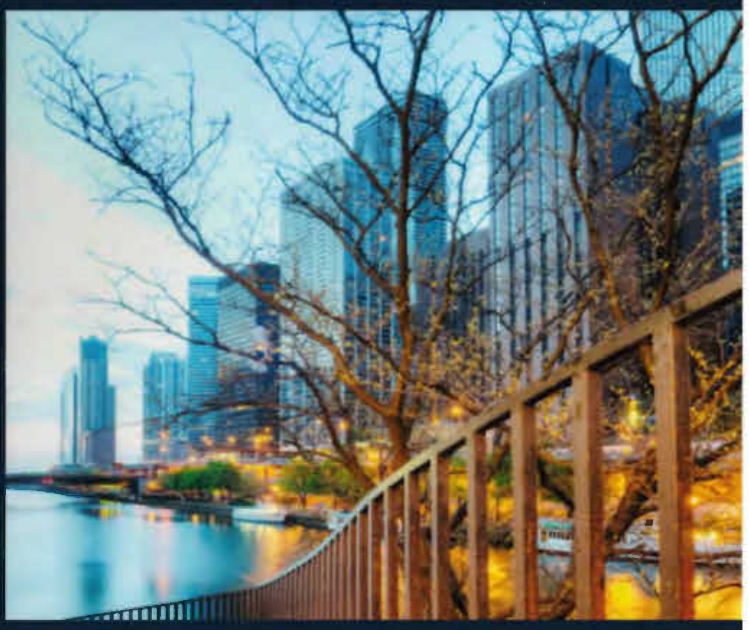
PROCEEDINGS AAVP

American Association of Veterinary Parasitologists



Membership Directory

58th Annual Meeting
July 20-23, 2013
Chicago, IL
Westin Chicago River North

Now and Tomorrow

Bayer HealthCare Animal Health
Elanco
Hartz
MERIAL
NOVARTIS ANIMAL HEALTH
SCYNEXIS
zoetis

2013 AAVP-Merial Distinguished Veterinary Parasitologist

Dan Zarlenga, DVM, PhD

Dan Zarlenga was born in Youngstown, Ohio and grew up in Girard, Ohio, a small, neighboring city. As a young boy he had a keen interest to understand how things worked. When items around the house broke, he liked to take them apart to see what made them tick (he once did this to his little brother's pet turtle). Following his junior year in high school, his family moved to Northern Ohio along the Great lakes. Dan stayed behind in Youngstown to finish his senior year and then attended Youngstown State University where he received a BS in Chemistry. He graduated Cum laude and was awarded the Youngstown State University Pin, an honor bestowed to only 5 graduates with combined highest scientific and academic achievement while maintaining a high level of extracurricular involvement in the University. He played AA baseball for a short while before deciding that the fun had gone out of the game. During his time at Youngstown State, he was elected student government vice-president. He was also inducted into Phi Lambda Upsilon, a nationally-recognized Honors Chemical Society. After graduating in 1976, he attended graduate school in the Department of Chemistry at the University of Cincinnati, and pursued a major in biochemistry wherein he synthesized and characterized ionene polymers that could be used to bind to and study DNA and RNA structure.

Dan received his Ph.D. in 1982 at which time he and his new wife, Gloria moved to Baltimore MD where he worked as a Post-doctoral fellow at The Johns Hopkins University. While at Johns Hopkins, he first ventured into the field of molecular biology and used his new found expertise to study the anti-proliferative effects of interferon-beta on human fibrosarcoma cells; a far cry from parasitology. He began teaching a course on molecular biology at the Center for Advanced Training in Cell and Molecular Biology at the Catholic University of America in Washington, DC. Following one of those lectures, Dan was approached by a participant, Dr. Ray Gamble who at the time was a staff scientist at the USDA in Beltsville, MD and very interested in advancing Trichinella research into the age of molecular biology. Ray explained the dearth of people working in molecular parasitology and convinced Dan that a change in research directions would

not go unrewarded; Dan never looked back. He worked as a post doc with the Agricultural Research Service at the USDA for 2 years and was eventually hired by Dr. Ralph Lichtenfels in the Biosystematics Lab where he continued his work on Trichinella and began a program on the molecular studies of Taenia spp.

Since Dan "crossed-over", he has applied recombinant DNA technologies to three broad research areas: DNA and protein-based diagnosis of parasitic diseases; reagent development and their application to studying host responses to parasite infections; and nematode systematics. In 1991, he received the Early-career Scientist of the Year award from the USDA. His pioneering work on the genetics of Trichinella resulted in the development of a test that was adopted by the International Trichinella Reference Center in Rome, Italy and has become the industry standard for genotyping Trichinella isolates. This test has underpinned all current knowledge of the worldwide distribution of organisms belonging to this genus. He was also a key contributor to sequencing the Trichinella genome which was recently published in Nature Genetics. He is an appointed board member of International Commission on Trichinellosis. Work performed by Dan on the molecular diagnosis of cattle GI nematodes led to establishing a regional testing facility to diagnose parasite infections from fecal eggs. This test was also used in the latest survey sponsored by the National Animal Health Monitoring System to assess the level of drug resistance in the US cattle population. His molecular studies on a newly identified Asian taeniid and the yearlong training that Korean scientists obtained while visiting his lab culminated in naming a new species of human Taenia. Recently, Dan collaborated with Chinese scientists to develop a novel mathematical model for simulating pathogen infection profiles. The algorithm has been successfully tested on H1N1, the emergence of drug-resistant bacteria, and the incidence and death rates of tuberculosis in the United States and Europe in the hope that the algorithm will be tested on zoonotic parasites when sufficient data sets become available. Dan was instrumental in developing novel reagents and transcriptional assays for multiple bovine and swine cytokines and receptors. These studies were among the first that quantified changes in bovine and swine immune-related genes resulting from nematode infections.

To date, Dan is the holder/co-holder of 8 patents, has authored/co-authored 18 book chapters, reviews and/or special issues for multiple scientific journals and coauthored >150 publications. He has appointments on the editorial boards of the Journal of Parasitology, Parasitology, Veterinary Parasitology and Comparative Parasitology (2000-04), and participated on the Scientific Advisory Council for the American Type Tissue Collection. Over the years, Dan has held adjunct positions with Department of Biochemistry, Howard University Graduate and Medical Schools, the Virginia-Maryland Regional College of Veterinary Medicine, and the University of Maryland College Park, Department of Graduate Studies, and was recently invited to join the adjunct faculty of the University of Delaware in the Department of Animal and Food Sciences. He is currently on staff with the "Foundation for Advanced Education in the Sciences" at NIH and took on the role as Research Leader for the Animal Parasitic Diseases Lab from 2007-2010. In is free time, he enjoys rebuilding old cars (British), golfing and spending quality time with his wife, Gloria and his two Korean adopted children, Kelsey and Alex.

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

AWARDS HISTORY

AAVP-Merial Distinguished Veterinary Parasitologist Award

1985	Jitender P. Dubey*
1986	Norman D. Levine
1987	E. J. Lawson Soulsby
1988	Jeffrey F. Williams
1989	K. Darwin Murrell
1990	William C. Campbell
1991	Jay Hal Drudge and Eugene T. Lyons
1992	Gilbert F. Otto
1993	Thomas R. Klei
1994	Peter M. Schantz
1995	James C. Williams
1996	T. Bonner Stewart
1997	J. Owen D. Slocombe
1998	J. Ralph Lichtenfels
1999	Roger K. Prichard
2000	Edward L. Roberson
2001	Byron L. Blagburn
2002	Sidney A. Ewing
2003	Louis C. Gasbarre
2004	David S. Lindsay
2005	Jorge Guerrero
2006	John W. McCall
2007	Ronald Fayer
2008	Dwight D. Bowman
2009	Ellis C. Greiner
2010	George A. Conder
2011	Thomas M. Craig
2012	James E. Miller

*National Academy of Sciences 2010

2013 AAVP-Merial Distinguished Veterinary Parasitologist Award Winner

DAN ZARLENGA

AAVP Distinguished Service Award

1976	Rurel R. Bell
1983	Terance J. Hayes
1987	Norman F. Baker
1988	Donald E. Cooperrider
1994	S. D. "Bud" Folz
1997	Honorico Rick Ciordia
2006	Raffaele "Raf" Roncalli
2008	Anne M. Zajac

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

AWARDS HISTORY

Hoechst-Roussel Agri-Vet Company Graduate Student Research Award

1987	Lora G. Rickard
1988	Debra A. Cross
1989	Stephen C. Barr
1990	Jim C. Parsons
1991	Carlos E. Lanusse
1992	David G. Baker
1993	Rebecca A. Cole
1994	Ray M. Kaplan
1995	Scott T. Storandt
1996	A. Lee Willingham III
1997	Carla C. Siefker
1998	Ryan M. O'Handley
1999	John S. Mathew
2000[1]	Sheila Abner
2001	Andrew Cheadle
2002	No recipient
2003	Mary G. Rossano
2004	Andrea S. Varela
2005	Alexa C. Rosypal
2006	Sheila M. Mitchell
2007	Martin K. Nielsen
2008[2]	Heather D. Stockdale
2009	Kelly E. Allen
2010	Stephanie R. Heise
2011	Aaron S. Lucas
2012[3]	Flavia A. Girao Ferrari

[1]2000, award renamed the "AAVP/Intervet Graduate Student Research Award"
[2]2008, award renamed the "AAVP/Schering-Intervet Graduate Student Research Award"
[3]2012, award renamed the "AAVP/Merck Animal Health Graduate Student Research Award"

2013 AAVP/Merck Animal Health Graduate Student Research Award

Lindsay A. Starkey

2013 AAVP-Companion Animal Parasite Council (CAPC) Graduate Student Award in Zoonotic Disease

2008	David G. Goodman
2009	Stephanie R. Heise
2010	Sriveny Dangoudoubiyam
2011	Jessica Edwards
2012	Lindsay Starkey

AAVP-CAPC Graduate Student Award in Zoonotic Disease

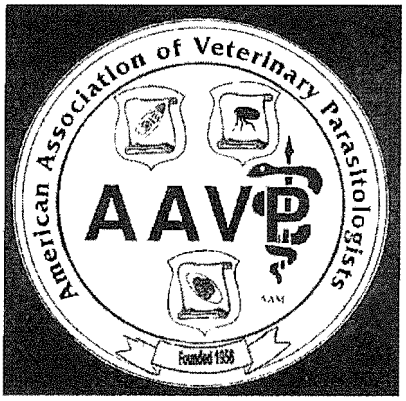
Gail M. Moraru

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

Founded 1956

Officers 2012-2013

President	**Alan A. Marchiondo** **Zoetis** **Kalamazoo, MI**
President-Elect	**Dwight D. Bowman** **Cornell University** **Ithaca, NY**
Vice-President	**Andrew Peregrine** **University of Guelph** **Guelph, ON**
Secretary/Treasurer	**Robert G. Arther** **Bayer HealthCare, Animal Health** **Shawnee, KS**
Immediate Past-President	**Patrick F. M. Meeus** **Zoetis** **Kalamazoo, MI**



2012-2013 AAVP Committee Chairs and Members (Term Date)

Nominations James Miller, Chair (2013), Tom Nolan (2013), Ray Kaplan (2014), Tom Craig (2014), Jenifer Edmonds (2015), and Craig Reinemeyer (2015)

Archives: Tom Nolan, Chair (2013), Raffaele Roncalli (2013), Miguel Suderman (2014)

Awards Committee: Doug Carithers, Chair (2013), Doug Hutchens (2013), Stephanie Heise (2013), Tom McTier (2014), Jeff Clark (2014), Sara Marley (2014), Dan Snyder (2014), William Ryan (2015), David Lindsay (2015), Susan E. Little (2016)

Constitution and Bylaws Committee: Wendell Davis, Chair (2013), Lori Carter (2013), Stephanie Heise (2013), Amy Edwards (2013); Tom Kennedy (2016)

Education Committee: Gary Conboy, Chair (2013), Doug Hutchens (2013), Araceli Lucio-Forster (2013), Pat Payne (2013), Tammi Krecek (2014), Christian Epe (2014), Tom Craig (2014), Andrea Varela-Stokes (2015), Tom Nolan (2015), Karen Snowden (2015), Roberto Cortinas (2016)

Finance Committee: Andrew Moorhead, Chair (2013), Ray Kaplan (2013), Robert Miller (2014), Kerry Powell (2014)

Historian: Raf Roncalli, Chair (2014), Tom Nolan (2014), Alan Marchiondo (2015)

Newsletter/Editorial Board: Jenifer Edmonds, Editor & Chair (2013), Ann Donoghue (2013), Claire Mannella (2013), Sue Holzmer (2014), Barbara Shock (2014), Theresa Hartwell (2014), Scott Bowdridge (2015), Tejbir S. Sandhu (2016)

Program Committee: Dwight D. Bowman, Chair (2013), Andrew Peregrine (2014), Alan Marchiondo (2013), Patrick Meeus (2013), Bob Arther (2014), Brian Herrin (2013), Melissa Beck (2014).

Publications/Internet Committee: Tariq Qureshi, Chair (2015), Richard Endris (2014), Kelly Allen (2014), Chris Adolph (2014), Miguel Suderman (2015),

Outreach/Research Committee: Lora Ballweber, Chair (2014), Charles Mackenzie (2013), Christine McCoy (2014), Kerry Powell (2014), Ray Kaplan (2015), Frederic Beugnet (2015), Sheila Mitchell (2015), Frederic Beugnet (2016)

PAST PRESIDENTS OF THE AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

1956-1958	L. E. Swanson
1958-1960	Fleetwood R. Koutz
1960-1962	Wendell H. Krull
1962-1964	Saeed M. Gaafar
1964-1966	E. D. Besch
1966-1968	George C. Shelton
1968-1970	John H. Greve
1970-1972	Harold J. Griffiths
1972-1973	Donald E. Cooperrider
1973-1975	Demetrice L. Lyles
1975-1977	Harold J. Smith
1977-1979	Norman F. Baker
1979-1981	Edward L. Roberson
1981-1983	Jeffrey F. Williams
1983-1985	John B. Malone
1985-1986	Robert M. Corwin
1986-1987	K. Darwin Murrell
1987-1988	Thomas R. Klei
1988-1989	Harold C. Gibbs
1989-1990	Bert E. Stromberg
1990-1991	Roger K. Prichard
1991-1992	J. Owen D. Slocombe
1992-1993	Ronald Fayer
1993-1994	George A. Conder
1994-1995	Charles H. Courtney
1995-1996	Byron L. Blagburn
1996-1997	Peter M. Schantz
1997-1998	James C. Williams
1998-1999	Louis C. Gasbarre
1999-2000	Robert S. Rew
2000-2001	Thomas J. Kennedy
2001-2002	Anne M. Zajac
2002-2003	Joseph F. Urban, Jr.
2003-2004	Craig R. Reinemeyer
2004-2005	Linda S. Mansfield
2005-2006	Ann R. Donoghue
2006-2007	Daniel E. Snyder
2007-2008	David S. Lindsay
2008-2009	Susan E. Little
2009-2010	Lora R. Ballweber
2010-2011	Karen Snowden
2011-2012	Patrick F.M. Meeus
2012-2013	Alan A. Marchiondo

PAST SECRETARY-TREASURERS OF THE AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS

Wendell H. Krull	1956-1959
Edward G. Batte	1960
Donald E. Cooperrider	1961-1969
Rurel Roger Bell	1969-1977
Terence J. Hayes	1978-1983
Vassilios J. Theodorides	1983-1986
S.D. "Bud" Folz	1987-1992
Thomas J. Kennedy	1993-1998
Daniel E. Snyder	1998-2004
Alan A. Marchiondo	2004-2010

PAST AAVP ANNUAL MEETINGS

1956	1st Annual Meeting – SAN ANTONIO, TX 16 OCT
1957	2nd Annual Meeting – COLUMBUS, OH 17 AUG
1958	3rd Annual Meeting – PHILADELPHIA, PA 18 AUG
1959	4th Annual Meeting – KANSAS CITY, MO 23 AUG
1960	5th Annual Meeting – DENVER, CO 14 AUG
1961	6th Annual Meeting – WEST LAFAYETTE, IN 20 AUG
1962	7th Annual Meeting – MIAMI BEACH, FL 12 AUG
1963	8th Annual Meeting – NEW YORK CITY, NY 28 JUL
1964	9th Annual Meeting – CHICAGO, IL 19 JUL
1965	10th Annual Meeting – PORTLAND, OR 11 JUL
1966	11th Annual Meeting – LOUISVILLE, KY 13-14 JUL
1967	12th Annual Meeting – DALLAS, TX 9 JUL
1968	13th Annual Meeting – BOSTON, MA 21 JUL
1969	14th Annual Meeting – MINNEAPOLIS, MN 13 JUL
1970	15th Annual Meeting – LAS VEGAS, NV 22 JUN
1971	16th Annual Meeting – DETROIT, MI 18 JUL
1972	17th Annual Meeting – NEW ORLEANS, LA 17 JUL
1973	18th Annual Meeting – PHILADELPHIA, PA 15 JUL
1974	19th Annual Meeting – DENVER, CO 21 JUL
1975	20th Annual Meeting – ANAHEIM, CA 13 JUL
1976	21st Annual Meeting – CINCINNATI, OH 19 JUL
1977	22nd Annual Meeting – ATLANTA, GA 11 JUL
1978	23rd Annual Meeting – DALLAS, TX 17 JUL
1979	24th Annual Meeting – SEATTLE, WA 22-24 JUL
1980	25th Annual Meeting – WASHINGTON, D.C. 20-22 JUL
1981	26th Annual Meeting – ST. LOUIS, MO 19-20 JUL
1982	27th Annual Meeting – SALT LAKE CITY, UT 18-19 JUL
1983	28th Annual Meeting – NEW YORK, NY 17-18, JUL
1984	29th Annual Meeting – NEW ORLEANS, LA 15-17 JUL
1985	30th Annual Meeting – LAS VEGAS, NV 22-24 JUL
1986	31st Annual Meeting – ATLANTA, GA 20-22 JUL
1987	32nd Annual Meeting – CHICAGO, IL 19-21 JUL
1988	33rd Annual Meeting – PORTLAND, OR 17-18 JUL

1989 34th Annual Meeting – ORLANDO, FL 16-18 JUL
1990 35th Annual Meeting – SAN ANTONIO, TX 21-24 JUL
1991 36th Annual Meeting – SEATTLE, WA 28-30 JUL
1992 37th Annual Meeting – BOSTON, MA 2-4 AUG
1993 38th Annual Meeting – MINNEAPOLIS, MN 17-20 JUL
1994 39th Annual Meeting – SAN FRANCISCO, CA 9-12 JUL
1995 40th Annual Meeting – PITTSBURGH, PA 6-10 JUL
(Joint meeting with the American Society of Parasitologists)
1996 41st Annual Meeting – LOUISVILLE, KY 20-23 JUL
1997 42nd Annual Meeting – RENO, NV 19-22 JUL
1998 43rd Annual Meeting – BALTIMORE, MD 25-28 JUL
1999 44th Annual Meeting – NEW ORLEANS, LA 10-13 JUL
2000 45th Annual Meeting – SALT LAKE CITY, UT 22-25 JUL
2001 46th Annual Meeting – BOSTON, MA 14-17 JUL
2002 47th Annual Meeting – NASHVILLE, TN 13-16 JUL
2003 48th Annual Meeting – DENVER, CO 19-23 JUL
2004 49th Annual Meeting – PHILADELPHIA, PA 24-28 JUL
(Joint meeting with the American Society of Parasitologists)
2005 50th Annual Meeting – MINNEAPOLIS, MN 16-19 JUL
2006 51st Annual Meeting – HONOLULU, HI 15-18 JUL
2007 52nd Annual Meeting – WASHINGTON, DC 14-17 JUL
2008 53rd Annual Meeting – NEW ORLEANS, LA 19-22 JUL
2009 54th Annual Meeting – CALGARY, CANADA 9-13 AUG
(Joint meeting with the World Association for the Advancement of Veterinary Parasitology and the International Commission on Trichinellosis)
2010 55th Annual Meeting – ATLANTA, GA 31 JUL – 2 AUG
2011 56th Annual Meeting – ST. LOUIS, MO 16-19 JUL
(Joint meeting with the Livestock Insect Workers Conference and the International Symposium of Ectoparasites of Pets)
2012 57th Annual Meeting – San Diego, CA 4-7 AUG

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AAVP 50th Anniversary Commemorative Silver Coins are available for purchase for $30.00.

AAVP 58th ANNUAL MEETING
The Westin Chicago River North Hotel

REGISTRATION

Grant Court Reception Desk
Saturday, 20 July 2013, 12:00 PM – 5:00 PM
Sunday, 21 July 2013, 8:00 AM – 5:00 PM
Monday, 22 July 2013, 10:00 AM – Noon

SPEAKER READY ROOM

Caucus Room
(No computer will be available for viewing slides)
Saturday, 20 July 2013, 1:00 PM – 5:00 PM
Sunday, 21 July 2013, 7:00 AM – 5:00 PM
Monday, 22 July 2013, 7:00 AM – 5:00 PM
Tuesday, 23 July 2013, 7:00 AM – Noon

SOCIAL PROGRAMS

Welcoming Reception – Bayer HealthCare, Animal Health
Astor Ballroom
Saturday, 20 July 2013, 7:00 PM – 9:00 PM

Merial Ltd Social
Grand Court
Sunday, 21 July 2013, 7:00 PM – 9:00 PM

Elanco Animal Health Social
Grand Court
Monday, 22 July 2013, 7:00 PM – 9:00 PM

DIPLOMATS, AMERICAN COLLEGE OF VETERINARY MICROBIOLOGISTS MEETING

Sunday, 21 July 2013, Noon to 1 PM
Executive Boardroom

MEETING OF AAVP COMMITTEES

Tuesday, 23 July, 2013, 6:30 AM – 8:00 AM
Executive Boardroom

STUDENT FUNCTIONS

AAVP Student Meeting, Luncheon, Dry Lab
Rogers Park
Saturday, 20 July, 2013, Noon – 1:15 PM

AAVP Student Luncheon/Mixer
Promenade Ballroom A
Sunday, 21 July, 2013, Noon – 1:00 PM

AAVP Student Luncheon / Speed Dating
Career Opportunities
Promenade Ballroom A
Monday, 22 July 2013, Noon – 1:15 PM

AAVP Student Zoetis Research Facility Tour - Kalamazoo, MI
(Need to have preregistered)
Tuesday, 23 July 2013, Noon to Wednesday, 24 July, 2013, 3:00 PM

SPECIAL SYMPOSIA

AAVP-American Society of Parasitologists Joint Symposium
Monday, 21 July 2013, Grand Ballroom AB, 8:00 AM – 9:00 AM
Gregarines and *Cryptosporidium*

AAVP-AVCM-NAVC Interactive Cases in Clinical Veterinary Parasitology
Tuesday, 22 July, Grand Ballroom AB

AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS 58th ANNUAL MEETING SPONSORS

SOCIAL EVENT SPONSORS

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[1]AAVP Best Student Paper Presentation Awards
[2]Honorarium for AAVP-CAPC Graduate Student Award – Zoonotic Diseases
[3]Honorarium for AAVP-Merck Graduate Student Award - Research
[4]Honorarium for AAVP-Merial Distinguished Veterinary Parasitologist Award

GENERAL MEETING SPONSORSHIP

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Box Lunches for DACVM Program Participants and Students on Tour

