hurricane Barbara in 2013) explain the assemblage of helminth communities of *P. opossum* in Agua Fria, Chiapas.

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ECHINOSTOME TREMATODES IN THE MUD SNAIL (*ILYANASSA OBSOLETA*), THE RIBBED MUSSEL (*GEUKENSIA DEMISSA*) AND GULLS (*LARUS* SPP.) IN A DELAWARE SALT MARSH

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The goal of our research is to elucidate the life cycle of a marine echinostome trematode in a Delaware Seashore State Park known as Savage's Ditch (SD hereafter) (38°37'43"N, 75°4'19"W). Our study site is a saltmarsh that borders the southeast region of Rehoboth Bay, where the echinostome *Himasthla quissetensis* has been recorded many times in the mud snail, *Ilyanassa obsoleta*, at >50% prevalence. Our previous research confirmed that echinostome cercariae and rediae were present in SD *I. obsoleta*. We also observed echinostome metacercariae infecting the ribbed mussel, *Geukensia demissa*. The echinostome larvae were identified morphologically as *H. quissetensis* using a key to trematode species in *I. obsoleta*. We confirmed the species identity of the mud snail and ribbed mussel hosts using molecular techniques but we were unsuccessful in identifying the echinostome larvae. Our current aims are to: 1) describe distribution patterns of echinostomes among snails and ribbed mussels; 2) obtain adult echinostomes from 3 gulls species that are known hosts for *H. quissetensis*: *Larus argentatus*, *L. marinus*, and *L. delawarensis*; and 3) identify the worms morphologically and compare them molecularly to the larvae that we collected from the snails and ribbed mussels. Between June and October, 2016, we visited SD once per month to collect *I. obsoleta*. Each month during low tide we collected 200-225 snails and examined them for parasites. In September we collected 80 mussels from 4 localities to enumerate metacercariae. Prevalence of *H. quissetensis* in snails ranged from 0.9-6%. Prevalence of *H. quissetensis* metacercariae in mussels ranged from 45-100%. Our current endeavor is to collect an identify echinostomes from 3 of the natural definitive hosts of *H. quissetensis*. We have recently acquired a euthanized great black-backed gull, *L. marinus*, which we necropsied for intestinal worms and, among other helminths, we have observed at least 2 echinostomes species that we are working to identify.

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PREVALENCE OF SCHISTOSOMIASIS AND WATER CONTACT PRACTICES AMONGST CHILDREN IN SENCHI, A RURAL COMMUNITY OF KEBBI STATE, NIGERIA

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A study of the prevalence of Schistosomiasis in Senchi town, located in Zuru local government area of Kebbi state, and the water contact practices of the children was carried out. This was aimed at boosting efforts to provide epidemiological data to help guide planning and control purposes. Schistosomiasis was assessed in Senchi town of Zuru local government area, examining children between the ages of 0-15 yrs of age, by parasitological diagnosis and questionnaire methods. Prevalence of *S. haematobium* amongst the 62 children surveyed in the Senchi town was 6.5% with a mean infection intensity of 4.25 eggs per 10 ml of urine. More males were infected than females, although there was no significant difference ($P>0.05$). Analysis of data supplied through questionnaire-interview method revealed that male contact with water was significantly higher than that of females ($P<0.05$), and all infected children in the study were within the ages 5-15 yrs of age. In addition, there was no detectable *S. mansoni* in the community examined, suggesting the absence of co-infections. Children between the ages of 5-15 yrs, especially males stand a higher risk of being infected with *S. haematobium*. Mass drug administration, health education, and poverty alleviation programmes need to be intensified in the control and elimination of schistosomiasis from Nigeria.

