Sharon Patton was born on a farm in Watertown, TN to Virginia Bland and Charles Patton. She was the middle child with an older brother and two younger sisters. Her father was an “egg peddler” and sold eggs door to door in what is now considered the “greater Nashville” area. In the summer, Sharon went with her father to sell eggs 2 days/week – leaving at 4 am and returning around 7 pm. It was hard work for a little girl carrying buckets full of eggs door to door, but she loved it and gradually began to deliver more eggs than her father (although she always slept during the 50-mile drive home). The family moved from the farm where they were living with her grandparents to the small town of Watertown, but the egg peddling continued as did raising Black Angus cattle, and her father tending to 10,000 chickens that he managed in chicken houses on the farm. Later the family returned to the farm and built a house across the road from the grandparents’ house. Her father died while she was in college.
Sharon attended Watertown public schools and graduated as salutatorian of her class in 1965 having majored in Home Economics and Science. She was rather shy and timid as a young girl, but her fifth grade teacher loved her and gave her major speaking parts in plays at the school, and she became known for her speaking ability. One never knows how actions can affect people’s lives. That teacher made her think she was intelligent. Sharon changed from a timid child to one who won speaking contests and had major roles in plays. Neither of her parents were college educated, but they always encouraged the children to do their best in school. Sharon kept up the family tradition by attending Middle TN State University in Murfreesboro, TN, graduating in 1969. Her grandmother had attended Middle TN Normal School to receive a teaching certificate, and her Aunt had attended Middle TN State College.

MTSU was a wonderful experience. Murfreesboro and the campus seemed large to this small town, farm-girl, but it was also exciting and full of opportunities she did not know were possible. She was assigned to the oldest dorm on campus which was originally a boy’s dorm. Her grandmother and aunt were teachers, and she had been teaching children in Sunday School since she was 14, so, naturally, she planned to major in elementary education. However, as much as she enjoyed children, she did not enjoy the Elementary Education curriculum. The second semester she was in the last group to register for classes, and the only Biology class left was one taught by Dr. Mary C. Dunn. On the way back to the dorm, several people told her how demanding Dr. Dunn was, and that was why only her section was still available! She went to the class in fear and trembling and sat in the middle of the 75 students there. Dr. Dunn explained that all exams, except the final, were pop quizzes and cumulative. Woe is me, she thought. I have to come every day ready for a final exam. She could not see from where she was sitting, so at the next class period she moved up to the front row. The class turned out to be exciting, and Sharon excelled. One day, Dr. Dunn asked about her major and suggested she change it to Biology. Actually, she suggested this almost every day for several weeks – and it worked.

As a Biology major Sharon loved all the classes, particularly parasitology taught by parasitologist, Dr. John Patten. The scholarship she had received from MTSU was a work scholarship, and she worked during those years for Drs. Dunn and Patten. Oddly enough, both were parasitologists. After graduation, Sharon worked for a summer in a veterinary practice in Murfreesboro, not knowing it was a foretaste of things to come.

Sharon enrolled in graduate school in the Biology/Zoology Department of the University of KY which offered her a teaching assistantship. She became proficient at dissection of all kinds of animals, vertebrate and invertebrate, preserved or fresh. Her major professor, Professor J.M Edney, a Middle TN graduate himself, provided her space to study a variety of parasitic organisms. (Prof too was a parasitologist.) Since he was considering retirement, he suggested that she run the laboratories and help write the exams. At first, Sharon taught the comparative anatomy laboratories and then the Parasitology and Helminthology laboratories. It was a wonderful teaching experience. It was always exciting to go on field trips collecting snails to study trematode stages or horse feces for various parasite eggs. Her Master’s and PhD research involved trematodes in snails, and the collecting of cercariae from hundreds of snails. The cercariae were fed to fish to try to complete a trematode life cycle. Later she studied the immune response of rodents to *Hymenolepis nana*. Upon graduation six years later, Sharon accepted a post-doctoral fellowship in what
was then called the parasitology wing of the Veterinary Science Department in the College of Agriculture at UK. She was to be mentored by Drs. Harold Drudge and Gene Lyons (famous parasitologists who also received this award).

The experience with Drs. Drudge and Lyons was indescribably wonderful! Sharon studied the immunopathology of doses of *Strongylus vulgaris* larvae on ponies, measuring a variety of parameters like temperature, immunoglobulin levels (IgGT at the time). She learned to inject ponies, take their temperatures, necropsy them etc. She became involved in all the exciting research that was going on there and found it unbelievably delightful. The hard work, mentoring, and teaching by Gene Lyons and Harold Drudge prepared her for a career in Veterinary Parasitology.

Unbeknownst to Sharon, the legislature of TN approved the formation of a School of Veterinary Medicine at the University of TN. Being a big blue fan, Sharon gave it little thought. Drs. Drudge and Lyons urged her to apply, and she was hired as the first female on faculty at the new UT College of Veterinary Medicine. Dr. Robert Scholtens, formerly with the CDC had moved to TN and accepted a parasitology position at UTCVM. He already had a good start on organizing the diagnostic parasitology laboratory and had been thinking about how best to teach the course and labs with so few specimens. Sharon’s UK mentors sent her away with lots of specimens that were used in those first few years until the UTCVM parasitologists were able to collect their own. Sharon found that she loved teaching Veterinary Parasitology, and she loved the veterinary students. For 38 years she taught parasitology and sections of epidemiology, organized labs, taught the Parasitology rotations for seniors, and gave lectures for other courses like gastrointestinal tract, infectious disease, epidemiology, cardiology etc. She had some wonderful parasitologists colleagues at UTCVM through the years like Drs. Robert Scholtens, Craig Reinemeyer, Charles Faulkner, and Rick Gerhold. She worked with some incredible technicians and student workers without whom she could not have met her teaching, research, and service commitments. She was privileged to serve as president of the American Society of Parasitologists.

Sharon always ended her lecture to the veterinary students with “Remember I love you”! This became folklore in the college, and one year after a couple of lectures in the new semester, a student approached her and said, “I heard that you always tell the students that you love them. Why do you not tell us that?” She replied, “I do not love you yet. I have not known you long enough.” On about the 4th lecture as time was winding down, she said – “well, have a good day, and remember I love you.” They cheered! All was well.

Sharon Patton loved teaching the veterinary students and won several awards for her teaching and service including the SCAVMA Outstanding Educator Award (6 times), University of Tennessee National Alumni Association Outstanding Teacher Award, Lindsay Young Outstanding Teacher Award (twice), Norden Distinguished Teacher Award (3 times) and the North American Norden Distinguished Teacher Award for the Outstanding Teacher in Veterinary Medicine in North America (1999). She credits her successful teaching career to the wonderful students she taught. Since retirement she has found many opportunities to use her love of teaching at First Presbyterian Church with children and adults.
Sharon credits any success she has had at UTCVM to her wonderful colleagues, particularly her Diagnostic Lab technicians and student workers. Her efforts were always supported by her family, particularly her husband, Raymond McCord, and daughter Rachel. Sharon thought she would never have time to get married and have a family, but fortunately Raymond changed her mind. She is now helping home school her six-year old grandson, Paul, during the pandemic.
AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS
AWARDS HISTORY

AAVP-Boehringer Ingelheim 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 1
      2
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
2013  Dante Zarlenga
2014  Timothy G. Geary
2015  Michael W. Dryden
2016  Anne M. Zajac
2017  Susan Little
2018  Ray M. Kaplan
2019  Carlos E. Lanusse
2020  Sharon Patton

1 National Academy of Sciences 2010
2 National Academy of Sciences 2002
3 Noble Prize in Physiology or Medicine, 2015
4 2017, award renamed the “AAVP-Boehringer Ingelheim
   Distinguished Veterinary Parasitologist Award”
Tom was raised on a dairy farm in Westport, near Madison, WI, the oldest of eleven children of Bernard and Louise Kennedy. He studied Latin in high school (to better understand parasite names) and French at the University of Wisconsin-Madison in a successful attempt to impress his future wife. An introductory class in veterinary parasitology by Professor Arlie Todd hit a nerve and led to a technician position in Todd’s lab for almost 2 years where he picked worms for Bud Folz and other veterinary parasitologists and AAVP luminaries. Really!! After Tom and Beverly Makaahilani Kong were married 21 June 1969 (see French lessons above) and a year of Army life in TX, GA and Panama’s Jungle School, Tom learned Vietnamese (more than just to order a beer), then spent 11 months knee deep in the Mekong Delta as an infantry advisor to the Vietnamese Army experiencing monsoons, leeches, mosquitoes and other buzzing sounds. After VN, Arlie Todd offered a research assistantship at the UW-Madison which led to MS (1973) and Ph.D. (1975) in Veterinary Parasitology. Tom learned poultry coccidiosis with Cornell Johnson at Hess & Clark, built a contract lab with Don Bliss that developed strongylid and ascarid models in horses, pigs and dogs, read at least 10,000 fecal samples and evaluated the efficacy and safety of numerous anticoccidials and anthelmintics in chickens, turkeys, cattle, horses, swine, dogs and cats. He joined the corporate veterinary pharmaceutical industry with increasing levels of responsibility at Boehringer Ingelheim, Mallinckrodt Veterinary, Bayer Animal Health (developed the drug Marquis® for equine protozoal myeloencephalitis) and Central Life Sciences. Tom owes a huge debt to Arlie Todd and to his extended Kennedy family for their professional and emotional support and examples of public service and has tried to repay that debt with service to his community and to veterinary parasitology. Tom has been the Secretary-Treasurer (encouraged by Bud Folz!), Vice-President and Program Chair, President and Past
President of the American Association of Veterinary Parasitologists. He is also past President and member of the Executive Committee of the World Association for the Advancement of Veterinary Parasitology, including the 5-year labor of love (?!?) to host the 27th meeting of the WAAVP in Madison in 2019 (thankful for family support!). He is a member of the American Society of Parasitologists, founding member and Past-President of the Equine Protozoal Myeloencephalitis (EPM) Society, serves on the Board of Visitors for the UW-Madison College of Agriculture, his church’s parish council, secretary/treasurer and president-elect of the local Rotary club. Discovering partial resistance to retirement, Tom consults to the industry as Eleven Bravo LLC and is a partner in Covenant Animal Health Partners. Tom and Beverly reside on the Wisconsin farm mentioned above, from where they hike, garden, mow grass, shovel snow (sometimes the same day) and visit the kids and 10 grandkids scattered from Illinois to Hawaii.

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
2017  William C. Campbell
2020  Thomas J. Kennedy

AAVP-Merck One Health Award

2018  Jitender P. Dubey
2020 AAVP–Merck Animal Health Graduate Student Research Award
Ashley E. Steuer, DVM, PhD

Dr. Ashley Steuer graduated from Michigan State University, Lyman Briggs College in 2012 with a BS in Animal Science and The University of Tennessee College of Veterinary Medicine in 2016 with a DVM. She started her PhD in August of 2016, working on the Host-Parasite interaction of cyathostomins in horses under Dr. Martin Krarup Nielsen. She defended her PhD in April of 2020 and graduated this past May. She is also the Zoetis Resident in Veterinary Parasitology through the NCVP and will sit the ACVM board examination in 2020. Ashley’s PhD research focused on the local and systemic inflammatory reactions caused by anthelmintic treatment in horses with naturally acquired cyathostomin infections. Her other research areas of interest include the development and validation of novel diagnostics, evaluation of novel anthelmintics and treatment protocols, epidemiological and prevalence surveys, and in vitro development and maintenance of equine helminths.
AAVP–Merck Animal Health Outstanding 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  
2000¹  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  
2008²  Heather D. Stockdale  
2009  Kelly E. Allen  
2010  Stephanie R. Heise  
2011  Aaron S. Lucas  
2012³  Flavia A. Girao Ferrari  
2013  Lindsay A. Starkey  
2014  Alice Che Yu Lee  
2015  Anne Barrett  
2016  Rachel Curtis-Robles  
2017  Brian H. Herrin  
2018  Russell Avramenko  
2019  Jeba Jesudoss Chelladurai  
2020  Ashley E. Steuer  

¹2000, award renamed the “AAVP–Intervet Graduate Student Research Award”
²2008, award renamed the “AAVP–Schering-Intervet Graduate Student Research Award”
³2012, award renamed the “AAVP–Merck Animal Health Graduate Student Research Award”
Dr. Jimenez Castro received his DVM from the College of Veterinary Medicine and Zootechnics at the National University of Colombia in 2013. Afterwards he started working at Novartis Animal Health and then Elanco Animal Health in regulatory affairs. In 2016, he enrolled in the dual PhD/veterinary clinical parasitology residency program of the Department of Infectious Diseases at the University of Georgia, College of Veterinary Medicine, Athens, Georgia. His research is focused on investigating the biology, epidemiology and genetics of multiple drug resistance in *Ancylostoma caninum*. His interests include clinical efficacy and safety trials, anthelmintic resistance and the epidemiology and control of parasites of veterinary and public health importance. He has several publications in peer-review journals and has given presentations at different scientific meetings. His talk this year is titled “Efficacy evaluation of anthelmintic products against an infection with the canine hookworm (*Ancylostoma caninum*) isolate Worthy 4.1F3P in dogs”.
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 A. Starkey
2013  Gail M. Moraru
2014  Anne Barrett
2015  Alice Che Yu Lee
2016  Brian H. Herrin
2017  Sarah Sapp
2018  Jeba R.J. Jesudoss Chelladurai
2019  Cassan N. Pulaski
2020  Pablo David Jimenez Castro
AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS  
Founded 1956

**Officers 2019-2020**

<table>
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<tr>
<th>Position</th>
<th>Name</th>
<th>Organization</th>
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<tr>
<td>President</td>
<td>Mason V. Reichard</td>
<td>Oklahoma State University</td>
<td>Stillwater, OK</td>
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<td>President-Elect</td>
<td>Doug Carithers</td>
<td>Boehringer Ingelheim</td>
<td>Duluth, GA</td>
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<tr>
<td>Vice-President</td>
<td>Martin Nielsen</td>
<td>University of Kentucky</td>
<td>Lexington, KY</td>
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<tr>
<td>Secretary/Treasurer</td>
<td>Adriano F. Vatta</td>
<td>Zoetis</td>
<td>Richland, MI</td>
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<tr>
<td>Immediate Past-President</td>
<td>John S. Gilleard</td>
<td>University of Calgary</td>
<td>Calgary, AB, Canada</td>
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2019-2020 AAVP Committee Chairs and Members (Term Date)

Nominations: Yoko Nagamori, Chair (2021), Joyce Login (2020), Ashley McGrew (2020), Lindsay Starkey (2021), Alice Lee (2022), Andrew Moorhead (2022)


Student Representatives: Grace VanHoy (2020), Kathryn Duncan (2021)
**PAST PRESIDENTS OF THE AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS**

<table>
<thead>
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<th>Year</th>
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<tr>
<td>1956-1958</td>
<td>L. E. Swanson</td>
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<td>1958-1960</td>
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<td>1960-1962</td>
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<td>Dwight D. Bowman</td>
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<td>Timothy G. Geary</td>
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<td>2017-2018</td>
<td>Dante S. Zarlenga</td>
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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
Thomas J. Kennedy  1993-1998
Daniel E. Snyder  1998-2004
Alan A. Marchiondo  2004-2010
Robert G. Arther  2010-2014
Doug Carithers  2014-2018
Adriano F. Vatta  2018-

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 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
1981  26th Annual Meeting – ST. LOUIS, MO  19-20 JUL
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<td>40th</td>
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<td>(Joint meeting with the American Society of Parasitologists)</td>
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<td>1996</td>
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<td>46th</td>
<td>BOSTON, MA</td>
<td>14-17 JUL</td>
</tr>
<tr>
<td>2002</td>
<td>47th</td>
<td>NASHVILLE, TN</td>
<td>13-16 JUL</td>
</tr>
<tr>
<td>2003</td>
<td>48th</td>
<td>DENVER, CO</td>
<td>19-23 JUL</td>
</tr>
<tr>
<td>2004</td>
<td>49th</td>
<td>PHILADELPHIA, PA</td>
<td>24-28 JUL</td>
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<tr>
<td></td>
<td>(Joint meeting with the American Society of Parasitologists)</td>
<td></td>
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<tr>
<td>2005</td>
<td>50th</td>
<td>MINNEAPOLIS, MN</td>
<td>16-19 JUL</td>
</tr>
<tr>
<td>2006</td>
<td>51st</td>
<td>HONOLULU, HI</td>
<td>15-18 JUL</td>
</tr>
<tr>
<td>2007</td>
<td>52nd</td>
<td>WASHINGTON, DC</td>
<td>14-17 JUL</td>
</tr>
<tr>
<td>2008</td>
<td>53rd</td>
<td>NEW ORLEANS, LA</td>
<td>19-22 JUL</td>
</tr>
<tr>
<td>2009</td>
<td>54th</td>
<td>CALGARY, CANADA</td>
<td>9-13 AUG</td>
</tr>
<tr>
<td></td>
<td>(Joint meeting with the World Association for the Advancement of Veterinary Parasitology and the International Commission on Trichinellosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>55th</td>
<td>ATLANTA, GA</td>
<td>31 JUL – 2 AUG</td>
</tr>
<tr>
<td>2011</td>
<td>56th</td>
<td>ST. LOUIS, MO</td>
<td>16-19 JUL</td>
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<tr>
<td></td>
<td>(Joint meeting with the Livestock Insect Workers Conference and the International Symposium of Ectoparasites of Pets)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>57th</td>
<td>San Diego, CA</td>
<td>4-7 AUG</td>
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<tr>
<td>2013</td>
<td>58th</td>
<td>Chicago, IL</td>
<td>20-23 JUL</td>
</tr>
<tr>
<td>2014</td>
<td>59th</td>
<td>Denver, CO</td>
<td>26-29 JUL</td>
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<tr>
<td>2015</td>
<td>60th</td>
<td>Boston, MA</td>
<td>11-14 JUL</td>
</tr>
<tr>
<td></td>
<td>(Joint meeting with the Livestock Insect Workers Conference and the International Symposium of Ectoparasites of Pets)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>61st</td>
<td>San Antonio, TX</td>
<td>6-9 AUG</td>
</tr>
<tr>
<td>2017</td>
<td>62nd</td>
<td>Indianapolis, IN</td>
<td>22-25 JUL</td>
</tr>
<tr>
<td>2018</td>
<td>63rd</td>
<td>Denver, CO</td>
<td>14-17 JUL</td>
</tr>
<tr>
<td>2019</td>
<td>64th</td>
<td>Madison, WI</td>
<td>7-11 JUL</td>
</tr>
<tr>
<td></td>
<td>(Joint meeting with the World Association for the Advancement of Veterinary Parasitology and the Livestock Insect Workers Conference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2020</td>
<td>65th</td>
<td>Virtual Meeting</td>
<td>20-23 JUN</td>
</tr>
</tbody>
</table>
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AAVP 65th ANNUAL MEETING
Virtual Meeting

CORPORATE PRESENTATIONS

Bayer HealthCare, Animal Health
Saturday, June 20, 2020 13:15-14:00
CVBD: Parasite and vector-borne disease concerns in rehomed dogs.
Susan Little,1 Andrew Peregrine,2 and Ian Wright3
1Oklahoma State University, 2University of Guelph, and 3Mount Veterinary Practice, Fleetwood, UK

Zoetis
Saturday, June 20, 2020 16:15-17:00
Maximizing moxidectin in a worsening heartworm disease landscape.
Chris Adolph, Jessica Rodriguez, Yoko Nagamori
Zoetis

Boehringer Ingelheim
Sunday, June 21, 2020 13:15-14:00
Risks Beyond Parasites: zoonoses, drug resistance, telehealth tools and you.
Sarah L. Babcock, DVM, JD
Animal & Veterinary Legal Services PLLC

Elanco Animal Health
Sunday, June 21, 2020 16:15-17:00
The DOG PARCS Study: Detection of Gastrointestinal Parasites at Recreational Canine Sites.
Susan Little
Oklahoma State University

AAVP- National Center for Veterinary Parasitology (NCVP)
Parasite Case Discussions
Moderators: Dwight Bowman1 and Andrew Peregrine2
1Cornell University, 2University of Guelph
AMERICAN ASSOCIATION OF VETERINARY PARASITOLOGISTS
65th ANNUAL MEETING SPONSORS

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BOEHRINGER INGELHEIM ²
COMPANION ANIMAL PARASITE COUNCIL³
ELANCO ANIMAL HEALTH⁴
MERCK ANIMAL HEALTH⁵

¹ AAVP- Bayer Best Student Oral Presentation Awards
² AAVP-Boehringer Ingelheim Distinguished Veterinary Parasitologist Award
³ AAVP-CAPC Graduate Student Award – Zoonotic Diseases
⁴ AAVP-Elanco Best Student Poster Presentation Awards
⁵ AAVP-Merck Animal Health Graduate Student Award
⁵ AAVP-Merck Animal Health One Health Award

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MIDWEST VETERINARY SERVICES, Inc. & VETERINARY & BIOMEDICAL RESEARCH CENTER, INC
Maximizing moxidectin in a worsening heartworm disease landscape

Please refer to the AAVP Virtual Calendar to join our presentation!

Presenters:

Chris Adolph
DVM, MS,
Diplomate ACVM
(Parasitology)

Jessica Rodriguez
DVM, PhD,
Diplomate ACVM
(Parasitology)

Yoko Nagamori
DVM, MS,
Diplomate ACVM
(Parasitology)
Parasite Case Discussion

A live interactive case-based clinical parasitology experience brought to you by Zoom and the NCVP

*With celebrity MCs Dr. Dwight Bowman and Dr. Andrew Peregrine!*

Monday June 22nd
2:35–4:35 EDT

Join your colleagues for entertaining and challenging clinical case quiz questions

Bring your diagnostic expertise!

Please watch your email for the secure meeting link.

Questions? Contact ncvp@okstate.edu
A million thanks.

Actually, over 220 million—that’s how many times NexGard® (afoxolaner) has been prescribed.¹

> The only flea & tick control product indicated for the prevention of Borrelia burgdorferi infections as a direct result of killing black-legged ticks

> Gentle protection in a bite-sized monthly dose

> Proven safety for puppies as young as 8 weeks, weighing 4 pounds or more

> The savory beef-flavored chew that makes compliance a treat

NexGard®
(afoxolaner) Chewables

IMPORTANT SAFETY INFORMATION: NexGard is for use in dogs only. The most frequently reported adverse reactions include vomiting, pruritus, lethargy, diarrhea and lack of appetite. The safe use of NexGard in pregnant, breeding, or lactating dogs has not been evaluated. Use with caution in dogs with a history of seizures or neurologic disorders. For more information, see the full prescribing information or visit NexGardClinic.com.

¹ Data on file at Boehringer Ingelheim.
after receiving the first dose and on the same day after receiving the second dose of NexGard.

2 Number of dogs in the control group with the identified abnormality.

CAUTION: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Table 1: Dogs With Adverse Reactions.

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>N</th>
<th>% (n=45)</th>
<th>N</th>
<th>% (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>26</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethargy</td>
<td>26</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea (with and without blood)</td>
<td>13</td>
<td>2.9</td>
<td>73</td>
<td>36.5</td>
</tr>
<tr>
<td>Itching</td>
<td>26</td>
<td>5.8</td>
<td>86</td>
<td>43.0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>26</td>
<td>5.8</td>
<td>52</td>
<td>26.0</td>
</tr>
<tr>
<td>Convulsions</td>
<td>26</td>
<td>5.8</td>
<td>23</td>
<td>11.5</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>26</td>
<td>5.8</td>
<td>71</td>
<td>35.5</td>
</tr>
<tr>
<td>Tremors</td>
<td>26</td>
<td>5.8</td>
<td>11</td>
<td>5.5</td>
</tr>
<tr>
<td>Seizures</td>
<td>18</td>
<td>4.0</td>
<td>86</td>
<td>43.0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>26</td>
<td>5.8</td>
<td>73</td>
<td>36.5</td>
</tr>
<tr>
<td>Vesiculation</td>
<td>13</td>
<td>2.9</td>
<td>68</td>
<td>34.0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>26</td>
<td>5.8</td>
<td>51</td>
<td>25.5</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>26</td>
<td>5.8</td>
<td>56</td>
<td>28.0</td>
</tr>
<tr>
<td>Appetite change</td>
<td>13</td>
<td>2.9</td>
<td>68</td>
<td>34.0</td>
</tr>
<tr>
<td>Other</td>
<td>26</td>
<td>5.8</td>
<td>51</td>
<td>25.5</td>
</tr>
</tbody>
</table>

3 Number of dogs in the afoxolaner treatment group with the identified abnormality.

4 Number of dogs in the control group with the identified abnormality.

In the US field study, one dog with a history of seizures experienced a seizure on the same day after receiving the first dose and on the same day after receiving the second dose of NexGard. This dog experienced a third seizure one week after receiving the third dose. The dog remained enrolled and completed the study. Another dog with a history of seizures had a seizure 15 days after the third dose of NexGard. The dog remained enrolled and completed the study. A third dog with a history of seizures received NexGard and experienced no seizures throughout the study.

Post-Approval Experience (July 2018):

The following adverse events are based on post-approval adverse drug experience reporting. Not all adverse events are reported to FDA/CVM. It is not always possible to reliably estimate the adverse event frequency or establish a causal relationship to product exposure using these data.

The following adverse events reported for dogs are listed in decreasing order of reporting frequency for NexGard:

Vomiting, pruritus, lethargy, diarrhea (with and without blood), anorexia, seizure, hyperactivity/ restlessness, panting, erythema, ataxia, dermatitis (including rash, papules), allergic reactions (including hives, swelling), and tremors.

Contact Information:

For a copy of the Safety Data Sheet (SDS) or to report suspected adverse drug events, contact Merit at 1-888-637-4251 or www.nexgardfordogs.com.

For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at http://vm.cfsan.fda.gov/animalveterinary/safetyhealth.

Mode of Action:

Afoxolaner is a member of the isoxazoline family, shown to bind to a binding site to inhibit insect and acarine ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), thereby blocking pre- and post-synaptic transfer of chloride ions across cell membranes. Prolonged afoxolaner-induced hyperpolarization results in uncontrolled activity of the central nervous system and death of insects and acarines. The selective toxicity of afoxolaner between insects and acarines and mammals may be inferred by the differential sensitivity of the insects and acarines to GABA receptors versus mammalian GABA receptors.

Effectiveness:

In a well-controlled laboratory study, NexGard began to kill fleas four hours after initial administration and demonstrated >99% effectiveness at eight hours. In a separate well-controlled laboratory study, NexGard demonstrated 100% effectiveness against adult fleas 24 hours post-infestation for 35 days, and was >95% effective at 12 hours post-infestation through Day 21, and on Day 35. On Day 28, NexGard was 91.1% effective 12 hours post-infestation. Dogs in both the treated and control groups that were infected with fleas on Day 1 generated flea eggs at 12- and 24-hours post-treatment (0-11 eggs and 1-17 eggs in the NexGard treated dogs, and 9-90 eggs and 0-118 eggs in the control group, respectively). At subsequent evaluations post-infestation, fleas from the treated group were essentially unable to produce any eggs (0-11 eggs) while fleas from dogs in the control group continued to produce eggs (1-141 eggs). In a 90-day US field study conducted in households with existing flea infestations of varying severity, the effectiveness of NexGard against fleas on the Day 30, 60 and 90 visits compared with baseline was 98.0%, 99.7%, and 99.9%, respectively. Collectively, the data from the three studies (two laboratory and one field) demonstrate that NexGard kills fleas before they can lay eggs, thus preventing subsequent flea infestations after the start of treatment of existing flea infestations.