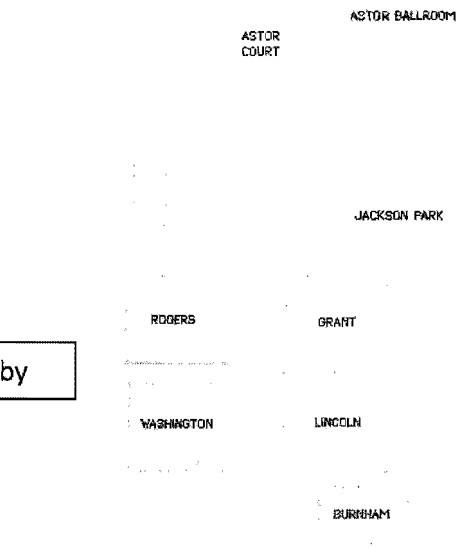
NATIONAL CENTER FOR VETERINARY PARASITOLOGY, OKLAHOMA STATE UNIVERSITY

MEETING OVERVIEW

	Saturday July 20		Sunday July 21			Monday July 22			Tuesday July 23
			Novartis Breakfast Grand Court			Novartis Breakfast Grand Court			Novartis Breakfast
		6:30	Promenade B	Promenade C	6:30	Promenade B	Promenade C	6:30	Grand Court
		6:45	VICH Meeting SA	VICH Meeting LA	6:45	VICH Meeting SA	VICH Meeting LA	6:45	AAVP Committee
		7:00			7:00			7:00	Meetings
		7:15			7:15			7:15	Executive Boardroom
		7:30			7:30			7:30	
		7:45			7:45			7:45	
8:00	Jackson Park	8:00	Grand Ballroom AB		8:00	Grand Ballroom AB	Grand Ballroom C	8:00	Grand Ballroom AB
8:15	AAVP	8:15	AAVP-ASP Joint Session		8:15	Nematode	Canine, Feline,	8:15	President's
8:30	Executive Committee	8:30	Gregarines & *Cryptosporidium*		8:30	Resistance	& Exotic	8:30	Symposium
8:45	Meeting and Luncheon	8:45			8:45		Parasitology	8:45	***Besnoitia***
9:00		9:00	Grand Ballrm AB	Grand Ballrm C	9:00			9:00	Lindsay
9:15		9:15	**Students**	**Students**	9:15			9:15	Ness
9:30		9:30	Apicomplexa	Nematode	9:30			9:30	Jacquiet
9:45		9:45		Large Animal	9:45			9:45	
10:00		10:00		Resistance	10:00			10:00	Brand Ballroom AB
10:15		10:15	Grand Court Scynexis Coffee Break		10:15			10:15	DACVM
10:30		10:30	Grand Ballrm AB	Grand Ballrm C	10:30	Grand Court		10:30	Preparation
10:45		10:45	**Students**	**Students**	10:45	Scynexis Coffee Break		10:45	
11:00		11:00	Small Animal	Treatment	11:00	Grand Ballroom AB		11:00	
11:15		11:15	Parasites	and Management	11:15			11:15	
11:30		11:30			11:30			11:30	
11:45		11:45			11:45			11:45	
12:00	Student Chapters	12:00	AAVP Members – lunch on your own		12:00	AAVP Members – Lunch on your own		12:00	NCVP Lunches
12:15	Meeting, Lab, Novartis	12:15	DACVM Meeting Exec Board Room		12:15	Students – Novartis Lunch and speed			
12:30	Lunch (Rogers Park)	12:30	Students – Novartis Lunch and Mixer		12:30	careers dating (Promenade Ballroom A)			
12:45		12:45	(Promenade Ballroom A)		12:45	Educator's Meeting (Exec Boardroom)			
13:00		13:00	Grand Ballroom AB		13:00				
13:15	Grand Ballroom AB	13:15			13:15	Grand Ballroom AB			
13:30	Opening Remarks	13:30			13:30	AAVP Business Meetings			
13:45	Alice Lee	13:45			13:45	Awards			
14:00		14:00	Grand Ballrm AB	Grand Ballrm C	14:00				
14:15	Alison Manchester	14:15	Heartworm	Wildlife	14:15				
14:30		14:30	Studies	Parasitology	14:30				
14:45	Meg Thompson	14:45			14:45				
15:00		15:00			15:00				
15:15		15:15			15:15				
15:30		15:30			15:30	Grand Court			
15:45	Grand Court	15:45			15:45	Scynexis Coffee Break			
16:00	Scynexis Coffee Break	16:00	Grand Court Scynexis Coffee Break		16:00	Grand Ballrm AB	Grand Ballrm C		
16:15	Grand Ballroom AB	16:15	Grand Ballrm AB	Grand Ballrm C	16:15	Small Animal/Avian	Large Animal		
16:30		16:30	**Students**	**Students**	16:30	Diagnosis &	Diagnosis,		
16:45		16:45	Epidemiology	Immunology	16:45	Treatment	Treatment,		
17:00		17:00	and Wildlife		17:00		& Management		
17:15	Grand Ballroom AB	17:15			17:15				
17:30		17:30			17:30				
17:45		17:45			17:45				
18:00	Grand Ballroom AB	18:00	Grand Ballroom AB		18:00	Grand Ballroom AB			
18:15		18:15	Merial Symposium		18:15	Elanco Symposium			
18:30		18:30			18:30				
18:45		18:45			18:45				
19:00	Astor Ballroom	19:00	Grand Court		19:00	Grand Court			
19:15	Bayer Welcome Social	19:15	Merial Sponsored Social		19:15	Elanco Sponsored Social			
19:30		19:30			19:30				
19:45		19:45			19:45				
20:00		20:00			20:00				
20:15		20:15			20:15				
20:30		20:30			20:30				
20:45		20:45			20:45				
21:00		21:00			21:00				

American Association of Veterinary Parasitologists
58th Annual Meeting, 20-23 July 2013
Westin Chicago River North, IL

HOTEL FLOOR PLAN



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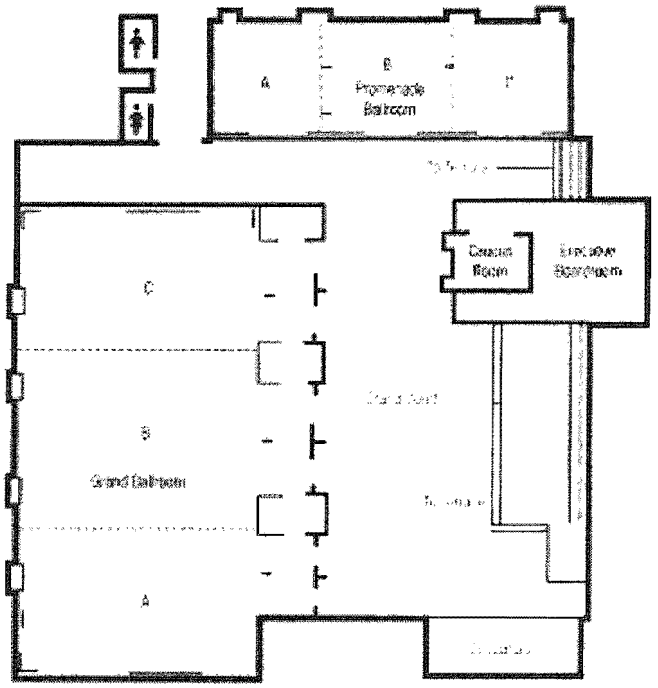

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Program for 2013 AAVP Meeting

Saturday July 20th, 2013

8:00-13:15	Jackson Park

AAVP Executive Committee Meeting & Luncheon
All AAVP officers and committee chairs please plan to attend!

12:00-13:15	Rogers Park

AAVP Student Chapter Meeting
All AAVP students and post-docs please plan to attend!

13:15-15:45	Plenary Session	Grand Ballroom AB

Opening Remarks
President: Alan Marchiondo
President-Elect and Program Chair: Dwight D. Bowman

Plenary Session: "Now and Tomorrow."
Moderator: Alan Marchiondo

13:45 1 A fresh perspective: in vivo imaging modalities for parasitic investigations.
Alice C. Y. Lee

14:15 2 Teaching principles of heartworm via a pedagogical approach.
Alison C. Manchester, Marc S. Kraus, Margret S. Thompson, Fred C. Brewer, Alice C.Y. Lee, Dwight D. Bowman.*

14:45 3 Imaging parasitic infection in the veterinary patient: a body system based review of imaging indications, modality choice, and findings in common and uncommon diseases.
*Margret S. Thompson**

15:45-16:15	Scynexis Coffee Break	Grand Court

16:15-17:15	AAVP-Merial Distinguished Veterinary Parasitologist Award	Grand Ballroom AB

16:15 AAVP-Merial Distinguished Veterinary Parasitologist Award to ***Dan Zarlenga***

17:15-17:45	AAVP-Merck Outstanding Graduate Student Talk	Grand Ballroom AB

Moderator: Doug Carithers

17:15 4 Persistent *Ehrlichia ewingii* infection in dogs
Lindsay A. Starkey*, *Anne W. Barrett, Melissa J. Beall, R. Chandrashekar, Brendon Thatcher, Phyllis Tyrrel, Susan E. Little.*

18:00-19:00	Bayer Symposium	Grand Ballroom AB

Moderator: Cristiano Von Simson

19:00-21:00	BAYER SOCIAL	Astor Ballroom

Sunday July 21st, 2013

6:30 to 8:00	Novartis Breakfast	Grand Court

6:30 to 8:00	VICH Resistance Discussions	Promenades B&C

6:30 Small Animal – Promenade B
Moderators: Michael Dryden and Alan Marchiondo

6:30 Large Animal – Promenade C
Moderators: Roger Prichard and Jenifer Edmonds

8:00-9:00	AAVP-ASP Joint Session	Grand Ballroom AB

Moderator: Dwight D. Bowman
8:00 5 What are the gregarines?
*John Janovy, Jr.**

8:30 6 *Cryptosporidium* species: Gregarine-like Coccidia or Coccidia-like Gregarines?
*John R. Barta**

9:00-10:15	Session 1 Students-Apicomplexa	Grand Ballroom AB

Moderators: Linda S. Mansfield and Tiffany Rushin

9:00 7 Detection and characterization of *Eimeria* sp in whooping cranes and sandhill cranes.
Miranda R. Bertram; Gabriel L. Hamer, Karen F. Snowden, Carolyn Hodo, Sarah A. Hamer.*

9:15 8 Loop-mediated isothermal amplification in the detection of *Cryptosporidium* in water samples
Emily G. Wessling, Janice L. Liotta, Araceli Lucio-Forster, Alice C. Y. Lee, Dwight D. Bowman.*

9:30 9 Development and experimentally induced reactivation of monozoic tissue cysts of *Cystoisospora canis* – A potential model for *Toxoplasma gondii.*
Alice E. Houk, David S. Lindsay.*

9:45 10 *In vitro* cultivation of *Cytauxzoon felis.*
Dana A. Pollard, Patricia J. Holman.*

10:00 11 *Eimeria* spp infecting turkeys: Utility of COI in DNA Barcoding and Phylogenetic Analysis.
Mosun E. Ogedengbe, Shiem El-Sherry, Mian A. Hafeez, Julia Whales, H. David Chapman, John R. Barta.*

9:00-10:15	Session 2 Students-Resistance	Grand Ballroom C

Moderators: Anne M. Zajac and Brian H. Herrin

9:00 12 New statistical model for analyzing fecal egg count reduction data
Xin Cao, Anand N. Vidyashankar, Martin K. Nielsen*

9:15 13 Efficiency of a genetic test to detect benzimidazole resistant *Haemonchus contortus* nematodes in sheep farms in Quebec
Virginie Barrère, Kathy Keller, Georg von Samson-Himmelstjerna and Roger K. Prichard.*

9:30 14 Characterization of *Haemonchus contortus* P-glycoproteins 2 and 9 expressed in mammalian cells and their possible role in macrocyclic lactone resistances
Pablo Godoy, Jing Lian, Hua Che, Robin N. Beech, Roger K. Prichard.*

9:45 15 Evaluation of the larval migration inhibition assay for detection of anthelmintic resistance in cattle
Melissa M. Miller, Lorraine S. Lopez, Sue Howell, Bob Storey, Ray M. Kaplan.*

10:00 15.5 Monitoring important parasites in naturally infected foals.
Jennifer L. Bellaw*, Eugene T. Lyons, Martin K. Nielsen.

10:15-10:30	Scynexis Coffee Break	Grand Court

10:30-12:00	Session 3 Students-Dog & Cat Parasites	Grand Ballroom AB

Moderators: Heather Stockdale-Walden and Jessica Rodriguez

10:30 16 *Rickettsia* spp. infection in domestic dogs in Oklahoma.
Anne W. Barrett, Jeff M. Gruntmeir, Susan E. Little.

10:45 17 Prevalence and assemblages of canine *Giardia duodenalis* isolates in southwestern Virginia
Meriam Saleh, Anne M. Zajac, Michael S. Leib.*

11:00 18 High prevalence of *Borrelia burgdorferi* in *Ixodes scapularis* from southwestern Virginia
Brian Herrin, Anne Zajac, Mauricia Shanks, Mary May, Susan Little.*

11:15 19 Gastrointestinal parasitological survey of shelter dogs in southern Louisiana.
Ntombi B. Mudenda, John B. Malone, Wendy Wolfson.*

11:30 20 Infection efficacy in mollusks with the nematode *Aelurostrongylus abstrusus*
Alexandra R. Kravitz, Janice L. Liotta, Chima Amadi, Dwight D. Bowman.*

11:45 21 *Mesocestoides* sp. infection in a cat: a case report.
Heather R. Rhoden, Eileen M. Johnson, Rebecca Decocq, Susan E. Little.*

10:30-12:00	Session 4 Students-Treatment & Management	Grand Ballroom C

Moderators: Karen Snowden and Alice Houk

10:30 22 Response of alpacas and sheep to experimental *Haemonchus contortus* infection.
Sarah J. Casey, Stephan Wildeus, Anne M. Zajac.*

10:45 23 Efficacy of injectable ivermectin on gastrointestinal helminths in captive wild elk (Cervus elaphus).
Alejandra Betancourt, Bryan M. Tom, Eugene T. Lyons, Martin K. Nielsen, John J. Cox.*

11:00 24 Effect of a single dose of Safe-Guard™ prior to weaning on parasite load of beef calves during the backgrounding phase.
Rush Holt, Alexandria Bay, Rebecca Price, Jesica Jacobs, Karen Sommers, and Scott A. Bowdridge.*

11:15 25 Evaluation of an integrated approach in controlling gastrointestinal nematodes in lambs
Sarah Orlik, James E. Miller.*

11:30 26 Abomasal gene expression of parasite-resistant sheep during a challenge Haemonchus contortus infection.
Karen N. Sommers, David R. Notter, Scott A. Bowdridge.*

11:45 27 Surfactant influence on the ovicidal activity of different short-chain fatty acids on *Ascaris suum* eggs.
Nuzhat Islam, Tammo S. Steenhuis, Janice L. Liotta, Michael P. Labare, Michael A. Butkus, Dwight D. Bowman.*

12:00-13:00	DACVM Meeting	Executive Boardroom

12:00-13:00	Students – Novartis Lunch and Mixer	Promenade A

13:00-14:00	Novartis Symposium	Grand Ballroom AB

13:00 28 Resistance and heartworm preventives: historical perspective and overview of research.
Byron Blagburn, James Carmichael, Ronald Kaminsky, Rudolf Schenker, Ray Kaplan, Andrew Moorhead, Roger Prichard, Timothy Geary, Catherine Bourguinat, John Malone, Dwight Bowman.*

14:00-16:00	Session 5 Heartworm Studies	Grand Ballroom AB

Moderators: Andrew S. Peregrine and Ablesh Gautam

14:00 29 Genomic search for macrocyclic lactone resistance markers in *Dirofilaria immitis*
Catherine Bourguinat, Timothy G. Geary, Kathy Keller, Byron Blagburn, Roger K. Prichard.*

14:15 30 Markers for macrocyclic lactone resistance in *Dirofilaria immitis* and utilization for management of Heartworm Disease
Roger K. Prichard, Catherine Bourguinat, Timothy G. Geary, Kathy Keller, Byron Blagburn, Dwight D. Bowman, Cassan Pulaski, John B. Malone, Ronald Kaminsky.*

14:30 31 Evidence of genetic selection following treatment of a heartworm-infected, microfilaremic dog with increasing dosages of ivermectin.
Byron Blagburn, Joy Bowles, Robert Loechel, James Carmichael, Rudolf Schenker, Linda Roycroft.*

14:45 32 Testing the efficacy of an injectable moxidectin formulation (ProHeart® 6) against a field isolate of canine heartworm.
Dwight D. Bowman,, Alice C.Y. Lee, Laura C. Harrington, Nicholas A. Ledesma, Marc S. Kraus, Janice L. Liotta, Byron L. Blagburn, Jason Drake, James Carmichael, Rudolf Schenker.*

15:00 33 The establishment of macrocyclic lactone resistant *Dirofilaria immitis* isolates in experimentally infected laboratory dogs
Cassan Pulaski, John B. Malone, Danielle Ward, Thomas R. Klei, Romain Pariaut, Catherine Bourguinat, Roger Prichard, James Carmichael, Rudolf Schenker.*

15:15 34 Efficacy studies in dogs demonstrate resistance of *Dirofilaria* against ivermectin and other macrocyclic lactones.
Ronald Kaminsky, Regina Lizundia, Byron L. Blagburn, Dwight D. Bowman, James Carmichael, Rudolf Schenker, Christian Epe, Heinz Sager.

15:30 35 Reversible interference with detection of antigen of *Dirofilaria immitis* in canine serum samples
Luisa Velasquez, Candace Munzing, Stephanie Heise, Kelly Allen, Lindsay Starkey, Jeff Gruntmeir, Eileen Johnson, Mason Reichard, Jim Meinkoth, Byron Blagburn, Susan Little.*

15:45 36 An analysis of time trends for canine heartworm in Grenada and its associated risk factors based on veterinary clinical pathology laboratory data base records between 2005 and 2009.
Richard M. Kabuusu, Donna F. Stroup, Calum Macpherson, Jennifer Chriestmon, Ruth Alexander, Carla Richards.*

14:00-16:00	Session 6 Wildlife	Grand Ballroom C

Moderators: Gary A. Conboy and Ashley M. Malmlov

14:00 37 Molecular characterization of *Sarcocystis* spp. isolates from marsupials of the genus *Didelphis* spp. through the analysis of mitochondrial and apicoplast genes, and surface antigen genes. Preliminary Results.
Samantha Y. O. B. Valadas; Juliana I. G. da Silva; Daniela P. Chiebao; Estela Galucci; Lara B. Keid; Ticiana Zwarg; Alice S. de Oliveira; Thaís C. Sanches; Adriana M. Joppert; Rodrigo M. Soares.*

14:15 38 Ecology of a potentially emerging tick-borne pathogen new to the US: *Ehrlichia muris* in the American Midwest and Texas
Sarah A. Hamer; Lisa Auckland; Isis M. Kuczaj; Ellen Stromdah; Jean I. Tsao.*

14:30 39 Virulence of Brazilian clonal genotypes of *Toxoplasma gondii* in mice orally infected with oocysts.
Daniela P. Chiebao, Aline Cabral, Hilda F. J. Pena, Estela G. Lopes, Samantha Y.O.B. Valadas, Lara B. Keid, Rodrigo M. Soares.*

14:45 40 Merogonic stages of *Theileria cervi* in mule and white-tailed deer.
Jason Wood, Eileen M. Johnson, Kelly E. Allen, Gregory A. Campbell, Grant Rezabek, Daniel S. Bradway, Louis L. Pittman, Susan E. Little, Roger J. Panciera.*

15:00 41 Parasite interactions and their potential to influence West Nile virus transmission.
Gabriel L. Hamer.*

15:15 42 Gastrointestinal parasites in commercial *Bison bison* herds from the U.S.A. central Great Plains.
Abdelhakim A. Eljaki, Jyotsna Acharya, John D. Bowron, Drew D. Grosz, Michael B. Hildreth.*

15:30 43 Molecular and parasitological detection of *Trypanosoma evansi* in Camels in Ismailia, Egypt.
Mahmoud M. Elhaig, Ahmed I. Youssef, Amal K. El-Gayar*

15:45 44 *Angiostrongylus cantonensis* in an orangutan (*Pongo pygmaeus*) in Florida
Heather D. Stockdale Walden, Jessica A. Emerson, Yvonne Qvarnstrom, Maniphet Xayavong, Henry S. Bishop, James F. Wellehan Jr, John D. Slapcinsky, Lisa L. Farina, Antoinette D. McIntosh.*

16:00-16:15	Scynexis Coffee Break	Grand court

16:15-18:00	Session 7 Students Epidemiology & Wildlife	Grand Ballroom AB

Moderators: Rhonda D. Pinckney and Anne W. Barrett

16:15 45 *Trypanosoma cruzi* genetic strain diversity in Texas wildlife and kissing bug vectors.
Rachel Curtis, Gabriel L. Hamer, Edward J. Wozniak, Karen F. Snowden, Sarah A. Hamer.*

16:30 46 Investigating the genetic diversity of muscular *Sarcocystis* species infection in raptors.
Tiffany P. Rushin, Alice E. Houk, David Scott, Alexa C. Rosypal, David S. Lindsay.*

16:45 47 Distribution and characterization of *Heterobilharzia americana* in dogs in Texas
Jessica Y. Rodriguez, Karen F. Snowden, Barbara C. Lewis.*

17:00 48 Surveillance of filarial nematodes in free ranging moose from Minnesota
Caroline Grunenwald, Richard Gerhold, Arno Wunschmann, Anibal Armien, Erika Butler*

17:15 49 Where's the risk?: Spatial epidemiology of gastrointestinal parasitism in Alberta beef cattle
Melissa A. Beck, Stefan W. Kienzle, Douglas D. Colwell, Cameron P. Goater.*

17:30 50 Invasion pathway and transmission dynamics of the invader *Dicrocoelium dendriticum* within cattle and wildlife in the Cypress Hills Park, Alberta.
Bradley J. van Paridon, Cameron P. Goater, Doug D. Colwell, John S. Gilleard.*

17:45 51 Confirmation of *Echinococcus canadensis* in wolves and elk in Idaho
Ashley M Malmlov, Lora R Ballweber*

16:15-18:00	Session 8 Students Immunology	Grand Ballroom C

Moderators: Andrew R. Moorhead and Miranda R. Bertram

16:15 52 Immunomodulation: The secret of *Haemonchus contortus* pathophysiology
Zia-ur-Rehman , H.V. Simpson, A. Pernthaner

16:30 53 Effects of feeding Celmanax™ on peripheral cellular responses of commercial crossbred lambs during *H. contortus* primary infection.
Rebecca C. Price, Megan Graham, Rush Holt, Jesica Jacobs, Karen Sommers, Scott A. Bowdridge.*

16:45 54 Comparison of immune responses during gastrointestinal helminth expulsion between resistant and susceptible breeds of sheep.
Javier J. Garza, James E. Miller.*

17:00 55 Cellular immune response to *Haemonchus contortus* in challenged sheep following extended rest period.
Jesica R. Jacobs, Rush M. Holt, Rebecca C. Price, Karen N. Sommers, Scott A. Bowdridge.*

17:15 56 Western blot analysis of IgG(T) antibody responses of horses to *Parascaris equorum* larval excretory-secretory proteins.
Steffanie V. Burk, Tammy Brewster-Barnes, Sriveny Dangoudoubiyam, Uneeda K. Bryant, Daniel K. Howe, Kevin R. Kazacos, Craig N. Robert J. Harmon, Eric S. Vanzant, Mary G. Rossano.*

17:30 57 Functional characterization of the surface antigens (SnSAGs) in *Sarcocystis neurona.*
Ablesh Gautam, Sriveny Dangoudoubiyam, Daniel K. Howe.*

17:45 58 Functional use of the *Eimeria* life cycle with live vaccination in caged replacement layer pullet production
Kayla Price, Julia Bulfon, Mian Hafeez, John R. Barta.*

18:00 to 19:00	Merial Symposium	Grand Ballroom AB

19:00 to 21:00	MERIAL SOCIAL	Grand Court

Monday July 22nd, 2013

6:30-8:00	Novartis Breakfast	Grand Court

6:30-8:00	VICH Resistance Discussions	Promenades B&C

6:30 Small Animal – Promenade B
Small Animal: Tom Kennedy and Jennifer Ketzis

6:30 Large Animal – Promenade C
Moderators: Craig Reinemeyer and Lora Ballweber

8:00-10:30	Session 9 Nematode Resistance	Grand Court AB

Moderators: Martin K. Nielsen and Melissa Beck

8:00 59 The heartworm refugia conundrum: Aren't refugia supposed to prevent the development of anthelmintic resistance?
Ray M. Kaplan.*

8:15 60 Why does there seem to be no spread of ML-resistant heartworms out of the Mississippi Delta region?
Ray M. Kaplan.*

8:30 61 Phenotype and genotype association in macrocyclic lactone resistance in nematodes
*Ludmel Urdaneta-Marquez, Joseph Dent, Patrick Janukavicius, Robin N. Beech, Roger K. Prichard**

8:45 62 *Haemonchus contortus* as a model system to study anthelmintic action and resistance: recent progress on developing genomic and genetic resources.
John Gilleard, Elizabeth Redman, Andrew Rezansoff, Neil Sargison, Roz Laing, Dave Bartley, Matt Berriman, James Cotton.*

9:00 63 Case history of a stocker operation using eggs per gram [EPG] and molecular identification of species to evaluate the source of resistant parasites and the use of two different classes of dewormers to control resistance in 2011 and 2012.
Larry L. Smith.*

9:15 64 Novel assay for the detection and monitoring of levamisole resistance in parasitic nematodes
Virginie Barrère, Roger K. Prichard, and Robin N. Beech.*

9:30 65 $\frac{dAR(ivm/mox)}{dt}$ A story of ivermectin/moxidectin resistance on goat and sheep farms in the eastern United States.
Sue B. Howell, Bob E. Storey, Ray M. Kaplan.*

9:45 66 Elimination of *Haemonchus contortus* from a llama farm in North Georgia.
Bob Storey, Lisa Williamson, Sue B. Howell, Michael Zager, Ray M. Kaplan.*

10:00 67 Novel anthelmintics and insecticides from filamentous fungi.
Jeffrey N. Clark; Lee Veasey, Meagan Tillotson, Blaise A. Darveaux, Nicolas H. Oberlies, Cedric J. Pearce.*

10:15 68 Anthelmintic drug discovery: a novel neurophysiology-based screening platform for nematode worms
Janis C. Weeks, Kristin Robinson, William M. Roberts, Shawn R. Lockery.*

8:00-10:30	Session 10 Canine, Feline, & Exotic Parasitology	Grand Court C

Moderators: David Lindsay and Alice C.Y. Lee

8:00 69 Hepatic alveolar echinococcosis in a dog in Ontario, Canada.
Alicia Skelding, Andrew Brooks, Margaret Stalker, Nicola Mercer, Eileen de Villa, Bruno Gottstein, Andrew S. Peregrine.*

8:15 70 Endemic *Crenosoma vulpis* infection in a dog in Wisconsin.
Gary A. Conboy, Helene Keily, Anne Kincaid.*

8:30 71 Prevalence of covert infections with intestinal helminths in dogs
Chris Adolph, Susan Little, Jennifer Thomas, Rebecca Duncan-Decoq, Eileen Johnson, Sharon Barnett.*

8:45 72 The efficacy of various imaging modalities to identify *Taenia pisiformis* cysticerci in rabbits.
Ruth M. Scott, Dwight D. Bowman, Michael A. Ulrich, Nathan C. Nelson, James P. Scott.*

9:00 73 Serological and molecular evidence of Leishmania parasite in dogs of visceral leishmaniasis endemic areas in Bangladesh.
Mohammad Z. Alam, Shirin Akter, Md. G. Yasin, Ryo Nakao, Tatsuya Sakurai, Hirotomo Kato, Ken Katakura.*

9:15 74 Epidemiological study of intestinal parasites in client-owned dogs of the Barcelona area and its evolution over a 6 month period
Anna Salas, Carmen L. Manuelian1, Nuria Sanchez, Joaquim Castellà, Sonia Fernandez, David Pumarola, Pedro Sancho, Jaume Avellaneda, Lluís Vilaseca, Celina Torre, Anna M. Ortuño.*

9:30 75 Molecular characterization of *Cryptosporidium* oocysts recovered from a Rock Pigeon (*Columba livia*) in central New York.
Janice L. Liotta, Araceli Lucio-Forster, James K. Morrisey, Dwight D. Bowman.*

9:45 76 Is an immunologic component required for *Brugia malayi* susceptibility in the domestic cat?
Erica J. Burkman, Bridget C. Garner, Kaori Sakamoto, Zack T. Moore, Andrew R. Moorhead.*

10:30-11:00	Scynexis Coffee Break	Grand Court

11:00-12:00	Scynexis Nematode Resistance / Control	Grand Ballroom AB

Moderators: Doug Colwell and Jesica Jacobs

11:00 77 Innovative solutions for anti-parasitic drug discovery
Kerrie Powell*.