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IDENTIFICATION AND DISTRIBUTION OF ASCARIDOID LARVAE IN MARINE FISHES ALONG THE COAST OF SOUTH CAROLINA, USA

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The distribution of ascaridoid nematodes in fishes has been well studied globally, particularly because some species are potentially zoonotic. However, there is limited taxonomic, host distribution, and smaller scale geographic distribution data in marine fishes along the US Atlantic coast. We surveyed 1,999 marine fishes belonging to 46 species of 23 families commonly caught along the coast of South Carolina. Fishes were captured from inshore estuaries, offshore shallow (5-10 m), and offshore deep (19-55 m) water. Larvae were first sorted and identified based on their morpho-anatomy, and final species identification was performed molecularly by sequencing the internal transcribed spacer ribosomal DNA and partial mitochondrial cytochrome c oxidase I DNA of representative sequences. Morphologically, individuals belonging to three anisakid genera (*Anisakis*, *Terranova* type 1, *Terranova* type 2, *Contracaecum*) and two raphidascarid genera (*Hysterothylacium*, *Raphidascaris*) were identified. Sequencing revealed five *Anisakis* species (*A. simplex* s.s., *A. typica*, *A. physeteris*, *A. paggiae*, an unknown *Anisakis* sp.), five species of *Contracaecum* (*C. rudolphii* and four unknown *Contracaecum* spp.), six species of *Hysterothylacium* (*H. deardorffoverstreetorum, H. relinquens,* and four unknown *Hysterothylacium* spp.), and two unknown species of *Terranova* type 1 and one of *Terranova* type 2. *Hysterothylacium* larvae were the most common and most broadly distributed and *A. paggiae* the least, being found only in mahi, *Coryphaena hippurus*. *Contracaecum rudolphii* and *Anisakis* sp. only infected inshore and offshore deep waters fishes, respectively. These results provide baseline data for better understanding the biology of these parasites in the US Atlantic.

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ISOLATION OF AND EXPERIMENTAL TRANSMISSION TO IFN- γ GENE KNOCKOUT MICE, AND MOLECULAR CHARACTERIZATION OF *SARCOCYSTIS* SPECIES FROM INTESTINAL CONTENTS OF RAPTORS FROM NORTH AND SOUTH CAROLINA, USA

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Sarcocystis species frequently infect muscles and intestines of raptors, but little information is available on the complete lifecycles of these parasites. When raptors serve as intermediate hosts, they have the potential to develop fatal neurological disease. As definitive hosts, raptors can transmit their infections to other avian species who may also develop fatal neurological *Sarcocystis* infections. The present study examined the intestinal contents from raptors for *Sarcocystis* sporocysts and oocysts. The intestines from 36 raptors (3 Cooper's hawks (CH), 8 red-tailed hawks (RTH), 7 red-shouldered hawks (RSH), 3 Eastern screech owls (ESO), 9 barred owls (BO), 3 great horned owls (GHO), 1 turkey vulture (TV), 1 sharp-shinned hawk (SSH), and 1 osprey (OSP)) submitted to the Carolina Raptor Center, Huntersville, NC, were collected at necropsy. Patients were either terminally-ill or were euthanized due to severity of their injuries. At necropsy, intestines were collected, placed in individual plastic bags, and refrigerated at 4°C until they were examined for parasites by microscopy. Smears of mucosa were made from multiple sections of intestines and examined for parasites as fresh preparations. Twenty-five (69.4%) of 36 intestine samples contained oocysts/sporocysts of *Sarcocystis* species. Intestines from 22 of 25 *Sarcocystis*-infected raptors were processed for oocysts/sporocysts isolation, in vitro developmental stages, and transmission studies by washing with commercial bleach and stored at 4°C. To date, sporocysts of 14 samples have been excysted in vitro and sporozoites were cultured using African Green monkey kidney cells. Presently, we have successfully grown and maintained *Sarcocystis* from 4 of 14 raptors, including 2 CH, 1 RTH, and 1 RSH. We were able to transmit sporocysts from 2 RTH and 1 BO to INF- γ gene knockout (KO) mice, but not to Swiss Webster mice and demonstrate sarcocysts in muscles of KO mice. Two isolates appear to be novel species based on ultrastructure of sarcocysts, molecular, and phylogenetic analysis of 18S rRNA, 28S rRNA, ITS-1, and cox1 genes. This work is part of a larger study to investigate the diversity of *Sarcocystis* species that use raptors as definitive and intermediate hosts. Supported by NSF grant # 1505407 to ARVD and an IRC grant to DSL.