In well-controlled laboratory studies, NexGard demonstrated >97% effectiveness against Dermacentor variabilis, >94% effectiveness against Ixodes scapularis, and >93% effectiveness against Rhipicephalus sanguineus, 48 hours post-infestation for 30 days. At 72 hours post-infestation, NexGard demonstrated >97% effectiveness against Amblyomma americanum for 30 days. In two separate, well-controlled laboratory studies, NexGard was effective at preventing Dermacentor variabilis infections after dogs were infected with ixodes scapularis vector ticks 28 days post-treatment.

Animal Safety:

In a margin of safety study, NexGard was administered orally to 8 to 9-week-old Beagle puppies at 1, 3, and 5 times the maximum exposure dose (6.3 mg/kg) for three treatments every 28 days, followed by three treatments every 14 days, for a total of six treatments. Dogs in the control group were sham-dosed. There were no clinically-related effects related to treatment on physical examination, body weight, food consumption, clinical pathology (hematology, clinical chemistry, or coagulation tests), gross pathology, histopathology or organ weights. Vomiting occurred throughout the study, with a similar incidence in the treated and control groups, including one dog in the 5x group that vomited four hours after treatment.

In a well-controlled field study, NexGard was used concomitantly with other medications, such as vaccines, anthelmintics, antibiotics (including topicals), steroids, NOAIDs, anesthetics, and antihistamines. No adverse reactions were observed from the concomitant use of NexGard with other medications.

Storage Information:

Store at or below 30°C (86°F) with excursions permitted up to 40°C (104°F).

How Supplied:

NexGard is available in four sizes of beef-flavored soft chewables: 11.3, 28.3, 68 or 136 mg afoxolaner. Each chewable size is available in color-coded packages of 1, 3 or 6 beef-flavored chewables.

NADA 141-406, Approved by FDA

Merck by Frontline Vet Labs™, a Division of Meril, Inc.

Duluth, GA 30096-4640 USA

Made in Brazil.

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Some things change.
Their heartworm prevention shouldn’t.

From puppy to senior, you’re there for your patients.*
And for 32 years, we’ve been there with you.

HEARTGARD® Plus (ivermectin/pyrantel) prevents
heartworm disease, and treats and controls
roundworms and hookworms, in the only
monthly real-beef chew dogs and puppies love.¹

Trusted at six weeks, trusted for 32 years.
Recommend it for a lifetime.

IMPORTANT SAFETY INFORMATION: HEARTGARD Plus (ivermectin/pyrantel) is well tolerated. All dogs should be tested for heartworm
infection before starting a preventive program. Following the use of HEARTGARD Plus, digestive and neurological side effects have rarely
been reported. For more information, please see full prescribing information or visit www.HEARTGARD.com.

¹Freedom of information: NADA 140-971 (January 15, 1993)
*For puppies 6 weeks of age or older.
HEARTGARD® Plus is recommended for dogs 6 weeks of age and older. For dogs over 100 lb use the appropriate combination of these chewables. 

**INDICATIONS:** For use in dogs to prevent canine heartworm disease by eliminating the tissue stage of heartworm larvae (Dirofilaria immitis) for a month (30 days) after infection and for the treatment and control of ascarids (Ancylostoma caninum, Uncinaria stenocephala, Ancylostoma braziliense). 

**DOSAGE:** HEARTGARD® Plus should be administered orally at monthly intervals at the recommended minimum dose level of 6 mcg of ivermectin per kilogram (2.72 mcg/lb) and 5 mg of pyrantel (as pamoate salt) per kg (2.27 mg/lb) of body weight. The recommended dosing schedule for prevention of canine heartworm disease and for the treatment and control of ascarids and hookworms is as follows:

<table>
<thead>
<tr>
<th>Dog Weight</th>
<th>Chewables Per Month</th>
<th>Ivermectin Content</th>
<th>Pyrantel Content</th>
<th>Color Coding on Foil Backing and Carton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 25 lb</td>
<td>1</td>
<td>68 mcg</td>
<td>57 mg</td>
<td>Blue</td>
</tr>
<tr>
<td>26 to 50 lb</td>
<td>1</td>
<td>136 mcg</td>
<td>114 mg</td>
<td>Green</td>
</tr>
<tr>
<td>51 to 100 lb</td>
<td>1</td>
<td>272 mcg</td>
<td>227 mg</td>
<td>Brown</td>
</tr>
</tbody>
</table>

HEARTGARD Plus is available in three dosage strengths (See **HOW SUPPLIED** section) for dogs of different weights. Each strength comes in convenient cartons of 6 and 12 chewables.

**Efficacy:** HEARTGARD® Plus (ivermectin/pyrantel) Chewables, given orally using the recommended dose and regimen, are effective against the tissue larval stage of *D. immitis* for a month (30 days) after infection and, as a result, prevent the development of the adult stage. HEARTGARD Plus Chewables are also effective against canine ascarids (*T. canis, T. leonina*) and hookworms (*A. caninum, U. stenocephala, A. braziliense*). 

**Acceptability:** In acceptability and field trials, HEARTGARD Plus was shown to be an acceptable oral dosage form that was consumed at first offering by the majority of dogs.

**Precautions:** All dogs should be tested for existing heartworm infection before starting treatment with HEARTGARD Plus which is not effective against adult *D. immitis*. Infected dogs must be treated to remove adult heartworms and microfilariae before initiating a program with HEARTGARD Plus. While some microfilariae may be killed by the ivermectin in HEARTGARD Plus at the recommended dose level, HEARTGARD Plus is not effective for microfilariae clearance. 

**Keep this and all drugs out of the reach of children.** In case of ingestion by humans, clients should be advised to contact a physician immediately. Physicians may contact a Poison Control Center for advice concerning cases of ingestion by humans. Store between 68°F - 77°F (20°C - 25°C). Excursions between 59°F - 86°F (15°C - 30°C) are permitted. Protect product from light. 

**Adverse Reactions:** In clinical field trials with HEARTGARD Plus, vomiting or diarrhea within 24 hours of dosing was rarely observed (1.1% of administered doses). The following adverse reactions have been reported following the use of HEARTGARD: Depression/lethargy, vomiting, anorexia, diarrhea, mydriasis, ataxia, staggering, convulsions and hypersalivation. 

**Safety:** HEARTGARD Plus has been shown to be bioequivalent to HEARTGARD, with respect to the bioavailability of ivermectin. The dose regimens of HEARTGARD Plus and HEARTGARD are the same with regard to ivermectin (6 mcg/kg). Studies with ivermectin indicate that certain dogs of the Collie breed are more sensitive to the effects of ivermectin administered at elevated dose levels (more than 16 times the target use level) than dogs of other breeds. At elevated doses, sensitive dogs showed adverse reactions which included mydriasis, depression, ataxia, tremors, drooling, paresis, recumbency, excitability, stupor, coma and death. HEARTGARD demonstrated no signs of toxicity at 10 times the recommended dose (60 mcg/kg) in sensitive Collies. Results of these trials and bioequivalence studies, support the safety of HEARTGARD Plus products in dogs, including Collies, when used as recommended.

HEARTGARD Plus has shown a wide margin of safety at the recommended dose level in dogs, including pregnant or breeding bitches, stud dogs and puppies aged 6 or more weeks. In clinical trials, many commonly used flea collars, dips, shampoos, anthelmintics, antibiotics, vaccines and steroid preparations have been administered with HEARTGARD Plus in a heartworm disease preventive program. In one trial, where some pups had parvovirus, there was a marginal reduction in efficacy against intestinal nematodes, possibly due to a change in intestinal transit time.

**How Supplied:** HEARTGARD Plus is available in three dosage strengths (See **DOSAGE** section) for dogs of different weights. Each strength comes in convenient cartons of 6 and 12 chewables. For customer service, please contact Merial at 1-888-637-4251.
Melarsomine dihydrochloride, the active ingredient in IMMITICIDE, is the ONLY FDA-approved heartworm adulticide. Give your canine patients the future they deserve with IMMITICIDE.

To get your supply of IMMITICIDE, call Boehringer Ingelheim Customer Care at 1-888-637-4251, contact your sales representative, or order instantly at BI-CONNECT.com

IMPORTANT SAFETY INFORMATION: IMMITICIDE should not be used in dogs with very severe (Class 4) heartworm disease. IMMITICIDE should be administered by deep intramuscular injection in the lumbar (epaxial) muscles (L3–L5) only. Do not use in any other muscle group. Do not use intravenously. Care should be taken to avoid superficial injection or leakage. Serious adverse reactions may occur in any dog with heartworm disease due to the killing of heartworms in the pulmonary arteries. Reactions may include thromboembolism, dyspnea, coughing, depression, right side heart failure, and death. Dogs should be cage rested following treatment due to possible thromboembolic disease. Post-injection site reactions (eg, pain, swelling) were the most commonly reported adverse events. See full prescribing information for dosing and administration directions prior to each use of IMMITICIDE.

For more information, please see full prescribing information.

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18625
INDICATIONS
IMMITICIDE Sterile Power is indicated for the treatment of stabilized Class 1, 2, and 3 heartworm disease caused by immature (4-month-old, stage L5) to mature adult infections of Dirofilaria immitis in dogs. See full package insert for Heartworm Disease Classification.

CONTRAINDICATIONS
IMMITICIDE is contraindicated in dogs with very severe (Class 4) heartworm disease. Patients in this category have Caval Syndrome (D. immitis present in the venae cavae and right atrium).

WARNINGS
(See boxed Warning). For use in dogs only. Safety for use in breeding animals and lactating or pregnant bitches has not been determined.

HUMAN WARNINGS
Keep this and all medications out of the reach of children. Avoid human exposure. Wash hands thoroughly after use or wear gloves. Potentially irritating to eyes. Rinse eyes with copious amounts of water if exposed. Consult a physician in cases of accidental exposure by any route (dermal, oral, or by injection).

PRECAUTIONS
Dogs with heartworm disease are at risk for post-treatment pulmonary thromboembolism (death of worms which may result in fever, weakness, and coughing). Dogs with severe pulmonary arterial disease have an increased risk and may exhibit more severe signs (dyspnea, hemoptyis, right heart failure and possibly death). Dogs should be restricted from exercise after treatment. Studies indicate that adverse reactions may occur after the second injection in the series even if no problems were encountered with the first injection. All patients should be closely monitored during treatment and for up to 24 hours after the last injection.

Special Considerations for Class 3 dogs: Following stabilization, severely ill (Class 3) dogs should be treated according to the alternate dosing regime in an attempt to decrease post-treatment mortality associated with thromboembolism. Post-treatment mortality due to thromboembolism and/or progression of the underlying disease may occur in 10 to 20% of the Class 3 patients treated with IMMITICIDE. Hospitalization post-treatment and strict exercise restriction are recommended. If the alternate dosing regime is used, expect increased injection site reactions on the side receiving the second injection since the skeletal muscles at the first injection site may not have fully recovered (healed). If persistent swelling is present at 1 month, the second injections may be delayed for several weeks up to 1 month.

Special Considerations for Older Dogs: In clinical field trials, dogs 8 years or older experienced more post-treatment depression/lethargy, anorexia/inappetence, and vomiting than younger dogs.

DOSEAGE AND ADMINISTRATION
Care must be taken to administer the proper dose deep into epaxial muscles only (see boxed WARNING). Accurately weigh the dog and calculate the volume to be injected based on the dose of 2.5 mg/kg (1.1 mg/lb). This is equivalent to 0.1 mL/kg (0.045 mL/lb). See full product insert for dosing table. Use a 23 gauge 1 inch needle for dogs equal to or less than 10 kg (22 lb) in weight. Use a 22 gauge 1½ inch needle for dogs greater than 10 kg (22 lb). Use alternating sides with each administration and avoid injecting at the same lumbar location.

Disease Classification: It is vital to classify the severity of heartworm disease to apply the appropriate dosage regime for IMMITICIDE. See full product insert for Heartworm Disease Classification criteria.

Class 1 and 2: IMMITICIDE should be given in two intramuscular injections of 2.5 mg/kg, 24 hours apart. Four months following treatment, a second treatment series (2.5 mg/kg twice, 24 hours apart) can be elected.

Class 3: Alternate Dosing Regime: Dogs with severe (Class 3) heartworm disease should be stabilized prior to treatment and then dosed intramuscularly in the lumbar (L3 - L5) muscles with a single injection of 2.5 mg/kg then approximately 1 month later with 2.5 mg/kg administered twice, 24 hours apart.

SAFETY
IMMITICIDE has a low margin of safety. A single dose of 7.5 mg/kg (3X the recommended dose) can result in pulmonary inflammation, edema, and death. Symptoms of overdose (2x recommended dose) may include excessive salivation, panting, restlessness, fever, vomiting and diarrhea. These symptoms were seen in the clinical trials and all signs resolved within 24 hours. Symptoms of up to 3x the recommended dose included tremors, lethargy, unsteadiness, restlessness, panting, shallow and labored breathing, pulmonary inflammation, edema, and vomiting which progressed to respiratory distress, collapse, and death. Daily administration of 2X and 3X the recommended dose for 14 days caused renal damage in healthy dogs.

In Case of Overdosage:
BAL in Oil Ampules (Dimercaprol Injection, USP) [Akorn, San Clemente, California, at 1-800-223-9851] is reported in the literature to be an antidote for arsenic toxicity and was shown in one study to reduce the signs of toxicity associated with over-dosage of IMMITICIDE. The efficacy of IMMITICIDE may be reduced with co-administration of BAL.

ADVERSE REACTIONS (SIDE EFFECTS)
In clinical field trials, the most common reactions seen in dogs treated with IMMITICIDE were coughing/gagging, depression/lethargy, anorexia/inappetence, fever, lung congestion, and vomiting. Hypersalivation and panting occurred more rarely, however, these signs may occur within 30 minutes of injection and may be severe. Significant irritation was also observed at the intramuscular injection sites, accompanied by pain, swelling, tenderness, and reluctance to move. Generally, injection site reactions were mild to moderate in severity and recovery occurred in 1 week to 1 month; however, firm nodules can persist indefinitely. Avoid superficial or subcutaneous injection and leakage. Heartworm disease may cause death in dogs with or without treatment, especially in the Class 3 dogs.

Post Approval Experience: There have also been rare reports of paresis and paralysis in dogs following administration of IMMITICIDE.

The information provided here is not comprehensive. The full FDA-approved product insert is available at http://www.merial.us/SiteCollectionDocuments/Immiticide_PI_8.5x11_version.pdf. Consult your veterinarian for further information. For technical assistance, to request a Safety Data Sheet or to report suspected adverse events, call 1-888-637-4251. For additional information about adverse event reporting for animal drugs, contact FDA at 1-888-FDA-VETS, or http://www.fda.gov/AnimalVeterinary.

IMMITICIDE STERILE POWDER (MELAROSMINE DIHYDROCHLORIDE)

Brief Summary: Before Using IMMITICIDE, please consult the product insert, a summary of which follows.

CAUTION
Federal law restricts this drug to use by or on the order of a licensed veterinarian.

WARNING
IMMITICIDE should be administered by deep intramuscular injection in the lumbar (epaxial) muscles (between L3 - L5) only.

DO NOT USE IN ANY OTHER MUSCLE GROUP DO NOT USE INTRAVENOUSLY.

Care should be taken to avoid superficial injection or leakage. (See SAFETY).
That sweet SQUIRRELL STALKER might be a TAPEWORM TASTER
96% of *D. Caninum* infections were missed using passive flotation*

**INTERCEPTOR**
PLUS
(milbemycin oxime/praziquantel)

**SO PROTECT AGAINST:**
- **✓ Tapeworms**
- **✓ Whipworms**
- **✓ Heartworms**
- **✓ Roundworms**
- **✓ Hookworms**

**READY TO TAKE ON ALL 5?**

**INDICATIONS**
Interceptor Plus is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of adult roundworm (*Toxocara canis, Toxascaris leonina*), adult hookworm (*Ancylostoma caninum*), adult whipworm (*Trichuris vulpis*), and adult tapeworm (*Taenia pisiformis, Echinococcus multilocularis, Echinococcus granulosus* and *Dipylidium caninum*) infections in dogs and puppies 6 weeks of age or older and 2 pounds of body weight or greater.

**IMPORTANT SAFETY INFORMATION**
Treatment with fewer than 6 monthly doses after the last exposure to mosquitoes may not provide complete heartworm prevention. Prior to administration of Interceptor Plus, dogs should be tested for existing heartworm infections. The safety of Interceptor Plus has not been evaluated in dogs used for breeding or in lactating females. The following adverse reactions have been reported in dogs after administration of milbemycin oxime or praziquantel: vomiting, diarrhea, depression/lethargy, ataxia, anorexia, convulsions, weakness, and salivation. For full prescribing information see Interceptor Plus package insert.


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Caution
Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

Description
INTERCEPTOR PLUS is available in four strengths in color-coded packages for oral administration to dogs and puppies according to their weight. Each chewable flavored tablet is formulated to provide a minimum of 0.23 mg/pound (0.5 mg/kg) of milbemycin oxime and 2.28 mg/pound (5 mg/kg) of praziquantel. Milbemycin oxime consists of the oxime derivative of 5-dihydrothienylbenzimidazoles in the ratio of approximately A4 (C23H29NO7, MW 451.63) and 20% A3 (C21H27NO7, MW 541.68). Milbemycin oxime is classified as a macrocyclic anthelmintic. Praziquantel is an isooquinoline anthelmintic with the chemical name 2-(Cyclohexylcarbonyl)-1,3,4,6,7,11b-hexahydro-4-thyropyrazino[2,1-a]isoquinol-4-one.

Indications
INTERCEPTOR PLUS is indicated for the prevention of heartworm disease caused by Dirofilaria immitis (Vetericyn sarcopticum, Toxascaris canis, Toxascaris leonina), adult hookworm (Ancylostoma caninum), adult whipworm (Trichuris vulpis), and adult tapeworm (Taenia pisiformis, Echinococcus multilocularis, Echinococcus granulosus, and Dipylidium canum) infections in dogs and puppies (see EFFECTIVENESS).

Dosage and Administration
INTERCEPTOR PLUS should be administered orally, once every month, at the minimum dosage rate of 2.3 mg/pound (0.5 mg/kg) milbemycin oxime and 2.28 mg/pound (5 mg/kg) praziquantel. For heartworm prevention, give once monthly for at least 6 months after exposure to mosquitoes (see EFFECTIVENESS).

For adult hookworm, give 2.3 mg/pound (0.5 mg/kg) milbemycin oxime per day. For whipworm and tapeworm, the dosage is 1.14 mg/pound (2.28 mg/kg) per day. The tablets containing 2.3 mg milbemycin oxime/22.8 mg praziquantel or 5.75 mg milbemycin oxime/57 mg praziquantel are available in color coded packages of one chewable tablet each. For heartworm prevention, give once monthly for at least 6 months after exposure to mosquitoes (see EFFECTIVENESS).

8.1 to 25 lbs. 2.3 mg 22.8 mg One
2.3 to 5 lbs. 1.14 mg 11.4 mg One
50.1 to 100 lbs. 23 mg 228 mg One
5.1 to 25 lbs. 5.75 mg 57 mg One
25.1 to 50 lbs. 11.5 mg 114 mg One

Palatability
The tablets contain 2.3 mg milbemycin oxime/22.8 mg praziquantel or 5.75 mg milbemycin oxime/57 mg praziquantel. The tablets containing 2.3 mg milbemycin oxime/22.8 mg praziquantel or 5.75 mg milbemycin oxime/57 mg praziquantel are also available in color coded packages of one chewable tablet each. Each strength is available in color-coded packages of six or twelve chewable tablets each.

Body Weight
2 to 8 lbs. 2.3 mg 22.8 mg One
8.1 to 25 lbs. 5.75 mg 57 mg One
25.1 to 50 lbs. 11.5 mg 114 mg One
50.1 to 100 lbs. 23 mg 228 mg One

Information for Owner or Person Treating Animal:
Echinococcus multilocularis and Echinococcus granulosus are tapeworms found in wild canids and domestic dogs. E. multilocularis and E. granulosus can infect humans and cause serious disease (alveolar hydatid disease and hydatid disease, respectively). Owners of dogs living in areas where E. multilocularis or E. granulosus are endemic should be informed how to minimize their risk of exposure to these parasites, as well as the dog’s risk of exposure. Although INTERCEPTOR PLUS (milbemycine oxime/praziquantel) was 100% effective in laboratory studies in dogs against E. multilocularis and E. granulosus, no studies have been conducted to show that the use of this product will decrease the incidence of alveolar hydatid disease or hydatid disease in humans. Because the present period for E. multilocularis may be as short as 26 days, dogs treated at the labeled monthly intervals may become reinfected and shed eggs between treatments.

Effectiveness
Heartworm Prevention:
In a well-controlled laboratory study, INTERCEPTOR PLUS was 100% effective against induced heartworm infections when administered once monthly for 6 consecutive months. In well-controlled laboratory studies, neither one dose nor two consecutive doses of INTERCEPTOR PLUS provided 100% effectiveness against induced heartworm infections. Intestinal Nematodes and Cestodes Treatment and Control:
Elimination of the adult stage of hookworm (Ancylostoma caninum), roundworm (Toxascaris canis, Toxascaris leonina), adult hookworm (Ancylostoma caninum), adult whipworm (Trichuris vulpis), and adult tapeworm (Taenia pisiformis, Echinococcus multilocularis, Echinococcus granulosus, and Dipylidium canum) infections in dogs was demonstrated in well-controlled laboratory studies.

Animal Safety
INTERCEPTOR PLUS:
In a repeated dose safety study, 40 ten-week-old puppies (10 per group) were dosed with either a sham dose (0X) or 1, 3, or 5X the maximum label exposure of INTERCEPTOR PLUS every 14 days for a total of seven treatments. Ataxia, lethargy, and salivation were seen in the 3X and 5X treated dogs following each of the seven doses. Vomiting was seen in all treatment groups but had a higher incidence in the 3X and 5X treatment groups.

In a repeated dose safety study, 64 six-week-old puppies (16 per group) were dosed with either a sham dose (0X) or 1, 3, or 5X the maximum label exposure of INTERCEPTOR PLUS every 14 days for a total of four treatments. Lethargy was observed in all groups. Ataxia was observed in the three treated groups, including one dog in the 3X treated group. For both lethargy and ataxia the incidence and duration increased in the 3X and 5X groups. These signs were observed during the first 24 hours following treatment. Salivation and tremors were observed in the 3X and 5X treated dogs beginning immediately after dosing and up to six hours post dose. Vomiting was only observed in the 5X treatment group on most, but not all, treatment days.

For INTERCEPTOR PLUS the maximum exposure based on product dosing is 2.5 mg/kg for milbemycin oxime and 25.1 mg/kg for praziquantel, which is higher than the minimum effective dose used in the safety studies for milbemycin oxime (see below).

Milbemycin Oxime:
Two studies were conducted in heartworm-infected dogs treated with milbemycin oxime. Mild, transient hypersensitivity reactions were observed in dogs with high microfilarial counts (see PRECAUTIONS).

Safety studies in pregnant dogs demonstrated that doses of 0.8X the maximum exposure dose of INTERCEPTOR PLUS (1.5 mg/kg of milbemycin oxime), administered daily from mating through weaning, resulted in measurable concentrations of milbemycin oxime in milk. Puppies nursing these females demonstrated milbemycin oxime-related effects (depression, decreased activity, diarrhea, dehydration, nasal discharge). A subsequent study, which evaluated the daily administration of 0.6X the maximum exposure dose of INTERCEPTOR PLUS, from mating until one week before weaning, demonstrated no effects on the pregnant females or their litters. A study, in which pregnant females were dosed once, at 0.6X the maximum exposure dose of INTERCEPTOR PLUS before, on the day of, or shortly after whelping, resulted in no effects on the puppies.

Some nursing puppies, at 2, 4, and 6 weeks of age, administered oral doses of 9.6 mg/kg milbemycin oxime (0.1X the maximum exposure dose of INTERCEPTOR PLUS) exhibited tremors, vocalization, and ataxia. These effects were all transient and puppies returned to normal within 24 to 48 hours. No effects were observed in puppies administered 0.5 mg/kg milbemycin oxime (maximum label dose). A rising-dose safety study conducted in rough-coated Collies resulted in ataxia, pyrexia, and periodic recumbency in one of fourteen dogs administered milbemycin oxime at 12.5 mg/kg (5X the maximum exposure dose of INTERCEPTOR PLUS). Prior to receiving the 12.5 mg/kg dose on day 56 of the study, all animals had undergone a dosing regimen consisting of 2.5 mg/kg milbemycin oxime on day 0, followed by 5.0 mg/kg on day 14, and 10.0 mg/kg on day 32. No adverse reactions were observed in any of the Collies treated with doses less than 12.5 mg/kg.

Storage Information
Store at room temperature, between 59° and 77°F (15-25°C).

How Supplied
INTERCEPTOR PLUS is available in four strengths, formulated according to the weight of the dog. Each strength is available in color coded packages of six or twelve chewable tablets each. The tablets containing 2.3 mg milbemycin oxime/22.8 mg praziquantel or 5.75 mg milbemycin oxime/57 mg praziquantel are also available in color coded packages of one chewable tablet each. Manufactured for: Elanco US Inc.
Greenfield, IN 46140, USA
Product of France
Approved by FDA under NADA # 141-338

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Revision date: October 2019

PA10228X
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Clinvet is a Contract Research Organization specializing in efficacy, safety and metabolism studies on veterinary medicines.

Studies are conducted under strict quality management systems ensuring compliance to the relevant quality guidelines (GCP/GLP) at our sites in South Africa, USA and Morocco.

With more than 20 years’ experience we are considered the world’s leading Animal Health CRO.

**PRODUCTION ANIMALS**

Clinvet sites can conduct research on a wide range of parasitic and infectious diseases commonly found in bovine, ovine, caprine, swine and poultry hosts. Research on these diseases can be conducted in the field or in containment facilities in Africa. Depending on the biosafety requirements for the specific pathogen, animals can be housed, in up to ABSL2+ facilities.

**RODENTICIDES, PESTICIDES AND REPELLENCY RESEARCH**

Studies are conducted according to appropriate guidelines (e.g. EU TNsG for PT 18 and PT 19; DAFF Guidelines for the Registration of Household Agricultural Remedies; WHO Guidelines; EPA OTTP Guidelines and OECD Environmental Directorate Guidelines) or any model can be developed on request.

**IN VITRO & IN VIVO SCREENING**

In vitro assays include contact and repellency bioassays, as well as artificial feeding models and systems. The former two use direct exposure of the parasites to an active ingredient, either in solution or on filter paper, glass or other materials. In artificial feeding assays, systemic compounds can be tested by allowing ectoparasites to feed on treated blood or another media using an artificial membrane or by allowing endoparasites to move through a medium and assessing behaviour.