12:00 to 13:15	Novartis Student Lunch and Speed Career Dating	Promenade A

13:15-15:30	AAVP Business Meeting & Awards	Grand Ballroom AB

AAVP Executive Committee, Student Officers, Corporate Award Sponsor representatives, and Awardees stay for pictures.

15:30-16:00	Scynexis Coffee Break	Grand Court

16:00-18:00	Session 11 Small Animal/Avian Diagnosis and Treatment	Grand Ballroom AB

Moderators: Robert G. Arther and Cassan Pulaski

16:00 78 ELISA detection of *Trichuris vulpis* coproantigen in dogs.
David A. Elsemore, Jinming Geng, Laurie Flynn, Larry Cruthers, Araceli Lucio-Forster, Dwight D. Bowman.*

16:15 79 Efficacy of Indoxacarb applied to cats against the adult cat flea, *Ctenocephalides felis*, flea eggs and adult flea emergence.
Michael W Dryden, Patricia A. Payne, Vicki Smith, Kathleen Heaney, Fangshi Sun.*

16:30 80 Efficacy of a 10% imidacloprid / 4.5% flumethrin collar for preventing the transmission of *Cytauxzoon felis* by *Amblyomma americanum* to domestic cats
Jennifer E. Thomas, Robert G. Arther, Joseph A. Hostetler, Kara L. Raetzel, James H. Meinkoth, Susan E. Little, Mason V. Reichard.*

16:35 81 Repellent effect of elimax (Oystershell Laboratories) against Ischnocera lice on poultry.
Jennifer K. Ketzis, Kris Honraet.*

17:00 82 An assay to detect tau-fluvalinate and coumaphos resistance in Varroa mites under tropical conditions and seasonal variations.
Rhonda D. Pinckney, Dwayne Mitchell, Jamie D. Ellis, David Westervelt, Jeffrey S. Pettis, Alizé Riff, Camille Simonis.*

17:15 83 Persistence of *Trichomonas gallinae* in clean and organic-debris contaminated water.
Richard W. Gerhold, Forrest Sturgill.*

17:30 84 A Study of Giardiasis in dogs in Lahore-Pakistan.
*Ayesha Sara, Jawaria Ali Khan, Muhammad Sarwar Khan, Muhammad Avais, Muhammad Hassan Saleem, Farhan Ahmad Atif and Zia-ur-Rehman**

16:00-18:00	Session 12 Large Animal Diagnosis, Treatment, Management	Grand Ballroom C

Moderators: Ray M. Kaplan and Sarah J. Casey

16:00 85 Effect of rifampicin and fosmidomycin on the causative agents of equine piroplasmosis, *Babesia caballi* and *Theileria equi in vitro*
W. Jacob Cobb, Patricia J. Holman.*

16:15 86 Development of *Strongylus vulgaris*-specific antibodies in naturally infected foals
Martin K. Nielsen, Anand N. Vidyashankar, Holli S. Gravatte, Jennifer Bellaw, Eugene T. Lyons Ulla V. Andersen.*

16:30 87 The McMaster method revisited – the critical role of detection limit
Martin K. Nielsen Xin Cao, Lene Larsen, Helena Carstensen, Ray M. Kaplan, Anand N. Vidyashankar.*

16:45 88 Incorporating parasite evaluation in a ram performance test.
Brandon C. Allen, Anne M. Zajac, Scott P. Greiner, D. Lee Wright, Mark A. McCann, David R.Notter*

17:00 89 Mitigating effects of gastrointestinal parasitism in organic sheep using protein supplementation.
Scott A. Bowdridge, Domingo Mata-Padrino, William Bryan.*

17:15 90 Efficacy Comparison of Two Injectable Formulations of Ivermectin Against Induced Gastrointestinal Nematode Infections in Cattle.
Craig R. Reinemeyer, C. David Nash, Julio C. Prado.*

17:30 91 Different strokes for different hosts: *Dicrocoelium dendriticum* metacercarial viability and survival in different host species.
Douglas D. Colwell, Cameron P. Goater, Melissa Beck, Kendra Sullivan, Dawn Gray, Fran Leggett, Alycia Ivarson*

18:00-19:00	Elanco Symposium	Grand Court

19:00-21:00	Elanco Social	Grand Ballroom AB

Tuesday July 23rd, 2012

6:30-8:00	Novartis Breakfast	Grand Court

6:30-8:00	AAVP Committee Meetings	Exec Boardroom

8:00-10:00	President's Symposium *Besnoitia*	Crystal I Ballroom

Moderator: Dwight D. Bowman

8:05 92 *Besnoitia*: (Apicomplexa: Sarcocystidae) developmental biology, chemotherapy, and immunodiagnosis.
*David S. Lindsay**

8:40 93 Clinical and serologic diagnosis of besnoitiosis in donkeys in the United States
Sally L. Ness, Gereon Schares, Jeanine Peters, Linda D. Mittel, Jitender P. Dubey, Dwight D. Bowman, Hussni O. Mohammed, Thomas J. Divers.*

9:15 94 Emergence of bovine besnoitiosis in Europe: a review.
Philippe Jacquiet, Jean-Pierre Alzieu, Emmanuel Liénard, Christelle Grisez, Françoise Prévot, Emilie Bouhsira, Michel Franc.*

10:00-12:00	AAVP-ACVM-NCVP DACVM Prep	Crystal I Ballroom

- American College of Veterinary Microbiology (Parasitology) exam process
  Susan Little, Anne Zajac, Lora Ballweber, Karen Snowden, and Linda Mansfield (ACVM Exam Committee)
- Parasites of small ruminants
  *Anne Zajac**
- Protozoan parasites of small animal patients
  *Karen Snowden**
- Equine clinical questions in veterinary parasitology
  *Craig Reinemeyer**
- Wildlife parasites in small animal patients
  *Mason Reichard**
- Parasites of pastured animals, or are they
  *Andrew Peregrine**
- Challenging small animal parasitic diagnoses
  *Byron Blagburn**
- Clinical parasitology cases in cattle
  *Lora Ballweber**
- **Boxed Lunches for Attendees**

12:00 – Meeting Adjourns – Happy Trails

AAVP

2013 ABSTRACTS

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ABSTRACTS

Plenary Session

1

A fresh perspective: in vivo imaging modalities for parasitic investigations.

Alice C. Y. Lee.* Department of Microbiology & Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY.

Traditionally, in-depth study of parasite-host interactions has relied heavily upon collection of tissues postmortem for histopathological evaluation, biochemical measurements, parasite recovery, and so on. While this approach has contributed valuable information to our body of knowledge, it also has distinct limitations. Technological improvements in biomedical imaging are now providing ways to detect and examine parasites in vivo. These non-terminal methods enable us to observe processes in real time and to follow the course of infection in individual subjects. Examples include whole-body bioluminescent imaging to determine malarial burden in the liver, endoscopy to study intestinal helminths within the definitive host, and PET-CT to trace biliary fluke migration in response to gallbladder contraction. This talk will touch on these and other techniques currently being utilized in parasitic investigations and how they have improved our ability to answer complex questions.

2

Teaching principles of heartworm via a pedagogical approach.

Alison C. Manchester[1]*, Marc S. Kraus[1], Margret S. Thompson[1], Fred C. Brewer[1], Alice C.Y. Lee[2], Dwight D. Bowman[2]. [1]Department of Clinical Sciences, [2]Department of Microbiology & Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY.

A teaching module based on the case of a naturally infected dog with stage 2a heartworm disease has been developed. Imaging modalities including echocardiography, radiography, 3D thoracic CT reconstruction, and interactive videos are utilized in evaluating heartworm-associated pathology and choosing a course of treatment. The non-linear module, which integrates multiple pathways or decision-making trees, will be demonstrated for the audience. Patient and case information is summarized. A 4-year-old female spayed Belgian Malinois was presented to her primary care veterinarian for a routine check-up. The dog had been adopted in Florida and relocated to New York one year prior. During that time, she received no heartworm preventive. A heartworm antigen ELISA test (SNAP® 4Dx® Test, IDEXX Laboratories) showed a positive result, and 3 microfilariae were observed on the Difil-Test® (Vétoquinol USA, Inc.). The case was referred to the Cornell University Hospital for Animals (CUHA) for staging and treatment of heartworm infection. Physical exam revealed no significant abnormalities. A second antigen test (SNAP® Heartworm RT Test, IDEXX Laboratories) was performed, yielding a positive result. No microfilariae were seen on Knott's test at this time. Thoracic radiography revealed focal enlargement of the main pulmonary artery and moderate enlargement of the caudal lobar arteries. Moderate pulmonary hypertension and a small PDA were observed on echocardiography with no detectable heartworms. The dog was started on 10 mg/kg doxycycline q12h and monthly Heartgard®Plus (Merial Limited). Six weeks later, CT imaging with contrast was performed. Multiple well-demarcated soft tissue nodules (1-2 cm in diameter)

centered around pulmonary arterioles were present in multiple lung lobes. Thirteen weeks after initial presentation to CUHA, a 3-dose regimen of melarsomine (2.5 mg/kg IM per dose) was initiated. Post-treatment imaging including radiographs, echocardiogram, and CT were performed 1 month after the final melarsomine administration, revealing resolution of the pulmonary nodules.

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Imaging parasitic infection in the veterinary patient: a body system based review of imaging indications, modality choice, and findings in common and uncommon diseases.

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In human medicine, tropical radiology, or more contemporaneously, imaging of infection, has been a challenging niche for the radiologist. Few individuals practice in tropical or sub-tropical parts of the world and until recently these diseases tend to be very localized. As these diseases have become more distributed to industrialized nations through world travel and immunosuppressive diseases and treatments, and remained important in developing regions, the need to recognize them via imaging has become more important. Patients often have non-specific or variable signs, and recognition of characteristic imaging patterns may point clinicians to test for these diseases. Additionally, imaging modalities have become more and more sophisticated venturing out of the morphologic, anatomic realm into function. In the veterinary profession, radiologists are typically generalists and minimal attention to the diagnostic imaging characteristics of parasitic diseases has occurred. Imaging descriptions are often limited to radiography or extrapolated from the human literature. A few diseases, such as canine heartworm, have been more thoroughly described, though often based on experimental cases. This talk will focus on describing the indications and value of all commonly used imaging modalities in the diagnosis of veterinary parasitic infection. A body system approach summarizing published imaging patterns and research on the most commonly imaged diseases will be combined with a description of modality (radiography, CT, MRI, nuclear medicine and ultrasound) value in each region. A brief discussion of the emerging functional modalities, particularly those now available clinically will be included. Cases will be utilized to facilitate group discussion of opportunities available for further characterization using these tools.

AAVP-Merck Outstanding Graduate Student Talk

4

Persistent *Ehrlichia ewingii* infection in dogs.

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To evaluate the persistence of natural infection with *Ehrlichia ewingii*, four beagles exposed to ticks via weekly walks through tick habitat in northcentral Oklahoma and positive for infection with *E. ewingii* by sequence-confirmed PCR and peptide-specific serology continued to be evaluated for a total duration of 733 days post initial infestation (dpi). Whole blood was collected once weekly for nested and real-time PCR. Serum was collected once monthly for

detection of antibodies to *E. canis* (p16), *E. chaffeensis* (IFA and VLPT), and *E. ewingii* (p28). All dogs (4/4) became infected with *Ehrlichia* spp. as evidenced by seroconversion on IFA to *E. chaffeensis* (4/4; maximum inverse titer =1,024-32,768; GMT_{MAX}=1,217.75); PCR detection of *E. ewingii* (4/4) and *E. chaffeensis* (2/4) DNA using both nested and real-time assays; and presence of specific antibodies to *E. ewingii* (4/4) and *E. chaffeensis* (2/4). Infection with *E. chaffeensis* was not detected after dpi 55. Intermittent *E. ewingii* rickettsemia persisted in 2 dogs from dpi 47-460, and in 1 dog from dpi 59-733. Doxycycline treatment was administered in 2/4 infected dogs from dpi 575-603. The infected, untreated dog continued to test positive for *E. ewingii* DNA in whole blood until the termination of the study (dpi 733). Clinical illness was never observed in any of the dogs. Our data suggest that dogs infected with *E. ewingii* from tick feeding are capable of maintaining the infection long-term, and may serve as a reservoir host for the maintenance of *E. ewingii* in nature.

AAVP-ASP Joint Session

5

What are the gregarines?

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The apicomplexan order Eugregarinorida ("gregarines") is arguably the most diverse eukaryotic taxon because its members parasitize virtually all invertebrate phyla, being especially numerous in arthropods and annelids. Traditionally these parasites have not attracted major attention because they are not of great economic importance, although evidence that *Cryptosporidium* species are actually gregarines has accumulated in recent years. Gregarines are typically large cells that develop in the host as trophonts and gamonts, the latter eventually undergoing syzygy, gametocyst formation, gametogenesis, fertilization, and subsequent zygotic meiosis within an oocyst. Gametocysts may be shed into the environment; oocyst development (sporulation) often continues within gametocysts after the latter are shed. Trophonts sometimes exhibit elaborate holdfast organelles. Oocysts also exhibit diverse structural features such as ridges and spines. Only a tiny fraction of all potential host species have been examined carefully for gregarines, but even that small number reveals enormous opportunities for studies of systematics, host-parasite co-evolution, morphological development, cell biology, biogeography, adaptations for transmission. Each of these areas of potential research will be illustrated by appropriate examples.

6

***Cryptosporidium* species: Gregarine-like Coccidia or Coccidia-like Gregarines?**

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For much of the time from their description over a century ago until now, *Cryptosporidium* have been considered 'unique' or 'unusual' coccidia that parasitize epithelial cells (primarily of the digestive tract) from a range of vertebrate hosts. Tyzzer's (1907) detailed original description of *Cryptosporidium muris* (the genotype) noted unusual epicellular development of these coccidia, complete endogenous sporogony, thick- and thin-walled oocyst production and the ability of this parasite to autoinfect through release of sporozoites from recently formed oocysts. It was only

following recognition of *Cryptosporidium* spp. as causative agents of diarrheal diseases of humans and other vertebrates and their roles in waterborne and zoonotic outbreaks that more extensive molecular and biological research was conducted on these tiny parasites. These recent molecular, morphological and biological observations on *Cryptosporidium* species have forced us to reconsider the "coccidian" nature of these small protists. Molecular phylogenies (most based on nuclear rDNA sequences) have demonstrated that *Cryptosporidium* species are actually much more closely related to archigregarines and eugregarines than they are to coccidia such as *Sarcocystis*, *Toxoplasma*, or *Eimeria* species. Comparison of the complete genomes from several *Cryptosporidium* species with the genomes of other Apicomplexa has highlighted dramatic genetic differences between *Cryptosporidium* and coccidia. *Cryptosporidium* species possess greatly simplified nuclear genomes and have lost both organellar genomes (plastid/apicoplast and mitochondrial) commonly possessed by other apicomplexan parasites, including coccidia. At least some of the genes originally located on these organelles are now associated with the nuclear genome suggesting the organelles have been secondarily lost during the evolution of the cryptosporidia. Whether ultimately determined to be sister taxa to gregarine parasites (and referred to as 'gregarines') or an early branching group of apicomplexan parasites with no known living relatives (and referred to as 'cryptosporidia'), *Cryptosporidium* spp. should no longer be referred to as "coccidia".

Session 1 – Students - Apicomplexa

7

Detection and characterization of *Eimeria* sp in whooping cranes and sandhill cranes.

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While the population of whooping cranes (*Grus americana*) has grown from a low of 15 individuals in 1941 to an estimated 278 birds present in the only natural migratory population today, this endangered species remains highly susceptible to stochastic events that could decimate the population. We hypothesize that disease may be limiting the population growth of whooping cranes. One disease of potential concern is disseminated visceral coccidiosis, caused by *Eimeria gruis* and *Eimeria reichenowi*. Unlike most species of *Eimeria*, which are localized to the intestinal tract, *E. gruis* and *E. reichenowi* can multiply systemically. Acute infections may result in death due to hepatitis, bronchopneumonia, myocarditis, splenitis and enteritis. Chronic infections are characterized by granulomatous nodules in various organs. To begin to characterize the parasites associated with cranes, we initiated a longitudinal study of crane feces in November 2012 at Aransas National Wildlife Refuge. Because we are limited to non-invasive sampling approaches (collections of voided feces) for the endangered whooping crane, our research approach also uses the sandhill crane (*Grus canadensis*) as a surrogate species. The sandhill crane is common in our study region, co-mingles with whooping cranes, and is actively hunted in other regions of the state, thereby allowing us to perform field necropsies in addition to fecal collections. Preliminary results indicate that 39.9% of 213 whooping crane fecal samples harbored *Eimeria* species. From the hunter-harvested sandhill cranes, feces showed a prevalence of 81.3% (n = 16) for oocysts via fecal flotation. Additionally, our necropsy results revealed that 83.3% (n = 18) of sandhill cranes showed granulomatous lesions in various organs. This study provides new information about the prevalence of *Eimeria* species infection

in cranes and lays the groundwork for future studies to determine the impact of coccidia infection on crane health.

8

Loop-mediated isothermal amplification in the detection of *Cryptosporidium* in water samples.

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Cryptosporidium is a unicellular eukaryotic parasite, which infects the human gastrointestinal tract, causing diarrhea. *Cryptosporidium* oocysts are important to detect because they are incredibly durable and resistant to external stressors, so they will not be destroyed on their own. A new, highly-sensitive method of detecting *Cryptosporidium* oocysts employs Loop mediated isothermal amplification, LAMP. LAMP uses a specific set of primers to amplify a gene, similar to PCR, but at a fixed temperature. The only technology the LAMP technique requires is a heating element (63°C), thus it is the optimal method of detection of *Cryptosporidium* in the field. For the interpretation of LAMP results, a field spectrophotometer can be used to determine turbidity or SYBR® Safe can be used, with UV or blue light. Both of these methods are battery operated and field safe. Cryptosporidium was used as an initial target pathogen; however, this project is to develop a format to test water samples in which the only minor alterations must be made to test for different pathogens. The LAMP technique is potentially a field-friendly method to test water samples for any target pathogen, which only requires minor changes to switch pathogens. Thus far, two buffers were tested to optimize the LAMP reaction, alternate methods of extraction have been explored, and methods of safe visualization have been examined. The LAMP reaction methods were adapted from "Sensitive and Rapid Detection of *Cryptosporidium* and *Giardia* by LAMP" by Momoda et. al.

9

Development and experimentally induced reactivation of monozoic tissue cysts of *Cystoisospora canis* – A potential model for *Toxoplasma gondii*.

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Cystoisospora canis is an Apicomplexan parasite of the small intestine of dogs that can cause diarrhea in young dogs. Biologically *C. canis* is similar to *C. belli* which causes intestinal coccidiosis in humans, especially those with AIDS. Relapse often occurs in *C. belli* patients due to reactivation of monozoic tissue cysts (MZT) in lymph nodes and re-colonization of the gut. *C. canis* also produces MZT that are similar to the polyzoic tissue cysts of *Toxoplasma gondii*, a parasite of medical and veterinary importance, which can reactivate and cause toxoplasmic encephalitis. We hypothesized that *C. canis* would be a novel model for studying tissue cyst biology. We used excystation solution on oocysts and the sporozoites were released and used for cell culture inoculations. We infected 8 cell lines (including 2 from dogs) and observed MZT formation but no multiplication in all cell lines studied. We were able to induce reactivation of MZT and egress of zoites. These zoites were infective for other host cells. We examined morphology using TEM and tissue cyst wall composition with *Dolichos biflorus* lectin staining and found both to be similar to *T. gondii*. We also conducted DNA analysis of *C. canis* using *Cystoisospora*-specific ITS1 primers and Alu1 restriction enzymes for RFLP. Using the Basic Local Alignment Search Tool (BLAST), we found *C. canis* is most similar to *C. ohioensis*-like

ITS1 sequences. We will do more sequencing of the genome of *C. canis* to study regulation of genes during induced tissue cyst reactivation events. Our system provides a simple model to produce tissue cysts and to study host factors that cause reactivation of tissue cysts which is vitally important in the effort to find effective therapeutics against these parasites. Supported by an IRG from the Office of Research & Graduate Studies, Virginia-Maryland Regional College of Veterinary Medicine.

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***In vitro* cultivation of *Cytauxzoon felis*.**

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Cytauxzoon felis, a tick-borne intraerythrocytic protozoan parasite, is the causative agent of feline cytauxzoonosis, which may cause effects ranging from clinically normal to anorexia and death. The disease progresses quickly with diagnosis often made during postmortem examination. At the present time, cytauxzoonosis research requires the use of infected cats. In vitro cultures of the parasite would provide an alternative to the use of animals, thus the goal of this study is to establish optimal conditions for continuous in vitro cultivation of *C. felis* in the erythrocytic stage. Modeled after the microaerophilous stationary phase cultivation method for *Babesia* spp., cultures of *C. felis* were initiated from infected cat blood in a variety of culture media and supplements and then monitored by daily microscopic examination of Giemsa-stained cultured erythrocyte smears. Fresh erythrocytes were added or the cultures were passaged at a 1:2 subculture ratio on a weekly basis. Cultures were initiated from *C. felis* infected blood from 7 different cats in combinations of 4 different media and serum from 6 different animal species, alone and in various combinations with different supplements. Parasites persisted at low levels in culture for as long as 140 days. The cultures that underwent subculture failed at the 7th passage or earlier. HL-1 medium supplemented with cat or fetal bovine serum has best supported the parasite in vitro. Achievement of our goal will allow propagation of this parasite in vitro to provide a continuous laboratory source of parasites for further research, thus reducing or eliminating the need to use infected cats.

Session 2 – Students – Large Animal Nematodes Resistance

11

***Eimeria* spp infecting turkeys: Utility of COI in DNA Barcoding and Phylogenetic Analysis.**

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Coccidiosis in turkeys is caused by enteric coccidia species belonging to genus *Eimeria* (including *E. meleagrimitis*, *E. meleagridis*, *E. dispersa*, *E. gallopavonis* and *E. adenoeides*). Identification methods, based mainly on morphometric and biological characteristics, are insufficient to reliably differentiate species. Although nuclear 18S rDNA sequences have been

used widely for phylogenetic reconstructions and species characterization, this gene has limitations as a molecular marker for species differentiation in the case of closely related coccidia. To address this, new nuclear 18S rDNA and mitochondrial COI sequences were obtained from single oocyst-derived lines from a number of *Eimeria* spp. infecting turkeys (*E. adenoeides* KCH, *E. adenoeides* USA; *E. adenoeides* Weybridge, *E. meleagridis* Arkansas, *E. dispersa* and *E. meleagrimitis* USMN08-01. Phylogenetic analysis of multiple 18S rDNA sequences obtained from single oocyst-derived lines of *E. meleagrimitis* show that divergent, paralogous 18S rDNA copies exist within the nuclear genome of this *E. meleagrimitis*. COI sequences of *E. meleagrimitis* and *E. adenoeides* were identical within the same species from multiple isolates and COI sequences from individual *Eimeria* species were easily distinguished from other *Eimeria* species infecting the same host or more distantly related hosts. COI sequences had much lower intraspecific genetic variation and higher interspecific genetic distances than nuclear 18S rDNA sequences. The COI locus is apparently free of divergent copies that were demonstrated for the nuclear rDNA loci. These observations demonstrate the utility of the mitochondrial COI locus as a species-level genetic marker for *Eimeria* spp. infecting turkeys. Oocyst morphometrics and histological observations of endogenous development during experimental infections *in vivo* are in agreement with the phylogenetic reconstruction for these parasites. We encourage COI-based DNA barcoding of coccidia as part of the species description or re-descriptions of any new, or newly isolated, species of *Eimeria* or related parasites.

12

New statistical model for analyzing fecal egg count reduction data

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Anthelmintic resistance is widespread in equine cyathostomin parasites and routine monitoring of anthelmintic efficacy is recommended in all equine establishments. However, equine populations are characterized by high diversities in breeds, ages, genders and management which all contribute to large levels of variability in the egg count data. To address these problems we constructed a statistical model for analyzing fecal egg count reduction test (FECRT) data and subsequently classifying farms into resistance categories. We applied this analysis to existing data set evaluating the efficacy of pyrantel embonate paste on 64 farms with a total of 614 horses in Denmark. We modeled the post treatment egg counts as a negative binomial distribution conditioned on the pre-treatment egg count. The model was then written as log(farm mean of post-treatment egg count)= log(farm mean of the pre-treatment egg count) + pre-treatment count for the horse + age +gender + presence of *Strongylus vulgaris* on the farm + farm + a geographical clustering effect defined by farm zip code. The farm and cluster effects were included as random effects. The overall anthelmintic efficacy is defined as (1 - (farm mean post-treatment count/farm mean pre-treatment count)). Based on our analyses, the pre-treatment egg count was statistically significant ($p<0.001$), while age, gender, and presence of *S. vulgaris* were not significant ($p>0.1592$). The variance of the farm effect was very small while cluster variance was determined to be 0.000031. Farm level efficacy and resistance classification was also calculated and comparisons show that they are similar to other previously published assessment methods. In conclusion, this analysis illustrates how FECRT data is significantly influenced by the pre-treatment egg count levels, and this needs to be carefully addressed when such data are analyzed.

13

Efficiency of a genetic test to detect benzimidazole resistant *Haemonchus contortus* nematodes in sheep farms in Quebec.