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TRENDS ON PREVALENCE OF MALARIA IN NIGERIA

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Malaria is the leading cause of morbidity and mortality in Nigeria. It is believed to be the number one killer, far surpassing HIV/AIDS. The most prevalent species which causes malignant sub-tertian malaria is *Plasmodium falciparum*. It accounts for about 90% of malaria infections in the country. The victims of malaria are mostly young children and pregnant women. Malaria in pregnancy is the leading cause of maternal anemia and low-birth weight babies. Malaria is one of the major tropical diseases. It is both in Nigeria and other countries south of the Sahara. The purpose of this paper is to review the prevalence of malaria in Nigeria during the period of 2005 to 2016. The study was conducted from an internet search and collection of publications from experts on malaria in Nigeria. A review of available literature shows that malaria is still a cause for concern in Nigeria. An examination of the yearly prevalence showed an irregular pattern. But the prevalence was highest in 2005. There was very little change between some years. The mounted control measures of getting people to use Insecticide-treated bed nets (ITNs) in their homes appears to be having some favorable impact in the prevalence of malaria in the country. It is reported that in Nigeria, about 14% of people used bed nets in 2006. This may be the reason why the

prevalence is getting lower. The fairly new and effective drug for treating malaria called artemether-lumefantrine is helping. Currently artemisinin-based combination therapy (ACT) is recommended for the treatment of *P. falciparum* malaria. Fast acting artemisinin-based compounds are combined with a drug from a different class. Companion drugs include lumefantrine, mefloquine, amodiaquine. sulfadoxine/pyrimethamine, piperaquine and chlorproguanil/dapsone. The benefits of ACTs are their high efficacy, fast action and the reduced likelihood of resistance development. This kind of review is recommended for monitoring the prevalence of malaria in Nigeria and to help in knowing its trend and controlling it.

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ENDOGENOUS DEVELOPMENT OF FIVE SPECIES OF TURKEY COCCIDIA

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To carry out the life-cycle study, turkeys were infected with graded doses from the highest (millions of oocysts) to the lowest (thousands) of oocysts. Infected birds were killed at 16 h intervals up to 128 h post-inoculation (p.i.). From each bird, ten samples were taken from all parts of the intestine, processed for paraffin histology and stained with hematoxylin-eosin. Three generations of asexual multiplication were observed in the parent strains of all five species. Except *E. innocua*, the first generation meronts were large and formed, according to species, 20-80 merozoites. The second and third generation, and also the first generation of *E. innocua*, formed smaller meronts that gave rise to about ten merozoites. The localization of the endogenous stages of *E. dispersa*, with emphasis on potential extraintestinal migration, was checked using quantitative PCR. Considerable number of parasite genome copies was detected in the spleen 16 and 32 h p.i. However, no significant amount of parasite cells was present neither in later intervals in the spleen nor in any interval in the liver.

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MORPHOLOGICAL CHARACTERIZATION AND MOLECULAR ANALYSIS OF *EIMERIA* SPP. (APICOMPLEXA: EIMERIIDAE) FOUND IN EASTERN GRAY SQUIRRELS (*SCIURUS CAROLINENSIS*) (RODENTIA: SCIURIDAE)

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D. Motriuk-Smith, **H.C. Lanier**, **H. McCurdy** and **S. Seville**, University of Wyoming
M.B. Connior, Northwest Arkansas Community College