**COMPANION ANIMALS**

Various challenge models are available to conduct studies at our facilities in Africa or the USA. Depending on the biosafety requirements, animals can be housed, in up to ABSL2+ facilities.

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- Field studies - Species covered: Cattle, pigs, poultry, sheep and goats

Standards
- AAALAC accredited BSL -2 facilities
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For further information, contact us at kelly@mvsinc.net
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East Tennessee Clinical Research, Inc. is a proud sponsor of the American Association of Veterinary Parasitologists (AAVP) annual meeting. ETCR is a veterinary contract research organization that specializes in GCP and GLP studies with horses, livestock, and companion animal species. Since 1997, clinical research conducted by ETCR has contributed to the approval of numerous NADAs and ANADAs.

ETCR offers unique expertise, excellent facilities, and dedicated, trained personnel to meet the challenges of clinical development and regulatory compliance for the pharmaceutical and biopharmaceutical industry.

To learn more about East Tennessee Clinical Research, Inc., please visit our website at www.easttenncr.com
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker(s)</th>
<th>Institution(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:00-16:00</td>
<td>AAVP Executive Committee Meeting</td>
<td>All AAVP officers and committee chairs please plan to attend</td>
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<tr>
<td>12:00-12:15</td>
<td>Opening remarks</td>
<td>AAVP President: Mason V. Reichard</td>
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<tr>
<td>12:15-15:10</td>
<td>PLENARY SESSION and Bayer Symposium</td>
<td>Moderator: Doug Carithers</td>
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<tr>
<td>12:15-13:00</td>
<td>1st Plenary Presentation</td>
<td>Ray Kaplan</td>
<td>University of Georgia</td>
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<tr>
<td>13:00-13:10</td>
<td>Question Period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:15-14:00</td>
<td>Bayer Sponsored Presentation</td>
<td>Susan Little, Andrew Peregrine, Ian Wright</td>
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<tr>
<td>14:00-14:10</td>
<td>Question Period</td>
<td></td>
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<tr>
<td>14:15-14:50</td>
<td>2nd Plenary Presentation</td>
<td>John Gillearder</td>
<td>University of Calgary</td>
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<td>15:00-15:10</td>
<td>Question Period</td>
<td></td>
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<tr>
<td>15:10-15:40</td>
<td>½ Hour BREAK</td>
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<tr>
<td>15:40-17:10</td>
<td>OGS and Zoetis Presentation</td>
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<td>15:40-16:10</td>
<td>2020 AAVP-Merck Animal Health Outstanding Graduate Student presentation:</td>
<td>Lindsay Starkey</td>
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<tr>
<td>16:15-17:00</td>
<td>Zoetis Sponsor Presentation</td>
<td>Maximizing moxidectin in a worsening heartworm disease landscape.</td>
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<tr>
<td>17:00-17:10</td>
<td>Question Period</td>
<td></td>
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<tr>
<td>17:10-17:15</td>
<td>Wrap up for the Day</td>
<td>Doug Carithers</td>
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</tbody>
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| 12:00-12:10 | Opening remarks  
President-Elect and Program Chair: Doug Carithers |
| 12:00-14:10 | DVP and Boehringer Ingelheim Sponsored Presentation |
| 12:10-13:10 | 2020 AAVP Boehringer Ingelheim Distinguished Veterinary Parasitologist  
Moderator: Doug Carithers  
Awarded to Sharon Patton  
Emeritus Professor, University of Tennessee |
| 13:10-13:15 | 5 Minute Stretch Period |
| 13:15-14:00 | Boehringer Ingelheim Sponsored Presentation:  
5. Risks Beyond Parasites: Zoonoses, drug resistance, telehealth tools and you. |
| 14:00-14:10 | Question Period |
| 14:10-14:40 | ½ Hour BREAK |
| 14:40-17:10 | AAVP Business Meeting and Elanco Sponsored Presentation |
| 14:40-15:40 | AAVP Business Meeting  
Moderator: Mason Reichard, President  
All members please plan to attend |
| 15:40-15:45 | 5 Minute Stretch Period |
| 15:45-16:30 | Elanco Sponsored Presentation:  
6. The DOG PARCS Study: Detection of Gastrointestinal Parasites at Recreational Canine Sites in the United States  
Susan Little  
Oklahoma State University |
| 16:30-16:40 | Question Period |
| 16:40-16:45 | Wrap up for the day  
Doug Carithers |
### Monday June 22, 2020

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
</table>
| 12:00-12:10 | Opening Remarks and Intro for Presidents Symposium:  
AAVP Past-President: Mason Reichard |
| 12:10-14:00 | **PRESIDENTS SYMPOSIUM**  
Moderator: Mason Reichard |
*Emily Jenkins*  
*University of Saskatoon*  
______________________________________  
Question Period |
| 12:55-13:05 | 5 Minute Stretch Period |
| 13:05-13:10 | 5 Minute Stretch Period |
| 13:10-13:55 | 8. *Clinical outcomes of ruminants and camelids with Parelaphostrongylus tenuis-associated cerebrospinal nematodiasis* presenting to two university teaching hospitals over a ten-year period  
*Grace VanHoy*  
*Ohio State University*  
______________________________________  
Question Period |
| 13:55-14:05 | Question Period |
| 14:05-14:35 | ½ Hour BREAK |
| 14:35-16:35 | **AAVP-NCVP Parasite Clicker-Session Case Discussions**  
Moderators: Dwight Bowman and Andrew Peregrine |
| 16:35-16:45 | **Wrap up for the day and Live Virtual Meeting Ends**  
Doug Carithers  
______________________________________  
Students: Please remember to join Grace and Kathryn via Zoom (invite sent by email) for the annual student meeting starting at 16:45! |

### Tuesday June 23, 2020

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
</table>
| All Day | **Student Judging**  
Awards Committee and Student Competition Participants |

Note: All other oral and poster presentations are accessible for viewing in your own time.
### ORAL SESSIONS

#### (Student Competition) Cestodes

9. Evaluation of the prevalence of *Echinococcus multilocularis* in dogs that visit off-leash dog parks in southern Ontario, Canada.
   - **Andrew Peregrine**  
   - Ontario Veterinary College

10. Alveolar echinococcosis in dogs in Western Canada.
    - **Temitope Kolopo**  
    - Western College of Vet Med

11. Canine peritoneal larval cestodiasis by Mesocestoides spp.: Two clinical cases.
    - **Silvia Carta**  
    - Universita’ degli Studi di Sassari, Italy

12. Retrospective investigation of *Echinococcus granulosus*: Emergence in translocated elk (*Cervus canadensis*) in Tennessee (USA) and examination of canid definitive hosts.
    - **BreeAnna Dell**  
    - University of Tennessee

13. Deep amplicon sequencing as a new tool to investigate the intraspecific diversity and the distribution of *Echinococcus multilocularis* in foxes and coyotes in western Canada.
    - **Maria Alejandra Santa**  
    - University of Calgary

#### Nematodes

#### Cattle Nematodes

    - **Candela Canton**  
    - National University of the Center of the Buenos Aires Province, Argentina

15. Seasonal epidemiology of major gastrointestinal nematodes in the northern semi-arid climatic zones of western Canada using the ITS-2 nemabiome barcoding approach.
    - **Tong Wang**  
    - University of Calgary

#### Dog/Cat Nematodes

16. Improving the accuracy of anthelmintic studies with certain nematode parasites of dogs and cats.
    - **Craig R. Reinemeyer**  
    - East Tennessee Clinical Research, Inc.

17. Efficacy and safety of a novel, oral chewable tablet containing sarolaner, moxidectin and pyrantel (Simparica Trio™) against immature and mature *Toxocara canis* in laboratory dogs and in veterinary patients in the USA.
    - **Susan Holzmer**  
    - Zoetis

18. Preventive efficacy of Bravecto Plus spot-on solution for cats (280 mg/ml fluralaner and 14 mg/ml moxidectin) against aelurostrongylosis in experimentally infected cats.
    - **Katharina Raue**  
    - University of Hannover, Germany

    - **Steven Maeder**  
    - Zoetis

20. Field efficacy and safety of a novel, orally administered combination product containing sarolaner, moxidectin and pyrantel (Simparica Trio™) for the prevention of heartworm disease (*Dirofilaria immitis*) in dogs presented as veterinary patients in Australia, Japan and the USA.
    - **Kristina Kryda**  
    - Zoetis
<table>
<thead>
<tr>
<th>(Student Competition)</th>
<th>Dog/Cat Nematodes (cont)</th>
</tr>
</thead>
</table>
| 21. Characterization of the prevalence of intestinal parasites in dogs in southern Ontario, Canada using sucrose double centrifugation, Fecal Dx® and Giardia antigen tests. | Andrew Peregrine  
Ontario Veterinary College |
| 22. Detection of heartworm antigen using heat treatment of serum without loss of specificity in dogs infected by gastrointestinal helminths and protozoa. | Jeff Gruntmeir  
University of Florida |
| 23. Characterizing microRNA populations of *Dirofilaria immitis* in vitro as potential diagnostic markers for early-detection of infection. | Gui Verocai  
Texas A&M University |
| 24. Evaluation of the effectiveness of fluralaner 280 mg/mL plus moxidectin 14 mg/mL spot-on solution for cats for prevention of *Dirofilaria immitis* infection (heartworm disease) in cats. | Byron Blagburn  
Auburn University |
Texas A&M University |
| 26. Comparison of development of JYD-27 and Missouri (MO) strains of *Dirofilaria immitis* in laboratory-reared *Aedes aegypti*. | Doyeon Park  
Auburn University |
| 27. Molecular and morphological characterization of *Thelazia californiensis* in dogs from New Mexico, USA. | Caroline Sobotyk  
Texas A&M University |
| 28. Microfilariae counts and antigen levels of macrocyclic lactone resistant *Dirofilaria immitis* during treatment with melarsomine dihydrochloride. | Rachel Beam  
Oklahoma State University |
| 29. Molecular detection of *Cercopithifilaria bainae* in brown dog ticks from across the southern United States. | Megan Lineberry  
Oklahoma State University |
| 30. Surveillance for pyrantel pamoate resistance in *Ancylostoma caninum* in north Florida shelter dogs. | Morgan Weldon  
University of Florida |
| 31. Comparative preventive efficacy of ProHeart® 12, Heartgard® Plus and Interceptor® Plus against a macrocyclic lactone-resistant strain (JYD-34) of heartworm (*Dirofilaria immitis*) in dogs. | Tom McTier  
Zoetis |
| 32. Efficacy evaluation of anthelmintic products against an infection with the canine hookworm (*Ancylostoma caninum*) isolate Worthy 4.1F3P in dogs. | Pablo Jimenez Castro  
University of Georgia |
(Student Competition)  Dog/Cat Nematodes (cont)

33. Retrospective analysis of canine endoparasites in 2019 with a focus on retired racing greyhounds.
   **Heather Yee**  
   Ohio State University

**Horse Nematodes**

34. Are we beating a dead horse? Modelling a failed anthelmintic in combination.
   **Jessica Scare Kenealy**  
   University of Kentucky

35. Equine parasite control protocols: An evaluation of health parameters.
   **Martin Nielsen**  
   University of Kentucky

36. Looking at the spectrum of cyathostomin disease through the lens of clinical cases.
   **Nicola Walshe**  
   University College Dublin, Ireland

37. Age, sex, and parasites: Is there an unusual predilection in foals?
   **Ashley Steuer**  
   University of Kentucky

   **Jennifer Bellaw**  
   University of Kentucky

39. Longitudinal survey of intestinal nematodes of Thoroughbred horses in Australia
   **Ghazanfar Abbas**  
   University of Melbourne, Australia

40. The local and systemic inflammatory response to anthelmintic therapy: Does killing encysted cyathostomins increase inflammation?
   **Ashley Steuer**  
   University of Kentucky

41. The microbiome of *Parascaris* spp.: A pilot study.
   **Jennifer Cain**  
   University of Kentucky

42. Multiple resistance in horses naturally infected by Cyathostomins in Mato Grosso do Sul, Brazil.
   **Mariana Green de Freitas**  
   University Federal of Mato Grosso do Sul, Brazil

**Nematodes (Probiotic and Fecal Sample Storage)**

43. Development of a paraprobiotic cure for gastrointestinal nematode parasites.
   **Raffi Aroian**  
   University of Massachusetts

44. Storage methods of cattle and horse faecal samples for egg-hatch test.
   **Mariana Green de Freitas**  
   University Federal of Mato Grosso do Sul, Brazil
<table>
<thead>
<tr>
<th>(Student Competition)</th>
<th>Swine Nematodes</th>
</tr>
</thead>
</table>
| 45. Microsatellite analysis – the useful tool to track transmission of *Trichinella* spp. | **Ewa Bilska-Zajac**  
National Veterinary Research Institute, Pulawy, Poland |
| 46. Complete mitochondrial genomes and ribosomal DNA sequences of *Trichinella spiralis* indicate that the split between Asian and European populations happened prior to the rise of agriculture. | **Peter Thompson**  
USDA |
| 47. *Ascaris suum* intestine: A new target site for cholinergic anthelmintic therapy. | **Mark McHugh**  
Iowa State University |

**Poultry Nematodes**

| 48. *Ascaridia* spp. of poultry provide an optimal model for evaluating the efficacy of anthelmintics and for studying the biology and genetics of anthelmintic resistance in ascarid nematodes. | **James Collins**  
University of Georgia |

**Small Ruminant Nematodes**

| 49. Effect of inactivated *Bacillus thuringiensis* with cytosolic Cry5B protein on *Haemonchus contortus* in experimentally infected sheep. | **John Sanders**  
Virginia-Maryland College of Veterinary Medicine |
| 50. Molecular characterization and immune-reactivity patterns of two novel *Haemonchus contortus* cathepsin Bs. | **Mariam Bakshi**  
USDA-Agricultural Research Service |
| 51. The use of deep amplicon sequencing in molecular diagnostics and molecular epidemiology of anthelmintic resistance. | **Camila Queiroz**  
University of Calgary |
| 52. Molecular epidemiological evidence for the spread benzimidazole resistance in the sheep parasitic nematode *Nematodirus battus* from a single source in NW England. | **Rebecca Chen**  
University of Calgary |
| 53. Evidence for wild cervids as transmission vectors of small ruminant drug resistant gastrointestinal nematode parasites. | **Libby Redman**  
University of Calgary |

**Wildlife Nematodes**

| 54. Detection of *Trichinella murrelli* and *T. pseudospiralis* in bobcats (*Lynx rufus*) from Oklahoma. | **Tiana Sanders**  
Oklahoma State University |
| 55. Phylogenetic relationships within the nematode subfamily Phascolostrongylinae (Nematoda; Strongyloidea) from Australian macropodid and vombatid marsupials. | **Tanapan Sukee**  
University of Melbourne, Australia |
<table>
<thead>
<tr>
<th>(Student Competition)</th>
<th>Education</th>
</tr>
</thead>
</table>
| 56. The future of Veterinary Parasitology in the classroom: Take-aways from the 2019 AAVP Educators Meeting. | Heather Walden  
University of Florida |
| 57. COVID 19-induced changes to teaching veterinary diagnostic parasitology. | Antoinette Marsh  
Ohio State University |

<table>
<thead>
<tr>
<th>Other (Fish and Reptiles)</th>
</tr>
</thead>
</table>
| 58. Fish Host Susceptibility Influences Myxozoan Community Composition In Proliferative Gill Disease Of Catfish Aquaculture. | Justin Stilwell  
University of Georgia |
University of Florida |

<table>
<thead>
<tr>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle Protozoa</td>
</tr>
</tbody>
</table>
| 60. Retrospective Analysis of Bovine *Eimeria* Cases Detected During 2010-19 at the Animal Health Diagnostic Center, New York State, USA. | Manigandan Lejeune  
Cornell University |
| 61. Arthropod surveillance and the detection of *Theileria orientalis* in host-seeking *Haemaphysalis longicornis* in Virginia, USA. | Alec Thompson  
University of Georgia |

<table>
<thead>
<tr>
<th>Dog/Cat Protozoa</th>
</tr>
</thead>
</table>
Istituto Superiore di Sanità, Italy |
| 63. Examining the relationship between dogs with overt clinical leishmaniasis, infectiousness to *Phlebotomus perniciosus* and its infectivity. | Manuela Gizzarelli  
University of Naples, Italy |

<table>
<thead>
<tr>
<th>Horse Protozoa</th>
</tr>
</thead>
</table>
| 64. Predicting surface antigen variation in *Sarcocystis neurona* isolates | Jamie Kaj Norris  
University of Kentucky |
| 65. Transplacental transmission of *Babesia caballi* from carrier mares to foals. | Yang Hu  
Heilongjiang Bayi Agricultural University, China |
### (Student Competition) Protozoa Environmental Survival

   **Perryn Kruth**  
   University of Guelph

### Poultry Protozoa

67. Back to basics: Genome replication dynamics in exogenous stages of *Eimeria tenella*.  
   **Taylor Lane**  
   University of Guelph

68. Who’s killing the Partridge Family? *Eimeria* species and clinical coccidiosis in commercial poultry.  
   **Jessica Rotolo**  
   University of Guelph

69. Macroscopic and microscopic lesions explain the pathogenicity of a ‘minor’ pathogen of turkeys, *Eimeria innocua*.  
   **Rachel Imai**  
   University of Guelph

### Wildlife Protozoa

70. *Toxoplasma gondii* prevalence and partial genotypes in North American river otters (*Lontra canadensis*) from the upper peninsula of Michigan.  
   **Stacey Cotey**  
   Michigan Technological University

71. Species diversity and geographic variation of piroplasms in striped skunks (*Mephitis mephitis*) and spotted skunks (*Spilogale* spp.) in the United States.  
   **Michael Yabsley**  
   University of Georgia

72. Prevalence of *Sarcocystis* spp. in North American river otters (*Lontra canadensis*) collected in Michigan.  
   **Ruth Scimeca**  
   Oklahoma State University

73. *Trypanosoma cruzi* infection in two meerkats (*Suricata suricatta*) at the Dallas Zoo.  
   **Brian Nguyen**  
   Oklahoma State University

74. *Enterocytozoon* sp. and *Ceratonova shasta* in the intestines of adult Chinook salmon.  
   **Michael Kent**  
   Oregon State University

75. Prevalence and partial genetic characterization of *Toxoplasma gondii* strains from 31 passerine species collected in north central Oklahoma.  
   **Alexis Hawton**  
   Oklahoma State University

### Ticks/Mites/Insects

#### Cattle Ticks/Mites/Insects

76. Genetic characterization and high-throughput screening of ticks and tick-borne pathogens infecting bovines in Pakistan.  
   **Abdul Ghafar**  
   University of Melbourne, Australia
<table>
<thead>
<tr>
<th>Student Competition</th>
<th>Dog/Cat</th>
<th>Ticks/Mites/Insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>77. Evaluation of a topical sarolaner-selamectin combination to control flea populations on naturally infested cats in private residences in West Central Florida.</td>
<td>Mike Dryden</td>
<td>Kansas State University</td>
</tr>
<tr>
<td>78. Laboratory and field efficacy of a novel, orally administered combination product containing sarolaner, moxidectin and pyrantel (Simparica Trio™) against experimentally and naturally acquired flea infestations in dogs.</td>
<td>Tammy Inskeep</td>
<td>Zoetis</td>
</tr>
<tr>
<td>79. Efficacy and speed of kill of a combination product containing sarolaner, moxidectin and pyrantel (Simparica Trio™) against five common tick species infesting dogs in the USA.</td>
<td>Sara Chapin</td>
<td>Zoetis</td>
</tr>
<tr>
<td>80. Seasonality of Ixodes species and stages infesting dogs and cats in the USA.</td>
<td>Parna Ghosh</td>
<td>Oklahoma State University</td>
</tr>
<tr>
<td>81. Geographic diversity of ticks collected from dogs and cats throughout the United States.</td>
<td>Meriam Saleh</td>
<td>Oklahoma State University</td>
</tr>
<tr>
<td>82. Has COVID-19 stay-at-home orders changed the risk of tick exposure for dogs?</td>
<td>Michael Yabsley</td>
<td>University of Georgia</td>
</tr>
<tr>
<td>83. Serologic evidence of select vector-borne pathogens in unowned dogs in the Southeastern United States.</td>
<td>Ali Perregrino</td>
<td>Auburn University</td>
</tr>
<tr>
<td>84. Do blacklegged ticks collected from domestic cats feed on feline blood and transmit Lyme disease.</td>
<td>Amira Ahmed</td>
<td>Cornell University</td>
</tr>
<tr>
<td>85. Diversity of Rickettsia spp. in Dermacentor spp. from across the United States.</td>
<td>Kathryn Duncan</td>
<td>Oklahoma State University</td>
</tr>
<tr>
<td>86. Sequential histologic comparisons of naïve and subsequent Amblyomma americanum bite lesions from induced infestations on dogs and cats.</td>
<td>Jennifer Thomas</td>
<td>Oklahoma State University</td>
</tr>
<tr>
<td><strong>Horse Ticks/Mites/Insects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87. Initiation of the National Equine Tick Survey as a novel method for tracking ticks on horses in the US.</td>
<td>Jana Gigliotti</td>
<td>Kansas State University</td>
</tr>
<tr>
<td><strong>Wildlife Ticks/Mites/Insects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>88. Studies on sarcoptic mange in black bears (Ursus americanus) in Pennsylvania.</td>
<td>Michael Yabsley</td>
<td>University of Georgia</td>
</tr>
</tbody>
</table>
## POSTER SESSIONS

### (Student Competition)  
**Acanthocephala**

89. *Oncicola canis* (Acanthocephala) in a rescued dog (Texas to Pennsylvania).  
**Thomas Nolan**  
University of Pennsylvania

### Cestodes

**Emilie Toews**  
University of Calgary

91. Polyomics of the neglected equine tapeworm *Anoplocephala perfoliata*.  
**Boontarikaan Wititkornkul**  
Aberystwyth University, Wales

### Nematodes

#### Cattle Nematodes

92. Gastrointestinal nematode fecal egg shedding intensity, prevalence, and predominant species in western Canadian cow-calf operations.  
**Eranga De Seram**  
University of Saskatchewan

93. Assessing the production impacts of gastrointestinal nematode parasites in stocker cattle in Western Canada.  
**Daniel Merchan**  
University of Saskatchewan

#### Dog/Cat Nematodes

94. Updates on serological diagnosis in heartworm infection in dogs.  
**Lavinia Ciucă**  
University of Naples, Italy

95. Evaluation of canine fecal samples using a *Toxocara* species-specific real-time PCR.  
**Todd Bezold**  
IDEXX

96. The difference of *Dirofilaria immitis* microfilaria concentrations in blood collected from the jugular and cephalic veins of dogs.  
**Elyssa Campbell**  
University of Georgia

97. The prevalence of *Ancylostoma* spp. in dogs in the Caribbean.  
**Jenny Kim**  
Ross University

98. P-glycoproteins are potential drugable targets in *Toxocara canis*.  
**Jeba Jesudoss Chelladurai**  
Iowa State University
**Horse Nematodes**

99. Analyst variability at the counting step for McMaster, Wisconsin, and automated equine fecal egg count methods.
   **Jennifer Cain**  
   University of Kentucky

100. Pooled fecal samples for diagnosis of *Strongylus vulgaris*.
    **Chelsea Facison**  
    Lincoln Memorial University

101. Spatial Variation of Cyathostomin Mucosal Larval Counts.
    **Avery Martin**  
    University of Kentucky

102. According to mitochondrial DNA evidence, *Triodontophorus* species belongs to the Cyathostominae.
    **Yuan Gao**  
    Heilongjiang Bayi Agricultural University, China

103. The prevalence of cyathostomin anthelmintic resistance on horse farms in Prince Edward Island, Canada.
    **Jaimie Butler**  
    University of Prince Edward Island

**Other (Molecular) Nematodes**

104. Using *C. elegans* to identify genes that affect ivermectin sensitivity of the filarial worm *B. malayi*.
    **Natalie Wilson**  
    University of Georgia

105. Parasitic nematode beta-tubulin alleles cause benzimidazole resistance and affect organismal fitness.
    **Clayton Dilks**  
    Northwestern University

**Small Ruminant Nematodes**

106. Carvone modulates *in vitro* and *in vivo* the kinetic behaviour and efficacy of abamectin.
    **Candela Canton**  
    UNICEN University, Argentina

107. Developing the nemabiome as an alternative to fecal egg counting: Absolute quantitation of parasitic nematode DNA in fecal samples.
    **Eleonore Charrier**  
    University of Calgary

108. Detection of levamisole resistance in *Haemonchus contortus* populations from the United States.
    **Jomar Patricio Monteiro**  
    Centro Universitário UNINTA, Brazil

109. 17 years of the FAMACHA© program in the United States.
    **Leonor Sicalo Gianechini**  
    University of Georgia
## Wildlife Nematodes

   **Araceli Lucio-Forster**  
   Cornell University

## Cattle Protozoa

11. Compounds with *in vitro* activity against *Trichomonas foetus*.  
   **Katy A. Martin**  
   Iowa State University

12. Long read metabarcoding of bovine *Eimeria, Neobalantidium* and *Buxtonella* communities.  
   **Venkateswara Rao Parimisetti**  
   University of Calgary

   **Hao-Xian Wang**  
   Heilongjiang Bayi Agricultural University, China

## Horse Protozoa

14. A molecular comparison of North American and South American *Klossiella equi* samples: One species spans the Americas?  
   **Elizabeth Zeldenrust**  
   University of Guelph

## Other Protozoa

15. An *in vitro* evaluation of ozonated water on cyst viability of the protozoon *Giardia duodenalis*.  
   **Maria Elena Morgoglione**  
   University of Naples, Italy

   **Perryn Kruth**  
   University of Guelph

## Wildlife Protozoa

17. Molecular and morphological characterization of an undescribed *Isospora* species infecting the introduced European starling (*Sturnus vulgaris*) in Ontario, Canada.  
   **Evelin Rejman**  
   University of Guelph
<table>
<thead>
<tr>
<th>(Student Competition)</th>
<th>Dog/Cat</th>
<th>Ticks/Mites/Insects</th>
</tr>
</thead>
</table>
| 118. Acaricidal efficacy of dinotefuran-pyriproxyfen-permethrin (Vectra® 3D)-treated dog hair against adult *Ixodes scapularis* and *Ixodes ricinus* ticks using an *in vitro* feeding assay. |  | Djamel Tahir  
Clinvet Morocco |
| 119. Seasonality of questing ticks in Claiborne County, TN: understudied risks associated with Ixodid ticks in rural Appalachia. |  | Barbara Shock  
Lincoln Memorial University |
| 120. Flea-Borne Bacterial Pathogens in Fleas and Tissues from Free Roaming Domestic Cats. |  | Charlotte Manvell  
North Carolina State University |
| **Horse** Ticks/Mites/Insects |  |  |
| 121. Equine tick infestation in fall and winter in northeastern Oklahoma: Diversity, seasonality, and attachment site preferences. |  | Kellee Sundstrom  
Oklahoma State University |
| **Wildlife - Ticks/Mites/Insects** |  |  |
| 122. Tracking an invader: Wildlife surveillance for *Haemaphysalis longicornis* in the Eastern U.S. |  | Alec Thompson  
University of Georgia |
| 123. Identification and molecular analysis of Ixodid ticks (Acari: Ixodidae) infesting wild boars (*Sus scrofa*) and tick-borne pathogens at the Meihua mountain of southwestern Fujian, China. |  | Xin Wang  
Longyan University, China |
| **TREMATODES** |  |  |
| 124. Proteomic analysis of *Clonorchis sinensis* ESPs interacting with serum of different periods by shotgun LC-MS/MS. |  | Xiao-Xiao Ma  
Heilongjiang Bayi Agricultural University, China |
Heilongjiang Bayi Agricultural University, China |
| 126. Complete mitochondrial genome of *Prosthogonimus cuneatus* (Trematoda: Prosthogonimidae), as the first representative from the Superfamily Microphalloidea. |  | Xinru Guo  
Heilongjiang Bayi Agricultural University, China |
| 127. Repurposing oxfendazole as a potential flukicidal compound. |  | Candela Canton  
UNICEN University, Argentina |
2020 ORAL ABSTRACTS

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Exploring the factors involved with the emergence of multiple anthelmintic resistance in *Ancylostoma caninum*: why in greyhounds and what is the risk to pet dogs?