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Haemonchus contortus is a haemophilic nematode which infects sheep and causes anaemia and death of lambs. Benzimidazole drugs are used to remove these parasites, but the phenomenon of benzimidazole resistance has arisen worldwide. Most parasitological tests used to detect anthelmintic resistance are applicable only after treatment. A sensitive test to detect resistance, even at low levels, before treatment would be a useful tool to enable farmers to anticipate the efficiency of the drug before drenching the flock. We compared a test to detect benzimidazole resistance based on detection of genetic markers at codons 167, 198 and 200 on β-tubulin isotype 1 gene in *H. contortus* (genetic test) before treatment with the common method of Fecal Egg Count Reduction Test (FECRT). Fecal samples from young sheep on pasture from 11 farms in Quebec (Canada) were collected per rectum before and after treatment in a control and a treated group (10 animals per group). The 10 sheep of the treated group were drenched with fenbendazole at the recommended dose rate. Five samples of fresh fecal samples were randomly collected from the pasture in 5 farms. Among the 11 farms tested, we found *H. contortus* in 8 of them, although egg counts were not high. Benzimidazole resistance was found in *H. contortus* in each of these 8 farms based on genetic test results. Benzimidazole resistance was detected with the FECRT in all 5 farms in which egg counts were sufficient to conduct the FECRT. Comparing the results from the genetic test for samples off pasture with results from collected from individual sheep, and results from the FECRT, we concluded that the genetic test can be applied to field samples to estimate the benzimidazole resistance status of a flock before treatment for *H. contortus*. Supported by MAPAQ, Quebec and NSERC, Canada.

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Characterization of *Haemonchus contortus* P-glycoproteins 2 and 9 expressed in mammalian cells and their possible role in macrocyclic lactone resistances.

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Haemonchus contortus affects small ruminant production worldwide. This parasitic nematode has developed resistance to all available anthelmintic classes including the macrocyclic lactones (MLs), such as ivermectin (IVM), abamectin (ABA) and moxidectin (MOX). One of the mechanisms involved in ML resistance in nematodes is efflux by cell-membrane proteins, called ABC transporters such as P-glycoprotein (Pgp). The goal of this work was to express and characterize the *H. contortus* Pgps (HcoPgp-2 and HcoPgp-9) in mammalian cells and to determine their interaction with the MLs. We have expressed HcoPgp-2 (Pgp-A) and HcoPgp-9 in LLC-PK1 pig kidney cells. Both transcript and protein profiles for these HcoPgp transfectants have been evaluated by qRT-PCR and Western-blot, providing evidence that these heterologous cells are expressing the transcript and the protein. We have identified these nematode transporters by antibody specific immunofluorescence assays (IFA) on the cell membranes. Furthermore, functional assays on the transfected HcoPgp expressing cells, incubated with a fluorophore such as Rhodamine 123 (Rho123), showed significantly reduced fluorescence accumulation of Rho123, compared with parental untransfected cells, indicating an

active efflux of Rho123 by these nematode pumps. We have then studied the inhibitory effect of the MLs on Rho123 transport by the HcoPgps. IVM and ABA produced a pronounced effect on the inhibition of Rho123 transport by the HcoPgps. In contrast, MOX had only a low inhibition of Rho123 accumulation. These data suggest that the avermectins, IVM and ABA, interact strongly with HcoPpg-2 and HcoPpg-9, but that MOX has markedly less effect on these transporters. The results suggest that these nematode transporters may be linked preferentially to avermectin resistance in *H. contortus* and provide an explanation for some of the differences between avermectin resistance and resistance to MOX. Supported by NSERC, Canada; MDEIE-PSR-SIIRI and FRQ-NT, Quebec and the Centre for Host-Parasite Interactions.

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Evaluation of the larval migration inhibition assay for detection of anthelmintic resistance in cattle.

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Anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) of cattle is becoming a serious problem worldwide. The fecal egg count reduction test (FECRT) is commonly used to detect resistance in GIN of cattle, however, it suffers from lack of standardized protocols, labor and cost intensity, and high variability. Thus, an in-vitro assay for detecting AR in cattle could provide a more convenient, cost-effective, and scientifically-standardized option. The objective of this study was to evaluate the larval migration inhibition assay (LMIA) for cattle GIN and compare results to the FECRT. FECRT and LMIA were performed on 2 cattle ranches in Georgia. Herd A showed resistance to ivermectin with -68.21% fecal egg count reduction (FECR) 14d post-treatment. Resistance to moxidectin was demonstrated in both herds A and B, with 82.87% and 35.65% FECR, respectively on d14 post-treatment. *Cooperia* were the predominant genus, with smaller numbers of *Haemonchus* present in post-treatment cultures. For the LMIA, L3 were incubated for 24h in 7 different concentrations of macrocyclic-lactone (ML) anthelmintics (eprinomectin, ivermectin or moxidectin), and transferred to migration chambers containing a 28 uM mesh sieve for 24h. Migrated and non-migrated larvae were counted and the half maximal effective concentration (EC50) was calculated using a logistic regression model. All results are for *Cooperia* spp. Herd A had an EC50 of 1.59 µM for eprinomectin, and Herd B had an EC50 of 0.23, 8.21, and 9.13 for eprinomectin, ivermectin, and moxidectin, respectively. An additional known resistant *C. punctata* isolate tested only with the LMIA showed similar results with EC50 of 0.26, 3.70, and 7.58 for eprinomectin, ivermectin, and moxidectin, respectively. Although lack of a ML-susceptible isolate did not allow for validation and calculation of resistance ratios, these data suggest that the LMIA may provide a practical means to detect anthelmintic resistance in-vitro.

15.5

Monitoring important parasites in naturally infected foals.

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Equine nematodes, including the strongyle and ascarid superfamilies, are ubiquitous around the world at increasing levels of anthelmintic resistance. *Strongylus vulgaris* and *Parascaris equorum* are the two most pathogenic species causing thromboembolic colic and small intestinal impaction, respectively. Despite the clinical importance of these parasites, current

diagnostic and parasite surveillance tools are crude and inefficient at best, requiring validation and further optimization. The goal of this study was to monitor parasite burdens using three common coprological methods over time in a cohort of six colts from a horse herd maintained without anthelmintic intervention at the University of Kentucky. The colts were followed until necropsy at 200-300 days of age. Biweekly, strongyle and *P. equorum* fecal egg counts, coproculture for speciation of strongyle-family parasites, and Real-Time PCR for detection of *S. vulgaris* were performed. Upon necropsy, gold standard information was generated by characterizing worm burdens by species, stage, and sex. Repeated FECs suggested that *P. equorum* was replaced by strongyle species as the dominant parasites at approximately 167 days of age and had disappeared entirely by about 218 days. Coproculture determined that *S. vulgaris* became present at an average of 206 days. PCR positives achieved occurred at an average of 189 days. In this study, coproculture was the most reliable method of diagnosing *S. vulgaris* infection. Although this herd may not be representative of regularly managed herds, this study suggests that the parasite profile changes drastically within the first 200 days of a foal's life, changes that must be considered in choosing appropriate foal treatments. Further studies utilizing a more typical herd are needed before treatment recommendations are qualified.

Session 3 – Students – Dog and Cat Parasites

16

Rickettsia spp. infection in domestic dogs in Oklahoma.

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Domestic dogs are susceptible to infection with a number of different spotted fever group (SFG) *Rickettsia* spp. To determine the prevalence of antibodies reactive to SFG *Rickettsia* spp. in a population of dogs and identify the organisms responsible for these infections, serum and whole blood samples were collected from 61 dogs from animal shelters in northeastern Oklahoma from September 2012 to April 2013 and evaluated via indirect immunofluorescence assays (IFA) for antibodies reactive to *Rickettsia rickettsii*, *R. montanensis,* and *R. amblyommii*, and by nested and real-time PCR assays for *17Kda, rompA,* and *23S* of *Rickettsia* spp., respectively. Antibodies reactive to *R. rickettsii, R. montanensis*, and *R. amblyommii* were identified by IFA (1/128 dilution) in 43/61 (70.5%), 33/61 (54.1%), and 36/61 (59.0%) dogs respectively. In total, 8/61 dogs were PCR positive for *Rickettsia* spp.; sequencing is ongoing, but initial results confirm the presence of *R. amblyommii.* While a complete prior tick infestation history is not available for these dogs, *Amblyomma americanum, Rhipicephalus sanguineus,* or *Dermacentor variabilis* were present on 6/61 dogs at initial examination, 5 of which were dogs that were also serologically or PCR positive for *Rickettsia* spp. Taken together, our data suggest infection with SFG *Rickettsia* spp. is common in dogs in areas with robust tick populations, and support the interpretation that these infections are likely due to SFG *Rickettsia* spp. other than *R. rickettsii.*

17

Prevalence and assemblages of canine *Giardia duodenalis* isolates in southwestern Virginia.

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The protozoan parasite *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*) can cause diarrhea in humans, cats, dogs and other animals. *Giardia duodenalis* consists of eight genetic assemblages that are morphologically identical. Most canine isolates belong to Assemblages C and D, but isolates of Assemblages A and B (predominantly found in humans) have also been recovered from dogs. The objectives of this study were to determine the prevalence and assemblages of *Giardia* in dogs at animal shelters in the New River Valley in southwestern Virginia. We hypothesized that both canine (C, D) and zoonotic assemblages (A, B) would be present in the area. Fecal samples were collected from dogs at local animal shelters. On the day of collection samples were examined for the presence of *Giardia* cysts using a centrifugal flotation test with ZnSO4 flotation solution and were cleaned and concentrated using $ZnSO_4$ density gradient centrifugation to allow for optimized recovery of cysts (if present) before DNA extraction. A 292 bp fragment of the SSU rRNA gene was amplified using nested PCR to determine the presence of *Giardia* in each fecal sample. Samples positive by PCR were further processed and sequenced to determine the assemblage(s) present. Preliminary results suggest a *Giardia* prevalence of approximately 17% (8/47) in shelter animals, and both common canine assemblages (C, D) have been identified to date in cyst positive samples.

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High prevalence of *Borrelia burgdorferi* in *Ixodes scapularis* from southwestern Virginia.

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Borrelia burgdorferi is the causative agent of Lyme disease in humans, and is known to infect many veterinary species including horses and dogs. Lyme disease is endemic in the northeastern United States as far south as northern Virginia, but has not traditionally been considered endemic in the mountainous regions of southwestern Virginia. Recent increases in the number of reported human Lyme disease cases and recognized canine infection with *B. burgdorferi* prompted an investigation into the prevalence of *B. burgdorferi* in ticks and dogs from this region. Review of canine medical records from 2008-2012 revealed prevalence of specific antibodies to *B. burgdorferi* in dogs, as determined by detection on commercial ELISA (4DxSNAP®), ranged from 15% in 2008 to 26% in 2012. Adult ticks were collected by dragging public and private natural areas on a semi-monthly basis from October 2012 through April 2013. A total of 194 adult ticks were tested from two locations, one a public park bordered by woody edge habitat and a river (Site A), and the second a wooded, low density residential area (Site B). All ticks were morphologically and molecularly confirmed to be *Ixodes scapularis. Borrelia burgdorferi* was detected by sequence-confirmed PCR of *flaB* in a total of 32/88 (36%) of ticks from site A and 65/106 (61%) of ticks from site B, for a total prevalence rate of 50% (97/194), confirming that the agent of Lyme disease is emergent or endemic in this region. Additional tick testing together with modeling efforts that take into account human case reports of Lyme disease and canine *B. burgdorferi* infection rates over time may help determine what environmental factors are driving the expansion of Lyme borreliosis.

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Gastrointestinal parasitological survey of shelter dogs in southern Louisiana.

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A parasitological survey was conducted among shelter dogs in nine parishes in southern Louisiana. Fresh fecal samples were collected from recently acquired dogs and subjected to a direct smear, saline sedimentation and double centrifugation flotation with zinc sulfate and sucrose on the same day. The most prevalent parasite was *Ancylostoma* sp, followed by *Trichuris vulpis, Cystoisospora ohioensis, Giardia, Cystoisospora canis, Toxocara canis, Dipylidium caninum, Alaria* and lastly *Capillaria* sp. Of 209 dogs in total, 163 were parasitized and multiple parasites were found in 86 dogs. The hookworm species was identified from eggs and cultured larvae using PCR-RFLP using *hinf*1 from eggs and larvae.

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Infection efficacy in mollusks with the nematode *Aelurostrongylus abstrusus*.

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Aelurostrongylus abstrusus infections have been detected across the U.S. and Canada with an infection rate of between 0.1%-1.1%. The infection has also been detected across the globe with unknown rate of infection. Infections in cats often present with subclinical symptoms and, as such, go undiagnosed. If the worm load is high enough, symptoms may include mild to severe coughing, sneezing, mucopurulent nasal discharge, severe dyspnea, and possibly death. Cats are commonly misdiagnosed with allergic respiratory disease and are treated with bronchodilators and corticosteroids, to which they respond fairly well. The Baermann technique is considered the most sensitive method in larval detection, but recent work in the use of IgG for detection of *A. abstrusus* in the serum of felines has been done. This research seeks to perfect the infection technique of various snail genera with the nematode *A. abstrusus*. The advantage to perfecting the infection technique is the ability to harvest consistently many L3 infective stage nematodes from snails which can be used to infect a cat. Infection methods and snail genii tested resulted in promising outcomes from group-infected *B. glabrata*.

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***Mesocestoides* sp. infection in a cat: a case report.**

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A case of *Mesocestoides* sp. infection in a cat is presented. An adult female, spayed, domestic short hair feline presented to the Boren Veterinary Medical Teaching Hospital, Oklahoma State University in April 2013, for an initial visit 2 days post adoption. The history provided included a one week stay at the shelter prior to adoption, where they had visualized loose, bloody, and mucoid feces containing nematodes and tiny rice grain like structures. The cat was treated with topical emodepside/praziquantel at the labeled dose. The patient also had a history of sneezing, dyspnea, and mild ocular discharge. The physical exam showed no evidence of ascites, weight loss, or pain on palpation and all parameters were within normal limits. A fecal sample was collected and flotation revealed *Mesocestoides* sp. eggs. Upon visual inspection of

the feces, several intact portions of *Mesocestoides* sp. tapeworms were identified. Praziquantel (23mg, 6.5mg/kg) was administered orally and repeated six weeks later. Radiographic and ultrasound evaluation was declined by the owner. *Mesocestoides* spp. are cestodes found worldwide in a variety of bird and mammal definitive hosts. Tetrathyridia in intermediate hosts are infectious to the definitive host upon ingestion. Once inside the definitive host, the tetrathyridia reproduce asexually. Clinical forms of *Mesocestoides* sp. infection include visceral and intestinal. The complete lifecycle of *Mesocestoides* spp. is not fully understood.

Session 4 – Students – Treatment & Management

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Response of alpacas and sheep to experimental *Haemonchus contortus* infection.

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The blood feeding nematode *Haemonchus contortus* infects the abomasum of small ruminants. Heavy infections may cause severe anemia and death. Alpacas were first introduced into the U.S. in the 1980s. Although not true ruminants, alpacas may become infected with *H. contortus* and develop the clinical symptoms. Lower fecal egg counts (FEC) observed in our previous research suggest that alpacas are more resistant to *H. contortus* infection than sheep and goats. We hypothesized that given equivalent experimental exposure to *H. contortus*, alpacas would be less susceptible than sheep. The study was conducted with adult male alpacas (24) and sheep (12) housed in pens to prevent additional exposure to *H. contortus*. All animals were orally dewormed. *Haemonchus contortus* infective larvae were administered orally to alpacas and rams in the following groups:1) 20,000 larvae as a single dose (bolus, n=6), 2) 20,000 larvae in daily doses of 4,000 larvae for 5 days (trickle, n=6) Two additional groups of alpacas received either 50,000 larvae as a bolus infection, or in daily doses of 10,000 larvae (trickle). Fecal egg counts were determined every 2 days from 14 to 42 days post infection and then at 5 day intervals until day 62 using the Modified McMaster test. Blood samples were collected weekly for determination of packed cell volume (PCV). FAMACHA anemia scores, weight, and body condition scores were also evaluated weekly. Mean FEC was lower in alpacas than sheep and mean alpaca PCV was affected less by infection than sheep PCV. The results of this study support the hypothesis that alpacas are less susceptible to *H. contortus* infection than sheep, although we were unable to determine whether alpacas FEC reflected fewer adult worms or only reduced *H. contortus* egg production compared to sheep. This research was supported by a USDA NIFA 1890 Capacity Building Grant.

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Efficacy of injectable ivermectin on gastrointestinal helminths in captive wild elk (*Cervus elaphus*).

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Kentucky supports the largest elk (*Cervus elaphus*) herd in the eastern United States subsequent to translocation of the Rocky Mountain subspecies. With wildlife translocations

increasing in number, health protocol establishment and evaluation is important for them to be successful. One of the main concerns during translocation is parasite introduction into naïve ecosystems, which is often addressed with anthelmintic treatment. In the case of elk, information regarding efficacy and kinetics of anthelmintics is not available, and assumptions about dosages are extrapolated from studies in domesticated ruminant species. In January of 2013, 51 wild elk were captured and kept in pens for health assessment and biological testing. During this quarantine period, fecal samples were collected to assess parasite prevalence before and after treatment with anthelmintics. Individual fecal samples were collected at time of capture and during individual workups. Subcutaneous ivermectin injections (0.2 mg/kg) were administered at the second workup. Elk were then divided into 2 pens, dependent on age; individuals over the age of 1 year were put into Pen 1, and those under the age of 1 were put into Pen 2. Fecal egg counts were then performed on the pooled fecal samples from each pen to assess effectiveness of the anthelmintic. Preliminary results suggest ivermectin was almost 100% effective against strongylid-type parasites in both groups, successfully suppressing FEC numbers throughout the sampling period, but post-treatment FEC's also showed an increase in *Moniezia* spp. in Pen 1, whereas *Trichuris* spp., *Nematodirus* spp., and *Capillaria* spp. numbers increased in Pen 2. Data acquired from this group of elk suggests that age may play a significant role in immune response following anthelmintic treatment, which should be taken into consideration when administering drugs, and can be especially important information when contemplating herd management tactics.

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Effect of a single dose of Safe-Guard™ prior to weaning on parasite load of beef calves during the backgrounding phase.

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Selective deworming in cattle is difficult as producers tend to handle beef animals less frequently. In an effort to mediate impacts of parasitism, many producers treat calves more frequently to maintain growth. The objective of this study was to determine the efficacy of a single dose of Safe-Guard prior to weaning on parasite load during backgrounding. A total of six farms in North-Central WV and South-Western PA participated in this study and 300 calves were treated with the anthelmintic Safe-Guard™ once 3 wk prior to weaning. Fecal sampling occurred 3 wk prior to weaning, at weaning and 45d after weaning and data were analyzed by Meyers Parasitology Service. Individual parasitic egg species were reported using a relative system in which + = 1-10, ++ = 11-50 and +++ = >51 eggs per slide. Parasite species-specific data were analyzed as the frequency of species in each individual calf. Results of total FEC data indicated that a single dose of Safe-Guard™ given 3 wk prior to weaning was effective in reducing parasite egg output by 79% at weaning ($P < 0.001$). Increases in total FEC were observed by 45 d after weaning but were not a result of *Ostertagia* or *Cooperia* infection. Rather resulting increases were largely due to *Monieza*, whereas 50% of calves had >51 eggs per slide (+++). Stomach worm species did increase to d 45, however, greater than 75% of calves had stomach worm egg counts that ranged from only + to ++. The majority of parasite infection after weaning was due to *Monieza* rather than *Ostertagia, Haemonchus,* or *Trichostrongylus*. Taken together these data indicate that Safe-Guard™ was not effective at managing *Monieza* yet a single dose of fenbendazole given to calves pre-weaning was sufficient to maintain low stomach worm egg count in calves throughout the backgrounding phase.

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Evaluation of an integrated approach in controlling gastrointestinal nematodes in lambs.

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Gastrointestinal nematode (GIN) parasites cause extensive damage to small ruminants, and *Haemonchus contortus* is a major concern to production worldwide. With the development of anthelmintic resistance, alternative methods to control GIN are needed. In this study, the long term use of combinations of strategies on animal health, changes in GIN population, and changes in the environment were examined. Fecal and blood samples were collected for fecal egg count (FEC), larval identification and blood for packed cell volume (PCV) for both control (normal production system) and treatment (integrated methods) groups. Control lambs were fed Sheep Chow ,allowed to graze Bermuda grass during the day and were selectively dewormed with levamisole/albendazole/moxidectin combo when FAMACHA© was categorized as a 4. Treatment lambs were fed sericea lespedeza (*Lespedeza cuniata*) pellets, allowed to graze sericea lespedeza pastures and were selectively dewormed when FAMACHA© was a 4 with 1.0 g Copasure® COWP. Over the duration of the study for year 1 (2011) and 2 (2012) the FEC for treatment lambs remained lower and PVC higher than for the control lambs and treatment lambs were treated less frequently. Furthermore, treatment lambs had less coccidia than control lambs. However, the treatment group had less weight gain than the control group. Year 1 GIN populations consisted primarily of *Haemonchus contortus* followed by *Trichostrongylus colubriformis,* and *Cooperia* spp. For year 2 GIN populations consisted primarily of *Trichostrongylus colubriformis* followed by *Haemonchus contortus,* and *Cooperia* spp. This population difference could be due the different temperatures and rainfall experienced during 2011 and 2012. The results suggest that an integrated approach is an acceptable way to control parasites while sustaining a small ruminant production system.

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Abomasal gene expression of parasite-resistant sheep during a challenge *Haemonchus contortus* infection.

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Full thickness abomasum and abomasal mucosa samples were collected in 2007 from 24 St. Croix sheep and 24 Dorset x (Finn-Rambouillet) sheep on d 0, 3, 5, and 7 following challenge infection with *Haemonchus contortus*. Genes, involved in the immune response to *H. contortus* infection, were selected and can be categorized as markers of inflammation, tissue remodeling, immune cells or transcription factors. Real-time PCR analysis revealed that significant differences between breeds were tissue and time-dependent. Abomasum samples of St. Croix sheep had higher expression of three tissue remodeling genes including *VEGF* on days 3 and 5 (2.365 vs. 1.950; $P = 0.007$; 2.514 vs. 2.199; $P = 0.029$, respectively), *MMP13* across all times (3.070 vs. 2.699; $P < 0.001$) and *PDGF* across all times (3.165 vs. 3.085; $P = 0.014$). Abomasum samples of crossbred sheep showed higher expression of immune cell recruiter, *MCP1*, across all times (3.022 vs. 2.769; $P = 0.008$). Mucosa samples of crossbred sheep had higher *MCP1* expression on day 7 (3.409 vs. 2.985; $P = 0.017$) and higher overall IL6 expression (3.498 vs. 3.268; $P < 0.001$). *IL4*, a key marker of T-helper 2 responses, was up-regulated in St. Croix mucosa from days 3-7, while IL4 expression was undetectable in mucosa

of crossbred sheep. St. Croix mucosa had greater expression of *arginase 1* (3.447 vs. 3.329; $P = 0.035$), *IL3* (3.749 vs. 3.390; $P < 0.001$), *TNFα* (3.612 vs. 3.412; $P = 0.003$), and *WC1* (3.309 vs. 3.141; $P = 0.044$). Increased expression of wound repair genes in the abomasum and immune response genes in the abomasal mucosa are associated with lower worm burdens in St. Croix sheep by d 7 after infection (250 vs. 875 $P < 0.05$). Collectively, these data may identify early immune mechanisms generated by St. Croix sheep that mediate parasite expulsion.

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Surfactant influence on the ovicidal activity of different short-chain fatty acids on *Ascaris suum* eggs.

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Ascaris suum eggs, due to their inherent environmental indestructability, are routinely used as bio-indicators to test the ovicidal activity of various manure and biosolids disinfection methods. Exposure to organic acid is a possible disinfection method for inactivating *A. suum* eggs. Previous research has shown the eggs could readily be killed when the pH of the acid solution was below the pK_a of the acid such that the majority of the acid was in the undissociated form. Expanding on this earlier work, various concentrations and contact times of butanoic, pentanoic, hexanoic and heptanoic acid at pH 4, with and without 18 mM Tween 20, were examined to determine minimum required times for total inactivation at 22°C and 37°C. Increasing the temperature by 15 degrees caused a significant decrease in the contact time required for total inactivation. The addition of the surfactant allowed 100% inactivation to occur within minutes. The results suggest that a higher molarity of pentanoic and hexanoic acids with the addition of the surfactant Tween 20 have potential for the rapid inactivation of helminth eggs on surfaces, manures and biosolids.

Novartis Symposium

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Resistance and heartworm preventives: historical perspective and overview of research.

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Reports of heartworm prevention failures to macrocyclic lactone (ML) drugs appeared to be escalating based on a 2005 report by the United States Federal Food and Drug Administration. Most were attributed to client compliance, but many were considered true prevention failures. We assembled a team of investigators to investigate selected cases of prevention failure.

Approaches and criteria included review of patient records, inability of veterinarians to remove microfilariae using traditionally effective drugs and methods, reduced response to MLs in *in vitro* microfilaricidal and L3 bioassays, genetic changes in target genes, and GPS analysis of putative failures. We have to date identified and maintained *Dirofilaria immitis* isolates in dogs that we consider candidates for resistance to ML preventives. Challenge studies in Beagle dogs confirmed resistance in the two isolates tested. *In vitro* bioassay evaluations of susceptibility of these isolates and genetic analysis of target genes yielded results that also were suggestive of ML resistance. Patent infections in experimentally infected dogs on ML therapy were produced from two different field dogs with suspected lack of efficacy, providing additional support for heartworm resistance. Studies will be presented and discussed in the context of support or lack of support for heartworm resistance.