There are few studies describing the morphology of eimerians found in North American eastern gray squirrels (*Sciurus carolinensis*). Earlier investigations reported *Eimeria lancasterensis* originally from Massachusetts and later from Arkansas and Texas, USA. *Eimeria ontarioensis* was described from Ontario, Canada, and later found in Massachusetts. The present investigation documents new geographic records of *E. lancasterensis* in Oklahoma and *E. ontarioensis* in Arkansas from *S. carolinensis*. *Eimeria lancasterensis* was found to be ovoidal in shape and a micropyle (M) and an oocyst residuum (OR) were absent. There were 0–2 polar granules (PG) and the mean oocyst size (L × W) was 25.4 × 15.2 µm. *Eimeria ontarioensis* oocysts were piriform shaped with a rough, golden-brown outer wall. Oocysts possessed a distinct M but lacked an OR and a PG. The mean oocyst size was 40.6 × 25.9 µm. DNA from 8 species of rodent eimerians was isolated and the 18S and/or the ORF 470 genetic markers were PCR-amplified, cloned, sequenced, and analyzed. Phylogenetic analysis was performed using 15 new sequences as well as those previously deposited in the GenBank. Maximum likelihood analyses of 18S and ORF 470 placed *E. lancasterenis* in a well-supported branch with other *E. lancasterensis* reported from widely-separated geographic locations. *Eimeria ontarioensis* represented a unique sequence forming branches

with weak bootstrap support. New ORF 470 rodent eimerian sequences from species lacking an OR formed a clade with other rodent oocysts lacking an OR. A second clade included those with an OR. These observations are consistent with previously published results. This study represents the first report of 18S and ORF 470 from *E. lancasterensis* and *E. ontarioensis* found in North American sciurid hosts.

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PHYLOGENETIC ANALYSIS OF FROG PARASITES OF THE GENUS *HAEMATOLOECHUS* (DIGENEA: PLATYHELMINTHES) FROM SOUTHERN MEXICO

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Universidad Nacional Autonoma de Mexico

Species of genus *Haematoloechus* Looss, 1899 are common parasites inhabiting the lungs of frogs. Most of the researches of this group have been made in the central plateau and western Mexico, where 11 species are known. In contrast only two species are recorded in southeastern Mexico: *Haematoloechus floedae* Harwood, 1932 from Yucatan and *Haematoloechus danbrooksi* León-Règagnon y Paredes-Calderón, 2002 from Veracruz. Clearly southern Mexico has been little explored and therefore its diversity is poorly characterized, not to mention the taxonomic problems of previous records. The main objective of the present work is re-investigating the phylogenetic relationships of species of genus, including a better representation of southern Mexico based on molecular and morphological evidence. One hundred thirty two *Lithobates* spp. were examined for parasites, (110 of *L. brownorum* and 22 of *L. Vaillanti*) collected in south of Veracruz, east Chiapas, Tabasco, Campeche, Quintana Roo, and Yucatan. In total 104 individuals were collected of genus, 66 were examined for morphological characters and 38 specimens were used to generate molecular data (Cytochrome C Oxidase 1 gene and ribosomal gene 28S). Preliminary analyses suggest the presence of at least four species, two mentioned before and two potentially new taxa. Molecular and morphological evidence suggest a clear biogeographic structure, which in turn appears to be directly correlated with the distribution of these hosts. This work complements previous records of this genus in the southeastern Mexico, and provides a phylogenetic framework to understanding of the diversity of the group in this country.

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PHYLOGENETIC POSITION OF TWO SPECIES OF *NYBELINIA* (CESTODA: TRYPANORHYNCHA) PARASITIZING ELASMOBRANCHS IN THE MEXICAN PACIFIC OCEAN

D.B. Adán Torres and **L. García Prieto**, National Autonomous University of Mexico