Ray Kaplan. University of Georgia, College of Veterinary Medicine

The development of anthelmintic resistance (AR) is a dynamic evolutionary process that involves a complex interaction of worm biology/genetics, host-parasite interactions, drug pharmacodynamics/pharmacokinetics, epidemiology of transmission, and treatment strategies used. Consequently, this dynamic process differs for each parasite and host species and for each drug class. Additionally, different sets of factors are involved with regard to the emergence of AR worms in a given suprapopulation, as compared to the increase and spread of resistant worms across different suprapopulations (i.e. from one farm or household to another). AR in gastrointestinal nematode (GIN) parasites of livestock was first reported >50 years ago and has been worsening for decades. In every case, AR is first reported for single drugs (or drug classes), increases in geographic diversity over time, and eventually leads to worms with multiple-drug resistance (MDR). AR in the canine hookworm, *Ancylostoma caninum* was first reported to pyrantel in a greyhound puppy imported from Australia in 1987. Several additional cases of resistance to pyrantel were subsequently diagnosed in Australia; however, subsequent to 2008 there were no further cases of AR reported in *A. caninum* anywhere in the world to any drug until 2019, when MDR to all three major anthelmintic classes was documented. Greyhounds are highly over-represented in these MDR cases, and evidence strongly suggests that greyhound breeding farms and training kennels are the original source of these MDR hookworms. In this talk, the following topics will be addressed: (1) the biological, genetic and epidemiological factors likely responsible for the emergence of MDR *A. caninum*, (2) why this problem seemed to appear rather suddenly, and why in greyhounds, (3) the role of monthly anthelmintic prophylaxis in the emergence and spread of AR, and (4) the likelihood of MDR hookworms becoming a national/international problem, and how this impacts recommendations for monthly anthelmintic prophylaxis.

Bayer Sponsored Presentation

CVBD: Parasite and vector-borne disease concerns in rehomed dogs

Susan Little*1, Frans Jongejan2, Mary Marcondes3, Andrew Peregrine*4, Ian Wright*5.

1Oklahoma State University, 2University of Pretoria, 3São Paulo State University, 4University of Guelph, 5Mount Veterinary Practice

The Companion Vector-Borne Diseases (CVBD) World Forum is a working group of leading international experts who meet annually to evaluate current scientific findings and future trends concerning the distribution, pathogenesis, clinical presentation, diagnosis and prevention of vector-borne infections of dogs and cats. At the 14th Symposium of the CVBD World Forum in Trieste, Italy (March 25-28, 2019), we identified the need to (a) bring attention to the potential spread of parasites and vectors with relocated dogs, and (b) provide advice to the veterinary
profession regarding the importance of surveillance and treatment for parasites and vector-borne infections when rehoming dogs. Animal welfare crises driven by economic, cultural, and environmental factors are resulting in global relocation of domestic dogs which is associated with dissemination of parasites and vectors, creating far-reaching veterinary and public health concerns. This presentation will share a draft consensus statement generated by a working group from the CVBD World Forum as well as a summary of the problem faced, including the role of veterinary professionals in parasite surveillance, causal issues, and the importance of interdisciplinary cooperation in addressing the problem. To limit opportunities for dissemination of parasites and vectors, whenever possible, underlying problems creating the need for dog rehoming should be addressed. However, when it is necessary to rehome dogs, this should ideally take place in the country and national region of origin. When geographically distant relocation occurs, veterinary professionals have a vital role to play in public education, vigilance for detection of exotic vectors and infections, and alerting the medical community to the risk(s) for pathogen spread. This includes the implementation of appropriate diagnostic tests and parasite preventive measures, ideally before relocation, where indicated. With appropriate veterinary intervention, dog welfare needs can be met without inadvertently allowing unfettered global spread of parasites, vectors, and vector-borne diseases.

Plenary

3

Improving and using molecular diagnostics for more targeted, effective and sustainable parasitic helminth control

John Gilleard. University of Calgary

Diagnostic techniques are still underutilized for directing the treatment and control of parasitic nematodes, particularly when compared to many viral, bacterial and protozoal pathogens. Instead, broad spectrum anthelmintic treatments are often used by default with insufficient assessment of need, benefit or effectiveness. This is partly due to the perceived high efficacy, and relatively low cost, of many anthelmintic drugs combined with the limitations of current diagnostic tests which are often insufficiently sensitive, quantitative, rapid and/or cost effective. However, this “treat first and ask questions later” culture is becoming increasingly unsustainable due to anthelmintic drug resistance as well as changes in regulatory and societal attitudes. There have been major developments in genomics and molecular diagnostics over the last decade that are revolutionising the management of many infectious diseases. However, these have yet to be widely adopted in helminth diagnostics. In this talk, I will focus on ruminant gastrointestinal nematode parasite control and discuss the need to change from a “treatment first” to a “diagnosis first” culture that embraces the new genomic and diagnostic technologies. I will discuss the barriers to the practical use of molecular genetic diagnostic tests, some of the research gaps that need to be bridged and the criteria for an “ideal” molecular diagnostic test. I will make the case that molecular diagnostic tests, even if they fall short of the “ideal,” can provide significant advances and should be adopted sooner than later to enable more targeted, effective and sustainable helminth control.
Cyathostomins: Their interaction with the equine host and with my life
Ashley Steuer. M.H. Gluck Equine Research Center, University of Kentucky

How does a parasite interact with its host? How does the host interact with its parasite(s)? How does an anthelmintic alter this relationship? These are questions that have led me on this path of veterinary parasitology. With recent advances, we are beginning to understand the complexity of this interaction. This is poorly understood in our domestic equids, which have one of the most diverse parasite faunae of all domestic species. While recent advances in large animal parasitology have pursued evaluation of novel diagnostics and further enhancement of our understanding of anthelmintic resistance, little research has evaluated the host-parasite interaction at the cellular level. In this talk, I will discuss the evaluation of the local and systemic immunologic response in horses following anthelmintic treatment of different drug classes and nonlarvicidal versus larvicidal products and these potential implications. I will also discuss the impact of veterinary parasitology, cyathostomins, and mentors have had on my journey along the way to unraveling this complex interaction, and its future directions.

Boehringer Ingelheim Sponsored Presentation

Risks Beyond Parasites: zoonoses, drug resistance, telehealth tools and you
Sarah Babcock. Animal & Veterinary Legal Services, PLLC

Recent events highlight the important role veterinarians serve as leaders on animals and public health. Society looks to veterinarians as experts on the prevention and response to zoonotic diseases. In order to be effective in this role, it is important to ensure that veterinarians are educated on zoonoses, the role veterinarian's play in preventing and treating zoonotic diseases, and the potential legal liabilities and ethical duties that may result based on these roles and responsibilities. Concurrently, the need for veterinarians to provide expertise remotely requires an analysis of the existing regulatory framework applicable to telehealth. During this time of social distancing, the use of telehealth tools in veterinary medicine is not only permitted but encouraged to prevent the spread of illness and conserve protective equipment. However, the veterinarian is required to use professional judgment to determine what is medically appropriate and maintain compliance with jurisdictional requirements. Specifically, veterinarians are responsible for ensuring there is an appropriate balance between enabling access to veterinary care while ensuring patient safety. Providing veterinarians with a stronger foundation in their applicable legal and ethical duties will help shift the veterinary mindset from fearing potential legal repercussions to embracing the opportunity to serve at the forefront of public health.
Elanco Sponsored Presentation

The DOG PARCS study: Detection of Gastrointestinal Parasitism at Recreational Canine Sites in the United States
Kristina Stafford¹, Todd Kollasch¹, Kathryn Duncan², Stephanie Horr³, Christine Heinz-Loomer¹, Troy Goddu³, Anthony Rumschlag¹, William Ryan⁴, Sarah Sweet³, Susan Little*.¹¹Elanco Animal Health, ²Oklahoma State University, ³IDEXX Laboratories, Inc., ⁴Ryan Mitchell Associates LLC

The rapid growth in off-leash dog parks provides opportunity for canine socialization activities but carries risk of exposure to infections, including parasites. To assess the prevalence of intestinal parasites in dogs visiting dog parks across the United States, fecal samples, signalment, brief history, and owner-reported use of heartworm/intestinal parasite control medication (HWCM) were collected from 3,006 dogs (87.9% ≥ 1 year of age) at 288 dog parks in 30 metropolitan areas in July and August, 2019. Fecal samples were tested by zinc sulfate centrifugal flotation (CF) and coproantigen immunoassay (CAI; Fecal Dx and Giardia Antigen Panel, IDEXX Laboratories, Inc.). Parasites were identified in 622/3,006 (20.7%) dogs, and nematodes were found in 263 (8.8%), with hookworm (7.1%), whipworm (1.9%), and ascarids (0.6%) the most common nematodes identified. Dogs with intestinal parasites were present at 245/288 (85.1%) parks, and dogs with nematodes at 143 (49.7%). Combined, CAI and CF detected 78.4% more intestinal nematode infections than CF alone. Hookworm and whipworm infections were detected in dogs in all age groups, but ascarids were only detected in dogs less than 4 years. Based on owner reports, 2,069/3,006 (68.8%) dogs currently received a HWCM. Dogs previously diagnosed with intestinal parasitism were more likely to be receiving a HWCM, and a significantly lower proportion of dogs receiving a HWCM were positive for intestinal nematodes (P < 0.001). Intestinal parasites, the most common of which were Giardia, Ancylostoma caninum, and Trichuris vulpis, were found in 1 in 5 dogs and more than 85% of dog parks across the United States. Optimal detection of canine intestinal parasitism was achieved by combining CF and CAI. Regular testing of canine fecal samples and routine administration of medications effective against the most common infections remain important components of canine wellness care.

President’s Symposium

Echinococcus multilocularis in North America: new threats and new tools
Emily Jenkins. University of Saskatchewan

Echinococcus multilocularis is a zoonotic taeniid cestode long established in arctic and prairie regions of North America, for which wild rodents serve as the usual intermediate host. Historically, outside of Alaska, most human cases of alveolar echinococcosis (AE) caused by E. multilocularis were thought to be acquired outside North America. Wild canids and domestic dogs serve as definitive hosts for the parasite, and a potential source of human exposure through shedding of immediately infective, environmentally resistant eggs in feces. As definitive hosts, dogs show little or no clinical signs, and diagnosis based on traditional fecal flotation is
notoriously insensitive. Increasingly, dogs in North America are also serving as aberrant intermediate hosts for *E. multilocularis*, and there is new evidence for local transmission to people in North America. Recent human and canine cases of AE in Canada and the USA, detection in new geographic locations, and molecular epidemiological evidence suggest that this parasite is emerging in North America and may be introduced into and translocated within North America through the largely unregulated movements of domestic dogs. It is important that both veterinary and human health professionals have this unusual, but potentially fatal, parasitic disease on their list of differential diagnoses. Definitive diagnosis will be greatly facilitated by recent advances in molecular tests for both AE and intestinal infections with *E. multilocularis* in dogs, and serology for AE in dogs. Finally, control of this serious zoonosis has traditionally relied on cestocidal treatment of dogs, but this will not prevent dogs developing AE, nor does it address human exposure directly from wild canids. Ultimately, management of *E. multilocularis* requires a One Health approach to address the ecological, anthropogenic, social, and environmental factors driving this wildlife reservoird disease, with spillover into pets and people.

Clinical outcomes of ruminants and camelids with *Parelaphostrongylus tenuis*-associated cerebrospinal nematodiasis presenting to two university teaching hospitals over a ten year period
Grace VanHoy*, Evelyn MacKay, Jeffrey Lakritz. ¹Ohio State University College of Veterinary Medicine, ²Texas A&M University Veterinary Medicine and Biomedical Sciences

*Parelaphostrongylus* species are protostrongylid nematodes causing rare clinical signs in white-tailed deer (*Odocoileus virginianus*), but severe clinical manifestations in aberrant hosts. After ingestion of the infected intermediate host, larvae are associated with cerebrospinal migration with the infected aberrant host showing proprioceptive (spinal) ataxia, varying degrees of paresis, and lateralizing cranial nerve dysfunction. “Meningeal worm” is an important differential for neurologic disease in large animals. Few large studies exist evaluating clinical outcomes and treatment strategies. Medical records from Ohio State University (OSU) and Texas A&M (TAMU) from 2009-2019 were reviewed. Postmortem morphology and cerebrospinal eosinophilia were used to diagnose *Parelaphostrongylus spp*. Records from 87 cases were evaluated for clinical and therapeutic variables. Ohio contained a greater case number, but over the 10 year period, case diagnosis increased at TAMU. The monthly presentation was highest from January-March, with a spike in August for Ohio. Average duration of clinical signs before presentation was 9.5 days with 76% presenting with spinal ataxia and 16% with signs of intracranial migration. 13% significantly improved following lumbar puncture. Overall, 49% of cases improved neurologically, whereas 35% were euthanized, 7% died, and 9% were treated as outpatients. Physical therapy resulted in a higher survival rate (69%). Survival for patients with intracranial migration was limited. Cerebrospinal eosinophilia was similar between cases that presented immediately (<24 hours) and those that presented >10 days after the onset of clinical signs. All cases received NSAID drugs, and the use of corticosteroids was not significantly associated with greater mortality. Only 10% of cases did not receive an anthelmintic. Histopathological lesion locations varied. Intraloesional nematodes were only detected in 26% of necropsied patients. Study analysis included incidence over time, presentation month and deer census data by county.
Cestodes

9

Evaluation of the prevalence of *Echinococcus multilocularis* in dogs that visit off-leash dog parks in southern Ontario, Canada

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Prior to 2012, *Echinococcus multilocularis* had not been diagnosed in Ontario. However, since that year, five cases of alveolar echinococcosis have been diagnosed in dogs that resided at the western end of Lake Ontario. Furthermore, *E. multilocularis* has been shown to be a common infection in wild canids across southern Ontario with a high-risk infection cluster in the area surrounding the western shores of Lake Ontario and northern shores of Lake Erie. In regions endemic for *E. multilocularis*, dog ownership is considered a risk factor for human alveolar echinococcosis. A study was therefore carried out to determine the prevalence of *E. multilocularis* intestinal infections in dogs within the high-risk infection cluster. From May to November 2018, fecal samples were collected from 477 dogs aged ≥6 months that visited 12 off-leash dog parks in the Halton, Hamilton, and Niagara public health units in southern Ontario. Fecal samples were analyzed via a semi-automated magnetic capture-probe DNA extraction and real-time PCR method for *E. multilocularis* DNA. Overall, 0% (95% CI: 0-0.96%) of samples tested positive. This result informs preventive recommendations for *E. multilocularis* infections in dogs in this region and will be discussed.

10

Alveolar echinococcosis in dogs in Western Canada

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Alveolar echinococcosis (AE) is caused by the larval stage of the cestode *Echinococcus multilocularis*. Wild and domestic canine definitive hosts harbour the adult parasite in their intestine and shed eggs in feces. When the eggs are ingested by the rodent natural intermediate host, the larval stage develops in the liver causing AE. Following ingestion of eggs of *E. multilocularis*, humans can develop AE, a debilitating condition. Rarely, dogs can also develop AE; the index case in North America was reported in 2009. Here, we review cases of canine AE reported over the last decade in Western Canada [British Columbia, Alberta (AB), Saskatchewan (SK), and Manitoba]. Cases were identified by contacting provincial, academic, and veterinary diagnostic laboratories, and through word of mouth. A standard set of questions on case history and presentation was administered to pathologists and veterinarians. Overall, 26 dogs of different breeds fit into the inclusion criteria for the study with no age (median 4 years, range 1-12) or sex (14 females, 12 males) predilection; almost all were from AB and SK. Clinical signs at presentation were non-specific; abdominal distension was the most common complaint and...
medical imaging commonly suggested neoplasia as the most likely initial diagnosis. On histopathology, protoscolices were observed in <40% of dogs. Definitive diagnosis was considered molecular identification using primers specific for the NAD1 region of mitochondrial DNA of *E. multilocularis*. Further characterization of NAD2 sequences from several canine AE cases were most similar to those circulating in coyotes in western Canada and in red fox in central Europe. This study provides important information for veterinary practitioners and diagnosticians on a rare, but serious disease, and suggests that dogs with AE may serve as indicators of range expansion of the parasite and sentinels of risk to humans in western Canada.

**Canine peritoneal larval cestodiasis by Mesocestoides spp.: two clinical cases**

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Introduction: *Mesocestoides* spp. are widespread cestode tapeworms whose first larval stage develops in coprophagous arthropods and the second in a wide variety of vertebrates. Dogs and cats could serve as both final and intermediate hosts and present a potentially life-threatening condition called “Canine Peritoneal Larval Cestodiasis” (CPLC). Cases presentation: Case 1: A 16-year-old, crossbreed, neutered, male dog was referred with a history of lethargy, anorexia, increased respiratory effort and progressive abdominal distension. The dog showed tachypnoea, fever and painful abdomen distension; haematological changes included non-regenerative anaemia, neutrophilia, hypoalbuminemia and hyperglobulinemia. Ultrasonography revealed ascites with several cystic structures. Morphological examination and molecular investigation (PCR) of abdominal effusion led to the identification of *Mesocestoides* spp.. Abdominal lavage and a treatment with praziquantel (10 mg/kg SID for 4 days) were performed leading to a complete recovery. One year later the dog relapsed and a new treatment with antibiotics, analgesics and oral fenbendazole (50 mg/kg, twice a day for 28 days) was initiated but the patient died 20 days later. Case 2: A 11-year-old, mixed breed, female dog was referred with a history of reduced appetite, polydipsia, vomitus and tachypnoea. Physical examination revealed weight loss, tachycardia, tachypnoea and abdominal distension. Haematology, ultrasound and an exploratory laparotomy showed the presence of peritonitis. Examination of abdominal fluid led to the diagnosis of CPLC and was confirmed by morphological and biomolecular investigations. A treatment with fenbendazole at 100 mg/kg twice daily for 28 days was administered. A periodic treatment cycle of fenbendazole was kept for one year and the patient seems to have undergone a full recovery. Results and conclusions: Mesocestodiasis is an uncommon, potentially life threatening and probably underestimated disease. For these reasons, research efforts should be made regarding the identification of effective therapies against CLPC as well as the development of early diagnostics and therapeutics measures. Keywords: Mesocestoidosis, *Mesocestoides* spp., dogs, peritoneal cestodiasis.
Retrospective investigation of *Echinococcus granulosus* emergence in translocated elk (*Cervus canadensis*) in Tennessee (USA) and examination of canid definitive hosts

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Few reports of *Echinococcus granulosus* have been described in the United States; however, the geographical distribution of *Echinococcus* spp. in wild hosts is increasing consequent to human activities. We investigated the prevalence of *Echinococcus* spp. in re-established free-ranging elk (*Cervus canadensis*) populations in the North Cumberland Wildlife Management Area (NCWMA) via a retrospective analysis of banked elk tissues and examination of intestinal contents from 11 coyotes (*Canis latrans*) from the NCWMA. Four banked elk tissues and one freshly obtained elk demonstrated histologic evidence or gross evidence of *E. granulosus* infection. Four elk were PCR and Sanger sequencing positive for *Echinococcus canadensis*. Each sequence had 98% or greater coverage and identity to multiple *E. canadensis* genotypes in Genbank. Adult *Echinococcus* spp. were not detected in any of the coyotes examined in this study collected in the regions where *Echinococcus*-positive elk were reported. Continued surveillance of this parasite in both definitive and intermediate hosts in these areas is warranted to confirm the establishment of a sylvatic transmission cycle in a region with no previous reports of *E. granulosus* in wildlife. These data further underscore the risk of pathogen introduction secondary to wildlife translocation.

Deep amplicon sequencing as a new tool to investigate the intraspecific diversity and the distribution of *Echinococcus multilocularis* in foxes and coyotes in western Canada.

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*Echinococcus multilocularis* (*Em*) is a zoonotic parasite considered as a global emergent pathogen. Recent findings indicate that the parasite is expanding its range in North America (NA) and that the European (EU) strain is the most prevalent in Alberta (Canada). However, genetic analyses are usually conducted only on a few parasites out of thousands from each definitive host, likely underestimating the prevalence of less common haplotypes. Moreover, mixed infections with several haplotypes in the same host have been reported, but their relative abundance was never estimated. Using deep amplicon sequencing (ILLUMINA® technology), we aim to: i. estimate the frequency of co-infections of different *Em* haplotypes in western Canada and their relative abundance within hosts, ii. to detect less prevalent haplotypes by sampling a larger proportion of the parasite subpopulation per host, and iii. to investigate differences in the distribution of *Em* haplotypes in the key definitive hosts, foxes and coyotes. So far, we extracted DNA from approximate 10% of the worm subpopulation per host (19 foxes and 47 coyotes) and performed deep amplicon sequencing of 4 loci, targeting the most polymorphic regions from the mitochondrial genes COX-1, NAD-1 and COB. As preliminary results, we confirmed the presence of mixed infections with multiple *Em* haplotypes and *Echinococcus* species per host, low intraspecific diversity of *Em*, and higher abundance of the EU-like...
haplotypes in both hosts foxes and coyotes. Next-generation sequencing technologies represent a valuable tool to further characterize Em in multiple hosts to assess the current distribution and possible origins of the European strain in North America. This is particularly important to understand the patterns of geographic expansion of the parasite, the differences between strains related to host specificity, infectivity, development, and virulence as well as the role of different hosts in the transmission of the parasite.

**Cattle Nematodes**

14

**Monepantel in cattle: pharmacokinetics and nematocidal efficacy**

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The increasing levels of resistance to all traditional anthelmintic classes have encouraged the search for molecules with alternative mechanisms of action. The broad-spectrum nematocidal drug Monepantel (MNP) was initially developed to use in sheep. The goals of the work described here were to evaluate the pharmacokinetic (PK) behaviour and anthelmintic efficacy of MNP given to calves naturally infected with gastrointestinal nematodes (GIN) resistant to ivermectin (IVM) on two commercial farms. Thirty (30) male calves were randomly allocated into two groups (n= 15) and treated with either MNP orally at 2.5 mg/kg or IVM subcutaneously at 0.2 mg/kg. Eight animals from the MNP treated group (Farm 1) were randomly selected to perform the PK study. Drug concentrations were measured by HPLC. The efficacy was determined at 15 days after treatment by the FECRT. MNP and MNPSO₂ were the main analytes recovered in plasma. MNPSO₂ (measured in plasma up to 216 h post-treatment) systemic exposure was markedly higher compared to that obtained for MNP (measured up to 120 h post-treatment). Higher Cmax and AUC values were obtained for the active MNPSO₂ metabolite (96.8 ± 29.7 ng/mL and 9220 ± 1720 ng.h/mL, respectively) compared to MNP (21.5 ± 4.62 ng/mL and 1709 ± 651 ng.h/mL, respectively). The MNPSO₂ AUC value was 6-fold higher compared to the parent drug. Efficacies of 99% (Farm 1) and 96% (Farm 2) demonstrated the high efficacy of MNP (P< 0.05) against GIN resistant to IVM in cattle (reductions between 43 and 68% in both farms). While IVM failed to control *Haemonchus* spp. and *Cooperia* spp., MNP achieved 100% efficacy against *Haemonchus* spp., *Cooperia* spp. and *Ostertagia* spp. on both farms. However, MNP failed to control *Oesophagostomum* spp. (efficacies ranging from 22 to 74%). In conclusion, the oral treatment with MNP should be considered for dealing with IVM resistant parasites in cattle.
Seasonal epidemiology of major gastrointestinal nematodes in the northern semi-arid climatic zones of western Canada using the ITS-2 nemabiome barcoding approach
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The prevalence of Gastrointestinal Nematode (GIN) parasites in northern semi-arid climatic zones is not as well understood as the other climatic regions. The population dynamics of GIN in these northern regions might be increased in the future for several reasons, including the emergence of anthelmintic resistance, global warming and changes in grazing management. This study investigated the species-specific seasonal prevalence of GIN free-living stages on pasture in northern semi-arid climatic regions. A field study was conducted on three organic farms in Alberta over the 2019 grazing season. Grass samples were collected every three weeks from June to October 2019 from around 72 fecal pats in each farm. Due to the rotational grazing patterns, each pasture was contaminated only once (3-7 days grazing) with calves not returning to the same pastures until next year. This experimental design allowed us to monitor the pasture larval ecology under natural climatic conditions without the confounding effect of recontamination and anthelmintic treatment. Detailed meteorological data at farm level were recorded by solar powered weather stations. Finally, ITS-2 nemabiome metabarcoding was used to determine the GIN species composition such that species-specific time series data were obtained. We found on most pastures, L3 could not be recovered until six weeks after fecal deposition and L3 count peaked at the 9th week after fecal deposition. A large number of larvae remained in fecal pats at the end of grazing season suggesting this is an important refuge for larvae under these climatic conditions. ITS-2 nemabiome metabarcoding showed that Cooperia oncophora and Ostertagia ostertagi were the two predominant species, with Nematodirus helvetianus and Trichostrongylus axei also present. Epidemiological patterns were the same for all four species. Output of this study will now be utilized to validate a mathematical model (GLOWORM-FL) that predicts pasture larval contamination based on various climatic conditions.