Session 5 – Heartworm Studies

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Genomic search for macrocyclic lactone resistance markers in *Dirofilaria immitis.*

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Macrocyclic lactone (ML) endectocides are used, mostly at monthly intervals, during the transmission season for chemoprophylaxis for heartworm (*Dirofilaria immitis*) in dogs and cats. Claims of loss of efficacy (LOE) of ML heartworm preventatives in recent years have become more common in some locations in USA. ML resistance is widely recognized in nematode gastrointestinal parasites of ruminants and horses and in the human filarial nematode *Onchocerca volvulus*. Previously a correlation between a P-glycoprotein genotype and ivermectin efficacy had been established. The goal of our project was to find markers that could reliably predict for ML resistance in *D. immitis*. We used a whole genome approach on phenotypically well characterized pooled samples to address this issue. A number of loci showed highly significant differences between pools of susceptible and LOE samples. From these loci, Sequenom analysis was conducted on 186 loci from 612 individual parasites (adult worms and microfilaria) which were well characterized with regard to the phenotypic response to treatment. From those candidate markers, after statistical analysis, a small number of genetic loci appear to be very promising as markers for predicting ML resistance in *D. immitis*.

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Markers for macrocyclic lactone resistance in *Dirofilaria immitis* and utilization for management of Heartworm Disease.

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Macrocyclic lactones (ML) are used as chemoprophylaxis to control for Heartworm diseases in dogs and cats. Claims of ML loss of efficacy (LOE) are being increasingly reported in USA. Currently it is sometimes difficult to know whether apparent LOEs are due to a failure of compliance or to genetically based ML resistance in *Dirofilaria immitis*. This causes problems in the veterinary clinic as decisions about appropriate prevention and control should be influenced by whether true genetic resistance is absent or present. We have identified a small number of DNA markers that can predict ML resistance in *D. immitis*. These markers are being validated on parasites collected from dogs in which ML resistance has been experimentally demonstrated. A temporary failure of compliance can sometimes explain apparent LOEs. However, in other cases compliance may not explain the apparent LOE and a simple test is needed to confirm the absence or presence of resistant parasites so that appropriate control strategies can be implemented. Such markers will be important tools to confirm true ML resistance, to establish the extent of resistance in the field and to determine the extent of the problem. This information will become critical if we are to make informed decisions which will allow heartworm control to continue and to stimulate renewed efforts to develop alternative control strategies.

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Evidence of genetic selection following treatment of a heartworm-infected, microfilaremic dog with increasing dosages of ivermectin.

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Resistance of heartworm to macrocyclic lactone (ML) preventives was postulated as early as 2005. It appeared from the data that reports were more common for the southeastern United States and specifically for the region referred to as the Mississippi delta. This region extends from Memphis, TN south to eastern Louisiana and follows a distribution in close proximity to the Mississippi River. We launched an investigation into the likelihood of resistance using the following criteria: compliant use of preventives (patient records), inability to eliminate microfilariae in heartworm positive dogs, results of our in vitro microfilariae susceptibility assay, and molecular characterization (single nucleotide polymorphisms [SNP) of a specific P-glycoprotein locus). Using these criteria, we identified and propagated several heartworm biotypes that we considered candidates for resistance. Among these isolates was a biotype we referred to as TD1. TD1 was shown in additional experiments to be resistant to the macrocyclic lactone class of heartworm preventives. The purpose of this study was to attempt to select for SNPs using a low, escalating dose of ivermectin in a microfilaremic dog experimentally infected with TD1. A microfilaremic dog infected with TD1 was treated orally at weekly intervals with doses of ivermectin ranging from 3 – 24 µg per kg of body weight. The change in AG vs. GG at SNP 11 of the Pgp-1 gene was determined at weekly intervals by sampling microfilariae at each time point. Results indicated a significant reduction in microfilariae (17,625/mL – 1,560/mL; $p<0.001$) over the 6-month study period. Results also indicated that there was a significant reduction in the AG SNP at the SNP 11 locus after 6 months of treatment with ivermectin ($P=0.017$). Based on the analysis of the Pgp-1 SNP 11 locus we conclude that repeated administration of ML can lead to genetic selection of heartworm biotypes with significantly reduced AG supportive of resistance to ML.

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Testing the efficacy of an injectable moxidectin formulation (ProHeart® 6) against a field isolate of canine heartworm.

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This study examined the effectiveness of an injectable moxidectin formulation (ProHeart® 6) to protect dogs experimentally challenged with *Dirofilaria immitis* third-stage larvae from a macrocyclic lactone-resistant field isolate, JD2009-1, from Earle, AR. The study utilized a total of 6 Beagle dogs from a Class A animal vendor. The dogs were housed under an approved IACUC in the AALAS-approved facilities of Cornell University. Following the same protocol as in the original NADA by which ProHeart® 6 was found to be fully efficacious, four dogs were treated with ProHeart® 6 on Day 0 and two dogs were left as untreated controls. The dogs were monitored for the next 6 months. On Day 180, all dogs were inoculated subcutaneously with 80 third-stage larvae from mosquitoes fed on the microfilaremic blood of a dog infected with isolate JD2009-1. Blood samples were collected from all dogs on Days -1, 150, 241, and 330. Echocardiography was performed on all dogs on either Day 328 or 329. The dogs were euthanatized and necropsied on Day 330. The four blood samples from each of the 6 dogs were all negative for heartworm antigen using the IDEXX SNAP® test. There were no worms seen in any of the dogs via echocardiography. At necropsy, all dogs harbored adult heartworms. One control dog had 15 worms and the other had 43 worms. From the four treated dogs, the worm recoveries were 15, 19, 24, and 33. The efficacy of the injectable moxidectin formulation as determined using geometric means was only 14.2%. It appears that ProHeart® 6 is not significantly protective against this isolate, JD2009-1, of macrocyclic lactone-resistant *Dirofilaria immitis*.

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The establishment of macrocyclic lactone resistant *Dirofilaria immitis* isolates in experimentally infected laboratory dogs.

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Strains of *Dirofilaria immitis* suspected of lack of efficacy (LOE) to macrocyclic lactone (ML) preventive drugs have been reported in dogs by practicing veterinarians in Louisiana and the Lower Mississippi Delta region. If proven, and not controlled in the early stages, the emergence of ML drug resistance threatens to become a widespread problem in the US that may limit the effectiveness of current preventive drug treatment methods. The present study utilized microfilariae (Mf) from two canine field cases from different state locations that fit criteria for a high index of suspicion of ML lack of efficacy against heartworms: a) history of failure of efficacy and full monetary compensation by a commercial pharmaceutical firm; b) location in an area identified as a 'hotspot' of suspected drug resistance in a statewide practitioner survey of LOE cases; c) persistence of Mf seven days after an accepted ML microfilaricidal dose, and d) high frequency of a genotype marker reported to be correlated with potential ML resistance.

Blood containing Mf from the two high index of suspicion dogs were used to infect and produce L3 in *Aedes aegypti* for experimental infection of two groups of laboratory dogs, each comprised of two dogs, one treated with prophylactic Ivermectin (12µg/kg) monthly and one untreated control. Both treated and untreated dogs from Group I and Group II developed patent *D. immitis* infections by 212 DPI and 187 DPI, respectively, as evidenced by a positive occult heartworm antigen test and microfilaremia by the Knott's test. Mf counts gradually increased post-patency in test and control dogs over the next 9 months (Group I) or 7 months (Group II). These experimental infection studies provide *in vivo* evidence of the existence of ML drug resistance in dogs infected by *D. immitis* L3 from suspect field LOE cases in the Lower Mississippi Delta.

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Efficacy studies in dogs demonstrate resistance of *Dirofilaria* against ivermectin and other macrocyclic lactones.

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Current products to prevent heartworm disease in dogs and cats are based on macrocyclic lactones (ML). Claims of ML loss of efficacy (LOE) are being increasingly reported in the USA. Whether this is due to irregular administration of products, or due to drug resistant parasites, has been disputed. Molecular work at McGill University and *in vitro* tests at Auburn University indicate that there is a genetic change in putative resistant isolates which correlates with changes of *in vitro* sensitivity of *D. immitis* for ivermectin. In order to elucidate the reason of non-responding isolates we have determined the efficacy of ivermectin and other ML in dogs in controlled laboratory studies. A total of 16 dogs were experimentally infected with *D. immitis* L3 larva derived from mosquitos fed on microfilaria collected from *Dirofilaria* infected dogs (2nd passage donor dogs). Donor dogs harbored *D. immitis* isolates that fulfilled established criteria for probable resistance. Thirty days after infection, dogs were treated with ivermectin/pyrantel (Heartgard Plus®) applied at the recommended dose or higher. Treatment was repeated at monthly intervals until the day of necropsy. Total worm counts showed an efficacy of ivermectin of 20.3% to 82.9% depending on the isolate. 15 out of 16 ivermectin treated dogs were positive for heartworms. Preliminary results of efficacy studies in dogs infected with the ivermectin-resistant isolates showed that these isolates were also resistant based on worm recovery to selamectin, milbemycin oxime and moxidectin. In all treated groups dogs with adult *D. immitis* were present. These results demonstrate that the 2 *Dirofilaria* isolates are resistant against ivermectin and other ML, and that the resistance is inherited. Thus, the reports of lack of efficacy of ML against certain *D. immitis* isolates despite appropriate testing and compliant heartworm prophylaxis are likely due to resistant heartworm isolates.

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Reversible interference with detection of antigen of *Dirofilaria immitis* in canine serum samples.

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Diagnosis of *Dirofilaria immitis* infection in dogs is largely dependent on detection of circulating antigen in canine serum, plasma, or whole blood. Serendipitously, we discovered that mixing serum from a dog not infected with *D. immitis* with serum from a microfilaremic, antigen-positive dog converted the positive sample to antigen-negative; detection of antigen was restored when the mixed sample was heat-treated. To determine the degree to which the presence of blocking antibodies or other inhibitors of antigen detection may interfere with our ability to detect circulating antigen in canine samples, plasma samples (n=165) collected from dogs in animal shelters were tested with up to 4 different commercial assays for *D. immitis* antigen (PetChek HTWM PF, DiroCHEK, SNAP 4Dx, and SoloStep CH) before and after heat treatment. All 165 samples were negative prior to heating, but 11 (7.1%) became positive after heat treatment, a conversion that was documented and quantified on spectrophotometric plate assays. An additional 99 plasma samples which tested positive prior to heating were evaluated by spectrophotometry; the optical density (OD) of 15/99 (15.2 %) significantly increased with heat treatment. A blood sample was also evaluated from a dog that was microfilaremic and for which microfilaria were identified as *D. immitis* by morphologic examination. Antigen was not detected in this sample prior to heating but the sample was strongly positive after heat treatment of whole blood. Our results indicate that blood samples from some dogs contain factors that inhibit detection of antigen of *D. immitis*, and that heat treatment of these samples prior to testing could improve the sensitivity of these assays in some patients.

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An analysis of time trends for canine heartworm in Grenada and its associated risk factors based on veterinary clinical pathology laboratory data base records between 2005 and 2009.

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Heartworm disease caused by *Dirofilaria immitis* and transmitted by mosquitoes in dogs and cats, affects primarily the cardiovascular and respiratory systems with the potential to cause heart failure and severe lung disease. This study is aimed at establishing the trends in the prevalence of heartworm disease in dogs in Grenada and the risk factors associated with it. We evaluated veterinary clinical pathology laboratory data base records to determine the number of cases of heartworm/ microfilariae diagnosed for each year between 2005 and 2009. All laboratory diagnosed cases of heartworm or microfilariae were frequency matched with records for dogs without a laboratory diagnosis of heartworm but, from the same year. Ninety three frozen serum positive samples were later analyzed for the presence circulating heartworm antigens using an antigen test kit (Heska solo® Step CH). We found 198 cases of heartworm and matched with 198 controls. Our analysis showed that the prevalence of canine heartworm increased gradually over the time period studied; from 59 cases per 1000 dogs in 2005 to 156 cases per 1000 dogs in 2009, [X^2 for linear trend =7.98, p=0.005]. Adult dogs (6 months - 7 years) and geriatric dogs (older than 7 years) had increased risks of heartworm disease, odds ratio (OR) = 5.5 (95% CI, 2.4 – 12.8) and OR= 3.3 (95% CI, 1.3 – 8.7) respectively, compared to puppies (less than 6 months). Neutered dogs had a statistically lower risk OR= 0.5 (0.3 – 0.8) compared to intact dogs. All cases (n=93) of microfilariae identified were confirmed as *Dirofilaria immitis* using the Heska antigen test kit. Our findings clearly show that the prevalence of heartworm disease in dogs in Grenada increased from 2005 to 2009.

Session 6 – Wildlife

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Molecular characterization of *Sarcocystis* spp. isolates from marsupials of the genus *Didelphis* spp. through the analysis of mitochondrial and apicoplast genes, and surface antigen genes. Preliminary Results.

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Sarcocystis neurona and *Sarcocystis falcatula* are very similar species of Apicomplexan protozoa that use marsupials of the genus *Didelphis* as definitive hosts. In a previous paper, we have shown extensive variation in surface antigens of *Sarcocystis* spp. shed by marsupials in the genus *Didelphis* and concluded that divergent alleles of SAG2, SAG3, and SAG4 coding genes might be exchanged in possible sexual recombination processes between different isolates of *S. falcatula*. Herein, we report preliminary results of a more extensive study of such variability. Gene sequencing of SAG2 and SAG3 fragments amplified from sporocysts isolated from ten South American opossums of the genus *Didelphis aurita* revealed that three of them shed sporocysts that have SAG3 fragments highly similar (although not identical) to homologous fragments of *S. neurona*. In contrast, SAG2 fragments obtained from these isolates have higher similarity to homologous sequences of *S. falcatula*. Surprisingly, sequence data obtained from gene coding for cytochrome B of all the samples revealed 100% identity. These results put light on the hypothesis that the species formally described as *S. falcatula* and *S. neurona*, may actually represent sexually driven recombinants of the same species.

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Ecology of a potentially emerging tick-borne pathogen new to the US: *Ehrlichia muris* in the American Midwest and Texas.

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Within the past five years, an *Ehrlichia muris*-like organism (hereafter *E. muris*) that is transmitted by ticks was for the first time associated with human and canine disease in the US. To begin to understand the ecology of this agent, we analyzed field samples collected from 2010-2012 at Fort McCoy army installation in west central Wisconsin, near the origin of the human infections. We subjected 674 samples, including ear biopsies from 4 small mammal species, *Ixodes scapularis* removed from hunter-harvested white-tailed deer, and questing *I. scapularis*, and, to three separate PCR assays to detect and characterize *E. muris* infection. Small mammals exhibited low *E. muris* infection prevalence, with 2% of 199 small mammals testing positive, including multiple infected white-footed mice. The infection prevalence of questing nymphs (10.9%, n = 190) was double that of questing adults (5.1%, n = 138), suggesting that larval bloodmeal hosts may be important reservoirs. Questing larvae were

characterized by 1.5% minimum infection prevalence indicating possible transovarial transmission. Adult ticks removed from deer had similar infection prevalence (4.8%) to questing adults, suggesting that deer are not important reservoirs. Sequencing of the citrate synthase gene fragment revealed variation with at least four strains present. To begin to assess the geographic distribution of this pathogen, we collected and analyzed 119 ticks from Texas wildlife including feral swine (*Sus scrofa*), bobcat (*Lynx rufus*), and white-tailed deer as well as pet dogs. Positive ticks were removed from all host types, with an average infection prevalence of 13%. The ecology of *E. muris* in the United States involves wild and domestic reservoir hosts and coinfections with other pathogens in the *I. scapularis* microbial guild; future studies must continue to elucidate the ecology of this 'emerging' pathogen.

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Virulence of Brazilian clonal genotypes of *Toxoplasma gondii* in mice orally infected with oocysts.

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Toxoplasma gondii is considered the primary cause of congenital disease, miscarriage and stillbirth in mammals. Toxoplasmosis is spread worldwide, but South America is dominated by genetically different genotypes, some of which are highly mouse-virulent. The model system that provided a wealth of data is the murine infection model. The aim of this study was to verify the virulence of the clonal genotypes BrI and BrII of *T. gondii* to mice. Oocysts of *T. gondii* genotype BrI (isolates TgCatBr74 and TgCatBr60) and BrIII (isolatesTgShBr8 and TgCatBr71) were obtained from faeces of cats fed with experimentally infected mice. After sporulation, 1,0 x 10^2 oocysts were orally administrated to swiss albino mice. Blood samples for antibody analysis using IFAT were collected seven days post inoculation and after death. After infected with BrI all mice died on days 9 to 11 p.i. regarding TgShBr8 (8/8) and on days 9 to 10 p.i. with TgCatBr74 (4/4). However, death by acute disease was also observed within genotype Br III: 75% (6/8) of mice inoculated with TgCatBr60 died on 11 to 21 days p.i. and 67% (4/6) from those which received TgCatBr71 oocysts died 12 to 20 days p.i. Clinical signs of disease as weight loss, apathy and bristly hairs were shown by all infected mice and tachyzoites were always observed within lungs during necropsy. Brain cysts were observed only in mice inoculated with BrIII types. Serum analyses were negative 7 days p.i., but there was seroconvertion soon before death resulting titles of 1:16 (TgShBr8), 1:32 (TgCat Br74), 1:512 (TgCatBr71) and 1:1024 (TgCatBr60). Genotype BrIII strains, previously considered avirulent, appear to be virulent when mice are inoculated orally with oocysts. This mode of infection is common in the natural life cycle and virulence of BrIII genotype have not been reported when mice were inoculated with tachyzoites.

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Merogonic stages of *Theileria cervi* in mule and white-tailed deer.

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In February, 2012, twelve farmed mule deer were moved from a facility in southwestern Oklahoma to a facility in southeastern Oklahoma that housed 100 farmed white-tailed deer. Between the third and fifth weeks, 9 of the 12 mule deer had succumbed, four of which were submitted for necropsy. The deer were heavily infested with *Amblyomma americanum* (lone star ticks). Hematologic data from one deer revealed severe anemia, leukocytosis and intraerythrocytic hemoparasites consistent with *Theileria* spp. Microscopically the liver, lymph nodes and spleen contained multifocally distributed, enlarged monocytic cells whose cytoplasm was replaced by developing meronts in various stages of merogony. In addition, there were renal tubular hemoglobin casts and uremic abomasitis. We hypothesize that, upon arrival, the *T. cervi*-naïve mule deer became infested with large numbers of *Theileria*-infected lone star ticks leading to massive exposure of the mule deer to sporozoites of the protozoan resulting in an acute hemolytic crisis and the fatalities. Further, we describe merogonic stages of *T. cervi* that are previously unreported. We attribute the lack of earlier reports of merogony to our presumption that only a single, short-lived, merogonic cycle follows exposure to sporozoites and thus merogonic stages are demonstrable for only a short period. PCR results of paraffin-embedded tissue show that the 507 bp amplicon sequence was 100% identical with the sequence of *T. cervi* previously reported from white-tailed deer in Oklahoma and from elk in Wisconsin and Indiana.

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Parasite interactions and their potential to influence West Nile virus transmission.

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Hosts are commonly infected with a suite of parasites, and interactions among these parasites can affect the size, structure, and behavior of host–parasite communities. During a long-term collaborative project in suburban, Chicago, Illinois studying West Nile virus, we explored the potential for co-circulating parasites to influence WNV transmission. We documented many parasites, including Haemosporidia, Trypanosomatida, and helminths in the key avian host species important for WNV amplification. These parasites will be described and patterns of association with WNV will be discussed. For example, we documented an inverse association between WNV serostatus and *Plasmodium* infection status, with an immune-mediated parasite interaction as a potential mechanism resulting in this pattern. Current studies are underway to gain a mechanistic understanding of the direct and immune-mediated interactions between multiple co-circulating parasites and WNV.

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Gastrointestinal parasites in commercial *Bison bison* herds from the U.S.A. central Great Plains.

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There have been no reported surveys of gastrointestinal parasites common to bison herds in the U.S.A. This study determined the prevalence and intensity of coccidian, trematode, cestode and nematode infections within bison age-categories covering 30 herds from 7 north-central states, and is also the first bison study using PCR to identify genera of trichostrongyles present. Fecal samples and questionnaires were collected from participating bison producers, and fecal egg counts were performed on 562 fecal samples. A PCR assay (based on the interspacial regions of rDNA) was performed on 96 DNA samples to determine if the 4 most common genera of cattle trichostrongyles (*Ostertagia, Cooperia, Haemonchus*, and *Trichostrongylus*) were present in these eggs. Samples were collected early in the grazing season to maximize detection of *Toxocara vitulorum* in calf samples. Trichostrongyles eggs and coccidian oocysts were common among most fecal samples (79.2% of cows, 75.5% of yearlings, 44.9% of calves), but *Nematodirus*, *Trichuris* and *Moniezia* eggs were found in fewer herds and in fewer egg numbers. Calves were more commonly infected with *Nematodirus* (19.1%), but yearlings were shedding many more trichostrongyle, *Trichuris* (11.9%) and *Moniezia* (9.9%) eggs and coccidian oocysts than the calves and adults. PCR identified at least 1 trichostrongyle genera in virtually all samples. *Cooperia* and *Haemonchus* were found in the vast majority of samples tested, and *Ostertagia* and *Trichostrongylus* were slightly less common. *Toxocara vitulorum* was found in calves from 2 out of 12 Minnesota bison herds that submitted calf samples. Microscopy revealed novel egg-like structures resembling *T. vitulorum* eggs that were commonly present in fecal samples from bison of various ages. Questionnaire results suggest that anthelmintic treatments were popular, but only half treated individual bison; the others provided their herd with opportunities to consume anthelmintics in the water, feed or mineral.

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Molecular and parasitological detection of *Trypanosoma evansi* in Camels in Ismailia, Egypt.

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Trypanosoma evansi (*T. evansi*) is an endemic disease of camels and other domestic animals in Egypt. This study aimed to determine the prevalence of clinical and sub-clinical *T. evansi* infection among camels in Ismailia, Egypt, as well as survey their owners for *T. evansi* infection. The diagnostic sensitivity of three different PCR assays for detection of *T. evansi* in blood samples was evaluated. Blood samples were collected from 100 camels and 20 of their owners from Ismailia governorate. Results revealed that the infection rate of camels with *T. evansi* varied with the detection method, ranging from 10 to 46% by PCR compared to 12% by microscopic examination of stained blood smears. Targeting the highly repeated sequence of mini-chromosome satellite DNA (TBR1/2 primer set) was the most sensitive PCR method (46% positive) compared to the primer sets targeting ITS 1 (16% positive) or RoTat 1.2 VSG (10%

positive) sequences. A partial sequence of RoTat 1.2 VSG gene was identical to *T. evansi* sequences reported from India and Kenya, but less similarity was seen when aligned with Egyptian *T. evansi* sequences. None of the camel owners were positive for *T. evansi* by microscopic examination of stained blood smears or PCR assays. Accurate diagnosis of surra disease in camels based on a highly sensitive PCR assay is considered an important measure for controlling the disease in camels and reducing economic losses.

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***Angiostrongylus cantonensis* in an orangutan (*Pongo pygmaeus*) in Florida.**

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Angiostrongylus cantonensis is a parasitic nematode in the Superfamily Metastrongyloidea, known as the lungworm of rats. The first stage larvae are shed in the feces of rats, and snails serve as intermediate hosts, harboring the infective third-stage larvae. If the infective larvae are ingested by other mammals or humans, they can cause serious neurotropic disease and rarely death. In November 2012, a six year old female orangutan (*Pongo pygmaeus)* from Miami, Florida presented to the University of Florida College of Veterinary Medicine teaching hospital with fever, inappetence, lethargy, ascending paresis/paralysis and cerebrospinal fluid eosinophilia. Disease progressed over the course of a week, and the orangutan was euthanized. At necropsy, within the dorsal brainstem, there were two dark red foci, measuring approximately 2-3 mm in the right side and approximately 5 mm on the left side. Histologically, there was necrosis and mixed eosinophilic, lymphoplasmacytic and granulomatous inflammation multifocally in the brain and spinal cord. Multiple cross sections of approximately 150-200 µm diameter nematodes were observed histologically in the brainstem. The owner noted that the orangutan often fed upon snails, which we identified as *Zachrysia provisoria* and *Bulimulus guadalupensis.* Snail specimens were collected from the orangutan habitat and were sent, along with scrolls of the histological blocks containing the nematodes, to the Centers for Disease Control and Prevention (CDC) for PCR verification. Histological sections, as well as the terrestrial snail, *Z. provisoria,* were positive for *A. cantonensis.*

Session 7 – Students – Epidemiology & Wildlife

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***Trypanosoma cruzi* genetic strain diversity in Texas wildlife and kissing bug vectors.**

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Trypanosoma cruzi is a protozoal parasite transmitted by triatomine bugs. Vectors for *T. cruzi* are prevalent throughout the tropical and subtropical regions of the Americas, where the parasite causes Chagas disease in animals and humans. Although *T. cruzi* is harbored in wild and domestic animals throughout the southern U.S., the public health burden of disease is not recognized to be as high as it is in Central and South America, and geographic variation in parasite genetic strains may contribute to the observed differences in human disease. Few studies have investigated these differences in the United States. Our research group is taking a comprehensive approach to study this disease system in Texas. Broadly, we hypothesize that the ecology at the nidus of Chagas disease in the U.S. includes vector phenology, wild and domestic animal population dynamics, and diverse parasite genetic strains. We initiated sampling of wild mammals and vectors across central Texas in spring 2013. Our sampling includes live-trapping of small rodents for blood collection, recovery of heart tissue from hunter-harvested mesomammals, and kissing bug collections from diverse ecoregions from bug traps and public submissions. We are assessing *T. cruzi* infection prevalence and strain diversity over space and time based on the amplification of a single polymorphic locus: the TcSC5D gene. We expect that our studies of the ecology of the disease system and parasite strain types in wild animal populations will provide critical information useful in efforts to predict and reduce *T. cruzi* infection in human and animal populations.

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Investigating the genetic diversity of muscular *Sarcocystis* species infection in raptors.