In order to establish the phylogenetic position of two *Nybelinia* spp. of elasmobranchs from the Mexican Pacific Ocean, we reviewed 36 stomachs and intestines of 3 sharks (*Sphyrna* sp., *Alopias* sp. and *Carcharinhus falciformis*) and 1 unidentified stingray, obtained by commercial fishing in two localities: La Reforma Lagoon, Sinaloa and San Blas, Nayarit, during August and November, 2016, respectively. Through of examination by light microscopy of whole mounts and histological sections, in combination with scanning electron microscopy, we analyzed the morphology of the cestodes found. Additionally, considering the most recent phylogenetic hypothesis available for the order Trypanorhyncha, we sequenced the *18s* and *28s* ribosomal genes. The sequences obtained were aligned together with the sequences of the same genes of 97 taxa available in Genbank for Trypanorhyncha. A phylogenetic parsimony and maximum likelihood analysis were performed. Excepting *C. falciformis*, the other 3 taxa of elasmobranchs were parasitized; two of the trypanorhynchs identified belong to Tentaculariidae and the other two to Eutetrarhynchiidae. The two Tentaculariidae were assigned to the genus *Nybelinia* and represent new records for both localities, since the other species recorded in Mexico (*N. anthicosum*) was

described from Sonora as parasite of *Heterodontus francisci*. Consensus phylogenetic tree show that all the sequences generated in this study nested together with the sequences of Tentaculariidae obtained from Genbank. However, the relationships within the species of *Nybelinia* are poorly resolved. For this reason, it is necessary to make a most detailed phylogenetic analysis of this genus.

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PHYLOGENETIC ANALYSIS OF OXYUROIDEA (NEMATODA: OXYURINA) INFERRED FROM RDNA

E.U. Garduño-Montes de Oca and **R. Mata-López**, Universidad Nacional Autónoma de México

Suborder Oxyurina contains two superfamilies: Oxyuroidea and Thelastomatoidea. Specifically, Oxyuroidea groups three families whose members are parasite of vertebrates: Heteroxynematidae, Oxyuridae and Pharyngodonidae. Previous phylogenetic analyses are poorly representative of Oxyuroidea, why it is necessary to increase the number of taxa of this group, adding mainly representatives of the family Pharyngodonidae and Heteroxynematidae, since they are the less considered in previous studies, although they present a high diversity. In the present study we analyzed the phylogenetic relationships between representatives of the superfamily Oxyuroidea using nucleotide sequences of the smaller ribosomal subunit (SSU); adding recently generated information. From several taxa representative of the family Pharyngodonidae the SSU gene was amplified by the DNA polymerase chain reaction, the obtained amplicons were sequenced. Subsequently, the sequences obtained were aligned together with 43 sequences of the same gene obtained from Genbank, belonging to representatives of the superfamily Oxyuroidea. Some representatives of the Thalastomatoid superfamily were considered as an external group. Phylogenetic analysis was performed under the Bayesian inference. The phylogenetic relationships obtained show that families do not form monophyletic groups; however, our results indicate that is necessary to carry out an analysis at a higher taxonomic level.

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TESTING SPECIES BOUNDARIES IN A WIDELY DISTRIBUTED FISH PARASITE *CREPIDOSTOMUM COOPERI* (TREMATODA: ALLOCREADIIDAE) USING MORPHOLOGY AND MOLECULAR DATA

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The genus *Crepidostomum* (Trematoda: Allocreadidae), is comprised of at least 12 species in North America, most of which are associated with particular families or even species of fishes. *Crepidostomum cooperi* is unusual because it is commonly reportedly in disparate hosts such as Yellow Perch (*Perca flavescens*, Percidae), several species of *Lepomis* sunfishes (Centrarchidae), and Trout-perch (*Percopsis omiscomaycus*, Percopsidae). Because most species show some host specificity, exceptions may indicate diversification events. We tested the hypothesis that worms in Trout-perch comprise a distinct species by first comparing them with worms from sympatric Yellow Perch hosts in Manitoba. We also examined specimens from different hosts and geographical locations (Wisconsin, Minnesota, and Manitoba) to determine geographical and host-induced effects. Worms from Trout-perch were generally larger but otherwise similar to Yellow Perch. Analysis of 28S rDNA and cytochrome oxidase *c* subunit 1 (*cox*-1) gene sequences indicates that worms from Trout Perch (in Manitoba) and Yellow Perch (in Minnesota and Manitoba) are conspecific but separate from '*C. cooperi*' found in *Lepomis* sunfishes across the upper Midwest of the U.S. This finding falsifies the hypothesis of host-specificity in '*C. cooperi*' but suggests that the worms in Yellow Perch and Trout-perch represents a separate entity.