Dog/Cat Nematodes

Improving the accuracy of anthelmintic studies with certain nematode parasites of dogs and cats

Abbott’s Formula reveals that anthelmintic efficacy is directly correlated to the magnitude of difference between group means of treated and control animals. But, group means are accurate only if all worms present in enrolled animals are recovered postmortem. In anthelmintic efficacy studies with hookworms (Ancylostoma or Uncinaria spp.), target nematodes are recovered not only from the gut contents but also by scraping the mucosa of the small intestine to dislodge adults. However, even aggressive mucosal abrasion is apparently incapable of dislodging all hookworms present. An additional step of incubating the scraped small intestine in 0.9% saline solution for 3 to 4 hours at ~100°F often yields worms that were missed by scraping. In six historical efficacy studies conducted at ETCR, saline incubation resulted in the recovery of
0 to 236 (mean = 10.5) additional hookworms from 45/54 (83.3%) control dogs and 0 to 15 (mean = 2.25) specimens from 16/96 (16.7%) treated dogs. Inclusion of these additional specimens altered the calculated efficacies of individual studies by a range of -0.3 to +0.4%, based on arithmetic means. Most efficacy protocols only allow worm fragments to be counted if EITHER a head OR a tail is present. However, examiners often encounter an excess number of one or the other body part. Assuming that One Worm = (One Head + One Tail), such stipulations will leave some individuals uncounted in ~50% of studies. Because an unmatched head or tail could only originate from an entire worm that was present at the time of treatment, this protocol stipulation can similarly result in systematic under-reporting of true worm numbers. Anthelmintic efficacy calculations will be more accurate if an additional saline incubation procedure is stipulated in hookworm protocols, and if worm counts are based on the greater number of head OR tail fragments recovered.

17

Efficacy and safety of a novel, oral chewable tablet containing sarolaner, moxidectin and pyrantel (Simparica Trio™) against immature and mature Toxocara canis in laboratory dogs and in veterinary patients in the USA
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Four laboratory studies and one field study evaluated the efficacy of a single, oral dose of Simparica Trio™ (1.2 mg/kg sarolaner + 24 µg/kg moxidectin + 5 mg/kg pyrantel) for the treatment of Toxocara canis, the dose limiting gastrointestinal nematode for pyrantel, in dogs. In the laboratory studies, dogs experimentally infected with T. canis were allocated to treatment with either placebo or Simparica Trio™ (n = 8). Infection was timed so that in two of the studies, T. canis were at the L5 stage and in the other two were at the adult stage at the time of treatment. Additional groups (n=8) were treated once orally with either 1.2 mg/kg sarolaner or 24 µg/kg moxidectin or 5 mg/kg pyrantel alone (in one adult study) or 1.2 mg/kg sarolaner + 24 µg/kg moxidectin only (in one L5 study). On Day 7, dogs were humanely euthanized in accordance with an approved IACUC protocol and necropsied for efficacy evaluation. In the USA field study, dogs naturally infected with T. canis, were treated once orally with either Simparica Trio™ or Heartgard® Plus (ivermectin/pyrantel) at the label dose. Efficacy was evaluated by comparing post-treatment reduction in egg counts per gram of feces (EPG), 8-14 days following treatment, to pre-treatment EPG. In the laboratory studies, efficacy of Simparica Trio™ was ≥95.2% against T. canis L5 stages and ≥97.3% against adults. Against adults, sarolaner or moxidectin or pyrantel alone provided 36.7%, 92.8% and 100 % efficacy, respectively. Against L5 stages, moxidectin + sarolaner provided 74.7% efficacy. In the field study, T. canis EPG were reduced by 99.2% for Simparica Trio™ and by 98.6% for Heartgard® Plus. All treatments were well-tolerated in all studies. These studies demonstrated that Simparica Trio™ was effective in the treatment of dogs with immature or adult T. canis.
Preventive efficacy of Bravecto Plus spot-on solution for cats (280 mg/ml fluralaner and 14 mg/ml moxidectin) against aelurostrongylosis in experimentally infected cats.

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Background: A negative-controlled, randomized, blinded laboratory study in 31 cats was carried out to evaluate the preventive efficacy of a topical parasiticide (Bravecto Plus spot-on solution for cats, MSD) against infections with the cat lungworm *Aelurostrongylus abstrusus*. The study was approved by the ethics commission of the Institutional Animal Care and Use Committee (IACUC). Material and Methods: The recommended minimum dose of 40 mg fluralaner and 2.0 mg moxidectin per kilogram bodyweight was administered either 12 (group [G] 1), 8 (G2) or 4 (G3) weeks before the infection with 300 third stage larvae of *A. abstrusus*. Starting on day 30 post infection (d.p.i.), the fecal larval shedding was monitored. On 47-51 d.p.i., the animals were humanly euthanized, the lungs were evaluated macroscopically for pathological findings and (pre-)adult *A. abstrusus* were counted. Results: All cats in the control group started continuous larval shedding between 32-40 d.p.i., whereas only in one animal of G1 larvae were present in the feces on several consecutive days. Another seven animals were copromicroscopically positive on one single day. Geometric mean (GM) of the maximum number of larvae shed on one day was 7574.29 in the control group compared to 1.10 (G1), 1.19 (G2) and 0.53 (G3). Thus, the administration of Bravecto Plus spot-on solution for cats reduced larval shedding by 99.98% (G2) to 99.99% (G1 and 3). All lungs in the control group showed severe to extremely severe pathological findings, whereas in 95.65% of the treated cats none or mild pathological findings were detected. The GM of isolated (pre-)adult *A. abstrusus* from the lungs was 26.57 in the control group compared to 0.09 (G1) and 0.00 (G2 and 3). Thus, the reduction of worm burden was 99.66% (G1) and 100% (G2 and 3). Conclusion: A single administration of Bravecto Plus spot-on solution for cats was effective in prevention of aelurostrongylosis for at least 12 weeks.

Simparica Trio™: Heartworm (*Dirofilaria immitis*) prevention in dogs using an optimized oral dose of moxidectin

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Simparica Trio™ is a novel, canine, oral combination parasiticide composed of sarolaner (1.2 mg/kg), moxidectin (24 µg/kg) and pyrantel (5 mg/kg). Initially, nine different strains of *Dirofilaria immitis* were evaluated in dogs using 3 µg/kg moxidectin administered 30 days after third-stage larvae inoculation. Five strains were determined to be macrocyclic lactone (ML)-susceptible, with 100% efficacy being achieved, while in 4 strains efficacy ranging from 19%-82% was obtained, suggesting ML resistance. The dose of moxidectin was then optimized to provide robust heartworm prevention against a range of heartworm strains representative of those to which most dogs in the US are likely to be exposed. This was done in three studies (5 or 8 dogs/group) using doses of 3-60 µg/kg moxidectin administered as single or 3 consecutive monthly doses with treatment initiated 28-30 days after inoculation of L3. The data from these
studies indicated that 3 consecutive doses of 24 µg/kg moxidectin provided ≥98.8% efficacy against three different ML-resistant strains (JYD-34, ZoeLA and ZoeMO), with 4 of 5 dogs in each group free of heartworms. Based on this high efficacy against ML-resistant strains and other commercial considerations, the 24 µg/kg moxidectin dose was selected to include in the Simparica Trio™ commercial product. Two pivotal placebo-controlled laboratory prevention studies (8 dogs/group) were conducted against two recently collected heartworm field isolates using study designs similar to those described above. In both studies, Simparica Trio™ was 100% effective in preventing heartworm disease when administered as a single, oral dose containing a minimum of 24 µg/kg moxidectin 30 days following D. immitis L3 inoculation, with mean control counts ≥30.7 worms/dog. All studies were conducted using IACUC approved protocols and animals were humanely euthanized prior to necropsy. Simparica Trio™ was well-tolerated in both studies. These studies demonstrated that Simparica Trio™ was effective in the prevention of heartworm disease in dogs.

Field efficacy and safety of a novel, orally administered combination product containing sarolaner, moxidectin and pyrantel (Simparica Trio™) for the prevention of heartworm disease (Dirofilaria immitis) in dogs presented as veterinary patients in Australia, Japan and the USA.

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Four field studies were conducted to evaluate the efficacy and safety of monthly treatment with a novel, oral chewable tablet containing moxidectin, sarolaner and pyrantel (Simparica Trio™) to prevent heartworm disease due to Dirofilaria immitis in dogs presenting as veterinary patients. Two field studies were conducted in Australia (one each in Southern and Northern Queensland), one in Japan, and one in the USA, in areas endemic for heartworm disease. Dogs were treated for 9-11 months with either Simparica Trio™ at the label dose of 24 to 48 µg/kg moxidectin in combination with sarolaner and pyrantel (184 dogs in the Australian studies, 45 dogs in the Japan study and 272 in the USA study), or with a positive control. In the Australian and USA studies, Heartgard® Plus (ivermectin/pyrantel) at the label dose was the positive control (62 dogs in Australia and 138 in the USA study). In the Japan study, NexGard Spectra® (afoxolaner/milbemycin oxime) at the label dose was the positive control (46 dogs). Efficacy was evaluated on Days 330 and/or 420 using heartworm antigen and microfilaria testing to assess adult heartworm infection. All dogs treated with Simparica Trio™ tested negative for adult heartworm infection on Days 330 and/or 420. Dogs treated with Heartgard® Plus in Australia and NexGard Spectra® in Japan were all negative for adult heartworm infection on study conclusion. In the USA field study, two dogs treated with Heartgard® Plus tested positive for adult heartworms, resulting in a statistically significant difference between prevention rates for heartworm disease for Simparica Trio™ vs Heartgard® Plus (p=0.0424) in this study. Simparica Trio™ was well-tolerated in all studies. These studies in 426 dogs, in heartworm-endemic areas of three countries, demonstrated the efficacy of Simparica Trio™ for the prevention of heartworm disease in veterinary patients.
Characterization of the prevalence of intestinal parasites in dogs in southern Ontario, Canada using sucrose double centrifugation, Fecal Dx® and Giardia antigen tests

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In southern Ontario there is a lack of information concerning the prevalence of intestinal parasites in dogs. As such, this study aimed to characterize the prevalence of intestinal parasites in dogs visiting off-leash parks in the region using sucrose double centrifugation, Fecal Dx® and Giardia plate ELISA. Additionally, data obtained via the sucrose double centrifugation method were used to evaluate the performance of the Fecal Dx® tests. Fecal samples were collected from 466 dogs aged ≥6 months from May to November 2018 (mean age = 3.7 years). Overall, 10 intestinal parasites were identified using the sucrose double centrifugation method. Roundworm eggs (i.e. Toxocara canis and Baylisascaris procyonis), whipworm eggs (Trichuris vulpis), and hookworm eggs (i.e. Ancylostoma caninum and Uncinaria stenocephala) were identified in 1.07% (95% confidence interval [CI] 0.38-2.56%) of samples, 5.15% (95% CI 3.33-7.57) of samples, and 5.79% (95% CI 3.85-8.31%) of samples, respectively. Using the Fecal Dx® tests, 1.07% (95% CI 0.38-2.56%), 2.15% (95% CI 1.03-3.91), and 4.29% (95% CI 2.64-6.55%) of the samples tested positive for roundworm, whipworm and hookworm antigen, respectively. In order to assess the level of agreement between the Fecal Dx® and sucrose double centrifugation methods, the prevalence-adjusted bias-adjusted kappa (PABAK) and Gwet’s first-order agreement coefficient (AC1) were used; both indicated almost perfect agreement for all of the Fecal Dx® components, ranging from 0.87 to 0.99. With respect to the Giardia test, 11.80% (95% CI 9.02-15.08%) of samples tested positive for Giardia antigen. This study provides valuable information on the prevalence of intestinal parasites in dogs in southern Ontario that will help guide parasite control recommendations for dogs in this region.

Detection of heartworm antigen using heat treatment of serum without loss of specificity in dogs infected by gastrointestinal helminths and protozoa.

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Detection of Dirofilaria immitis antigen in sera from dogs is the primary means of diagnosing infections. Heat treating serum prior to antigen testing has improved detection of some heartworm infections otherwise testing false negative on antigen tests. In prior publications, it has been postulated that heat treatment may allow cross-reactions with parasitic infections of dogs to occur using antigen tests. We evaluated the use of heat treatment for improved detection of heartworm antigen and assessed the potential for cross-reactivity with parasitic infections using canine serum (n=146) and the commercially available DiroChek® assay. The heartworm status and gastrointestinal helminth infections were confirmed by necropsy. Additional parasitic infections were detected by modified Knott’s technique, acid phosphatase staining, PCR, and
centrifugal Sheather’s sugar flotation. Prevalence of heartworm infection by necropsy was 37% (54/146). All 92 confirmed heartworm negative dogs remained unchanged before and after heat treatment, despite confirmed infections of *Acanthocheilonema reconditum*, *Ancylostoma caninum*, *Ancylostoma braziliense*, *Trichuris vulpis*, *Toxocara canis*, *Dipylidium caninum*, *Spirometra mansonoides*, *Macracanthorhynchus ingens*, *Cystoisospora* sp., *Giardia* sp., and *Sarcocystis* sp. Results of this research suggests that heat treatment can be used prior to antigen testing to improve detection of mature heartworm infections without the likelihood of induced cross-reactivity with certain parasites.

23 **Characterizing microRNA populations of *Dirofilaria immitis* in vitro as potential diagnostic markers for early-detection of infection**

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The American Heartworm Society recommends annual testing of all dogs in order to ensure the achievement and maintenance of successful prophylaxis. This recommendation includes the concurrent use of an antigen-detection test and microfilariae-detection test. Despite the high sensitivity and specificity of available tests, diagnosis can be only achieved approximately 6 months post-infection, coinciding with the pre-patent period of heartworm. There is a need to characterize diagnostic markers that are able to detect early stages of infection. Our objective was to assess the consistency of the excretory/secretory miRNA profiles of L3 and L4 of two different *D. immitis* strains *in vitro*. We cycled one macrocyclic lactone susceptible (Missouri) and one resistant (Yazoo) for obtention of L3s, and cultured L3s and L4s *in vitro*. RNA of media was extracted in triplicate for each strain and stage, and submitted to miRNA deep-sequencing and bioinformatic analysis. We identified two candidate miRNA markers representing novel sequences that are abundantly secreted by L3s and L4s of both strains. These miRNAs were previously detected in the secretions of heartworm microfilariae, L3s, L4s, adult males and females. One miRNA was previously identified in blood from heartworm-infected dogs. These markers have not been discovered in the secretions of other nematodes, including related filarial worms. It is necessary to further characterize the serum miRNA profile of dogs experimentally infected with *D. immitis* throughout the course of infection. This way, we will be able to assess how early and for how long these miRNA markers can be detected in canine sera, as well as their abundance and consistency. We believe that these heartworm-specific miRNAs have strong potential as early detection and multi-stage diagnostic markers.

24 **Evaluation of the effectiveness of fluralaner 280 mg/mL plus moxidectin 14 mg/mL spot-on solution for cats for prevention of *Dirofilaria immitis* infection (heartworm disease) in cats**

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Heartworm infection can result in serious and sometimes fatal disease in cats. This notwithstanding, fewer than 5% of cats receive a heartworm preventive. Longer acting, broad spectrum products would improve parasite protection for cats. The objective of this study was to
determine the efficacy of a topical combination of fluralaner and moxidectin (F/M) administered either two or three months prior to *D. immitis* infection. Thirty, healthy domestic short-hair cats (16 males and 14 females), approximately 7 to 9 months of age, and 2.6 to 5.3 kg were randomly assigned to one of three groups (n=10/group): Group 1 - non-treated controls; Group 2 – F/M treatment on day 0 (90-day efficacy); Group 3 – F/M treatment on day 30 (60-day efficacy). All cats were infected subcutaneously with 100 *D. immitis* infective larvae on day 90. Blood samples were collected and tested for heartworm antibody, antigen (heat treatment) and microfilariae on days -7/8, 89 (before infection) and for antigen (heat treatment) and microfilariae on days 180, 210, 240, and 271 (after infection). Cats were humanely euthanized per IACUC approval and examined for adult heartworms at necropsy on day 272 or 273. No heartworms were recovered from any of the cats treated with F/M 60 or 90 days before infection. The geometric mean number of heartworms recovered from the control cats was 3.5 (range = 2-16; 7/10 control cats were infected). One cat in the 60-day treatment group died from hypertrophic cardiomyopathy unrelated to treatment on study day 89. 4/7 infected control cats were antigen positive on day 240; 6/7 infected control cats were positive on day 271. Cats were negative for antibodies (days -7, 89) and microfilariae (all samples). This study demonstrates that F/M can be effective against experimentally induced *D. immitis* infections in cats for 2 to 3 months after treatment.

25

**Retrospective occurrence of canine endoparasites across veterinary parasitology diagnostic laboratories, United States, 2018**

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Infections with endoparasites, especially gastrointestinal helminths and protozoans, remain a significant and relatively common clinical finding among client-owned dogs in the United States. Current recommendations of year-round, broad-spectrum parasite prevention should decrease the prevalence of gastrointestinal nematodes. However, there remains a need for routine fecal diagnostics, as commercially available products do not control all canine endoparasites, and to monitor treatment efficacy in the face of emerging anthelmintic resistance in canine gastrointestinal helminths. The aim of this study was to assess the occurrence of canine endoparasites using retrospective fecal flotation data available through veterinary parasitology diagnostic laboratories within academic institutions and state laboratories. Data were obtained from 9 diagnostic laboratories across 8 states in the United States from 01 January to 31 December 2018. Of the 4,573 fecal samples analyzed, 944 (20.64%) were positive for at least one parasite. The most common parasites detected were *Giardia* sp. (8.44%; 386/4,573), followed by *Ancylostoma* sp. (5.31%; 243/4,573), *Cystoisospora* spp. (4.5%; 206/4,573), *Toxocara canis* (2.41%; 110/4,573) and *Trichuris vulpis* (2.16%; 99/4,573). Other endoparasites detected in less than 1% of samples included *Sarcocystis* sp., *Eucoleus* spp., *Dipylidium caninum*, *Strongyloides stercoralis*, *Taeniidae*, and *Toxascaris leonina*. The occurrence of endoparasites was highest among dogs younger than 1-year old (35.05%; 334/944) (p<0.05), and among intact male (25.21%; 238/944)
and intact female (18.75%; 177/944) dogs \( (p<0.05) \). In summary, we report a moderately high occurrence of endoparasites in dogs tested across academic and state diagnostic laboratories in the United States. At least 17 different endoparasites were detected, including zoonotic agents. These data illustrate the importance of routine fecal screening and a treatment program that would protect dogs from parasites all year long. Veterinarians should continue to reinforce client education about endoparasite diagnostics, treatment, and prevention to reduce the risk of environmental contamination and transmission.

26
Comparison of development of JYD-27 and Missouri (MO) strains of *Diro*filaria immitis* in laboratory-reared *Aedes aegypti*
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Canine heartworm disease (CHD) is an important mosquito-borne disease of dogs in many parts of the world. Generally, CHD is prevented by administering prescribed macrocyclic lactones (ML)-based HW preventives at monthly, semi-annual or annual intervals. However, infrequent but demonstrable resistance to ML preventives has been reported. Resistant strains of *D. immitis* may have genetic-based characteristics that affect their development in mosquito vectors. We compared development of resistant (JYD-27) and susceptible (MO) strains of *D. immitis* in *Aedes aegypti*. Laboratory-reared mosquitoes were fed for equal amounts of time on blood containing comparable numbers of microfilariae (mff; 2,853–3,160/mL). Sixteen days after feeding, female mosquitoes were examined for numbers and location of L1, L2 and L3 larvae (infection rate [IR]). Mortality rate (MR) was also assessed by counting both live and dead mosquitoes. Developmental success rate (DSR), which we define as \( \frac{L3}{L1+L2+L3} \times 100 \), was also calculated. IR, MR and DSR were analyzed by linear regression in R Studio \( (P \leq 0.05) \). 462 mosquitoes from 5 batches of JYD-27 and MO were collected and analyzed. Overall, higher percentages of MR, IR, and a higher DSR was observed for mosquitoes infected with MO vs. JYD-27. However, the differences were not statistically significant. No differences were observed for either heartworm strain in the timing or location of development stages. We conclude that under the conditions of this study there were no statistical differences in IR, MR and DSR in laboratory-reared mosquitoes experimentally infected with JYD-27 and MO strains of *D. immitis*.

27
Molecular and morphological characterization of *Thelazia californiensis* in dogs from New Mexico, USA
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Thelaziosis is a zoonotic ocular infection caused by nematodes of the genus *Thelazia*. This arthropod-borne disease has been increasingly reported in different hosts from several countries. *Thelazia californiensis* is endemic to western North America, and has been reported in companion animals, wildlife and humans. Nevertheless, information on this parasite is scarce.
We described two cases of thelaziosis in dogs from New Mexico with no history of travel. The first case was a 1-year-old female spayed Siberian Husky mix from Bernalillo county, presented to a private veterinary clinic for unrelated clinical symptoms of vomiting. Small nematodes were observed in the tear film bilaterally on physical examination. Twenty-one nematodes (14 females, 7 males) were collected in the dorsal and ventral conjunctival fornix. The second case was a seven-year-old male neutered Belgian Malinois from Santa Fe County with a pigmented iridial mass of the right eye. During surgical biopsy of the mass, a single female worm was incidentally noted in the dorsal conjunctival fornix and collected. The specimens were morphologically confirmed as *T. californiensis*, and analysis allowed for the expansion of the morphometric data on the species. We characterized for the first time the partial sequences of two mitochondrial genes: cytochrome oxidase c subunit 1 (COI) and 12S. Phylogenetic analysis for each gene was performed in MEGA X, comparing to homologous sequences of other *Thelazia* species (*Thelazia callipaeda*, *T. lacrymalis*, and *T. gulosa*). To our knowledge, this is the first case report of *T. californiensis* infection in dogs from New Mexico confirmed by integrated classical and molecular approaches. These two molecular markers will be valuable for assisting in the diagnosis of *Thelazia* cases in both animals and humans, and screening of vectors to develop a better understanding the biology of this nematode.

28

**Microfilariae counts and antigen levels of macrocyclic lactone resistant *Dirofilaria immitis* during treatment with melarsomine dihydrochloride**

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A 5-year-old, spayed mixed breed dog was presented for evaluation 14 months after receiving a melarsomine dihydrochloride treatment series for *Dirofilaria immitis*. The patient remained consistently microfilaremic (median = 3,676.6/mL), though was no longer *D. immitis* antigen positive. Milbemycin oxime was reportedly administered monthly for ≥ 40 months prior to presentation. Moxidectin (≥ 2.5 mg/kg) was started as heartworm preventive and microfilaricidal treatment at time presentation (Day 0). We quantified *D. immitis* microfilariae and antigen, before and after heat-treatment of serum, throughout the entire treatment course. Microfilariaemia did not decrease until doxycycline (10 mg/kg q12hr, days 29–58) was added to the treatment protocol. After doxycycline was included, *D. immitis* microfilaria numbers dropped precipitously (median = 476.0/mL; U = 10,000, p = 0.054) but remained detectable until Day 175. Melarsomine dihydrochloride (2.5mg/kg) was administered on Days 90, 119, 120. Significantly higher OD values for *D. immitis* antigen (*Di*-Ag) were measured after heat-treatment of serum (W = 136.0, p < 0.001) than before. OD values for *Di*-Ag without heat-treatment were below those of the negative control at all but 2 time points when there was a spike in levels prior to initiation of the second arsenical treatment series. OD values for *Di*-Ag with heat-treatment remained above those of the negative control until 147 days after the last dose of melarsomine dihydrochloride (day 267). Due to the documented clinical history from the rDVM and the lack of response to initial treatment for microfilariae, the dog was most likely infected with a macrocyclic lactone resistant strain of *D. immitis*. To the authors’ knowledge, this is the first macrocyclic lactone resistant *D. immitis* reported from Oklahoma. Significant differences in the OD-values of *Di*-Ag before and after heat-treatment of serum demonstrate the usefulness of disrupting immune complexes to measure adulticide treatment response.
Molecular detection of *Cercopithifilaria bainae* in brown dog ticks from across the southern United States
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*Cercopithifilaria bainae* is a filarial nematode infecting the skin of dogs that is transmitted by brown dog ticks (*Rhipicephalus sanguineus* sensu lato). Despite the ubiquity of brown dog ticks, the discovery of *C. bainae* in the United States is recent; reports include a single pet dog in Florida in 2017 and 6 shelter dogs in Oklahoma in 2019. Studies conducted to assess geographic distribution of the emerging parasite in the U.S. are lacking. Here, brown dog ticks collected from across the nation were tested for molecular evidence of infection. Ticks were submitted to researchers at Oklahoma State University during March 2018–March 2020 by veterinary clinics, animal shelters, and other participants as part of an ongoing national tick survey study (showusyourticks.org). Received ticks were morphologically identified, dissected, extracted for DNA, and tested by PCR to amplify a 330-bp region of the filarioid 12S rRNA gene; resulting amplicons were sequenced. A total of 1,400 brown dog ticks from 23 states were assayed. Ticks were collected from 311 dogs, 8 cats, and 2 additional species. Due to high numbers of ticks submitted from some animals, up to 20 ticks per animal were tested. *Cercopithifilaria bainae* DNA (99–100% sequence homology) was detected in 73 (5.2%) of brown dog ticks tested, which included larval, nymphal, and adult instars. Positive ticks were removed from 54 dogs (17.4%) residing in 11 states, primarily in southern regions. To the authors’ knowledge, this is the first study conducted in the U.S. to better understand the distribution of *C. bainae*. We document molecular evidence of the filarial parasite in ticks from 9 additional states, indicating it is more widespread in dogs here than previously known. Veterinarians across the southern United States should consider *C. bainae* as a differential diagnosis when evaluating canine dermatitis and polyarthritis.

Surveillance for pyrantel pamoate resistance in *Ancylostoma caninum* in north Florida shelter dogs
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*Ancylostoma caninum*, the canine hookworm, is the most common intestinal helminth of dogs in the United States, particularly in the southeast. Infection can lead to anemia and also poses a public health risk as larvae can cause cutaneous larva migrans in people. Recently, multi-drug resistant *A. caninum* has been independently isolated from 4 dogs from Florida and Georgia. The purpose of this study was to determine whether resistance to pyrantel pamoate, an anthelmintic commonly used in shelters, is present in *A. caninum* in north Florida shelter dogs. Fecal samples were examined by sugar centrifugal flotation for *Ancylostoma* eggs. Dogs were treated with 22 mg/kg of pyrantel suspension PO according to the shelter’s normal dosing protocol, and post-treatment fecal samples were collected 6-11 days later. Egg counts were performed on all pre- and post-treatment samples using the Mini-FLOTAC device in order to calculate the fecal egg count reduction (FECR) after treatment. Of 51 dogs in the study, only 10 were fully cleared of infection (i.e. FECR of 100%). Mean FECR was 76.8%. PCR was performed for the ribosomal
ITS region of 31 egg samples to confirm species identity. All 25 samples that successfully amplified were verified to be *A. caninum* through sequencing. RFLP analysis using HinfI enzymatic digestion did not reveal evidence of a mixed infection. These results demonstrate the presence of pyrantel-resistant *A. caninum* in the north Florida dog population, and should prompt further investigation into the extent and distribution of anthelmintic resistance in this parasite.