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Protozoal encephalitis is increasingly being reported in raptors from the United States. *Sarcocystis falcatula* DNA has ben identified using molecular methods in Great Horned Owls (*Bubo virginianus*), Golden Eagles (*Aquila chrysaetos*) and Bald Eagles (*Haliaeetus leucocephalus*) from rehabilitation centers in Indiana, Minnesota and Virginia. We examined the heart and breast muscles of raptors submitted to the Carolina Raptor Center in Huntersville, NC for the prevalence of *Sarcocystis* species. Birds that died or were humanely euthanized due to poor clinical prognosis were used in the present study. Hematoxylin and eosin (H&E) stained tissue sections of heart and breast muscle were examined microscopically for parasites and sarcocysts. DNA was isolated from frozen heart and breast muscle of H&E positive samples and examined using PCR primers ITS primers 18S 9L/1H for *Sarcocystis* species and JNB 34/55 for *Sarcocystis neurona* or *S. falcatula*. Samples that tested positive for ITS and negative for JNB (N=15) were also tested with primers to identify *Sarcocystis calchasi* using SCa1/2, *S. columbae* using SCo1/2, and *Sarcocystis sp. Ex. A. nisus* using SNi1/2. Of the 106 DNA samples examined 19 were positive for ITS and 4 were positive for JNB34/55 and ITS. Examination by RFLP using DraI and HinfI indicated that *S. falcatula* was present in 1 bird while RFLP testing on the remaining 3 is underway. DNA from *S. falcatula, S. neurona* and *S. lindsayi* was amplified by SNi (*S. sp. Ex. A. nisus*) primers but not primers designed to detect *S. calchasi* (SCa1/2) or *S. coulmbae* (SCo1/2). Our early molecular studies indicate that *S. falcatula* and other *Sarcocystis* species occur in the muscles of raptors from North America. Supported in part by the Historically Black Universities and Colleges-Undergraduate Program (HBCU-UP) and Smith Institute for Applied Research grant to ACR and an IRC grant from Virginia Tech to DSL.

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Distribution and characterization of *Heterobilharzia americana* in dogs in Texas.

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Heterobilharzia americana is a trematode parasite (family Schistosomatidae) that infects a wide range of wild mammalian hosts, and a small number of cases have been reported in dogs and horses. A total of 238 canine *H. americana* cases in Texas were retrospectively collected for a period of approximately 22 years from case records at the Texas Veterinary Medical Diagnostic Laboratory and the Veterinary Medical Teaching Hospital, pathology service, diagnostic parasitology service, and Gastrointestinal Laboratory at TAMU CVM. Of these cases, 26 patients had 1 to 2 repeat positive tests with a median of 51 days (14 days to 4 years) between testing making a total of 266 positive tests (26 biopsies, 39 necropsies, 160 fecal examinations, and 43 PCR). There were 12 households with multiple dogs infected, ranging from 2-4 dogs. The median dog age was 5.6 years (2.7 mos. to 17.2 years) and median weight was 45 pounds (2.3 to 135.6). Most AKC breed groups were well-represented: Crossbreed (20%), Herding (17.8%), Sporting (16.1%), Toy (10.8%), Hounds (10.8%), Working (10.1%), Terrier (8.5%), Non-sporting (4.9%), and Miscellaneous (1%). Diagnosis was made year round with no seasonal pattern. Clinical signs included diarrhea (67%), weight loss (38%), anorexia/hyporexia (27%), vomiting (22%), hematochezia (20%), lethargy (17%), polyuria /polydipsia (6%), and collapse (3%).Cases were distributed primarily in the eastern region of Texas from 40 counties and appeared more frequent in metropolitan areas with higher populations and in coastal counties. Bexar, Galveston, and Harris counties contained more than half of all cases. Cases were also seen in northern counties bordering Oklahoma. Our data indicate that this parasite is more widely distributed and more common than is generally believed. Increased awareness may aid in more diagnoses and timely therapy.

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Surveillance of filarial nematodes in free ranging moose from Minnesota.

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Moose (*Alces alces*), a culturally and economically valued species, have historically occupied northern forests of Minnesota. However, since 2006 total population numbers have decreased by approximately 50%. The cause of the decline is currently unclear. *Elaeophora schneideri*, a filarial parasite of deer, is associated with moose morbidity and mortality and although not commonly reported in Minnesota, may contribute to the population decline. To detect filarial parasites in moose with suspected nematode infections, brain tissue and blood samples were tested using a PCR assay with nematode-specific primers targeting the 18S rRNA gene or internal transcribed spacer regions. To investigate the prevalence of filarial nematodes, banked blood samples from hunter-killed and live-caught moose were screened microscopically for microfilariae utilizing a modified Knott's test. From hunter-killed moose and moose that died of natural causes, DNA sequences of brain tissue PCR products revealed 19 (65.5%, n=29) moose contained sequences similar to *E. schneideri*. Within 2 (40%, n=5) arterial blood samples, sequences of an unidentified filarial parasite most closely related to *Mansonella*

ozzardi (80% maximum identity) were identified. At present, it is unknown if the *Mansonella*-like parasite is pathogenic in moose. Microscopic screening of hunter-killed and live-caught moose blood revealed 22.4% (n=125) contained microfilariae, with size ranges of 150-185µm (length) and 2-5µm (width) and a single sample containing additional microfilariae measuring 280x13µm, similar to the size range reported for *E. schneideri*. These data suggest *E. schneideri* is harbored within the Minnesota population; however, further surveillance is needed to better understand the eco-epidemiology and transmission of both filarial parasites and their role in moose populations.

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Where's the risk?: Spatial epidemiology of gastrointestinal parasitism in Alberta beef cattle.

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Temporal and spatial heterogeneity in risk of exposure to gastro-intestinal (GI) parasites on pasture has long been recognized. Regardless of this variation, along with the high cost of semi-annual treatment and the threat of anthelminthic resistance, 'blanket treatment' programs continue to be the norm for Alberta beef cattle even in years and in regions where rates of transmission are low. In this study, we assessed geographical variation in GI parasitism in 1700 beef calves sampled from October through December across Alberta, Canada over 3 years. Faecal egg counts and ELISA detection of serum antibody concentrations were used to screen for the presence and estimated exposure to *Nematodirus*, other trichostrongyles (primarily *Ostertagia* and *Cooperia*) and *Trichuris* in individual hosts. Standard multiple regression analyses and Geographical Information Systems (GIS) technology were used to associate variation in antibody concentrations with region-specific environmental characteristics (e.g. moisture, humidity and temperature) that are known to influence the survival and infectivity of nematode larvae. Predictive maps were generated by projecting the output of our regression model. Significant spatial variation in mean serum antibody concentrations was documented in two of the three sampling years. Results indicated that variation in annual accumulated precipitation, the number of growth degree days above 5°C, and maximum humidity are important in predicting risk of transmission. The risk maps identified a relatively small hotspot of transmission located in the central region of the province in two wet years (2008, 2010). While antibody levels in cattle from southeastern and northwestern Alberta were relatively low in all years, concentrations increased in these regions in the two wetter years. Application of this multi-pronged approach for other host-parasite systems, particularly in regions influenced by climate change, will aid in the development of management initiatives that tie treatment recommendations to actual risk of infection.

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Invasion pathway and transmission dynamics of the invader *Dicrocoelium dendriticum* within cattle and wildlife in the Cypress Hills Park, Alberta.

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Invasive parasites can cause severe economic losses to livestock producers and due to increased animal movement they are often hard to prevent. Once established in a new host population, chemical control becomes impractical. Thus, the identification of historical invasion pathways can be a more effective management strategy for prevention and control of introduced parasites. Evaluation of the population genetic structure of introduced parasites provides an invaluable tool for the characterization of invasion pathways. These data can also be used to investigate epidemiological and ecological questions concerning the invasive population and its potential for further spread. *Dicrocoelium dendriticum* invaded southeastern Alberta, Canada, in approximately 1990 and is now present in most cattle, elk, and deer in the region. It is not known whether this invasion occurred via single or multiple introductions from Europe directly or stepwise from earlier invasion sites in North America. We use two genetic marker systems (Amplified Fragment Length Polymorphism and cytochrome oxidase 1 gene sequences) to genotype flukes collected from hosts in North America and Europe to determine the invasion history of this parasite. We also genotype *D. dendriticum* metacercariae populations in different populations of intermediate host (ants of the genus *Formica*) to estimate rates of transmission among and between populations of final hosts within our study area. Our preliminary data indicate multiple European sources of the invasive population and higher than expected levels of genetic diversity within final hosts in the Cypress Hills which suggest no barriers to transmission within the park.

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Confirmation of *Echinococcus canadensis* in wolves and elk in Idaho.

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Echinococcus granulosus was first identified in a scavenged goat carcass found in Idaho in 2006. Since then, a survey of wolves in Idaho revealed a 62% prevalence of *E. granulosus,* suggesting that this organism is endemic to the area. It was postulated that the Northern biotype was the most likely biotype involved, but the genotype had yet to be confirmed. Colorado State University Veterinary Diagnostic Laboratory recently evaluated lung tissue from two elk, and small intestines from three wolves legally harvested from Idaho suspected of having an *Echinococcus* infection. Hydatid cysts were identified on histological examination of both elk lung tissues. *Echinococcus* was diagnosed via gross and microscopic examination in all three wolf samples and *Taenia serialis* was identified in two of the samples. All samples were confirmed positive by conventional PCR, and sequence analysis of the NAD gene identified *Echinococcus canadensis* genotype G8 and G10. G10 was identified in a wolf and both elk samples, demonstrating infection in a definitive host and intermediate host. These hosts play a role in the complete natural life cycle of *E. canadensis*, and the results lend evidence to the endemicity of *E. canadensis* in Idaho. While domestic livestock are typically not good intermediate hosts for the cestode, there is a risk of spillover into domestic canids. This type of spillover is of public health concern as it potentially increases human exposure to this organism.

Session 8 – Students – Immunology

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Immunomodulation: The secret of *Haemonchus contortus* pathophysiology.

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Haemonchus contortus is a highly pathogenic and probably most studied gastrointestinal nematode of small ruminants worldwide. But many aspects of pathophysiology associated with parasite are still unknown. Helminths being strong immunomodulators achieve long term survival in hostile environment by skewing the host immune response. Several immunomodulators have been identified in excretory secretory molecules of different helminths. Present study was aimed to explore if *H. contortus* excretory secretory molecules have ability to modulate host immune response. For this, excretory secretory molecules of *H. contortus* were tested on a human monocyte-derived dendritic cell (mdDC) model. Multi colour flow cytometry revealed the ability of excretory secretory molecules to modulate CD32 (Fcγ receptor II) and lesser extent to CD86 and CD40 expression, while no change in expression of CD305, galectin, CD80 and MHCII. Unveiling the interaction between antigen presenting cells and *H. contortus* adult ES could result in developing alternative control strategy for this parasite.

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Effects of feeding Celmanax™ on peripheral cellular responses of commercial crossbred lambs during H. contortus primary infection.

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Commercial breeds of sheep fail to generate an effective immune response capable of clearing Haemonchus contortus infection. Coupled with data indicating that drugs used to treat these infections have reduced efficacy, producers have limited options for parasite control. Immunomodulation using prebiotics may serve as a means to stimulate immunity in parasite-susceptible sheep, thus limiting the effects of trichostrongylid parasitism. Preliminary studies conducted in 2012 with challenge infected crossbred lambs using the prebiotic, Celmanax™, reported a one week delay in fecal egg output and a 62% reduction in fecal egg count (FEC) by six weeks after infection. The aim of this study was to determine the effect of Celmanax™ supplementation on FEC reduction during primary infection with H. contortus. Parasite-naïve, fall-born Dorset-Texel crossbred lambs were limit-fed a 16% CP corn-soybean grain including Celmanax™ (1g/hd/day) (n=20) or without (n=19). After feeding Celmanax™ for one week, 15 lambs in each group were infected with 10,000 L3 H. contortus larvae, leaving five and four uninfected control lambs in each group respectively. Blood samples were taken every 2 days for 14 days, then weekly until 35 days post infection for blood cell analysis. Fecal samples were taken weekly for 35 days post infection. Fecal egg counts between the two treatment groups did not differ ($P > 0.05$), as an increase was observed to d35 (2221 eggs/g) followed by a reduction to d42 (1316 eggs/g). No significant differences in white blood cell differential counts were observed between the groups. Taken together, these data indicate that Celmanax supplementation was not effective in reducing FEC during a primary H. contortus infection.

Subsequent studies have been designed evaluate the effects Celmanax on similar sheep during a challenge infection.

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Comparison of immune responses during gastrointestinal helminth expulsion between resistant and susceptible breeds of sheep.

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The immune response to the self-cure phenomenon seen during gastrointestinal nematode (GIN) parasitism of small ruminants was compared between sheep breeds that are resistant or susceptible to *Haemonchus contortus* infection. Fifty-four Gulf Coast Native (resistant) and Suffolk (susceptible) lambs were allowed to acquire a natural GIN infection in pasture, and were then allocated into 4 groups. After being moved to parasite free housing for 2 months, lambs were given a challenge infection of 20,000 *H. contortus* L_3. Fecal egg counts (FEC) were monitored throughout the study and animals were necropsied at 0, 1, 3, and 7 days post infection(DPI). Gastrointestinal contents, venous blood, and abomasal tissue samples were collected at necropsy for worm counts, complete blood counts, and histopathology respectively. Additionally, abomasal mucosa, as well as prescapular and abomasal lymph node samples were taken and processed for quantitative PCR analysis of IL-4, IL-5, IL-13, IFN-γ, IL-10, and Foxp3 gene expression. FEC decreased beginning at 3 DPI in both breeds, with Native lambs having a higher percent reduction in egg counts at 3 and 7 DPI compared to Suffolk lambs. Circulating eosinophil counts increased in both breeds over time, with no difference observed between breeds at any time point. Additionally, lymphocyte counts for Native lambs increased over time and were higher than Suffolk lamb counts at both 3 and 7 DPI. At the site of infection, eosinophil counts increased at 3 DPI in Native lambs but did not increase in Suffolk lambs until 7 DPI. This coincides with a decreased number of adult worms within the abomasums of inoculated animals. Similarly, increased neutrophil and mast cell counts in the abomasums of Native lambs at 7 DPI coincide with decreased numbers of larval worms when compared to Suffolk lambs. The results suggest a possible role of neutrophils and mast cells in the expulsion of larval GIN, while eosinophils may play a role in the expulsion of adults.

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Cellular immune response to *Haemonchus contortus* in challenged sheep following extended rest period.

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A two-year study was conducted to determine peripheral cellular responses to *Haemonchus contortus* in parasite resistant St. Croix and parasite susceptible Suffolk crossbred sheep. In year one, transient increases of immune cells in blood were observed on specific days and were associated with a decrease of FEC in challenged St. Croix sheep. Therefore, the second experiment specifically targeted challenged lambs at d 0, 3, 4, 5, 7, 8, 11, 12 and 14 after infection using 10,000 L_3 *H. contortus* larvae. St. Croix and Suffolk crossbred sheep were randomly assigned to one of two infections groups: naïve (n=5/breed) and challenge (n=6/breed). Challenged animals were allowed a 12 week rest period between *H. contortus* infections. Consistent with the previous experiment, reduced FEC was observed in challenge St. Croix sheep on day 21 (216 eggs/g vs. 1750 eggs/g, $P = 0.06$), however, in the previous experiment no FEC was observed in St. Croix lambs which may have resulted from a shorter

rest period. Challenged St. Croix sheep had higher total circulating neutrophils (3.28 x 10^3 cells/µl vs. 2.97 x 10^3 cells/µl; $P = 0.007$) and higher total circulating basophils (0.22 x 10^3 cells/µl vs. 0.17 x 10^3 cells/µl; $P < 0.001$) through day 21. Challenged crossbred sheep generated higher monocytes (0.812 x 10^3 cells/µl vs. 0.554 x 10^3 cells/µl; $P < 0.001$) and higher eosinophils (0.237 x 10^3 cells/ µl vs. 0.141 x 10^3 cells/ µl; $P < 0.001$) than challenge St. Croix sheep. Despite elevated cell counts, crossbred sheep remained unable to reduce FEC to St. Croix levels. Previous work from mouse studies indicates a role for innate immune cells in parasite expulsion. Thus, these data indicate that recruitment of innate immune cells, specifically granulocytes and neutrophils, may be associated with the rapid parasite expulsion observed in St. Croix sheep.

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Western blot analysis of IgG(T) antibody responses of horses to *Parascaris equorum* larval excretory-secretory proteins.

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Parascaris equorum is a nematode parasite that frequently infects young horses. Infections are diagnosed late in the developmental life cycle by detection of eggs in feces. The goal of this study was to develop an antibody-based test that would allow for prepatent diagnosis of *P. equorum* infection. To produce western blot antigen, *P. equorum* larvae were hatched and cultured in vitro for collection of excretory-secretory proteins (ESP). Sera were collected from 18 pregnant broodmares and from their foals pre-suckle, post-suckle, and weekly until diagnosis of patent infection by fecal flotation test. Additionally, sera were collected from 12 older mares and geldings negative for patent *P. equorum* infections. Lastly, sera were obtained from rabbits inoculated with *Baylisascaris procyonis* or *Toxocara canis* eggs, and from rabbits immunized with *B. procyonis* or *P. equorum* ESP. In western blot analysis, sera from 17 of 18 broodmares showed IgG(T) antibody that recognized *P. equorum* larval ESP antigens (94% at 19 and 34 kDa, 89% at 26 kDa, and 83% at 22 kDa). Foals showed no antibodies pre-suckle, but post-suckle and subsequent samples exhibited antibodies similar to the dams. Of the older mares and geldings, 58% had antibodies that recognized molecules at 26 and 34 kDa, and 50% at 19 and 22kDa. Sera from rabbits immunized with *B. procyonis* ESP did not recognize the 19 or 34 kDa antigens. Sera from rabbits challenged with *B. procyonis* or *T. canis* eggs did not recognize the 19 kDa, 22 kDa, or 34 kDa molecules. Proteins at 19 kDa and 34 kDa appear to have the highest potential for distinguishing between *P. equorum*, *B. procyonis*, and *T. canis* infections. Results indicate that IgG(T) antibodies against larval *P. equorum* ESP are common in mature horses, and are transferred from mare to foal, limiting the diagnostic potential of an antibody-based test.

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Functional characterization of the surface antigens (SnSAGs) in *Sarcocystis neurona*.

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Sarcocystis neurona is a protozoan parasite that causes the serious neurologic disease equine protozoal myeloencephalitis (EPM). The *S. neurona* merozoite surface is covered by multiple related proteins, which are orthologous to the surface antigen (*SAG*) gene family of *Toxoplasma gondii*. The SAG surface antigens in *T. gondii* and another related parasite *Neospora caninum* seems necessary for parasite transmission and persistence of infection. The present study was conducted to assess the role of SnSAGs in host cell attachment and/or invasion by *S. neurona*. Serum neutralization assays were conducted using heat inactivated polyclonal serum raised against SnSAG1, SnSAG2, SnSAG3, and SnSAG4. Preliminary data obtained from these assays suggest a potential role of SnSAG1 and SnSAG4 in host cell attachment and/or invasion. However, results obtained for SnSAG2 and SnSAG3 were inconsistent. Studies are underway to develop alternative methods to examine antibody inhibition of surface antigen function. In addition, SnSAG1 and SnSAG2 gene knockout (KO) plasmids were constructed and transfected into *S. neurona* in order to produce parasites that are deficient for these two proteins. The transfected parasites are presently being grown in selection medium to isolate transgenic clones. The SnSAG-KO parasites will provide additional data about the role of SnSAGs in host cell attachment and/or invasion. Collectively, the information acquired will help to understand the importance of the SnSAG proteins for parasite survival and could lead to improved methods for EPM prevention and/or treatment.

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Functional use of the *Eimeria* life cycle with live vaccination in caged replacement layer pullet production.

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Live vaccination stimulates immunity using a small dose of vaccine oocysts enhanced through fecal-oral cycling. Successful live vaccination likely occurs with uniform vaccine administration and environmental control. Day old chicks are frequently vaccinated in coarse spray cabinets that may lead to non-uniform ingestion of vaccinal oocysts. Without adequate cycling to complement vaccination, limited immunity puts birds at risk of coccidiosis. Studies examined variation in vaccine uptake and the level of protection against challenge depending on cage-environment modifications. The first experiment confirmed variation in spray vaccine ingestion at 6 days post inoculation via counting oocysts shed. A second trial indicated that low relative humidity (<25%) during the initial vaccination period had a negative impact on vaccine success. For the third experiment, birds were separated into 4 vaccine ingestion groups and reared on 3 cage floor modifications to examine protection against challenge. Treated groups were: gavage (*E. acervulina*, *E. brunetti* and *E. tenella*); fecal-oral transmission, "contact-vaccinated"; positive (sham-vaccinated, challenged) and negative (sham-vaccinated, sham-challenged) controls. Cage floor coverage (CFC) groups were: 0%; 40% with thick paper; and 40% with fibre trays. In general, vaccinated and "contact-vaccinated" birds reared on 40% CFC during the treatment phase had higher body weight gains than positive controls during challenge infection with *E. acervulina* at 3 weeks of age. Birds challenged with *E. brunetti* or *E. tenella* did not follow this pattern, which may be due to a decrease in relative humidity during initial oocyst sporulation in

the barn. Understanding the *Eimeria* life cycle and its practical application can improve live vaccination success in caged replacement layer pullets.

Session 9 – Nematode Resistance

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The heartworm refugia conundrum: Aren't refugia supposed to prevent the development of anthelmintic resistance?

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Based on our current understanding of the various factors involved in driving the development of anthelmintic resistance (AR) in gastrointestinal nematodes (GIN) of livestock, levels of refugia are considered the most important. This is demonstrated in both field trials with sheep and in computer simulation models. Consequently, farms and/or regions where GIN refugia are highest are also the slowest to develop AR. Based on this understanding of the role of refugia, it has often been suggested that the high levels of refugia that exist for *Dirofilaria immitis* would prevent the development of AR in heartworm. However, given the current compelling evidence that resistance in *D. immitis* to the macrocyclic drugs (ML) is real, the assumptions about the role of refugia in preventing ML-resistance in heartworm are clearly flawed. Although the clinical and epidemiological picture is far from complete, it appears that the overwhelming preponderance of cases of resistance are reported in the Mississippi Delta region. This region of the USA has the most optimal combination of epidemiological factors that drive high levels of heartworm transmission, and this is reflected by the very high heartworm infection prevalence. Given this epidemiological scenario, levels of refugia in the Mississippi Delta are greater than any other region of the USA. Furthermore, only a relatively small percent of the canid population at risk is on compliant monthly prophylaxis, thus treatment coverage is relatively low. High refugia and low treatment coverage are thought to be the ideal for preventing the development of AR. This begs the question: why has resistance developed and is concentrated almost exclusively where refugia are the greatest? Critical analysis of the biology and genetics underlying the development and detection of AR suggests that there are important differences between filarioid and GIN parasites, and these produce very different dynamics with regard to the development and detection of AR. These relevant differences and why this conundrum exists will be explained in detail.

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Why does there seem to be no spread of ML-resistant heartworms out of the Mississippi Delta region?

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In gastrointestinal nematodes (GIN) of ruminants and horses, once anthelmintic resistance reaches clinically recognizable levels in a particular region, it rapidly spreads to other farms and other regions, and the prevalence continually increases over time. In fact, various surveys of resistance prevalence performed on livestock farms across different geographic regions demonstrate variability only in relative magnitude; patterns are almost always quite similar. However, the epidemiological pattern of anthelmintic resistance in heartworms stands in stark

contrast to this. It has been 10-15 years since macrocyclic lactone (ML) lack of efficacy (LOE) reports began to greatly increase in the Mississippi Delta region, and 8 years since Hurricanes Katrina and Rita, when thousands of heartworm-infected rescue dogs from Louisiana and Mississippi were re-located throughout the US. In fact, the first published report claiming to document a case of ML resistance in heartworm was in a Katrina rescue dog in Ontario, Canada that originated from Louisiana (Bourguinat et al., 2011). Despite the passage of many years, which should have provided great opportunities for amplification and spread of resistant heartworms, LOE cases seem to still be concentrated in the Delta. Although the epidemiological picture is far from complete, it appears based on empirical evidence that large clusters of cases still are not being diagnosed outside of the Mississippi Delta region, and that numbers of cases in the Delta region may have decreased in recent years. This begs the question: what is different about the biology and epidemiology of anthelmintic resistance in heartworms as compared to GIN that is responsible for these differences? In this presentation, hypotheses will be presented that may explain the apparent epidemiological contradictions seen with heartworms as compared to GIN of livestock.

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Phenotype and genotype association in macrocyclic lactone resistance in nematodes.

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Resistance to macrocyclic lactones (MLs) has been confirmed in many places worldwide and represent a big challenge for parasitic control in farm animals. The gold standard test to detect and monitor for ML resistance is the fecal egg count reduction test (FECRT) which requires considerable parasitological expertise, has low sensitivity and provides the information on resistance status after animals have been treated. The detection of genetic changes which are implicated in the mechanism of resistance offers the possibility of developing a rapid, sensitive and inexpensive DNA based assay for ML resistance. We have analyzed ML resistant and susceptible isolates of *Caenorhabditis elegans* and *Haemonchus contortus*, for genetic differences, including using Next Generation sequencing. Similar genetic changes were revealed in strains of the free-living nematode and parasites which had been selected with MLs. These changes have been confirmed in field isolates of the parasite from Africa, Australia, Europe and North and South America. Rescue studies with a gene implicated in ML resistance have been conducted in *C. elegans*, confirming the involvement of these genetic changes in ML resistance. These findings represent significant advances in understanding the mechanism of ML resistance and have led to a DNA based assay to detect ML resistance in nematodes.

This research was supported by funding from NSERC, Canada; FRQNT and MDEIE-PSR-SIIRI, Québec and the Centre for Host-Parasite Interactions. We are grateful to many colleagues who have provided us with ML sensitive and resistant isolates of *H. contortus* and to Dr Catherine James for an ivermectin selected strain of *C. elegans*.

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Haemonchus contortus as a model system to study anthelmintic action and resistance: recent progress on developing genomic and genetic resources.