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MOLECULAR IDENTIFICATION OF DIGENEAN PARASITES IN AQUATIC SNAILS IN THE UK

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Lymnaeid snails, *Radix auricularia*, *R. balthica*, *Lymnaea stagnalis* and *Stagnicola palustris* collected from freshwater sites in the UK were screened for digenean parasites. DNA sequencing of nuclear and/or mitochondrial gene markers revealed a total of 13 species belonging to six families. *Diplostomum pseudospathaceum* and *Tylodelphys clavata* (Diplostomatidae), *Cotylurus brevis* (Strigeidae), *Plagiorchis maculosus* (Plagiorchiidae), *Trichobilharzia szidati* (Schistosomatidae), *Echinostoma revolutum*, *Echinoparyphium aconiatum,* and *Hypoderaeum conoideum* (Echinostomatidae) are molecularly identified for the first time in the UK. *Australapatemon* sp. (Strigeidae) and *Echinoparyphium* sp. could only be identified to genus level and require further investigation. *Trichobilharzia franki* (Schistosomatidae) was molecularly identified for the first time from Scotland while *Echinoparyphium recurvatum* and *Lecithodendrium linstowi* (Lecithodendriidae) were molecularly identified for the second time in the UK. *Trichobilharzia* species are causative agents of human cercarial dermatitis (swimmers itch) which is currently regarded as a re-emerging disease in Europe and *E. revolutum*, *Ep. recurvatum* and *H. conoideum* are zoonotic agents of echinostomiasis. *Diplostomum pseudospathaceum* and *T. clavata* cause diplostomiasis in aquaculture. The cercaria of the bat trematode *L. linstowi* is reported for the first time and its molluscan host identified as *R. balthica*.

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COMPARATIVE PATHOGENICITY AND CROSS PROTECTION ANALYSES OF TWO DIFFERENT *EIMERIA TENELLA* ISOLATES FROM CHINA

X. Tang, **X. Liu** and **X. Suo,** China Agricultural University

Recent studies have shown that *Eimeria tenella*, one of the causative agents of coccidiosis, collected from different regions possessed a genetic diversity. Better understanding of the pathogenicity and cross-protective immunity among the local strains will provide a guideline for vaccine development. In this study, we compared the differences in the pathogenicity and cross-protective immunity between two *E. tenella* isolates in China. 0, 5×10^2, 1×10^3, 5×10^3, 1×10^4, 2.5×10^4 and 5×10^4 sporulated oocysts of XJ isolate (isolated from Xinjiang Autonomous Region, China), ZJK isolates (isolated from Zhangjiakou, Hebei Province, China) and Houghton strain were used for pathogenicity test with SPF and broiler chickens. All surviving birds were weighed, and euthanized for necropsy on 5.5 day post-inoculation. An assessment of the comparative pathogenicity was based on cecal lesion scores, survivor weight gain, and mortality percentage. To determine the cross-protective immunity between XJ and ZJK isolates, 200 XJ or ZJK oocysts were used for immunization, 2000 ZJK or XJ oocysts for challenge 7 days later. Fecal oocysts of all groups were counted daily from the fifth day post immunization. Body weights were measured on day 0, 7, 13 and 16 post immunization. High mortality was occurred in XJ-infected birds with 60% (3/5) SPF chickens in 5×10^4 inoculation dosage and 20% (1/5) in 1×10^4. 20% (1/5) chickens inoculated with 1×10^4 of H isolate were died 5 days after inoculation. 33% (1/3) birds inoculated with 2.5×10^4 oocysts of XJ and 5×10^4 of H isolate were died in broiler chicks. However, all chickens infected with ZJK isolate were survived. Significant difference in mean body weight gain (BWG) was present in the chicken inoculated with 1×10^4 oocysts of XJ or H strains, but absent even with 5×10^4 ZJK isolate. In contrast with severe cecum bleeding in XJ and H group, very few scattered petechiae were seen on the cecal mucosal tissues even in the serious infected ZJK groups. In cross-protection test, based on the number of fecal oocysts, a single immunizing dose of 200 ZJK oocysts can provide 18% immune protection against XJ challenge with a dosage of 2000 oocysts. Our study provides a solid evidence of the heterogeneity in *E. tenella* in different regions. Further understanding of the mechanisms underlying these diversity could help to find dominant antigen epitope, which has an important significance on vaccine development and coccidiosis