31

Comparative preventive efficacy of ProHeart® 12, Heartgard® Plus and Interceptor® Plus against a macrocyclic lactone-resistant strain (JYD-34) of heartworm (*Dirofilaria immitis*) in dogs

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ProHeart® 12, an extended release injectable suspension (0.5 mg/kg moxidectin) is approved for prevention of heartworm disease in dogs for 12 months in the USA. Twenty-four beagles free of adult heartworm infections were allocated to 4 groups (n=6): placebo control and three treatments; ProHeart 12 (PH 12), Heartgard® Plus (ivermectin/pyrantel) (HGP) and Interceptor® Plus (milbemycin oxime/praziquantel) (INTP) all at the label dose. All dogs were inoculated with 50 third-stage *D. immitis* larvae (JYD-34 strain) on Day -30. The PH 12 dogs were administered a single subcutaneous (SC) injection on Day 0. The HGP and INTP dogs were treated orally on Days 0, 30, 60, 90, 120 and 150. On Day 185, dogs were humanely euthanized in accordance with an approved IACUC protocol and necropsied for recovery of adult heartworms. All control dogs had adult heartworms at necropsy with a geometric mean of 29.9 worms per dog (range: 23-37). Compared to control, PH 12 (n=5 at necropsy) was 100% effective in preventing the development of adult JYD-34 heartworms after a single SC dose 30 days post-inoculation. After 6 consecutive monthly doses, all HGP and INTP treated dogs had adult heartworms with geometric mean counts of 26.8 (range: 9-34) and 25.5 (range: 13-35), and efficacies of 10.5% and 14.6% respectively. Mean worm counts for the PH 12 group were significantly lower (P<0.0001) than the control, HGP and INTP groups. The mean worm counts of the HGP or INTP groups were not significantly different (P>0.2719) from the control nor were they significantly different (P=0.7405) from each other. In conclusion, a single SC injection (0.5 mg/kg) of ProHeart 12 was 100% effective and in this study was significantly better than either 6 consecutive monthly doses of Heartgard Plus or Interceptor Plus in preventing the development of the ML-resistant JYD-34 heartworm strain in dogs, when inoculated 30 days before treatment initiation.

32

Efficacy evaluation of anthelmintic products against an infection with the canine hookworm (*Ancylostoma caninum*) isolate Worthy 4.1F3P in dogs

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*Ancylostoma caninum*, the dog hookworm, is the most prevalent and medically important intestinal nematode parasite of dogs, and is also zoonotic. Recently, multiple-drug resistance (MDR) to all anthelmintic classes currently approved for its treatment in the US has been
diagnosed in this parasite. Emodepside, a cyclooctadepsipeptide, is not registered for use in dogs in the US. However, it is registered for the treatment of *A. caninum* in a number of other countries/regions. The objective of this study was to evaluate the efficacy of emodepside, as well as three drugs that are commonly used in the US for treatment of hookworms, against a confirmed MDR isolate of *A. caninum*. 40 dogs were infected on day 0 with 300 L3s of the isolate Worthy 4.1F3P, and allocated randomly to 1 of 5 treatment groups: pyrantel pamoate, fenbendazole, milbemycin oxime, emodepside + praziquantel and non-treated control. Fecal egg counts (FEC) were performed on study day (SD) 19, 20, 22, 27, 31 and 34. All treatments were administered as per label instructions on SD 24. Dogs were necropsied 10 days after treatment and the digestive tract was removed and processed for worm recovery and enumeration. The geometric mean with 95% CI worm counts for the control group were 97.4 (81.6, 116.4), and for the pyrantel pamoate, fenbendazole, milbemycin oxime, and emodepside + praziquantel groups were 74.8 (65.8, 85), 72 (62.1, 83.6), 88.9 (74, 106.8), and 0.4 (0.04, 0.9), respectively. These yielded efficacies of 23.2%, 26.1%, 8.8%, and 99.6%, respectively. FEC reductions with 95% CI for pyrantel pamoate, fenbendazole, milbemycin oxime and emodepside + praziquantel groups were 13% (-91, 60), 46% (17.5, 64.9), -50% (-286, 42), and 100% (99.7, 100), respectively. These data conclusively demonstrate the high-level MDR status of the *A. caninum* Worthy isolate to pyrantel, fenbendazole, milbemycin oxime, and susceptibility to emodepside.

33
**Retrospective analysis of canine endoparasites in 2019 with a focus on retired racing greyhounds**
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Infections with endoparasites, especially gastrointestinal helminths, are a common finding in client-owned dogs. The Community Practice (CP) section at the Ohio State University College of Veterinary Medicine (OSU-CVM) follows Companion Animal Parasite Council (CAPC), American Animal Hospital Association (AAHA), and American Veterinary Medical Association (AVMA) guidelines for parasitology by recommending annual fecal analyses of dogs and prescribing year-round, broad-spectrum parasite preventatives. There is increasing interest in determining if drug resistant helminths are present in canine populations as there are reports of Greyhounds with drug resistant hookworms. A student club rehabilitates and rehomes retired racing greyhounds. Many new greyhound owners wish to participate in the OSU-CVM volunteer blood bank program. An initial fecal analysis is performed when screening dogs as potential blood donors. Once a dog enters the program, an annual fecal analysis is performed as part of ongoing health screening. Our 2019 study retrospectively analyzed the fecal analysis results from varying dog breeds. We report 885 total canine fecal samples submitted during 2019 from 678 dogs of varying breeds. Of the 883 canine fecal samples, 232 (26.27%) of these samples had a positive fecal analysis for gastrointestinal parasites. We included in this analysis 128 fecal samples derived from 63 greyhounds and used client interviews to evaluate their compliance with monthly broad-spectrum parasite control. These data demonstrate the importance of educating clients on the appropriate administration of monthly parasite preventatives and performing annual fecal examinations.
Horse Nematodes

Are we beating a dead horse? Modelling a failed anthelmintic in combination
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Combination deworming utilizes two or more anthelmintics with different modes of action to target the same parasite. It has been used against drug resistant parasites, but few studies have explored the effects against cyathostominis. In a previous study, we reported that oxibendazole (OBZ) and pyrantel pamoate (PYR) combination was not sustainable against cyathostominis harboring high-levels of resistance to OBZ and PYR. The current study utilized a moxidectin-oxibendazole (MOX-OBZ) combination against the same cyathostomin population in a field study and two computer simulations. In the field study, treatments were administered when 10 horses exceeded 100 eggs per gram. Fecal egg counts and efficacy evaluations were performed every two weeks. The two simulations implemented local weather data and parasite population parameters from the field study. The first simulation repeated the field study’s treatment schedule over a 40 year period. The second study evaluated efficacy over a 40 year period using selective therapy. In the field study, the efficacies of MOX single active and MOX-OBZ were both 100%. The egg reappearance period for MOX was 16 weeks and the two combination treatments were 12 and 18 weeks. The OBZ single-active efficacies were 46.7% and 40.1% for the first and last treatments, respectively. They were not significantly different from each other. In the simulation study, MOX-OBZ delayed MOX resistance compared to when the single active was used alone. This occurred despite the low OBZ efficacy. The second simulation identified the MOX-OBZ combination to be most effective at delaying MOX resistance. In conclusion, this study supports the use of combination treatment when one of the actives exhibits high efficacy against drug-resistant cyathostominis despite the low efficacy of the other active.

Equine parasite control protocols: an evaluation of health parameters.
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Strongylid and ascarid parasites are omnipresent in equine stud farms, and ever-increasing levels of anthelmintic resistance are challenging veterinarians, farm managers, and horse owners with finding more sustainable and yet effective parasite control programs. The objectives with this study were to evaluate egg count levels, body weight, and equine health under defined parasite control protocols in cohorts of foals and mares in two Standardbred and two Thoroughbred stud farms in New Zealand. Foals (n=93) and mares (n=99) were enrolled into two and three treatment groups, respectively. Parasite egg count data, body weight estimates, and health information was collected from all horses. Horses underwent a health examination at each fecal collection date, and episodes of colic or diarrhea were recorded. Body weights were assessed using a weight tape, and mares were body condition scored. In group FA, foals were dewormed at two and five months of age with a fenbendazole/ivermectin/praziquantel product, while group
FB foals were dewormed on a monthly basis, alternating between the above-mentioned product and an oxfendazole/pyrantel embonate product. Group MA mares were dewormed twice with fenbendazole/ivermectin/praziquantel, group MB mares were dewormed with the same product, when egg counts exceeded 300 strongylid eggs per gram, and group MC mares were dewormed every two months, alternating between the two products. There were no differences between treatment groups with regards to body weight and body condition scores. Health status was very good throughout the study with very few incidents recorded. Foals in group FA had significantly higher ascarid and strongylid egg counts, whereas no egg count differences were observed between mare groups. In conclusion, anthelmintic treatment intensity was lowered from the traditional intensive regimes without measurable negative health consequences for mares and foals.

36

**Looking at the spectrum of cyathostomin disease through the lens of clinical cases**

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Cyathostomins are recognised as pervasive and pathogenic intestinal helminths of horses worldwide. Within the cyathostomin life cycle, hypobiosis of encysted larvae in the large intestinal mucosa can occur. Larvae can remain here for two or more years, allowing for accumulation of large burdens within the host. Cyathostominosis has traditionally been presented as either a chronic insidious disease associated with lethargy, weight loss or an acute severe, sometimes fatal, colitis associated with mass emergence of larvae – acute larval cyathostominosis (ALC). Here we present retrospective clinical and clinicopathological data from 18 cases of suspected cyathostominosis that presented over the Autumn-Spring period of 2018/19, that suggest that cyathostominosis can in fact present as a spectrum of disease. Chronic burdens can persist, leading to non-specific clinical signs, or, in the presence of exacerbating factors can lead to acute disease, on occasion in a relapsing/remitting pattern. We suggest that where cyathostomin burdens are high, chronic cyathostominosis in a herd may be accompanied in individual horses by various intensities of disease from per-acute to chronic, with some horses suffering from repeated episodes. We suspect that horses that presented with acute severe clinical signs were either overwhelmed by infection burdens and/or had experienced a trigger, most likely adulticidal treatment, to induce synchronised larval re-emergence and an acute severe typhlocolitis. Furthermore, we postulate that the resulting waxing and waning disease seen in some individuals is due to waves of emergence of previously hypobiotic larvae. This indicates the need for continuing vigilance and observation, particularly after anthelmintic administration, where cyathostominosis occurs adapting treatment protocols as necessary.

37

**Age, sex, and parasites: Is there an unusual predilection in foals?**

Ashley Steuer*, Jessica Scare-Kenealy, Martin Nielsen. M.H. Gluck Equine Research Center

Horses are host to one of the most diverse parasite faunae in domestic species. Over the course of the first months of life, parasite infections follow distinct patterns that appear to be orchestrated by route of infection, exposure, and age-dependent immunity. We have previously reported differences between fillies and colts in *Parascaris* egg shedding, as well as associations between
age and *Parascaris* spp. and *Strongyloides westeri* egg shedding. However, detailed data are missing from the first few weeks of life to unravel the course of parasitic events in naturally infected foals. This study aimed to evaluate trends in fecal egg output and worm burdens with age in foals. In 2018, 14 foals born into a parasitology research herd were followed from April to December until humanely euthanized under the University of Kentucky’s IACUC protocol 2012-1046. Fecal egg counts were determined biweekly from all foals, and intestinal stages of *Parascaris* spp., mucosal cyathostomins, *Anoplocephala perfoliata*, *Strongylus vulgaris*, and *Strongylus edentatus* were identified and enumerated. Age was significantly associated with fecal egg counts of *Strongyloides westeri*, *Parascaris* spp., and strongylid type eggs (p=<0.0001). Significantly higher ascarid egg counts were identified at 17-19 weeks of age in colts, compared to 20-22 weeks of age in fillies (p<0.001). Colts also incurred a higher peak of strongylid eggs at 6 weeks of age, compared to fillies (p=X). These differences associated with sex have been previously reported from this herd, but have not been widely found in other equine studies. Factors causing colts to have higher worm burdens and egg counts have yet to be determined, but hormonal or behavioral explanations may apply.

38

**Prevalence and relative abundance of cyathostomin species in domestic horses from 1975-current: Meta-analysis of geographic region and specimen collection method.**

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Cyathostomin parasites (Strongylidae: Nematoda) of horses comprise a complex of ~40 currently recognized species. Infections are ubiquitous across virtually all grazing horses regardless of host demographics, and prevalence often approaches 100%. Concomitant infections of 10 or more species in individual horses is standard. Complexity of cyathostomin infections is appended by the predisposition of immature cyathostomins to encystment and hypobiosis within the large-intestinal lining. Prolonged hypobiosis facilitates large accumulations of encysted stages that, upon mass synchronous emergence, potentiates the rare but often fatal clinical disease syndrome of larval cyathostominosis. Additionally, cyathostomins exhibit widespread or emerging resistance to currently available drug classes. Thus, cyathostomins are the most important gastrointestinal parasites of horses weaning age and beyond. Despite this, species-specific research from basic ecology to the contribution of individual species to clinical disease and/or anthelmintic resistance is wanting. This meta-analysis reviews current knowledge of cyathostomin species at the adult meta-population level to emphasize critical limitations of currently available species-specific information and identify future research needs. Specifically, this meta-analysis describes regional differences in and the influence of specimen collection method on the prevalence and relative abundance (RA) reported for individual cyathostomin species’ adults. Thirty-nine publications, published 1975-current and representing seven geographic regions and three specimen collection methods, were included, comprising 52 datasets for which prevalence and/or RA was reported for individual species. Definitively, *Clycostephanus* (*Cys.*) *longibursatus*, *Clycoclyclus nassatus*, and *Cyathostomum catinatum* are statistically the most prevalent (~90%) and relatively abundant (~21-22%) cyathostomin species of represented domestic horses globally. The most notable regional difference is the statistically higher prevalence and RA of *Cys. longibursatus* in North America (~100% and ~36%) (n=17 and n=13) than in Eastern Europe (~67% and ~9%) (n=22 and n=16) (P>0.0001). Analysis of the
influence of specimen collection method on prevalence and RA is confounded by regional use of 
single method types.

39 Longitudinal survey of intestinal nematodes of Thoroughbred horses in Australia
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Very little is known about the epidemiology of horse parasites from Australia. Currently, a 
comprehensive research project is underway to (i) understand the epidemiology of intestinal 
nematodes of horses, (ii) assess worm control practices used by Thoroughbred breeders as well 
as veterinarians and (iii) determine the efficacy of commonly used anthelmintics against 
testinal parasites of horses. This study aimed to understand the epidemiology of intestinal 
nematodes of Thoroughbred horses in four climatic zones of Australia using a longitudinal 
survey. The study started in November 2019 and 16 Thoroughbred breeding farms from five 
climatic zones were recruited. Approximately, 25 faecal samples were tested from each farm 
every 1-2 month using the modified McMaster technique, with a sensitivity of 15 eggs per gram 
(EPG). To date, a total of 897 samples has been tested from foals/weanlings, yearlings, mares. 
Overall, the prevalence of intestinal nematodes was 57.3%, with 46.6% being for strongyles and 
10.7% for Parascaris. The mean faecal egg count (FEC) of strongyles was higher (403±661 
EPG) than Parascaris (327±1073 EPG), with the highest FECs of 9,555 and 35,955 EPG, 
respectively. Parascaris eggs were only detected from foals, weanlings and yearlings, with the 
highest mean FEC in foals (584±1444) followed by yearlings (49±192). In adult horses, wet 
mares had a higher burden of strongyles (358±738 EPG) than dry mares (198±272 EPG). These 
preliminary results indicate that the young horses have higher burden of intestinal nematodes 
than adult horses. Findings of this project will help to develop effective worm control strategies 
for Thoroughbred horses in Australia.

40 The local and systemic inflammatory response to anthelmintic therapy: Does killing 
encysted cyathostomins increase inflammation?
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Cyathostomins are pervasive parasites of horses and in rare cases, mass excystment of larval 
stages within the mucosal walls can lead to life-threatening disease. Anthelmintics that are 
adulticidal only, such as ivermectin, have been implicated in triggering this condition. However, 
concerns have also been raised that killing large numbers of encysted larvae in situ could lead to
adverse inflammatory reactions as well. This study aimed to evaluate the local and systemic inflammatory reaction to moxidectin, which is both adulticidal and larvicidal, and ivermectin, which is adulticidal only. Briefly, 36 horses were allocated into one of 3 groups: moxidectin treated with 0.4mg/kg orally, ivermectin treated with 0.2 mg/kg orally, and an untreated control group. Half the horses from each group were humanely euthanized at 2 weeks post treatment, and the other half humanely euthanized at 5 weeks post treatment per the University of Kentucky’s IACUC protocol 2018-3134. Weekly blood samples were collected throughout the study for gene expression evaluation. Tissue samples were collected from the cecum, ventral, and dorsal colon for histopathology and gene expression at the 2 and 5 week post treatment intervals. The moxidectin treated group had a significantly lower inflammatory response (such as with IFN-\( \gamma \) \( p<0.05 \) ), followed by the ivermectin treated group, and then the untreated controls. The data suggests that removal of cyathostomins will reduce the proinflammatory response associated with cyathostomin infections. In conclusion, proinflammatory reactions to anthelmintic treatment was minimal, but lowest for moxidectin-treated horses.

41 The microbiome of Parascaris spp.: A pilot study
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Parascaris spp. is the most pathogenic parasite in juvenile horses, causing clinical symptom as severe as colic and death. Overuse of anthelmintics has led to a serious situation where Parascaris spp. is resistant to macrocyclic lactones and has emerging resistance to benzimidazoles and pyrimidines – this represents all available anthelmintic drugs available for treatment in horses. Previous research in other species of parasitic nematodes has shown that they have diverse microbiomes differing in composition from their hosts as well as within the parasites themselves. The goal of this pilot study was to analyze the microbiome of Parascaris spp. gonad and intestinal samples from both male and female parasites. A total of 92 adult parasites were collected from three foals in accordance with IACUC protocol 2012-1046, dissected, DNA extraction performed, and next generation sequencing library preparation completed. Samples were sequenced using the Illumina MiSeq platform and all data analysis was completed in R. Preliminary data indicate that bacterial taxa present in the samples include Weissella, Sarcina, Clostridium, Lactobacillus, Hydrotalea, and Aminobacter. A completed analysis of all data will be presented at the conference comparing relative abundance of bacterial taxa between male and female parasite gonads and intestines.

42 Multiple resistance in horses naturally infected by Cyathostomins in Mato Grosso do Sul, Brazil

Cyathostomins are the most prevalent gastrointestinal nematodes in horses and its resistance to benzimidazole is widespread globally, while resistance to macrocyclic lactone compounds is emerging. The aim of the study was to report a case of multiple resistance to ivermectin, moxidectin and the association of trichlorfon and albendazole in a horse farm in Bela Vista-MS,
Brazil, with animals of different breeds, ages and purposes and intense transit of horses. The animals remained housed and were fed fresh forage cut daily. The area used for forage production was fertilized daily with untreated feces collected from the stalls of the horses. There were no technical criteria for deworming the animals. All horses were treated every 3 months, without prior weighing. For this study, thirty adult animals naturally infected were selected according to Fecal eggs counts (FECs). FECs and pre- and post-treatment (14 days) coprocultures were performed. The animals were randomly assigned to the treatments (n=10): ivermectin (0.2mg/Kg), moxidectin (0.4 mg/Kg) and the association of trichlorfon (35mg/Kg) and albendazole (5mg/Kg). The mean efficacies were ivermectin 57% (CI95%: 38-71), moxidectin 47% (CI95%: 15-67) and trichlorfon +albendazole 56% (CI95%: 29-73). Only cyathostomin larvae were detected by morphological identification pre and post-treatment. Thus, this study characterizes phenotypically a cyathostomin isolate with multiple resistance in a farm with intensive use of anthelmintic drugs, inappropriate use of feces as fertilizer and intense transit of horses.

Nematodes (Probiotics and Fecal Sample Storage)

43 Development of a paraprobiotic cure for gastrointestinal nematode parasites
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Gastrointestinal nematode (GIN) parasites are common and serious infectious agents of humans and livestock. For humans and livestock, GIN infection has serious health consequences (e.g., stunting, malnutrition, anemia). Routine deworming is a mainstay in human and veterinary medicine. However, GIN resistance to all deworming drugs (anthelmintics) is rampant or growing. New anthelmintics are needed but they must be safe, effective, inexpensive, stable without a cold chain, and scalable. Here we propose a new modality for deworming-- a paraprobiotic (dead probiotic) that expresses vertebrate-safe Bacillus thuringiensis (Bt) crystal proteins (Cry) with activity against nematodes. We have engineered asporogenous Bt to express Bt Cry proteins during the vegetative growth phase, forming cytosolic crystals. These Bacteria with Cytosolic Crystals (BaCC) are rendered inviable (inactivated BaCC or IBaCC) with food-grade essential oils. Bioactivity of IBaCC against GINS of humans and of livestock were tested in vitro. Rodent studies in vivo demonstrate high activity against hookworm. IBaCC production was successfully scaled up to 350 liters at a contract manufacturing facility; bioactivity was confirmed in vitro. This allowed for large animal studies in pigs and horses. For example, 10 foals infected with Parascaris were enrolled in a single-dose treatment study (average eggs per gram or EPG of feces = 770). Six were treated single dose with 30 mg/kg IBaCC; four were left untreated. One week later, all six treated foals had 0 EPG whereas all four untreated foals still had significant EPGs. IBaCC treated horses remained at 0 EPG for 28 days, the duration of the study. Initial histopathology safety studies in rodents were also carried out and results will be presented. These studies form a foundation for the development and launching of a new modality of effective, achievable, and cost-friendly anthelmintics.
Storage methods of cattle and horse faecal samples for egg hatch test
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The egg hatch test (EHT) is a reliable test for the diagnosis of resistance to benzimidazoles, however the sensitivity to this drug decreases with the age of the eggs, which is a limitation to the use of this test routinely in the field. The objective of this study was to evaluate the influence of cold and anaerobic storage of bovine and equine feces on the hatchability (viability) and sensitivity of nematode eggs to thiabendazole in EHT. Eighteen calves naturally infected predominantly by Cooperia sp. and a horse naturally infected predominantly by Cyathostomins, both previously phenotypically characterized as susceptible to benzimidazoles were used as donors. Pooled fecal samples were submitted to 30 treatments: three anaerobic or aerobic methods (anaerobic storage in plastic bottle, anaerobic storage in vacuum sealed bags or aerobic plastic bags), under two temperature conditions (cooled or ambient temperature), and five different time-point evaluations (24, 48, 72, 96 and 120 hours). Additionally, an assay was carried out until 3 hours as a standard test. The reproducibility of the storage methods was tested by repeating the tests three times in different days. To evaluate the effect of the storage methods, two criteria were used: hatchability in the control group (water) and the sensitivity of the eggs to thiabendazole, compared by the confidence interval of the half maximal effective concentration. For horse faecal samples, no storage method can be recommended due to the large inter-day variation and reduced egg sensitivity to thiabendazole, which can generate false resistance results. For cattle, anaerobic samples storage allowed the EHT to be performed up to 96 hours after collection.

Swine Nematodes

Microsatellite analysis – the useful tool to track transmission of Trichinella spp.
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The microsatellite analysis is becoming a popular tool to identify subpopulations. In this study it was used to recognize the genetic structure of 29 isolates of Trichinella spiralis (n.15) and T.britovi (n.14) circulating in Poland. Thirty-six larvae from each isolate collected from farm-pigs, wild boars, foxes and rats, were genotyped at 9 (T.spiralis) and 4 (T.britovi) microsatellite loci. Allele and genotypic frequencies were obtained by GenePop4.0 and GenAlEx6.501 softwares, while Wright’s indexes (Fis and Fst) were compute by GenePop4.0 software. The multilocus genotypes (MLGs) were used to identify the membership among single larvae and Bayesian analysis larvae and isolates. The genetic variability(average number of alleles per locus and Ho) in the T.spiralis isolates was lower than in T.britovi. The genetic differentiation (Fst index) among the isolates showed a wide range of values, both among the T.spiralis (0.006-0.956) and T.britovi (0.02–0.542) isolates. Interestingly the T.spiralis isolates from pigs and a rat from the same farm showed minimal Fst values (range 0.000-0.050), indicating similar genetics content in term of alleles and their frequencies. The Bayesian MLG analysis confirmed that the allelic profiles of T.spiralis isolates' from pigs and
rats collected on the farm were almost identical, and at the same time were different from other *T. spiralis* isolates derived from wild boars. The Bayesian analysis of MLGs membership of individual larvae showed low level of genetic admixture in *T. spiralis*, and opposite in *T. britovi*, where most of the isolates showed a MLG largely admixed. This and previous study suggest that microsatellite MLGs could be a useful tool to investigate transmission of *Trichinella* spp. However, the low genetic variability of *T. spiralis* and low number of polymorphic loci in *T. britovi* available at present indicate the necessity to discover more polymorphic microsatellites in order to draw a more ‘deep’ MLGs in the future which could strengthen the results of analysis.

46

**Complete mitochondrial genomes and ribosomal DNA sequences of *Trichinella spiralis* indicate that the split between Asian and European populations happened prior to the rise of agriculture.**

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Available evidence suggests that *Trichinella spiralis* first originated in Asia and subsequently spread to the rest of the world. Notably limited genetic diversity in European *T. spiralis* isolates indicates that the parasite went through a dramatic genetic bottleneck at some point in its history. Did this genetic bottleneck result from the transport of a limited number of *T. spiralis* infected pig hosts from Asian centers of domestication? Or was the parasite resident in Europe far earlier than the domestication of pigs there? In order to answer this question, we generated complete mitochondrial genomes and ribosomal DNAs from seventeen European, six North American and seven Asian isolates. A total of 13,858 base pairs of mitochondrial DNA and 7431 nucleotides of ribosomal sequence were aligned and subjected to phylogenetic analysis using other *Trichinella* species as outgroups. We confirmed that North American and European isolates were tightly clustered within a single “western clade”. All Chinese *T. spiralis* were placed within a well-supported sister clade. These results indicate that European *T. spiralis* did not recently descend from Chinese parasite populations. Furthermore, considerable distinctions between European and Asian parasites suggests that they diverged before pigs were domesticated. The distinct geographic distributions of these two clades suggest that these parasite populations have remained separate ever since. We conclude that the genetic bottleneck diminishing variation in European *T. spiralis* did not result from recent migration of founders from China, but instead most likely occurred earlier, near the end of the last glacial maximum.

47

**Ascaris suum intestine: a new target site for cholinergic anthelmintic therapy**

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*Ascaris suum*, is a gastrointestinal parasite that has negatively impacted the health of pigs and caused significant constraints and economic losses in the swine industry. In the absence of vaccines, anthelmintics have been the only effective method for the treatment of infections. However, the intensive use of these drugs has given rise to concerns about the development of resistance. Anthelmintics like levamisole and pyrantel selectively activate a subgroup of
nicotinic acetylcholine receptors (nAChRs) in the nematode nerves and muscle, yielding therapeutic effects. nAChRs are transmembrane proteins comprised of five subunits that surround a central cation-permeable pore. Different combinations or stoichiometry of subunits gives rise to a wide diversity of receptor pharmacology. Numerous studies have characterized the physiological roles of these receptors in muscle cells and neurons, but their role in non-neuronal tissues remain mostly unknown. Here we focus on the nematode intestine, which lacks muscles and neurons and is involved in digestion, absorption, defense against environmental toxins, and many other essential processes, making it an attractive site for anthelmintic intervention. RT-PCR was used to confirm the expression of nAChR subunits: Asu-unc-38, Asu-unc-29, Asu-unc-63 and Asu-acr-8 that constitute the putative levamisole receptor in adult female *A. suum* intestine. We then validated these findings by using RNAscope *in situ* hybridization to localize the subcellular distribution of the subunits in the intestine, and qPCR to compare mRNA levels in both muscle cells and intestine. Our calcium imaging results also demonstrated that both acetylcholine and levamisole elicit intracellular calcium responses in the intestinal tissue. These findings suggest that the presence of nAChR in the intestine may not be limited to neuromuscular transmission, but an acetylcholine paracrine function. Further studies on the mechanisms involved in intestinal nAChR signaling are paramount for therapeutic exploitation.