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Anthelmintic resistance is essentially a complex quantitative trait in which multiple mutations contribute to the resistance phenotype in an additive manner. Consequently, a combination of genetic and genomic approaches are needed to identify the causal mutations and quantify their contribution to the resistance phenotype. Therefore, there is a need to develop genetic and genomic approaches for key parasite species identified as relevant models. *Haemonchus contortus,* a gastro-intestinal parasite of sheep, has shown a remarkable propensity to develop resistance to all the drugs used in its control. Partly because of this, and partly because of its experimental amenability, research on this parasite has already contributed more than any other to our understanding of anthelmintic resistance. *H. contortus* offers a variety of advantages as an experimental system including the ability to undertake genetic crosses; a prerequisite for genetic mapping. Recent progress in producing a high quality draft reference genome sequence and in developing a forward genetic approaches to study anthelmintic resistance in this parasite will be discussed.

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Case history of a stocker operation using Eggs per Gram [EPG] and molecular identification of species to evaluate the source of resistant parasites and the use of two different classes of dewormers to control resistance in 2011 and 2012.

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In 2011 resistant *Cooperia* and *Haemonchus* were diagnosed in calves arriving at a conditioning facility in the Southeastern US. The resistance was identified by a lack of EPG reduction in an egg reduction, followed by the molecular identification of DNA from the eggs using amplification with the polymerase chain reaction [PCR]. In the early summer of 2011, these parasites were prevented from being transported with the animals to Wisconsin by treating the animals with a deworming combination of oral levamisole and injectable moxidectin at a dose for animals weighing about 100 pounds more than the average weight of the treated animals harboring the resistant worms. In samples collected 14 to 20 days after treatment, and monthly thereafter, EPG counts through September were 0, and at the end of November, the mean EPG was only 3.5. Thus no other deworming was necessary during the six months grazing season in WI. In 2012, resistant *Cooperia* and *Haemonchus* were again diagnosed by EPG and PCR in the Southeastern conditioning facility. The same deworming protocol was used, but because levamisole was in short supply, levamisole was only dosed at per label dosages, and some animals may even have been under dosed. The 14 to 21 day EPG count was very low (0.3 EPG), but increased slowly each month, and by December 2012 the average EPG was 34.5. At this time, the worms present were identified using PCR as *Ostertagia* and *Cooperia*. Is it possible that more worms were present due to the underdosing with levamisole?

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Novel assay for the detection and monitoring of levamisole resistance in parasitic nematodes.

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Levamisole is an antinematodal drug which interacts with acetylcholine receptors. The acetylcholine receptor in *Caenorhabditis elegans* is composed of five subunits, the α-subunits UNC63, UNC38 and LEV8 and two β subunits LEV1 and UNC29, formed in a ring in the membrane with the pore in the center. Several studies have investigated the level of expression of the homolog's subunits in the parasitic nematode *Haemonchus contortus*. Relationship between reduced transcription of three subunits (UNC63a, UNC38 and UNC29) and levamisole resistance have been proposed. In addition, truncated isoform Hco-unc-63b and Hco-acr-8b expression have been found in levamisole resistant isolates and were absent in levamisole susceptible isolates of *H. contortus*. The measure of transcript expression requires RNA extraction, cDNA synthesis and PCR amplification. The use of RNA as a diagnostic tool for detecting resistance poses technical problems that could be avoided if DNA polymorphism causing, or linked with reduced production of a transcript, or the production of a truncated transcript, could be identified. We investigated genomic DNA sequences from a wide range of susceptible and levamisole resistant *H. contortus* isolates to highlight genetic changes related to levamisole resistance. Sequences potentially involved in generating a pattern described in previous publications and related to levamisole resistance were analysed from individuals and pools of parasites. We found a genetic marker easily detectable and statistically linked to levamisole resistance (Chi^2 test). This is the first report of a putative DNA marker for levamisole resistance detection. This marker can be used for the detection and monitoring of levamisole resistance in parasitic nematodes of animals. Supported by NSERC, Canada and MDEIE Quebec. Research at the Institute of Parasitology is supported by the Centre for Host-Parasite Interactions – FRQ-NT, Quebec.

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$\frac{dAR(ivm/mox)}{dt}$ A story of ivermectin/moxidectin resistance on goat and sheep farms in the eastern United States.

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We performed a study in 2001 to investigate the prevalence of anthelmintic resistance on goat farms in Georgia. The study showed that ivermectin resistance in *Haemonchus contortus* was detected on 90% of farms, but moxidectin resistance was not detected by the FECRT. Moxidectin use became increasingly common around this time, as did the development of resistance. To investigate the changes in susceptibility to ivermectin/moxidectin over time in *H. contortus*, we performed a retrospective examination of DrenchRite© LDA data collected from 2001 through 2012 on 107 goat and 82 sheep farms in the eastern, southern and midwestern regions of the United States. These data were grouped by species into 3 year increments (2001-2003), (2004-2006), (2007-2009) and (2010-2012). The prevalence of resistance and the EC_{50} and EC_{95} values for ivermectin/moxidectin were calculated. The prevalence of ivermectin resistance for goats remained relatively stable over time at approximately 90%, whereas the prevalence of resistance to moxidectin increased from 24% to 67%. However, the resistance

prevalence for sheep was lower. The prevalence of ivermectin resistance showed an erratic increase from 48% to 89%, while the prevalence of resistance to moxidectin only increased from 5% to 12%. In goats, the substantial increase in resistance prevalence to moxidectin was accompanied by a very large decrease in moxidectin susceptibility; mean EC_{50} and EC_{95} values increased from 128 to 640 and from 396 to 2500 nM, respectively. In contrast, only a modest increase in mean EC_{50} and EC_{95} values from 39 to 51 and from 127 to 243 nM, respectively were observed for sheep. Likewise, the highest EC_{95} value recorded for goats was 10,000 nM as compared to 1317 nM for sheep. It is not known why EC_{50} and EC_{95} values for *H. contortus* increased so dramatically in goats and not in sheep, but this may be partly related to the differences in the metabolism of anthelmintics in these two species.

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Elimination of *Haemonchus contortus* from a llama farm in North Georgia.

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A llama farm, located in North Georgia, was established in 1989 with the acquisition of 1 llama (*lama glama*) and 100 acres (40.47 hectares) of land. Herd size was increased through further acquisitions and breeding, reaching 150 animals by 2005. No problems with clinical parasitism were noticed until spring of 2005; the situation then worsened leading to a major parasite-related event in July. Thirty-three animals developed lethargy, anorexia, hypoproteinemia, and severe anemia, (hematocrit ranged from 6-14% in affected animals) and 13 died. Fecal examinations performed by the attending veterinarian yielded trichostrongyle type eggs too-numerous-to-count. A diagnosis of haemonchosis was rendered, which was supported by necropsy. Immediately, strict biosecurity measures were established, and both an environmental control and treatment plan were instituted. All animals were removed from pasture and held on a gravel dry lot for 8 weeks, and were fed hay and a commercial llama ration. The owner, after veterinary consultation, treated all llamas with Quest gel (2% moxidectin) at the dose rate of 400 ug/kg. Treatment was repeated 14 days later. Communal dung piles were removed and all pastures raked. Pastures were then burned, with particular attention paid to areas near the communal dung piles. Composting of feces and daily communal dung pile removal were instituted and has been continued since. In 2008 FEC performed on herd females yielded a maximum of 100 epg; no eggs were found in samples from 89% (99/111) of the herd (mean epg = 4.3 epg). Concurrent coproculture yielded a mix of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. Subsequent FEC and coproculture surveillance in 2009, 2011, and 2013 showed similar results. *Haemonchus contortus* has not been detected on this farm since the 2005 event, suggesting that the intervention measures taken together with the changes in biosecurity and dung/pasture management were successful in eliminating *H. contortus* from this herd.

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Novel anthelmintics and insecticides from filamentous fungi.

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Mycosynthetix owns one of the largest libraries (55,000 strains) of filamentous fungi in the world which were isolated from substrates sourced from a wide variety of ecosystems and includes many strains from tropical rain forests where there is a high degree of biodiversity. Our primary purpose is to exploit the medicinal and pharmaceutical potential of this large library. Screening efforts target cancer, bacteria, fungi and several human and animal parasites. Parasite targets include malaria, trypanosomes, leishmania, soil transmitted helminths, animal health GI tract nematodes and filarial worms which infect humans and animals. We have established validated whole organism assays to determine activity of both pure compounds and semi-purified extracts of fungi against GI tract nematodes, filarial worms, adult arthropods and mosquito larvae. Pure compounds have been shown to have high activity against one or more of the following; *Haemonchus contortus*, *Cooperia onchophora*, *Strongyloides stercoralis*, *Brugia pahangi* (Bp) and *Dirofilaria immitis* (Di) microfilaria (Mf) and L3 and L4 stages and *Aedes aegypti* larvae. There is little crossover of activity between the endo- and ectoparasites. Several compounds not only had high potency against BpMf but also caused high mortality within 24 hours. A small library of carefully selected purified fungal metabolites was tested in the BpMf assay at 50 ppm giving an overall hit rate (ED100) of 96.1% at 120 hrs. Upon down titration, 30/58 compounds were fully active at 3.125 ppm (3-6 μM) or less, and a number had nanomolar activity. Several of these 30 were also active against DiMf. One compound showed potent activity against Bp L3 and L4 and DiL3, and several others are being tested. Results from several mammalian cell lines coupled with associated anti-parasitic data have identified a number of compounds with high potency and a promising *in vitro* therapeutic index.

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Anthelmintic drug discovery: a novel neurophysiology-based screening platform for nematode worms.

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To accelerate development of new and effective anthelmintic drugs for veterinary use, we combined the fields of microfluidics and neurophysiology to develop the first semi-automated, medium-throughput screening platform that assays test compounds' ability to disrupt neuromuscular function in nematodes (Lockery et al. 2012 doi:10.1039/c2lc00001f). This mode of action underlies many anthelmintic drugs currently in use. Our patent-pending microfluidic device ('chip') non-invasively records electrical signals [electropharyngeograms (EPGs)] emitted by rhythmic contraction of the nematode pharynx (muscular throat), much as an electrocardiogram records the human heart. Anthelmintic bioactivity is indicated by disruption or cessation of pharyngeal pumping, or perturbation of EPG waveforms, detected by customized computer algorithms. Currently, each microfluidic chip records simultaneously from 8 worms, which can be readily scaled up. The method has been validated using the non-parasitic nematode, *Caenorhabditis elegans*, including dose-response analysis of various drug classes; the use of resistance mutants to 'fingerprint' compounds based on mode of action; and demonstration that the method can distinguish closely-related compounds (e.g., macrocyclic lactones), as desired for structure-activity relationships. This *C. elegans* platform can help

reveal the mode of action of uncharacterized 'hits' from high-throughput primary screens, before testing compounds on parasitic species of interest. Importantly, we are working to adapt the technology for parasitic nematodes, including L4 canine heartworm, *Dirofilaria immitis*. JC Weeks and SR Lockery co-founded NemaMetrix LLC in 2011 to provide neurophysiology-based screening services to academic and industry researchers undertaking antiparasitic drug development. NemaMetrix is also developing a plug-and-play 'EPG kit,' allowing researchers to implement built-to-order, EPG-based screens in their own laboratories. We welcome input on how our offerings can best facilitate anthelmintic drug discovery and development for parasitic nematodes that damage animal health. Support: Oregon Nanoscience & Microtechnology Institute; Bill & Melinda Gates Foundation.

Session 10 – Canine, Feline, & Exotic Parasitology

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Hepatic alveolar echinococcosis in a dog in Ontario, Canada.

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In June 2012, a 2-year old dog presented to a veterinary clinic in southern Ontario with a 12-hour history of acute-onset inappetance and lethargy. The cranial abdomen was tense and painful on palpation. Radiography revealed loss of serosal detail throughout the entire abdomen. Exploratory laparotomy revealed hemoabdomen. In addition, the liver contained generalized, multifocal, white nodules; a space–occupying lesion, approximately 10 cm in length, was located within the liver that had ruptured, leaving a cavity with uncontrollable hemorrhage. Due to the poor prognosis the dog was euthanised. Histopathology demonstrated the presence of multi-loculated cystic structures, containing eosinophilic fragmented hyaline membranes, and prominent chronic inflammation that was both eosinophilic and granulomatous. Testing of formalin-fixed tissue confirmed the presence of *Echinococcus multilocularis* on the basis of characteristic morphological changes on histopathology, sequence data for a PCR-generated fragment of the mitochondrial 12S rRNA gene, and restriction fragment length polymorphism analysis of PCR-generated fragments from the mitochondrial 12S rRNA and NADH dehydrogenase 1 genes. The final diagnosis was hepatic alveolar echinococcosis (HAE). *Echinococcus multilocularis* was previously not known to occur in Ontario. However, the dog described here had only lived in southern Ontario and vacationed on Manitoulin Island, Ontario. Cases of HAE typically are only reported in highly endemic areas. Data from Europe indicate that some dogs with HAE may also have patent intestinal infections, and therefore constitute a potential zoonotic threat. Thus, in light of the size of the dog's hepatic lesion, 13 people that came in contact with this dog at 2-6 months of age were tested for antibody to *E. multilocularis* using three different screening ELISAs and a Western blot method. On the basis of blood samples collected in September 2012, all in-contact people tested seronegative. Additional confirmatory testing is planned.

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Endemic Crenosoma vulpis infection in a dog in Wisconsin.

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A 6-yr-old, female-spayed, dachshund-terrier cross from Chetek, Wisconsin was presented for veterinary care due to signs of chronic cough of 1 month duration. The dog had been treated by a previous veterinarian with doxycycline but the cough condition had persisted. The only abnormality detected on physical exam was an easily induced cough on tracheal palpation. CBC and chemical profiles were normal; radiographs revealed a generalized bronchial pattern. The cough resolved in response to a course of treatment with a combination of Clavamox, prednisone and hydrocoedone but returned soon after treatment ceased. The dog was placed back on prednisone; a transtracheal wash sample was taken for culture and cytology and a fecal sample was collected for Baermann examination. TTW cytology revealed a mild catarrhal, eosinophilic/histiocytic inflammation. Baermann fecal examination detected first-stage nematode larvae (L1) approximately 270 microns in length. The larvae were identified as *Crenosoma vulpis* based on size and morphology. A 3-day course of daily of fenbendazole (50 mg/kg, PO) was started, however, the severity of the cough greatly worsened after the first dose. Therefore, the prednisone dosage was increased and the 2 remaining doses of fenbendazole were administered every other day followed by a course of daily fenbendazole for 3 days. Clinical improvement began after the third dose of fenbendazole with complete resolution after completion of the treatment. The dog lives in a lake cottage (Prairie Lake) and had a history of eating a large number of freshwater aquatic snails. The dog had not been out of the state in the last 5 years. Due to this travel history and the life span of *C. vulpis* (10 mo.), the infection could only have occurred through endemic exposure. Whether *C. vulpis* has been newly introduced or is only newly recognized in this region are unknown.

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Prevalence of covert infections with intestinal helminths in dogs.

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Intestinal helminths are considered common in dogs, particularly dogs in animal shelters, but surveys by fecal flotation routinely underestimate their prevalence. To determine the prevalence of intestinal helminths in dogs and to investigate the utility of fecal flotation in identifying those infections, 97 adult (> 1 year) dogs humanely euthanized at two animal control shelters in northeastern Oklahoma were examined externally for evidence of flea infestation and presence of tapeworm proglottids. Fecal flotation of samples collected per rectum was performed using both passive sodium nitrate and centrifugal sugar solution techniques. Contents of the entire gastrointestinal tract were screened and all helminths recovered were washed in saline and fixed prior to enumeration and identification to genus and species. *Ancylostoma caninum* was identified in 47/97 (48.4%), *Toxocara canis* was identified in 11/97 (11.3%), and *Trichuris vulpus* was identified in 37/97 (38.1%) dogs. Cestodes were identified in 53/97 (54.6%) dogs. *Dipylidium caninum* was present in 48/97 (49.5%) and *Taenia* sp. was present in 7/97 (7.2%) dogs. Presence of helminths was not associated with body condition score, age of dog, or presence of flea infestation. Centrifugal fecal flotation with sugar solution identified 38/47

(80.9%) *A. caninum*, 7/11 (63.6%) *T. canis*, 28/37 (75.7%) *T. vulpis*, 4/7 (57.1%) *Taenia* sp., and 3/48 (6.3%) *D. caninum* infections. Although infection with cestodes was present in over half the dogs examined in this study, the majority of these infections, and in particular, *D. caninum*, were not evident by the recovery of characteristic stages on fecal flotation or by detection of proglottids on external examination.

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The efficacy of various imaging modalities to identify *Taenia pisiformis* cysticerci in rabbits.

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Taenia pisiformis is one of the most common intestinal cestodes of domestic dogs. Gravid taeniid segments are shed from the definitive host and ingested by lagomorph intermediate hosts. The taeniid larva, the cysticercus, consists of a fluid-filled bladder with one or more scolices that encysts in the peritoneal membranes after migration through the liver parenchyma. An increase in specific antibody levels to *Taenia pisiformis* in infected intermediate hosts can be effective in determination of exposure, but antibody levels are not effective in determining the number of viable cysticerci in an infected intermediate hosts. In this study, several imaging modalities were used to determine which was most effective in confirming and enumerating the presence of cysticerci in rabbits. Rabbits were inoculated with 200 *Taenia pisiformis* ova on day one. On day 182 abdominal radiographs and abdominal ultrasound was performed. The radiographs were unable to confirm the presence of cysticerci. Abdominal ultrasounds did confirm the presence of the cysticerci with the fluid filled bladder and scolices being visualized and measured. CT was performed on one rabbit at day 191 of infection and showed numerous calcium-containing scolices. This same rabbit was euthanized and a necropsy was performed to confirm the presence of and number of cysticerci. In conclusion, ultrasound is effective in determination of the presence of cysticerci in the peritoneal membrane, but not determination of numbers. CT was effective in determination of both the presence and numbers of cysticerci, but may be cost prohibitive.

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Serological and molecular evidence of *Leishmania* parasite in dogs of visceral leishmaniasis endemic areas in Bangladesh.

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The *Leishmania* parasite is transmitted by the bite of a corresponding sand fly vector and primarily maintained in nature by wild and domestic reservoirs, including dogs, foxes and jackals. Infected dogs are the primary reservoir host of *Leishmania infantum*, the etiological agent of visceral leishmaniasis (VL) in Europe and South America. However, VL is considered to be anthroponotic in the Indian subcontinent, although the only known vector, *Phlebotomus*

argentipes is zoophilic in nature. In the present study, we examined stray dogs for serological and molecular evidence of *Leishmania* infection in VL endemic areas of Bangladesh. Anti-*Leishmania* antibodies were detected in 6 dog serum samples (12%) using rk39 immunochromatographic test. Five samples (10%) were positive to *Leishmania* DNA with ITS1-PCR. *Leishmania* species was identified as *L. donovani* by sequencing of the PCR products. Results from our study support the assumption that dogs are apparently the probable animal reservoirs for *Leishmania* parasite in Bangladesh. To clarify the exact role of dogs in *L. donovani* transmission cycle, it is necessary to directly isolate the parasite from the dogs and also to prove that these animals can transmit the *Leishmania* parasite to the vector naturally.

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Epidemiological study of intestinal parasites in client-owned dogs of the Barcelona area and its evolution over a 6 month period.

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Intestinal parasite prevalence was evaluated in healthy adult pet dogs considered at low risk of parasitism. Ninety-four client-owned dogs were recruited by 4 private clinics (22-25 cases/center) in the Barcelona county. Only dogs that were not dewormed over the last 3 months were included in the study. Faecal samples were evaluated at the beginning of the study and after 2, 4, and 6 months using a coprological technique (flotation-centrifugation). Positive dogs were dewormed (Giardiasis: Fenbendazole, Panacur®; Helminthiasis: Praziquantel, Pyrantel and Febantel, Drontal Plus®). At time of enrollment, 4.26% (4/94) of dogs were parasitized. At 2 and 4 months, intestinal parasite prevalence was 4.35% (4/92) and 3.4% (3/88), respectively. The prevalence at 6 months was 10.5% (8/76). Throughout the study, 16 samples showed single infection [*Giardia sp. (n=7)*, *Toxocara canis (n=5)*, hookworms (n=2), *Trichuris vulpis (n=1)* and *Toxascaris leonina* (n=1)]. Co-infection was observed in 3 samples (*T. canis* and hookworms). After deworming, reinfection was observed in 3 dogs (2 with *T. canis*; 1 with *Giardia* sp.); one dog infected by *T. canis* and hookworms at T2 was positive to *T. leonina* at T4. Time until first infection was evaluated by a survival analysis (Kaplan-Meier and Actuarial Methods).The probability of not being infected decreased drastically at 6 months (< 6 months, 0.92; ≥ 6 months, 0.75). The 50% of the positive cases were infected at ≤5.23 months. In conclusion, deworming protocol in dogs at low-risk of infection should include a previous coprological examination to determine either deworming treatment is necessary or the right type of treatment, as to avoid unnecessary use of antihelmintics that may promote drug resistance.

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Molecular characterization of *Cryptosporidium* oocysts recovered from a Rock Pigeon (*Columba livia*) in central New York.

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An injured Rock Pigeon (*Columba livia*) was presented at the Avian and Exotic Pet Service at Cornell University Hospital for Animals. A fecal sample was collected and used with Cornell

University veterinary students in the Zoological Medicine clinical rotation as teaching material in fecal diagnostic techniques and parasite identification. The sample was processed for parasite egg and (oo)cyst recovery by 1.18 spg zinc sulfate centrifugal flotation and 1.3 spg sugar centrifugal flotation. *Cryptosporidium* oocysts were observed and collected. Respiratory and enteric cryptosporidiosis is a major parasitic disease in wild and domesticated birds. Currently, *C. baileyi, C. meleagridis*, and *C. galli* are the accepted avian *Cryptosporidium* species. Although fecal surveys have indicated *Cryptosporidium* prevalence as high as 14.6% in *Columba livia*, few studies have examined the diversity of *Cryptosporidium* spp. present in *C. livia*. In the few studies in which the species of *Cryptosporidium* present has been determined, *C. baileyi* and *C. hominis* have been reported. *Cryptosporidium hominis*, a human pathogen, has been responsible for many of the human cryptosporidiosis outbreaks worldwide. Pigeons have a wide distribution and live in close proximity to humans, increasing the potential for human contact with their feces and fostering concerns regarding public health. The *Cryptosporidium* species present in this animal was determined through DNA amplification and sequencing of 18S rRNA gene by nested PCR. A BLAST search of the resulting sequence in GenBank® revealed it to be highly similar (99%) to that of *C. baileyi*. Although *C. baileyi* may not cause human disease, it is the most common cause of cryptosporidiosis in birds. This fact, along with the pigeon's wide distribution, suggests pigeons may act as a vector in disseminating cryptosporidiosis to avian hosts.

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Is an immunologic component required for Brugia malayi susceptibility in the domestic cat?

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The filarial parasite *Brugia malayi* is one of the causative agents of lymphatic filariasis and is used in laboratory studies of the disease. Maintenance of the life cycle in a laboratory setting requires a competent vertebrate definitive host from which a sufficient quantity of microfilaremic blood can be obtained. Currently, the domestic cat is the most suitable animal for this purpose. However, the unpublished rate of suitable infections that can be induced in this host is relatively low, approximately 10 to 20%. Due to the high cost associated with infecting and maintaining these animals and the low infection rate, incorporating a method to determine which cats would be susceptible to infection would improve the efficiency of maintaining this parasite life cycle. For these reasons, we chose to examine cats during the course of infection, by monitoring total white blood cell (WBC) counts and WBC differential counts as well as different pro-inflammatory cytokines, such as TNF-alpha, to determine whether there are notable biomarkers for efficient *B. malayi* infection. Ten *B. malayi* negative, six-month-old, intact male kittens were each infected by subcutaneous injection of 400 *B. malayi* infective third-stage larvae. Pre- and post-infection, cats were bled every two weeks in order to run a complete blood count (CBC) and to collect serum for analysis. Should any or all of these cats become patent after 6 to 8 months, their CBCs and serum cytokine profiles will be evaluated to determine whether total WBC counts and WBC differential counts, cytokines, and immunoglobulins are predictive/indicative of infection susceptibility of cats for *B. malayi*.

Scynexis Symposium

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Innovative solutions for anti-parasitic drug discovery.

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SCYNEXIS has a long-standing commitment to the discovery of anti-parasitic drugs for Human Health, Animal Health and Public Health. Over the past nine years, SCYNEXIS has partnered directly with various organizations including: Product Development Partnerships such as Drugs for Neglected Diseases *initiative*, Medicines for Malaria Venture and Innovative Vector Control Consortium, large pharmaceutical companies, animal health companies, biotechs and academic institutions for purposes of screening and optimizing compounds for these indications. SCYNEXIS has developed an innovative business model that allows for the evaluation of novel chemical libraries against over thirty parasite species thus allowing for the efficient alignment of a chemical series to the appropriate human or animal therapeutic area. A case study of the model for the identification of novel chemical series targeted at key veterinary therapeutic areas will be presented.

Session 11 – Small Animal/Avian – Diagnosis & Treatment

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ELISA detection of *Trichuris vulpis* coproantigen in dogs.

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Infection of *Trichuris vulpis*, the dog whipworm, is often detected by identification of the characteristic egg by fecal flotation. Diagnosis by flotation may be challenging because of a denser egg and a lengthy prepatent period. New technology may shed light on detection of adult whipworms in the absence of positive egg flotation data. Here we describe the development of a coproantigen capture ELISA that detects whipworm infections in canines. Monoclonal antibody on both the solid and detection phase of the ELISA allows specific detection of prepatent and patent whipworm infections in laboratory-infected canines. Lack of cross reaction with non-whipworm experimental nematode infections demonstrates assay specificity. Analysis of >1000 canine field samples shows application of the whipworm ELISA may double the frequency of whipworm detection.

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Efficacy of Indoxacarb applied to cats against the adult cat flea, Ctenocephalides felis, flea eggs and adult flea emergence.