control. This work was supported by the National Natural Science Foundation of China (31330076, 31572507 and 31472180) and the Beijing Natural Science Foundation of China (6152011).

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COMPARISON OF THE ENDOGENOUS DEVELOPMENT OF TWO *EIMERIA TENELLA* ISOLATES WITH SIGNIFICANT DIFFERENCE IN PATHOGENICITY

X. Tang, **X. Suo** and **X. Liu**, China Agricultural University

Eimeria tenella, one of the most pathogenic species of chicken coccidia, causes hemorrhage in ceca or even death in heavy infected birds. As dramatically pathogenic difference was observed between two *E. tenella* geographic isolates, we conducted the investigation of the endogenous development of these two parasites. Fifty eight 2-day-old broiler chicks were randomly assigned to two groups. Birds in one group were inoculated with XJ isolate (isolated from Xinjiang Autonomous Region, China) while ones in the other group inoculated with ZJK isolate (isolated from Zhangjiakou, Hebei Province, China). One chick from each group was humanely euthanized for collecting the cecal samples at four-hour interval from 40 h to 148 h. 1 cm pieces of caecum in length were rapidly fixed immediately after each sacrifice. Transverse sections of about 5 μm in thickness were cut and stained with hematoxylins and eosin. Sections are observed and pictured under microscope. Mature first-generation meronts of ZJK and XJ isolates, observed in cells of the crypts at 48 h and 40h respectively without morphological difference between these two parasites. Numerous colonies of trophozoites observed from the crypts and lamina propria developed into mature second-generation schizonts at 68 h and 88 h PI of ZJK and XJ isolates, and the generation of schizogony of ZJK isolate still can be seen at 136 h, while later than 148 h for XJ isolate. Mature meronts of ZJK isolate were significantly smaller than those of XJ isolate (mean size of 20.99 μm ± 4.04 μm × 17.67 μm ± 2.49 μm [n=31] and 37.26 μm ± 12.11 μm × 28.57 μm ± 6.39 μm [n=31]) and contained fewer merozoites (22.91 ± 4.40 compared with 47.08 ± 12.20 in sections). Notably, the second-generation schizonts of XJ isolate invaded deeply in the lamina propria even in submucosa. Third-generation meronts of ZJK isolate were observed at 92 h in crypts cells, tunica propria and the surface epithelium, while similar numbers were not seen for XJ isolate until 104 h. Sexual stages of ZJK and XJ isolates localized either in the crypts or the surface epithelium was initially observed at 104 h and 132 h, respectively. The severe pathogenicity of XJ isolate, may be due to the deep localization of large second-generation schizonts with a great number of merozoites, which severely disrupt the intestinal mucosa, and lead to fatal hemorrhagic diarrhea. Knowledge defining the differences in *Eimeria* isolates can improve understanding of *Eimeria* evolution and facilitate biological studies of different geographic strains. This work was supported by the National Natural Science Fundation of China (31330076, 31572507 and 31472180) and the Beijing Natural Science Foundation of China (6152011).

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* With American Society of Tropical Medicine; since 1952, American Society of Tropical Medicine and Hygiene
† With American Institute of Biological Sciences
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