**Poultry Nematodes**

48

*Ascaridia* spp. of poultry provide an optimal model for evaluating the efficacy of anthelmintics and for studying the biology and genetics of anthelmintic resistance in *Ascarid* nematodes.

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*Ascaris lumbricoides* is the most prevalent and one of the most important soil transmitted helminths (STH) of humans, with more than one billion people infected worldwide. Mass drug administration (MDA) programs for STH using benzimidazole anthelmintics provide many public health benefits; however, a major concern with MDA program scale-up is the development of anthelminthic resistance, which is a longstanding and severe problem in nematode parasites of animals. Despite these concerns there still are no reports of benzimidazole resistance in *A. lumbricoides*. Currently, the most commonly used model for *A. lumbricoides* is *A. suum* in swine, but this model is both difficult and expensive to maintain, and no known benzimidazole-resistant isolates exist. In contrast, we recently confirmed benzimidazole resistance in two isolates of *Ascaridia dissimilis*, a closely related Ascarid nematode of turkeys. Here we propose an alternative model for the study of benzimidazole resistance in Ascarids using *Ascaridia* spp. of poultry, parasites that are very similar to *A. lumbricoides* and *A. suum*, both biologically and genetically. This model is also well suited for measuring the efficacy of new candidate anthelmintics against Ascarids. Major benefits of the poultry model include ease of animal handling, minimal space requirements, and dramatically lower costs. For a similar cost to purchase a single weanling pig, up to 300 birds can be acquired, and daily housing cost for the 300 birds would be less than for the single pig. Also, *Ascaridia* reaches patency 2-3 weeks earlier than *Ascaris*, further decreasing time, effort and costs. Lastly, these differences permit the use of much larger sample sizes and treatment group replication, yielding much higher statistical power for detecting differences in efficacy among treatment groups. We are currently using this
model system to investigate whether mutations in the beta-tubulin gene are associated with the benzimidazole-resistant phenotype in Ascarid nematodes.

**Small Ruminant Nematodes**

49  
**Effect of inactivated *Bacillus thuringiensis* with cytosolic Cry5B protein on *Haemonchus contortus* in experimentally infected sheep**  
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The widespread anthelmintic resistance seen in trichostrongyle nematodes of ruminants creates a pressing need for new control methods. Cry5B, a crystal protein produced by the bacterium *Bacillus thuringiensis (Bt)*, has displayed significant anthelmintic effect both *in vitro* and *in vivo* against multiple nematode parasites, including *Haemonchus contortus*. In this study an inactivated asporogenous *Bt* strain expressing cytosolic Cry5B (IBaCC), was tested against *H. contortus* in sheep. Six female and six castrated male lambs aged 7-8 months were removed from pasture and housed for the duration of the study. Each animal was dewormed to remove existing trichostrongyle infection and then infected with 10,000 *H. contortus* third stage larvae. Six weeks later, the sheep were divided into control and treatment groups balanced for sex and mean FEC determined by Mini-FLOTAC© (detection limit 5 epg). The treatment group received IBaCC (60mg Cry5B /kg orally once daily for three days). The control group was given an equivalent volume (200mL) of water for three days. FEC were determined daily for eight days post treatment until animals were euthanized and abomasa removed to determine worm burden. A 72% reduction (P=0.05) was seen in mean total worm burden of the treated animals relative to controls, with a 95% reduction in female worm numbers in treated animals compared to controls. The mean FEC of treated animals was reduced by 91% relative to controls after three doses and remained significantly lower until euthanasia. These results indicate that live *Bt* is not required for efficacy of Cry5B against *H. contortus* and provide additional evidence that *Bt* Cry5B protein shows promise as a treatment for *H. contortus* in sheep. Research supported by USDA-NIFA Project #1008776: Engineered Probiotics for Farm Animals and Human Nematodes.

50  
**Molecular characterization and immune-reactivity patterns of two Novel *Haemonchus contortus* cathepsin Bs**  
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*Haemonchus contortus* is a pathogenic, gastrointestinal nematode parasite of small ruminants that causes huge economic losses to the industry worldwide. Disease caused by *H. contortus* leads to high morbidity and mortality due to increasing resistance to anthelmintics and the lack of a long lasting, cost efficient vaccine. Despite extensive efforts, our understanding of the molecular mechanisms that these parasites use to evade host immune responses remains in question. Cathepsin B (CBP) is one member of the cysteine protease family that plays an important function in worm invasion, migration, molting and immune escape. CBP is unique due to its dual activities as both an endopeptidase and carboxy-exopeptidase. Previously, we
identified *H. contortus* CBPs by *in silico* comparative sequence analysis of conserved proteins among clade V parasitic nematodes. To this end, we have cloned, expressed, and functionally characterized two novel CBPs from *H. contortus*, designated Hc-CBP-1 and Hc-CBP-2. The corresponding genes encode proteins of 350 and 346 amino acids in length, respectively. Functional and degradative roles showed activity over a broad pH range. Immunohistochemistry showed the binding of Hc-CBP-1 to the brush borders of the intestine and, HC-CBP-2 interacted with longitudinal muscles and intestinal regions consistent with differences in localization and expression patterns. Using peptide displays incubated with sera from immunized rabbits and experimentally infected sheep, we identified dominant but non-overlapping epitopes on both proteins. ELISA results showed that Hc-CBP-1 was present in *H. contortus* adult excretory secretory products. Hc-CBP-2 specific antibodies neutralized the Hc-CBP-2 activity by ~40%; however, no effect was observed on the activity of Hc-CBP-1 when incubated with Hc-CBP-1 specific antibodies. These results suggest that the two Hc-CBPs have differences in function and expression during the infection in the host and are promising candidates for developing vaccines against haemonchosis.

51

**The use of deep amplicon sequencing in molecular diagnostics and molecular epidemiology of anthelmintic resistance**

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Benzimidazoles anthelmintics are the class of drugs for which we have an understanding of the molecular basis of resistance, particularly in gastrointestinal nematodes of sheep. Consequently, this provides an excellent system to explore the use of next-generation sequencing approaches to improve anthelmintic resistance diagnosis and undertake molecular epidemiology studies. We harvested populations of L1 larvae from ovine fecal samples, before and after treatment with several anthelmintics, from over 90 sheep flocks across western Canada. We used deep amplicon sequencing to screen for known resistance associated mutations in the isotype-1 beta-tubulin locus of the major gastrointestinal nematode species. F200Y alleles were present at very high frequencies (>95%) in almost all *Haemonchus contortus* populations but at much more variable frequencies of F200Y alleles in *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. The F167Y, E198A or E198L mutations were essentially absent from all three species. Other than those populations with already very high pre-treatment F200Y frequencies, there was a significant increase in the F200Y mutation frequency following treatment with benzimidazoles but not with other drug classes, such as ivermectin, supporting its important role in benzimidazole resistance. Analysis of fecal egg count reduction test data showed good agreement when parasite populations were categorized as susceptible or resistant by phenotype compared to isotype-1 beta-tubulin F200Y mutation frequency, illustrating the diagnostic value of this marker. We are undertaking haplotype network analysis of this large isotype-1 beta-tubulin dataset to provide insights into the origins and spread of resistance. Finally, we are also using the same approach described above to investigate the potential role and relative importance of mutations in the isotype-2 beta-tubulin gene which has yet to be explored in detail.
52 Molecular epidemiological evidence for the spread benzimidazole resistance in the sheep parasitic nematode *Nematodirus battus* from a single source in NW England

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Routine anthelmintic drug use has resulted in widespread drug resistance in many parasitic nematode species. Due to the high cost of drug development, it is unlikely that new drugs will be produced in the foreseeable future. Consequently, it is crucial to minimize the development of resistance and maximize the life of the few available drugs. In order to achieve this, we need a better understanding of how drug resistance develops and spreads. Currently, our understanding of benzimidazole resistance in small ruminants is the most advanced: resistance is associated with single nucleotide polymorphisms (SNPs) of isotype-1 β-tubulin gene at codons 167 (F167Y), 198 (E198A), and 200 (F200Y). The lack of sensitive diagnostic tools means that anthelmintic drug resistance is typically only detected at a late stage when resistance is already widespread. This makes it challenging to study its evolution and spread. The situation with *Nematodirus battus* in UK sheep flocks provides a rare opportunity to study the emergence of anthelmintic resistance at early-stage. Short-read deep amplicon sequencing was performed on the isotype-1 β-tubulin gene from fecal samples from over 150 UK sheep flocks. Haplotype network analysis was performed to determine the distribution and relationship of benzimidazole resistant and susceptible alleles mutations in *Nematodirus battus* across the UK regions. The data suggest that the major codon 200 and 167 resistance alleles have each spread from single sources in NW England. A variety of rarer resistance alleles have been detected, and we are currently investigating approaches to determine the validity of rare alleles. This unique data set illustrates the importance of animal movement and resistance allele spread in the early emergence of anthelmintic resistance and provides us with an opportunity to explore analytical approaches for investigating the validity of rare alleles in deep amplicon sequencing data sets.

53 Evidence for wild cervids as transmission vectors of small ruminant drug resistant gastrointestinal nematode parasites

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Anthelmintic resistance is widespread in gastrointestinal nematode (GIN) parasites of small ruminants making control increasingly difficult. Wild cervids (e.g. roe deer, red deer, mule deer and elk) are competent hosts for many small ruminant GIN species and often range over pastures grazed by domestic sheep. Here we investigate the role of wild cervids in translocating drug resistant parasites between sheep pastures and farms. We sampled three wild cervid populations in France; one population with overlapping grazing with domestic sheep (Aurignac) and two
populations with no opportunity for such interaction (Chize and Trois-Fontaines). Relative species abundance, determined by ITS-2 nemabioime metabarcoding, revealed that for deer with no access to sheep pastures, GIN communities comprised mainly of species typically found in wild cervid hosts such as *Spiculopteragia spiculoptera*. In contrast, for deer that were sympatric with sheep, the GIN communities had similar compositions as those of sheep of the same region with *H. contortus*, *T. colubriformis* and *C. curticei* predominating. Deep amplicon sequencing of the isotope-1 beta tubulin gene revealed resistance mutations in four GIN species (*H. contortus, C. curticei, T. circumcincta* and *T. colubriformis*) in both sheep and wild deer from the co-grazed samples. 198A and 200Y mutations predominated in *H. contortus* and *T. colubriformis* respectively from both sheep and co-grazing deer and haplotype network analysis revealed shared resistance alleles (with low frequencies of the 200Y mutation in *H. contortus*, *C. curticei* and *T. circumcincta*). The close similarity of GIN species abundance and the sharing of benzimidazole resistance alleles proves strong evidence for the co-transmission of benzidiazole resistant GIN parasites between sheep and wild cervids sharing pasture.

**Wildlife Nematodes**

54

**Detection of Trichinella murrelli and T. pseudospiralis in bobcats (Lynx rufus) from Oklahoma**

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*Trichinella* spp. infect wild carnivores throughout the world. We determined the prevalence and mean intensity of *Trichinella* spp. in bobcats (*Lynx rufus*) from 41 counties in Oklahoma. Tongues from 301 bobcats were examined using artificial tissue digestion. The prevalence (95% confidence interval) of *Trichinella* spp. was 5.9% (3.7%–9.2%) in which 18 of the 301 bobcats were infected. Bobcats infected with *Trichinella* spp. were detected in 10 of the 41 (24.4%; 13.7%–39.5%) counties sampled. Although variable, a statistically significant difference was not detected in the prevalence of *Trichinella* spp. among counties where bobcats were collected. The mean intensity of *Trichinella* sp. larvae ranged from 0.6–119.9 larvae per gram of tissue examined. Genotyping results demonstrated that 17 bobcats were infected with *Trichinella murrelli* and one bobcat was infected with *Trichinella pseudospiralis*. This is the first report of *T. pseudospiralis* in bobcats and in Oklahoma. Considering that bobcats are infected over several different counties, the obligate carnivore likely plays an important role in maintaining sylvatic cycles of *T. murrelli* in Oklahoma.

55

**Phylogenetic relationships within the nematode subfamily Phascolostrongylinae (Nematoda; Strongyloidea) from Australian macropodid and vombatid marsupials**

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The strongyloid nematode subfamily Phascolostrongylinae are endemic parasites of the forestomachs and large intestines of macropodid (kangaroos and wallabies) and vombatid
(wombats) marsupials. Current understanding of the taxonomy and evolution of the Phascolostrongylinae is based on phenotypic data. Previous molecular studies have detected the presence of cryptic species and examined phylogenetic relationships at the generic level, but none has determined the relationships at the subfamily level. We characterised the first and second internal transcribed spacers (ITS+) of the nuclear ribosomal DNA of *Paramacropostrongylus* spp. from grey kangaroos (*Macropus giganteus* and *Macropus fuliginosus*), *Oesophagostomoides* spp. and *Phascolostrongylus turlei* from wombats (*Vombatus ursinus* and *Lasiorhinus latifrons*). We conducted phylogenetic analyses of these ITS+ sequences along with published sequences of *Macropostrongyloides* spp., *Hypodontus macropi* and *Macropicola ocydromi* from macropodid and vombatid marsupials. Our results suggest that Phascolostrongylinae is monophyletic. However, *Macropostrongyloides* and *Paramacropostrongylus* appear to be paraphyletic. *Macropostrongyloides dissimilis* parasitic in the swamp wallaby (*Wallabia bicolor*) formed a clade with *Paramacropostrongylus iugalis* and *P. typicus*, to the exclusion of *P. toraliformis* from eastern grey kangaroos which was placed in a clade with other species of *Macropostrongyloides*. These phylogenetic associations probably relate to the predilection sites within the hosts, with *P. iugalis*, *P. typicus* and *M. dissimilis* occurring in the stomach and *P. toraliformis* and the remaining species of *Macropostrongyloides* in the large intestine. The phylogenetic data are further discussed in light of previous morphological hypotheses and their implications for the current classification of the Phascolostrongylinae.

**Education**

56

The future of Veterinary Parasitology in the classroom: Take-aways from the 2019 AAVP Educators Meeting

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The Educators Meeting, founded by Dr. Dwight Bowman, has become an integral part of AAVP, bridging a valuable relationship with CAPC, and bringing together faculty dedicated to veterinary parasitology education. Themed ‘The Future of Veterinary Parasitology Education: Maximizing Significant Learning’, the 2019 AAVP Educators Meeting incorporated hands-on, interactive and creative ways to focus on how we can emphasize parasitology across the veterinary curricula, in a time when the curriculum is being limited for many educators. Thirty faculty representing 22 universities came together for this full day meeting. We introduced a variety of different out-of-the-box ways to energize students and faculty, alike. From podcasts, to Escape Boxes, to developing a mock lab using powerpoint – there is something available for anyone trying to move away from traditional didactic lectures. We also discussed ways to better assess clinical reasoning of students and other resources available to help keep students engaged and abreast of current parasitology topics. Participants began with limited experience and uncertainty about what could change and left with excitement and eagerness to try something new. Participant feedback was encouraging, demonstrating the need for this invaluable meeting for years to come.
COVID 19 induced changes to teaching veterinary diagnostic parasitology.
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In the preclinical years at The Ohio State College of Veterinary Medicine (OSU CVM) Parasitology instruction occurs over three years with applied diagnostic laboratory components taught in small groups (17 to 21 students) in two-hour laboratories with a total of 15 to 16 laboratories in the course. During Fall semester of the 2019-20 academic year, 35 students participated in a block schedule whereas, in the Spring, 119 students participated once weekly. The course used the same teaching objectives and exercises in both semesters. On March 9, COVID-19 restrictions required OSU CVM to change all courses to remote online instruction. This significantly disrupted traditional courses including, applied components. For the applied diagnostic parasitology laboratory course, we converted our in laboratory exercises to the online, remote delivery. This included “class wrap-up sessions,” “VM I-III pairing,” “unknown slide reading,” and “final examinations.” Online teaching provides some unique and attractive alternatives. While appealing the online teaching environment does not mimic applications performed in the teaching laboratory or fully develop the classroom discussion environment. These student cohorts participated in a hybrid online/laboratory course during their prior VM II year. Thus, an online parasitology course was not novel to this class. We compared student evaluations of instruction, assessments and grades. We anticipate following this cohort of students through their clinical year where students will perform their own parasitology examinations and interpret laboratory test results from samples collected from their patients.

Other (Fish and Reptiles)

Fish Host Susceptibility Influences Myxozoan Community Composition In Proliferative Gill Disease Of Catfish Aquaculture
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_Henneguya ictaluri_, the cause of proliferative gill disease (PGD) in channel and hybrid catfish, is an important myxozoan parasite of commercial catfish aquaculture in the southeastern United States. Research indicates there is arrested sporogenesis in channel (_Ictalurus punctatus_) × blue (_Ictalurus furcatus_) hybrid catfish, yet PGD persists in hybrid production systems. These reports suggest other myxozoans besides _H. ictaluri_ may be associated with PGD. Further, it is hypothesized host susceptibility drives myxozoan diversity in catfish pond aquaculture systems. This work investigated the influence of catfish host on myxozoan community composition within 1) naturally infected gill tissues and 2) pond water associated with channel and hybrid catfish monoculture. For three years, DNA extracted from gills of diagnostic case submissions with clinical PGD and water from experimental ponds dedicated to either channel or hybrid catfish monoculture were submitted for metagenomic sequencing to compare myxozoan community
composition and diversity between catfish species and assess year-to-year trends. Myxozoan community composition significantly differed between channel and hybrid systems in gill and pond water datasets. Channel gills had greater relative abundance of *H. ictaluri* in 2017 and 2019 and unclassified taxa in 2018 compared to hybrids. *H. ictaluri* was present in all channel and hybrid PGD cases but was not the most prevalent taxon in nearly half. In the pond experiment, *H. ictaluri* relative abundance was greater in channel ponds in years 2 & 3. In hybrid ponds, *H. ictaluri* never exceeded 20% average relative abundance. Both datasets revealed hybrid catfish monoculture selectively suppresses *H. ictaluri* proliferation. This work suggests crop rotation strategies could mitigate disease by preventing *H. ictaluri* from reaching levels associated with catastrophic losses. Detection of numerous unclassified taxa may further indicate PGD involves mixed species infections. Future work will investigate the potential contribution of other myxozoans to gill pathology in PGD outbreaks.

59

*Raillietiella orientalis*, an invasive pentastome, in a banded water snake (*Nerodia fasciata*) in north central Florida

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Pentastome parasites are found in a variety of definitive hosts, and utilize intermediate hosts including mammals, reptiles, amphibians, fish and arthropods, depending on the species. In Florida, the Burmese python (*Python bivittatus*) was introduced in the 1990s and are known in the southern most regions of the state. In recent years, a common respiratory pentastome of the pythons, *Raillietiella orientalis*, has been found in numerous native snake species. This includes native snake species not only in the python’s known geographical range in Florida, but also in central Florida in pygmy rattlesnakes (*Sistrurus miliarius*) and most recently in an adult, female, free-ranging banded water snake (*Nerodia fasciata*) in north central (Alachua County), Florida. Pentastomes of *R. orientalis* were recovered from the lungs, trachea, oral cavity, and esophagus, of this animal. Additionally, the snake was infected with *Ochetosoma* sp. trematodes and had ophidiomycosis (snake fungal disease). Pentastomiasis was presumed to have contributed to the death of this snake. This is the first report of *R. orientalis* in north central Florida, indicating movement northward outside of the known geographical range of this pentastome in Florida. While the exact clinical significance of this parasite is unknown, the variety of species it can infect and its relatively rapid movement northward is of great concern for reptile conservation.
Cattle Protozoa

60 Retrospective Analysis of Bovine Eimeria Cases Detected During 2010-19 at the Animal Health Diagnostic Center, New York State, USA
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Bovine coccidiosis is a disease of economic importance to the cattle industry worldwide. It is caused by members of the Genus: Eimeria, of which, thirteen valid species are known to infect cattle in North America. Among them, Eimeria bovis and E. zuernii are highly pathogenic while others are almost always of moderate to very low pathologic significance. Fecal analysis to confirm coccidiosis must identify and differentiate the pathogenic from the non-pathogenic species. This is necessitated by the fact that the anticoccidial drugs available on market are labeled specifically for E. bovis and E. zuernii. Classical detection based on morphology and morphometry of Eimeria oocysts remains the mainstay for species differentiation. Although it requires considerable diagnostic parasitology expertise, the fecal test (modified Wisconsin) offered at the AHDC Parasitology lab allows differentiation of Eimeria to species. In this study, we retrospectively analyzed the frequency of occurrence of bovine Eimeria species diagnosed at the AHDC during 2010-2019, with main focus on pathogenic species. A total of 16,563 bovine fecal floatation test results were retrieved from the current laboratory management system (VetView) and analyzed. The frequency of occurrence of various Eimeria species were as follows: E. bovis (4408, 26.6%), E. ellipsoidalis (3195, 19.3%), E. auburnensis (1971, 11.9%), E. zuernii (1921, 11.6%), E. cylindrica (1832, 11.1%), E. alabamensis (1782, 10.8%), E. subspherica (1248, 7.5%), E. canadensis (1161, 7.0%), E. bukidnonensis (506, 3.1%), E. brasiliensis (134, 0.8%), E. wyomingensis (47, 0.3%), E. pellita (18, 0.1%) and E. illinoisensis (0, 0%). Despite the limitations of classical techniques for accurate differentiation of Eimeria species, especially the closely related ones, the data clearly indicate that the highly pathogenic E. bovis occurs in higher frequency. This suggests that E. bovis poses a continued threat to cattle industry in the NE United States.

61 Arthropod surveillance and the detection of Theileria orientalis in host-seeking Haemaphysalis longicornis in Virginia, U.S.A.
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The spread of non-native parasites with the movement of animals is a major concern for disease emergence and native species health and conservation. Haemaphysalis longicornis (Asian longhorned tick) is native to eastern Asia, but has become invasive in several countries including Australia, New Zealand, and recently the United States. Within its established range, H. longicornis is a vector of the protozoan parasite, Theileria orientalis Ikeda genotype, which until recently was not known to occur in the United States. In 2017, clinical disease due to T. orientalis Ikeda resulting in cattle mortality was reported in a cattle herd in Virginia U.S.A. Further investigation at the index site revealed H. longicornis infestations in early 2018, but it is
not yet known if *H. longicornis* transmitted *T. orientalis* to the cattle. From May 2019 – September 2019, we conducted environmental surveillance for *H. longicornis* at the cattle farm where *T. orientalis* Ikeda genotype was first detected to determine the possible role of *H. longicornis* in the transmission of *T. orientalis*. We also screened white-tailed deer (*Odocoileus virginianus*) from this region in Virginia to investigate their potential role as reservoirs for *T. orientalis*. We document the detection of exotic *T. orientalis* Ikeda genotype in environmentally collected *H. longicornis* using both the 18S rRNA and major piroplasm surface protein (MPSP) gene targets from the Virginia index site. Native ticks collected from this site were all negative for exotic *T. orientalis*, but we detected other native protozoan parasites. No cervids sampled during this study were positive for *T. orientalis*. This detection offers new insight into the risks associated with the introduction of this exotic tick to North America.

**Dog/Cat Protozoa**

62 *Dinofuran, pyriproxyfen and permethrin combination abrogates Leishmania infectiousness by sick dogs: a potential leishmaniasis control tool*  
Gioia Bongiorno*1, Antonio Bosco2, Riccardo Bianchi1, Laura Rinaldi2, Valentina Foglia Manzillo2, Manuela Gizzarelli2, Daniela Giaquinto2, N El Houda BenFayala2, Marie Varloud3, Alessia Crippa3, Gaetano Oliva2, Luigi Gradoni1, Giuseppe Cringoli2. 1Istituto Superiore di Sanità, 2University of Naples Federico II, 3Ceva Santé Animale

Introduction: Dogs are reservoir hosts of leishmaniasis caused by *Leishmania infantum* and transmitted by competent phlebotomine vectors. This study assessed the efficacy of dinofuran, pyriproxyfen and permethrin spot-on solution (Vectra®3D, Ceva Santé Animale) to reduce *Leishmania* transmissibility by infected dogs via *Phlebotomus perniciosus*. Animals naturally affected by clinical leishmaniasis were enrolled and xenodiagnosis was performed using colonized sand flies to evaluate antifeeding and insecticidal activity of the topical treatment.

Materials and Methods: Leishmaniasis diagnosis was based on clinical examination, serology and LAMP analysis. Infected dogs served as their own control through infectiousness tests performed by xenodiagnosis 7-28 days before treatment. Only dogs infecting ≥10% of sand flies were treated with Vectra®3D on Day 0. Caged animals were exposed to 85-141 sand flies for 1.5h on Days 1, 7 and 28 post-treatment. Blood feeding and mortality rates were assessed at 24h post exposure, whereas promastigote detection and assessment of stage maturation (transmissibility rate) were performed by dissection of live blood-fed sand flies up to 96h post blood meal. Results and Conclusions: Among 17 infected dogs evaluated, 6 were enrolled showing infectiousness rates in a 25.5-78.9% range. Pre-treatment feeding rates ranged 39.3-90.7% and transmissibility rates 19.6-63.6%. On Day 1, anti-feeding efficacy ranged 80.6-100% (>90.0% in 4 dogs) and insecticidal efficacy 75.9-100% (100% in 4 dogs). Anti-transmissibility effect was 100% in all animals. Day 7 assessments resulted in efficacy rates very similar to Day 1. On Day 28, anti-feeding efficacy was in a variable range of 32.6-100% (>90.0% in 1 dog); insecticidal efficacy was also variable (18.0-100%) (100% in 3 dogs). Transmissible infections were demonstrated in sand flies fed on 2 dogs affected by generalized cutaneous lesions, however 78.9-93.6% reduction in transmissibility rate was observed. Altogether the tested product abrogated by 95.4% the *Leishmania* transmissibility of the examined pool of infected dogs.
Examining the relationship between dogs with overt clinical leishmaniasis, infectiousness to *Phlebotomus perniciosus* and its infectivity

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Introduction: Infected dogs are considered the main domestic animal reservoirs for *Leishmania infantum* parasite. Infectiousness to competent phlebotomine vectors has been associated with many factors, the main being the severity of the disease exhibited by infected dogs. This study examines the relationship between different clinical parameters and the infectiousness to colonized *Phlebotomus perniciosus* sand flies having a blood meal on dogs. Data obtained in the present study come from an untreated group of *Leishmania* sick dogs submitted to xenodiagnosis for the evaluation of a spot on insecticide solution. Methods: 17 dogs were diagnosed as affected by leishmaniasis through clinical examination, IFAT serology and LAMP-PCR. The disease severity (clinical score) was staged by using a numeric value derived from 8 clinical and parasitological parameters. Xenodiagnosis was performed on caged dogs exposed for 1.5h to sand flies (n. 85-141) bites. The following parameters related to sand flies were examined: blood feeding (% of engorged females), promastigote detection (% of promastigote-positive sand flies), promastigote burden, and the promastigote stage maturation (transmissibility rate). Statistical relationship between the clinical score and entomological parameters was investigated, as well as the possible correlation between each clinical and laboratory parameters and sand fly infection/infectivity. Results and conclusions: The severity of clinical score may influence the blood feeding by, and the promastigote detection in, sand flies (r²: 0.614 and 0.493); skin lesions seem to be the main factor that influences the blood feeding (r²: 0.543). Promastigote burden is related to IFAT titer, skin lesions and clinical score (Cramér’s V: 0.553; 0.758; 0.761). All entomological parameters are strongly related among them. This study confirms that both *P. perniciosus* infection and infectivity are influenced by dog’s clinical condition.