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A study was conducted to evaluate the effect of indoxacarb applied to cats on adult cat fleas, *Ctenocephalides felis*, flea egg production and adult flea emergence. Sixteen cats were selected for the study and allocated to two treatment groups. Eight cats were treated with a 19.5% w/v topical spot-on solution of indoxacarb on day 0 and eight cats served as untreated controls. Each cat was infested with 50 fleas on Days -2, 7, 14, 21, 28, 35 and 42. On Days 1, 2, and 3, and at 2 and 3 days after each post treatment reinfestation flea eggs were collected from the pan under each cat cage. Eggs were counted and viability assessed by evaluating adult flea emergence 28 days after egg collection. Three days after treatment or infestation, each cat was combed to remove and count live fleas. Treatment with indoxacarb provided 100% efficacy following infestations on day -2, 7, 14, 21 and 28 and efficacy was 99.6% following infestations on days 35 and 42. Egg production from indoxacarb treated cats was reduced by 99.9% within 72 hours of treatment. For subsequent infestations no eggs were produced from treated cats from day 8 through day 30. Egg production was still reduced by $\geq$95.8% through day 45. Indoxacarb treatment also reduced adult flea emergence from eggs for 5 weeks after treatment. The combination of reduction in egg numbers and egg viability from indoxacarb treated cats reduced predicted flea emergence by 100% from days 2 – 31 and 99.9%, 100%, 96.4% and 99.0% on days 37, 38, 44 and 45, respectively. A topical spot-on formulation of indoxacarb provided $\geq$99.6% efficacy against flea infestations on cats for 6 weeks following a single treatment and eliminated or markedly reduced egg production for the entire evaluation period.

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Efficacy of a 10% imidacloprid / 4.5% flumethrin collar for preventing the transmission of *Cytauxzoon felis* by *Amblyomma americanum* to domestic cats.

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Infection of *Cytauxzoon felis* in domestic cats produces a severe disease characterized by fever, lethargy, inappetence, anorexia, depression, dehydration, icterus, and often death. Transmission of *C. felis* to cats is dependent upon the feeding of an infected *Amblyomma americanum* (lone star tick). The purpose of the present study was to determine if application of a 10% imidacloprid / 4.5% flumethrin collar to cats (Seresto™) prevents transmission of *C. felis* by repelling ticks. Twenty cats were randomized to either a treated (n = 10) or non-treated control group (n = 10) based on their susceptibility to ticks. Cats of high, medium, and low tick susceptibility were represented in both groups. Treated cats were fitted with 10% imidacloprid / 4.5% flumethrin collars on study day 0 and then both groups were infested with *C. felis*-infected *A. americanum* on study day 30. Tick thumb counts were performed at 24 and 48 hours post-infestation. Transmission of *C. felis* was determined by examining blood of cats by DNA extraction followed by PCR amplification with piroplasm-specific primers. Ticks did not attach to any of the 10% imidacloprid / 4.5% flumethrin treated cats. However, ticks attached and fed on

all the non-treated control cats. The geometric mean number of ticks attached to the non-treated control cats at 24 and 48 hours was 14.2 and 13.1, respectively. *Cytauxzoon felis* was transmitted to 9 of 10 (90%) untreated control cats; *C. felis* was not transmitted to any of the treated cats. Transmission of *C. felis* to the non-treated cats occurred between 8 and 16 days post-infestation. Our results indicate that application of the 10% imidacloprid / 4.5% flumethrin collar to cats prevented ticks from attaching, feeding and transmitting *C. felis.*

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Repellent effect of elimax (Oystershell Laboratories) against Ischnocera lice on poultry.

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Head lice (*Pediculus humanus capitis)* are a growing issue in many countries. School age children, after treatment, can become re-infested if the outbreak has not been controlled at the school. Elimax, a head louse treatment *and infestation deterrent, combines a short-acting pediculicidal oil known to dehydrate lice and a natural-based product shown to repel lice in in vitro tests. However, an in vivo model was needed to demonstrate efficacy. The purpose of this study was to determine if chickens could be used as a model to assess repellency and evaluate the repellent effect of elimax. Three groups of chickens were co-housed for 24h. Group 1 (3 chickens) had their lice infestation treated with elimax. Group 2 (2 chickens) were used to assess lice repopulation. Group 3 (6 chickens) were lice infested.* Pretreatment and +4, +6, +8 and +12h post-treatment +/- 30min and +24h +/- 1h, lice counts were conducted on 4 areas (approximately 2cm x 2cm; the ventral and dorsal surface of each wing, the breast and the tail feathers) and totaled. None of the Group 1 chickens became re-infested during the 24h period. Lice were slow to repopulate the Group 2 chickens, but lice (1-3) were seen on both chickens at the +4 to +12h counts and an average of 6 lice were seen at +24h. The Group 3 chickens maintained their lice throughout the study with an average of 30.5. Based on this study, chickens can be used as a model to test repellency. In future development of the model, direct application of lice to lice free chickens as well as exposure to co-housing will be assessed. In addition, the study showed that elimax could be an effective means of protecting children from lice while at school.

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An assay to detect tau-fluvalinate and coumaphos resistance in Varroa mites under tropical conditions and seasonal variations.

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The objective of this study was to develop a simple screening field assay that can be used by bee inspectors and bee keepers to determine if honey bee (*Apis mellifera*) colonies contain tau-fluvalinate (Apistan®, Vita Europe Ltd. UK) and coumaphos (CheckMite+®, Bayer HealthCare) resistant Varroa mites. The assay relies upon a comparison of mites decreasing in numbers when exposed to 2.5% and 10% tau-fluvalinate and coumaphos using paired samples of live adult honey bees from the same colony. Two samples from each of the ten to twelve colonies were collected per apiary and honey bees were held in jars for 24h at 75-95°F. After 24h, the

number of dead mites and the number of those remaining on the honey bees were determined. Test results from four agriculture districts in Grenada determined that Varroa mites were susceptible to Apistan® and CheckMite+® in three to four weeks. The percentage mortality was more than 85% for 2.5% and 10% concentrations of coumaphos. In contrast, mites were deemed resistant from colonies where tau-fluvlinate did not provide control. The mite percentage mortality was less than 30% and 50% for the 2.5% and 10% concentrations, respectively. These results suggest that insecticide resistance may exist however confirmation would require additional testing over a period of time. We suggest that the health of honey bees in Grenada may be affected by the indiscriminate use of products, no monitoring or control of pests and disease in addition to low incomes associated with bee keeping situations. The field assay is relatively simple and "user friendly". Education of bee keepers regarding the proper use of acaricides and performing a simple field assay will help to determine if Grenada honey bees are infected with Varroa mites and if resistance exists in their hives.

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Persistence of *Trichomonas gallinae* in clean and organic-debris contaminated water.

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Trichomonas gallinae is a protozoan parasite commonly found in columbids, passerines, and raptors. In passerines and columbids, trichomonosis causes significant morbidity and mortality associated with contaminated bird feeders and waters. However, there has been little work on the persistence of *T. gallinae* in water to determine if artificial waters are a likely source of infection for naïve birds. To examine bird waters as a source of *T. gallinae* transmission, we inoculated 1 X 10^6 trichomonads, from one of two separate isolates, into Tupperware containers filled with 500 ml of either clean distilled water or distilled water containing 15 g organic matter consisting of leaves, soil particles, and organic debris. Aliquots of 0.5 ml were collected from each container at 0, 1, 5, 10, 20, and 60 min, following initial trichomonad inoculation, and inoculated into Diamond's media. The flasks were incubated at 37 C and observed daily for 5 days. Persistence was greatest in the presence of organic matter with positive samples at 60 min post inoculation; whereas persistence in clean distilled water did not exceed 20 min. These results indicate that bird waters are important in the transmission of *T. gallinae* in birds and organic material often observed in waters with poor sanitation can increase the persistence of the trichomonads.

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A Study of Giardiasis in dogs in Lahore-Pakistan.

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Giardiasis is a protozoon disease of zoonotic importance in humans and animals worldwide. Present study was designed to determine prevalence of giardia in dogs in metropolis of Lahore and to find suitable remedy with least detrimental effects on host. Five hundred faecal samples were collected from dogs suspected for giardiasis from veterinary teaching hospital (Pet Center), University of Veterinary and Animal Sciences, Lahore and private pet clinics of Lahore

city belonging to different age groups and sex. Coprological examination using zinc sulphate floatation and sucrose gradient centrifugation methods revealed prevalence of 10.4%. Among positive animals, 40 were selected randomly for chemotherapy trial, which were kept under optimal conditions of housing and feeding. The experimental animals were divided in four groups A, B, C and D, comprising 10 dogs in each group. Metronidazole was given to Group-A @ 25 mg/kg body weight BID orally for 5 days and Group-B received albendazole @ 25 mg/kg body weight BID orally for 5 days whereas, Group-C and Group-D acted as positive and negative control respectively. The faecal examinations were performed on the 3rd, 5th and 7th post-treatment days. In the present study the efficacy of metronidazole and albendazole was recorded as 85 % and 74% respectively on 7th post-treatment day. The results showed that the metronidazole was better in efficacy than albendazole. The haematological parameters showed a mild increase in total leukocytic count whereas; differential leukocytic count did not show a significant difference among groups. It was concluded that metronidazole was better in efficacy than albendazole and experimental animals well tolerated these drugs with no significant adverse effects on blood parameters.

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Effect of rifampicin and fosmidomycin on the causative agents of equine piroplasmosis, *Babesia caballi* and *Theileria equi in vitro.*

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Equine piroplasmosis is a disease that impacts the horse industry worldwide. The United States is considered nonendemic for the causative agents, *Babesia caballi* and *Theileria equi,* and strict regulations are in place to prevent their introduction. Because treatment options are limited in both quantity and efficacy and there is no vaccine, there are serious regulatory repercussions if a horse is identified as infected in the U.S. Rifampicin and fosmidomycin are anti-microbial agents with efficacy against plasmodium and other protozoan parasites closely related to *T. equi* and *B. caballi*. These drugs were screened for activity against the erythrocytic stage of *T. equi* and *B. caballi* using *in vitro* inhibition assays. Parasite growth was evaluated over a 72 h treatment period and was significantly reduced for both species with notable morphological changes. Rifampicin was shown to possess more potent effects at lower concentrations than fosmidomycin for both *T. equi* and *B. caballi*. The combination of fosmidomycin and rifampicin did not enhance their efficacy against either parasite. These results show that rifampicin and fosmidomycin do exhibit anti-parasitic activity against both *T. equi* and *B. caballi* and suggest a need for investigation into the efficacy of other currently available pharmaceuticals against these agents of equine piroplasmosis.

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Development of *Strongylus vulgaris*-specific antibodies in naturally infected foals.

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Strongylus vulgaris is regarded as the most pathogenic helminth parasite infecting horses. Migrating larvae cause pronounced endarteritis and thrombosis in the cranial mesenteric artery and adjacent branches, and thromboembolism can lead to ischemia and infarction of large intestinal segments. A recently developed serum ELISA assay allows detection of *S. vulgaris*-specific antibodies during the six-month-long prepatent period. A population of horses has been maintained at University of Kentucky without anthelmintic intervention since 1979, and *S. vulgaris* has been documented to be highly prevalent. In 2012, 12 foals were born in this population, and were studied during a 12-month period (March-March). Weekly serum samples were collected to monitor *S. vulgaris* specific antibodies with the ELISA assay. Nine colts underwent necropsy at different time points between 90 and 300 days of age. At necropsy, large strongyle parasites and *Parascaris equorum* were identified to species and stage and enumerated. Initial statistical findings indicate a significant interaction between foal age and ELISA results ($p<0.042$). All foals had initial evidence of maternal antibodies transferred in the colostrum, but then remained ELISA negative during their first three months of life. Foals born in February and March became ELISA positive at about 12 weeks of age, while those born in April and May went positive at about 15 and 21 weeks, respectively. This could be explained by age-dependent differences in parasite exposure. One foal remained ELISA-negative throughout the course of 30 weeks during the study. A significant association was found between ELISA values and larval *S. vulgaris* burdens ($p=0.0387$) as well as a three-way interaction between *S. vulgaris*, *S. edentatus*, and *P. equorum* burdens ($p<0.001$). More detailed analyses are needed to analyze these findings further. A plateau with a subsequent decline in ELISA values corresponded with *S. vulgaris* larvae leaving the bloodstream and migrating back to the intestine.

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The McMaster method revisited – the critical role of detection limit.

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Usage of fecal egg counts (FEC) for parasite surveillance and evaluating anthelmintic efficacy has led to an increased focus on performance of egg counting methods. Eggs are being lost during the process, and the observed FEC might not reflect the true FEC in the feces. The aims of this study were to a) evaluate the extent of strongyle egg loss with a modified McMaster egg counting technique with a 20 eggs per gram (EPG) detection limit, and b) to analyze whether increasing the sensitivity of the technique by counting the entire McMaster chamber will cause a significant increase in the reliability of the method. Approximately 15,000 mixed species cyathostomin eggs were collected from a donor horse and added to strongyle-free 4-gram samples to achieve true egg counts of 0, 5, 10, 15, 20, 40, 60, 80, 100, 200, 500, 1000, and

2000 EPG, respectively. Ten repeated counts were performed for each true EPG. For each count, both the area defined by the McMaster grid and the entire chamber were counted. The entire protocol was repeated three times. Egg loss rates varied between 14 and 48% across the true egg count levels evaluated, with a mean of 37%. Counting the entire chamber improved the accuracy of the estimation of the egg count by 50-100% across the true egg count levels. Performing three repeated egg counts improved the estimation by 70-100%. A simulated fecal egg count reduction analysis was performed by randomly setting 5, 10, 15, and 20 true EPG as post-treatment values and keeping 2000 true EPG as pre-treatment count. Here, counting the full chamber significantly improved the coverage by 50-100%. This study illustrates the importance of the sensitivity of egg counting methods. Egg loss can leave the lower post-treatment egg counts undetected, causing an overestimation of anthelmintic efficacy.

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Incorporating parasite evaluation in a ram performance test.

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Alternative strategies for control of *Haemonchus contortus* are needed as anthelmintic efficacy continues to decline. Selecting parasite resistant breeding stock can improve overall herd or flock status. Parasite monitoring in a ram performance test allows evaluation of relative parasite susceptibility in animals of multiple origins. A test for hair sheep ram lambs was conducted at the Virginia Tech Southwest Agricultural Research and Extension Center in summer, 2012. Eighty-five rams from 13 flocks were evaluated. Rams grazed fescue pasture and received 3% body weight grain supplement daily. Following arrival all lambs were dewormed to reduce fecal egg counts (FEC) to <50 eggs/g. Because test pastures are minimally naturally parasite contaminated, all lambs were administered 5000 *H. contortus* third stage larvae. Lambs were divided into 3 management groups based on initial body weight (low, medium and high). An additional, separately grazed group of ram lambs from the SWAREC flock received 10,000 larvae. At 2, 4, 6, 8 and 10 weeks after infection rams were weighed and fecal samples were collected. At the same intervals FAMACHA score was determined and blood samples were collected for packed cell volume analysis. Mean FEC for rams over the course of the study ranged from 10 to 3480 eggs/g. Final average daily gain at the end of the test did not differ among the 3 weight groups, but mean FEC in the high weight group was significantly lower than in the other two weight groups. Use of 5000 vs 10000 larvae in the initial infection had no significant effect on mean FEC over the course of the study. Experimental infection helps ensure similar parasite exposure in a performance test, but parasite susceptibility may also be affected by individual animal age and weight at the start of the test in addition to genetic factors.

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Mitigating effects of gastrointestinal parasitism in organic sheep using protein supplementation.

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The objective of this research was to determine effects of concentrate supplementation on gastrointestinal trichostrongylid parasitism (GTP) in organic lambs. Four treatments were compared during 2010, 2011 and 2012 which included: TR1) Control, TR2) Dewormed, TR3) Supplemented, TR4) Dewormed and Supplemented. Suffolk crossbred lambs were managed using a grazing period of 3 d per paddock and rest of 56 d between successive grazings. TR3 and TR4 received supplementation at 1% of BW daily. Supplement contained 10% CP in 2010 and 16% in 2011 and 2012. Results from 2010 indicated no difference in ADG or FAMACHA score in any treatment group, however, FEC was higher in TR1 and TR3 when compared to TR2 and TR4. In 2011, lambs in TR3 had higher ADG when compared to TR1 and TR2 (0.03 vs 0.02 and -0.01 kg.day^{-1} respectively; $P < 0.01$), no difference in FAMACHA scores was observed. Lambs in TR1 and TR3 had higher FEC than those in TR2 and TR4. In 2012, TR3 gained equally well as TR2 (0.3 vs. 0.4kg.day^{-1}, ; $P > 0.05$) and maintained equivalent FAMACHA scores while having significantly higher FEC (2227 vs. 91 eggs/gram; $P < 0.001$). Analyzing data across three years revealed greater ADG in lambs receiving 16% CP when compared to 10% and control (0.03 vs. 0.01 and 0.01kg.day^{-1}; $P < 0.001$). Lambs receiving 16% CP had lower FAMACHA scores then control (2.7 vs 3.1; $P < 0.04$), indicating that supplementation is at least reducing pathological effects of GTP. No differences were observed when evaluating FEC between supplemented and non-supplemented lambs regardless of protein level. Taken together these studies demonstrate that protein supplementation can positively affect organic lamb production by improving post-weaning gain of parasitized lambs without anthelmintic treatment.

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Efficacy Comparison of Two Injectable Formulations of Ivermectin Against Induced Gastrointestinal Nematode Infections in Cattle.

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A masked, randomized, controlled study was conducted to compare the efficacies of two injectable ivermectin solutions in cattle against induced gastrointestinal nematode infections. Thirty-four Holstein calves (104.5 to 228 kg) were treated with oral fenbendazole suspension (10 mg/kg) and confined in communal pens. On Day -30, each calf was inoculated orally with ~10,000 *Ostertagia* spp., ~49,800 *Cooperia* spp., ~8,160 *Trichostrongylus* spp., and ~13,460 *Oesophagostomum radiatum* infective, third-stage larvae. The inoculum had a history of minimal anthelmintic exposure. Fecal samples were collected 25 to 27 days after infection, and calves were ranked by egg counts and allocated randomly to three treatment groups: 1) Noromectin® (Norbrook, Ltd.), 200 µg/kg; 2) Ivomec® (Merial), 200 µg/kg; or 3) placebo (0.9% saline, 1 mL/50 kg). All doses were administered by subcutaneous injection on Day 0. On Days 10 to 12 post-treatment, equal numbers of calves from each treatment group were euthanatized and necropsied. Duplicate, 5% aliquots of abomasal and small intestinal contents and saline soaks of the abomasum, along with 10% aliquots of the large intestinal contents were collected and preserved in 10% formalin. Complete aliquots were inspected microscopically,

and nematodes were recovered, counted, and identified to genus, species, or stage. Adult worms of seven species achieved adequacy of infection (*i.e.* ≥100 specimens in six or more control calves). Both products exhibited >99.8% efficacy against *O. ostertagi* and *O. radiatum* (P<0.0001). Efficacies of Noromectin and Ivomec were 77.7% and 89.4% (P=0.3381) against *Nematodirus helvetianus*, 93.1% and 96.9% (P=0.016) against *Cooperia oncophora*, and 87.8% and 98.9% (P=0.0007) against *C. surnabada*, respectively. Neither product was effective against *Haemonchus* spp. (≤46.4%) or *Cooperia punctata* (≤ -6.04%), indicating probable macrocyclic lactone resistance in the isolates tested. This study demonstrated no significant differences in the anthelmintic efficacies of two injectable, 1% ivermectin solutions against induced nematode infections in cattle.

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Different strokes for different hosts: *Dicrocoelium dendriticum* metacercarial viability and survival in different host species.

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The survival and development of larval parasites often differs markedly in different definitive hosts. Although this pattern is frequently observed, especially for trematode metacercariae, underlying mechanisms are rarely known. The survival of *Dicrocoelium dendriticum* metacercariae in their definitive hosts differs according to host species, with a higher proportion of flukes surviving in smaller hosts; i.e. hamsters > sheep > cattle. Recovery of adult worms in sheep, regardless of the number of metacercariae administered, is 16-20%; recovery in beef rarely exceeds 7%. These are in comparison to published results of hamster infections where recovery exceeded 38%. Does this phenomenon reflect differential response to host factors, or is it a result of differences in the flukes themselves? Two contrasting morphotypes of *D. dendriticum* metacercarial cysts have been observed in ants (*Formica aserva*) from Cypress Hills Interprovincial Park, Alberta, Canada. MT1 cysts contain metacercariae with clear structural detail while MT2 appear amorphous. The types differ in their response to artificial excystation medium with over 80% of MT1 excysting while less than 20% of MT2 excyst. Furthermore, a small but consistent proportion of metacercariae excyst prior to ingestion by grazing herbivores. These unexpected observations imply that processes occurring within intermediate hosts are key in determining the recovery of adult worms in different definitive hosts.

President's Symposium

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***Besnoitia*: (Apicomplexa: Sarcocystidae) developmental biology, chemotherapy, and immunodiagnosis.**

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Besnoitiosis is a severe disease of cattle in Africa, Asia and southern Europe caused by *Besnoitia besnoiti.* It appears to be expanding in importance into regions of France, Germany and Spain in the last 10 years. It has been considered an emerging disease of cattle by the

European Food Safety Agency since 2010. It is caused by Apicomplexan tissue cyst forming coccidia in the genus *Besnoitia*. Caribou and reindeer can develop clinical disease due to *B. tarandi* infection in North America in their arctic and subarctic ranges. Clinical besnoitiosis due to *B. bennetti* has been reported from donkeys from several areas of the United States. *Besnoitia* species have diverse life cycles using 2 hosts. Cats serve as the definitive host for 4 species (*B. darlingi, B. neotomofelis, B. oryctofelisi, B. wallacei)*. The complete life cycles of the remaining 6 species (*B. akodoni, B. bennetti, B. besnoiti, B. caprae, B. jellisoni, B. tarandi*) are not known. Researchers have begun investigating hematophagous flies to determine if they can be mechanical vectors. Tachyzoites are rapidly dividing stages that disseminate the infection. Tachyzoites are the stage used in immunodiagnosis. Large, often grossly visible, tissue cysts containing bradyzoites are produced in connective tissue cells. These large tissue cysts are often readily visible in the sclera, conjunctiva, nares, and larynx. Treatment of clinical disease is most often done using sulfonamides. The efficacy of treatment for chronic disease is minimal. Diagnosis of infection can often be made by grossly observing tissue cysts in infected tissues. Serological methods including MAT, IFAT, ELISA and WB can be used. PCR can be used to detect parasite DNA in blood and in tissue samples. It is conceivable that *Besnoitia* may emerge as a disease in livestock in North America. Veterinarians should be on the look out for *Besnoitia* in the future.

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Clinical and serologic diagnosis of besnoitiosis in donkeys in the United States.

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Besnoitiosis is an emerging infectious disease of donkeys in the United States caused by infection with the protozoan parasite *Besnoitia bennetti.* Clinical disease is characterized by miliary dermatitis with parasitic cysts in the skin, nares, and sclera. The nasopharynx and testicles of infected animals are also affected. Currently the only method of diagnosis is histopathologic identification of *Besnoitia* organisms within tissues. Here we describe the clinical features of besnoitiosis and evaluate the utility of clinical examination and three serologic assays for the diagnosis of *Besnoitia* spp. infection in donkeys. A prospective study of 416 donkeys in 6 states was performed. Donkeys were examined for clinical lesions suggestive of besnoitiosis and evaluated for antibodies against *B. bennetti* using an immunofluorescent antibody (IFA) test and 2 immunoblot assays specific for bradyzoite and tachyzoite antigens, respectively. Donkeys confirmed to be infected with *B. bennetti* by histopathology (cases; n=32) were compared to those with no clinical signs of besnoitiosis (controls; n=384). Mean age of infected donkeys was 4.5 years (range, 1mon – 28yr). No sex predilection was found. Identifying clinical lesions in 2 or more locations correctly identified infected donkeys 80% of the time. Donkeys with besnoitiosis had significantly higher IFA titers ($P<.001$) and numbers of bradyzoite ($P<.001$) and tachyzoite ($P<.001$) immunoblot bands than control donkeys. The sensitivity and specificity of the serologic assays for detecting besnoitiosis was 88% and 100% for IFA, 81% and 96% for bradyzoite immunoblot, and 90% and 100% for tachyzoite immunoblot, respectively. Immunofluorescent antibody and immunoblot assays are effective at identifying donkeys with besnoitiosis and provide a more efficient and less invasive diagnostic alternative to histopathology. Knowledge of the clinical and serologic features of besnoitiosis will assist clinicians in the identification and diagnosis of infected donkeys in the United States.

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Emergence of bovine besnoitiosis in Europe: a review.

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Bovine besnoitiosis (BB), which is caused by the cyst-forming apicomplexan *Besnoitia besnoiti*, is an emerging disease in Europe. Known since the 19th century in small areas of the French Pyrenees and in southern Portugal, this disease was thought to have disappeared until the 1990s. On the contrary, BB showed a sharp spread since 2000 firstly in southern France and secondly in Spain, Italy, Germany, Belgium and Switzerland. The reasons of this extension are discussed. The biological cycle of *B. besnoiti* remains a mystery: up to now, no definitive host has been identified and the more likely route of contamination seems to be a mechanical transmission by horse flies and stable flies. However, the precise modalities of this cattle-to-cattle transmission need to be better understood. For unknown reasons, most of the infected animals remain asymptomatic whereas clinically affected cattle may present pyrexia, oedema, nasal and ocular discharges in the acute phase of the disease. The subsequent chronic phase is characterized by the formation of numerous tissue cysts in skin, upper respiratory tract and sclera conjunctiva leading to scleroderma and cachexia. This review describes the entire set of clinical manifestations of bovine besnoitiosis in Europe. Since its emergence in Europe, more attention has been given to this infection during the last ten years. Several seroprevalence and risk factors studies have been performed in areas of emergence in southern Europe. The putative involvement of wild fauna has been taken into account. Moreover, recent developments of diagnostic tools (real-time PCR, ELISA and Western Blots) have been proposed. As no efficient treatment or registered vaccines are available in Europe, control strategies are focused on vector control and selective culling of infected animals. This review presents the interests and the limits of these strategies in the control of BB in Europe and especially in France.

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