Horse Protozoa

Predicting surface antigen variation in *Sarcocystis neurona* isolates

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*Sarcocystis neurona* is an obligate intracellular parasite and is the causal agent of equine protozoal myeloencephalitis (EPM). Surface Antigen (SAG) proteins of *S. neurona* have been shown to be useful in developing assays for the diagnosis and timely treatment of EPM. These SAGs and SAG-related sequences (SRSs) represent a gene family of paralogous proteins that have arisen through gene duplication and deletion events. Importantly, some of these SAG/SRS proteins have been shown to be omnipresent among all *S. neurona* isolates examined while others are isolate specific. *S. neurona* is currently represented by reference genomes derived from two strains, SoSN1 and SN3.e1, which are available in public databases. At present, the S.
The genome of the parasite *S. neurona* contains 23 annotated SAG/SRS genes. Recent next generation sequencing (Illumina) and subsequent annotation efforts have been undertaken to document the genomes of additional *S. neurona* isolates (SN4, SN-OT1, and SN138). In silico homology-guided searches are being conducted to identify additional SAG/SRS differences between the SN3.e1, SoSN1 SN4, SN-OT1, and SN138 isolates. Greater understanding of these antigenic variances may provide insights into the biology of this parasite while potentially improving the ability of clinicians to detect and treat horses suffering from EPM.

### Transplacental transmission of *Babesia caballi* from carrier mares to foals

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Equine piroplasmosis is a globally significant intra-erythrocyte protozoa disease caused by *Babesia caballi* (*B. caballi*) and *Theileria equi* (*T. equi*) and is considered endemic in most countries worldwide. The major modes of EP transmission are tick vector transmission, iatrogenic transmission and transplacental transmission. Transplacental transmission of *T. equi* has been reported at present, evidence of *B. caballi* infection via transplacental transmission in utero is lacking. In this study, a case of equine piroplasmosis due to *B. caballi* was diagnosed. A pregnant mare extremely thin, powerless, unwilling to feed, fever and showed symptoms of anemia and icteric. Blood from this mare was collected and blood smears showed that there were piroplasms with different shapes in the red blood. There was no improvement after treatment and the mare died after premature delivery. The foal kept indoors after birth, not able to stand upright, weak, no strength, no other specific symptoms and there was not any tick bite being detected after a whole-body examination, but it died three days after birth. We didn’t find the piroplasms in the blood smears (collected within 12 hours of birth) of the foal. Molecular diagnosis using 18S rRNA based cPCR was performed to detect parasites in the mare and the foal, clone and sequence analysis revealed that the etiology is *B. caballi*. This is the molecular evidence that *B. caballi* can occur via transplacental transmission from carrier mares to foals. The results lay a foundation for preventing transplacental transmission and establishing effective control measures. * This work was supported by the National Key Research and Development Program of China (2017YFD0500404), the Young Talents Project of Heilongjiang Bayi Agricultural University (CXRC2016-08) and Heilongjiang Bayi Agricultural University Graduate Innovative Research Project (YJSCX2019-Y30). Keywords: *B. caballi*, transplacental transmission, cPCR, horse
Protozoa Environmental Survival

66

**Battening down in times of stress and adversity – how Apicomplexa survive hostile environments**
Perryn Kruth*, Taylor Lane, John Barta. University of Guelph

Metabolic downregulation – for both brief and extended periods of time – is an important feature of apicomplexan lifecycles. Hypobiosis of endogenous stages can facilitate transmission by minimizing immune activation, thereby prolonging infection. In exogenous stages, dormancy promotes transmission by extending the window of viability of infectious parasites. Oocysts of *Eimeria* spp. can remain infectious in the environment for years. One of the largest impacts of members of this genus is to the commercial poultry industry, where coccidiosis is one of the biggest biological threats to production. *Eimeria* spp. that infect chickens are easy to work with and are ubiquitous, making them attractive model organisms for the study of apicomplexan hypobiosis. We have applied transcriptomics to examine the strategies used by *Eimeria tenella* sporozoites to persist in the environment and become quickly reactivated upon ingestion by the host. Research suggests a reliance on the sequestration of mRNAs, stalled transcription pre-initiation complexes, and sporozoite proteins for the ability to become rapidly activated to enter host cells. Another likely feature of this stress-like response is a global reduction in metabolic activity to preserve limited resources, with only a minimal set of genes being expressed. Apicomplexan control of gene expression is primarily achieved via chromatin-level genome reorganization and post-transcriptional regulation, with transcriptional regulation having a lesser role. A “what you see is what you get” approach to interpreting the apicomplexan transcriptome is therefore inappropriate – presence of non-coding RNAs and stored mRNAs must be taken into consideration. In this talk, a theoretical model for control of gene expression in sporozoites is discussed. Preliminary transcriptomic data is presented, and ongoing and future directions of research are shared.

Poultry Protozoa

67

**Back to basics: Genome replication dynamics in exogenous stages of Eimeria tenella**
Taylor Lane*, John Barta, Perryn Kruth. University of Guelph

The phylum Apicomplexa includes a number of notorious protozoan pathogens. Apicomplexan lifecycles include both haploid and diploid stages that, when combined with short generation times, contribute to rapid evolution across the phylum. One of the apicomplexan genera with the greatest health impact to non-human hosts is *Eimeria*. Species within this genus infect a wide range of vertebrate hosts; high population density makes poultry, cattle, and sheep in production farming particularly susceptible. Effective, accessible, and responsible measures for controlling the disease caused by these parasites, coccidiosis, are therefore of great importance to the security and sustainability of food-animal production. Loss of anticoccidial drug efficacy, concern for possible environmental impacts, legislative changes, and shifting consumer demand have all contributed to the increased reliance on immunological coccidiosis control strategies. Live vaccines use oocysts (infective parasite stages) to establish mild and self-limiting infection
that circulates throughout a flock or herd, resulting in robust protective immunity. Although live vaccines are attractive on paper, significant optimization is required before they can replace the use of prophylactic drugs. The eimerian oocyst, the lifecycle stage on which live vaccines depend, is a black box. Before effective immunological control of coccidiosis can become a reality, we must improve our foundational biological understandings of this enigmatic parasite stage. We have applied quantitative PCR to characterize the replication dynamics of the apicoplast, mitochondrial, and nuclear genomes of *Eimeria tenella* throughout the exogenous portion of the lifecycle during the largely unexplored process of sporulation. Understanding the relationships between genome copy numbers during exogenous development is the first step towards expanding our understanding of the roles of extranuclear genes during this critical phase. With our research we aim to expand fundamental biological understandings of these and related parasites that could help inform the optimization of live vaccines.

68

**Who’s killing the Partridge Family? *Eimeria* species and clinical coccidiosis in commercial poultry.**

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Clinical coccidiosis in commercial partridge flocks is responsible for high flock mortalities and associated welfare and production impacts. Reported attempts of standard live-vaccination employing small numbers of infective oocysts given to day-of-age birds to elicit protective immunity has proven unsuccessful when reared on the ground. Partridge chicks appear unable to acquire protective immunity fast enough to prevent clinical coccidiosis resulting from high environmental challenge with these parasites. *Eimeria* species were isolated and characterized from European and North American samples from commercial partridge flocks. Oocyst and sporocyst morphometrics indicated the presence of *E. kofoidi* and *E. legionensis* from red-legged partridge (*Alectoris rufa*) in France. Two *Eimeria* species present in a commercial flock of chukar partridge (*Alectoris chukar*) in Ontario, Canada, were morphometrically distinct from *E. kofoidi* and *E. legionensis* as well as all previously described *Eimeria* species from related galliform birds. Sequence-based genotyping of the complete mitochondrial genome and partial nuclear 18S rDNA was used for molecular characterization of all species. Experimental infections with coccidia-free chukar partridges were used to complete life cycle descriptions of these two species. Endogenous development was determined histologically from samples collected at 8 locations along the intestinal tract every 6 hours throughout prepatency. Daily fecal collection post-inoculation was used to define this prepatent period, the duration of shedding and the fecundity of each *Eimeria* species. Molecular and biological observations confirmed that neither *Eimeria* species has been reported previously; both will require formal species descriptions. Understanding of the biology of these parasites will inform development of vaccination methodologies designed to elicit protective immunity such as: 1) live oocyst vaccination followed by a carefully monitored 2-step partial house brooding; or, 2) a ‘bioshuttle’ (vaccination/anticoccidial combination). Effective coccidiosis control will enhance flock health and increase profitability for commercial chukar producers.
Macroscopic and microscopic lesions explain the pathogenicity of a ‘minor’ pathogen of turkeys, *Eimeria innocua*.
Rachel Imai*, Jessica Rotolo, Ryan Snyder, John Barta. University of Guelph

Historically, only *Eimeria meleagrimitis* and *Eimeria adenoeides* were considered highly pathogenic to turkeys. The recent observation that all 6 *Eimeria* species (including *Eimeria innocua*) that infect turkeys could be found in commercial flocks raises the question of the pathogenic potential of ‘minor species’. Detection of these ‘minor’ species has only become possible recently through application of a nested, species-specific PCR assay that can differentiate all 6 species in fecal samples. Endogenous development of *Eimeria innocua* had not been described previously; in order to properly characterize the damage caused by this parasite, each asexual generation was identified histologically along the GI tract. Animal experimentation was approved by the University of Guelph's Animal Care Committee (AUP e4314).

Macroscopic damage assessed using a lesion scoring system and histopathological observations provided direct evidence that pathogenic changes were attributable to this parasite. Female poults were reared coccidia-free and infected at 14, 23, 30 or 40 days-of-age by oral gavage with 10^2 to 10^6 oocysts per bird. Five days post-inoculation, turkeys were necropsied for description of macroscopic lesions and tissue collection for histopathology. *Eimeria innocua* produced macroscopically obvious pathological changes that, at higher doses, extended from the duodenal loop into the ileum (20cm or more beyond Meckel’s). Bleaching, ballooning and thinning of the intestinal mucosa were evident; unsurprisingly, lesion severity and length of affected intestinal tract increased with higher numbers of oocysts inoculated. However, dose-dependent increases in lesion severity were not consistent for younger poults; turkey gut maturation could be a potential mitigating factor impacting severity of infections to be considered when challenge experiments are planned. Microscopic lesions were extensive with villar atrophy evident in regions displaying macroscopic lesions and in adjoining regions. Body weight gains of poults inoculated with the highest challenge doses were up to 30% less than uninfected sham controls. Pathogenicity of *Eimeria innocua* may have been grossly underappreciated in past literature.

**Wildlife Protozoa**

70

*Toxoplasma gondii* Prevalence and Partial Genotypes in North American River Otters (*Lontra canadensis*) from the Upper Peninsula of Michigan
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*Toxoplasma gondii* is a ubiquitous parasitic protozoon that poses a health threat to human, domestic, and wild animals. A felid host is required for sexual reproduction of *T. gondii* and production of oocysts. Oocysts are shed into the environment and may persist for several years. Persistence in the environment coupled with runoff from rainfall and snowmelt carries the oocysts into waterways. Once in the water, oocysts may be concentrated and transported over great distances. Semi-aquatic mammals such as the Northern American river otter are particularly at risk of exposure as they may ingest the oocysts and become infected from both the
terrestrial and aquatic environments. Despite these risks, only a small number of studies have examined the prevalence of *T. gondii* in river otter populations in the United States. We sampled tongue tissue from otter heads submitted from the Upper Peninsula by trappers to the Michigan Department of Natural Resources in the 2018-19 harvest season. We extracted DNA from the tongue tissue and amplified a portion of the B1 *T. gondii* gene with PCR. We genotyped a subset of positive samples for comparison with known *T. gondii* sequences. Of the 124 samples tested, 35 (28.2%; 95% C.I: 20.7–37.1%) were positive for *T. gondii*. No significant differences were found in comparisons of *T. gondii* prevalence with host sex (X² = 0.6922; p=0.403), age (X² = 5.56; p = 0.8534), or region of the peninsula where the otter was harvested (X² = 5.238; p = 0.0697). Our results suggest that *T. gondii* infection in otter populations is widespread in the Upper Peninsula of Michigan.

71

**Species diversity and geographic variation of piroplasms in striped skunks (Mephitis mephitis) and spotted skunks (Spilogale spp.) in the United States**

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*Babesia* species are intraerythrocytic protozoan piroplasm parasites that infect a high diversity of hosts, including striped skunks (*Mephitis mephitis*). Previously, a single species, *Babesia mephitis*, was morphologically described from striped skunks in Maryland and a *B. microti*-like sp. sequence was detected in a striped skunk from Massachusetts. We aimed to determine the prevalence and diversity of piroplasm species in striped skunks and spotted skunks (*Spilogale* spp.) in selected areas of the United States. We also obtained partial 18S rRNA and cytochrome oxidase subunit 1 (*cox1*) gene sequences to investigate intraspecific variation. We tested DNA isolated from spleen and/or blood samples from Georgia, Kentucky, Missouri, Texas, Pennsylvania, Florida, Louisiana, South Carolina, and California for piroplasms. We used two PCR assays to screen skunks for infection with *Babesia* sensu stricto (s.s.) and/or *Babesia microti*-like sp. piroplasms. Positive samples were further tested by amplifying and sequencing partial 18S rRNA and cytochrome oxidase subunit 1 (*cox1*) genes to evaluate diversity and intraspecific variation. We tested 59 skunks (46 striped skunks, 5 western spotted (*S. gracilis*) and 8 eastern spotted (*S. putorius*)) and 66% [39/59] were positive, all for a *Babesia microti*-like sp. The 18S and *cox1* analyses indicate that there are two distinct *Babesia microti*-like sp. in skunks and they are different from other *B. microti*-like spp. from carnivores (e.g., fox, raccoons, badgers, etc.). Also, based on *cox1* sequences, one piroplasm species had ‘eastern’ and ‘western’ lineages which were not associated with specific skunk species (i.e., the ‘western’ lineage was found in striped and spotted skunks from California). Our data show that piroplasms are common in skunks and that striped and spotted skunks can host multiple piroplasm species. Additional work is needed to determine if there are any morphological differences between these two piroplasm species and if one of them represents *B. mephitis*. 

103
Prevalence of *Sarcocystis* spp. in North American river otters (*Lontra canadensis*) collected in Michigan

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*Sarcocystis* is a protozoan parasite with an indirect life cycle. Sexual development and oocyst formation of *Sarcocystis* spp. take place in the intestinal mucosa of the definitive host. Whereas in the intermediate host, asexual multiplication occurs in vascular endothelial cells and striated muscles. River otters can serve as intermediate hosts for *Sarcocystis* spp., but little is known about the occurrence or geographical range of the parasites in the United States. The aim of this study was to determine the prevalence of *Sarcocystis* spp. in river otters collected in Michigan. DNA was extracted from tongue tissue of 147 river otters submitted to the Michigan Department of Natural Resources by trappers from January to April 2018. PCR with primers that amplify the 18s rRNA gene fragment was used to test for the presence of *Sarcocystis* DNA. The overall prevalence (95% confidence interval) was 29% (22%–37%) in which 43 of the 147 otters were infected. Prevalence in males (37%) was significantly higher (p=0.026) than in females (20%). Significant differences were not found in the prevalence of *Sarcocystis* spp. infection according to otter age nor geographic region of origin (p=0.4339 and p=0.3583, respectively). Sequence analysis from a subset of 10 positive samples showed a 100% similarity to *S. caninum* and *S. artica* in 5 of the samples and 99.5% similarity in 4 of the samples. Only one sequence showed 100% similarity to *S. falcatula*, *S. neurona* and *S. speeri* when compared to the sequences available in Genbank. Results of this study indicate that infection with *Sarcocystis* spp. in northern river otters from Michigan is common.

Trypanosoma cruzi infection in two meerkats (*Suricata suricatta*) at the Dallas Zoo.

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CASE DESCRIPTION: Five meerkats have been maintained since 2013 at the Dallas zoo. In October 2019, a 9.5-yr-old female was presented to zoo veterinarians for ataxia and lethargy before succumbing to respiratory arrest. Three months later, a 7.5-yr-old male presented for depression and tachypnea secondary to hypothermia but recovered after supportive care and antibiotics; two weeks later he was found dyspneic and moribund, and died before arrival to the hospital. CLINICAL FINDINGS: At presentation, bloodwork on the female revealed electrolyte abnormalities, elevated ALT/ALP, monocytosis, and neutrophilia. Radiographs indicated pleural effusion; thoracentesis yielded straw-colored and blood-tinged fluid. Cytology of fluid revealed flagellated forms of *Trypanosoma cruzi*, and PCR confirmed morphologic identification. Histopathology documented pulmonary congestion, edema and atelectasis, and severe lymphocytic myocarditis with intralesional pseudocysts of *T. cruzi* amastigotes; amastigotes were also found in skeletal muscle. Bloodwork on the male (two weeks prior to death) showed elevated ALT and amylase. At necropsy, dilation of the right ventricle was noted. Histopathology showed mild interstitial pneumonia, and severe lymphocytic myocarditis with rare pseudocysts. PCR of preserved cardiac tissue from both meerkats amplified *T. cruzi* DNA.
TREATMENT AND OUTCOME: Zoo staff enhanced insecticidal measures taken to reduce triatomine vector populations. The three surviving meerkats did not show clinical signs and tested negative by PCR, but precautionary benznidazole was administered for 60 days. Post-treatment blood smear and PCR evaluation were negative, and to date, these three meerkats have remained clinically normal. CLINICAL RELEVANCE: *Trypanosoma cruzi* readily infects a variety of mammals. Sporadic reports have occurred in other captive wild animal species. Zoos should practice stringent vector control in habitats conducive to triatomine infestation to reduce risk of *T. cruzi* transmission.

74

**Enterocytozoon sp. and Ceratonova shasta in the intestines of adult Chinook salmon**

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Salmon, *Oncorhynchus tshawytscha*, like most species of Pacific salmon species are semelparous; they return to freshwater from spring to early fall, spawn in the fall, and then die shortly after spawning. Prespawning mortality (PSM) in freshwater during the summer can exceed 50% in certain populations of Pacific salmon. We have been investigating the underlying causes of PSM in spring run Salmon in the Willamette River system. Adult Pacific salmon are severely immune compromised before they spawn in freshwater. High burdens of some well-recognized salmon parasites have been documented prior to and after spawning, and some have been associated with PSM. We have been using histology as primary diagnostic method to investigate PSM in the Chinook Salmon for the last decade, and we have examine a variety of life stages of the adult fish; midsummer healthy and PSM fish, and post-spawned fish in the autumn. The salmon do not feed after returning to freshwater and they show severe degeneration of the gastrointestinal epithelium, which progresses through the summer. We discovered a novel microsporidium associated with the lesions, and along with *Ceratonova shasta* (Myxozoa), these parasites and lesions were particularly prevalent in PSM and post-spawned fish. The microsporidium is consistent with members of the genus *Enterocytozoon*, in that it has small spores (about 1.5 - 2.0 um) that infect the intestinal epithelium of an immune compromised host. We examined the parasite using Correlative Light and Electron Microscopy from paraffin sections, and consistent with this genus, sporogony occurs within a syncytium. Also, rDNA sequence of this parasite shows that it is in a clade with other members of Enterocytozoidae. We are now conducting a large, retrospective study of our histological material to elucidate correlations of this parasite and *C. Shasta* to these degenerative intestinal changes and PSM.

75

**Prevalence and partial genetic characterization of Toxoplasma gondii strains from 31 passerine species collected in north central Oklahoma**

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Passerine birds are commonly exposed to *Toxoplasma gondii*. The purpose of our project was to determine the prevalence and genotypes of *T. gondii* in 31 different species of passerines collected as mortalities due to presumptive building window strikes in north central Oklahoma. DNA was extracted from pectoralis muscles and subjected to PCR with primers that amplify a
portion of the *T. gondii* B1 gene. Genotyping was based on a subset of the infected birds using a secondary, multiplex PCR followed by RFLP of 12 *T. gondii* markers. Of 103 birds comprising 31 species, the overall prevalence (95% confidence interval) of *T. gondii* infection was 33.0% (24.1%–42.6%). Significant differences were not observed in the proportion of *T. gondii* in birds according to taxonomic family, sex, nor weight. However, samples size of each species were small and prevented a robust analysis of *T. gondii* according to those biological variables. Genotyping of *T. gondii* from a subset of 13 infected birds comprised of 7 species, revealed 4 partial ToxoDB genotypes. These partial genotypes included #54, #139, #200, and #220. Our results, while hampered by a small sample sizes for each of bird species, suggest that infection with *T. gondii* is common in Oklahoma passerines.

**Cattle Ticks/Mites/Insects**

76

**Genetic characterization and high-throughput screening of ticks and tick-borne pathogens infecting bovines in Pakistan**

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Ticks and tick-borne pathogens (TTBPs) are a major constraint to livestock production in Pakistan but knowledge on the diversity of TTBPs in Pakistan is limited. The present study investigated the prevalence and diversity of bovine TTBPs in five agro-ecological zones (AEZs) of Pakistan. A total of 774 ticks were collected from cattle (*n* = 242) and water buffaloes (*n* = 200) on small-holder farms. Ticks were identified morphologically and then genetically using two mitochondrial (cytochrome *c* oxidase subunit 1 and 16S) and one nuclear ribosomal RNA (second internal transcribed spacer) loci. The novel microfluidic real-time PCR was used to test the individual ticks for the presence of 27 bacterial and eight parasitic microorganisms. The overall tick prevalence was 46.1% (204/442), which varied slightly between cattle (47.9%; 116/242) and buffaloes (44%; 88/200), and significantly across different AEZs. Five tick species were identified including *Hyalomma anatolicum*, *Hy. hussaini*, *Hy. scupense*, *Rhipicephalus microplus* and *R. annulatus*. Out of 234 ticks tested using microfluidic real-time PCR, 94.4% tested positive for DNA of at least one of the 14 microorganisms present as single (43.4%), double (38.9%), triple (14.5%), quadruple (2.3%) and quintuple (0.9%) mixed infections. Piroplasms (i.e., *Babesia* and *Theileria*) were the most prevalent (31.6%), followed by *Ehrlichia* spp. (20%) and *Anaplasma marginale* (7.7%). The highest diversity of microorganisms was detected in *Hy. anatolicum* ticks (14/14 microorganisms), followed by *R. microplus* (4/14), *Hy. hussaini* (3/14) and *R. annulatus* (2/14). Ticks from cattle carried more frequently piroplasms (41.2%, 54/131) than those from buffaloes (19.4%, 20/103). However, the overall prevalence of microorganisms did not vary significantly among ticks from the two host species as well as across different AEZs. This study provides useful insights into the diversity of TTBPs in Pakistan which could be useful in designing future control strategies for TTBPs.
**Evaluation of a topical sarolaner-selamectin combination to control flea populations on naturally infested cats in private residences in West Central Florida.**

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An in-home study was conducted in West Central Florida USA to evaluate the efficacy of a topically applied selamectin-sarolaner formulation to control flea infestations, reduce pruritus and minimize dermatologic lesions in naturally flea infested cats over a 12-week period. A topically applied fluralaner formulation was used as a positive reference control. Any dogs present in the households, regardless of treatment group were administered oral sarolaner.

Thirty-seven cats in 21 households were treated once monthly with the selamectin-sarolaner topical solution. In the topical fluralaner treatment group forty-three cats in 20 households were treated once on day 0. There were thirty dogs total in both groups treated once monthly with oral sarolaner. Fleas on cats were counted by flea combing, fleas on dogs were counted using visual area counts and fleas in the indoor premises were assessed using intermittent-light flea traps. Blinded-assessments of feline dermatologic lesions (SCORFAD) were conducted monthly by a boarded-dermatologist and pruritus severity was evaluated by pet owners. Three consecutive monthly treatments of selamectin-sarolaner reduced flea populations on cats by 96.3% within 7 days and by 100% from week 6 to the end of the 12-week study. The topical application of fluralaner reduced flea populations by 98.1% within 7 days and efficacy reached 100% by week 12. At the end of the study, fleas were completely eradicated (from cats, dogs and homes) in every home regardless of treatment group. Owner reported cat pruritus was reduced by > 87% in both treatment groups by week 12. Significant improvements in dermatologic lesion scores (> 81%) were achieved by both products by the end of the study. Monthly applications of topical selamectin-sarolaner or topical fluralaner to cats living in the heavy flea challenge environment of west-central Florida USA were effective in eradicating flea infestations, reducing pruritus and improving dermatologic lesions.

**Laboratory and field efficacy of a novel, orally administered combination product containing sarolaner, moxidectin and pyrantel (Simparica Trio™) against experimentally and naturally acquired flea infestations in dogs**

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Four laboratory studies and a field study evaluated the efficacy of Simparica Trio™ against fleas. In the laboratory studies, dogs (8-10/group) were allocated based on pre-treatment flea counts to treatment with a single dose of either placebo or Simparica Trio™ on Day 0. In each study, dogs
were infested with ~100 unfed *Ctenocephalides felis* prior to treatment and weekly for 5 weeks. In Studies 1 and 2, viable fleas were comb-counted at 24h after treatment and each infestation. Study 2 included groups treated with either sarolaner-alone (1.2 mg/kg), moxidectin-alone (24 µg/kg), or pyrantel-alone (5 mg/kg). In Study 3, counts were conducted at 3, 4, 8 and 12h after treatment and weekly infestations. In Study 4, dogs were housed in an enclosure for collection of flea eggs after each infestation. The field study was conducted in 18 US veterinary practices in which dogs were allocated to treatment with either Simparica Trio™ or Nexgard® (afloxiolane) at the label dose. Flea counts and examination for clinical signs of flea allergy dermatitis (FAD) were performed at the initial visit prior to treatment/Day 0 and Days 30 and 60. Efficacy against *C. felis* was ≥99.7% at 24h after treatment and after subsequent infestations for at least 35 days. Treatment with sarolaner-alone had similar efficacy to Simparica Trio™, while moxidectin-alone and pyrantel-alone did not show flea efficacy. Flea egg-laying was completely eliminated for 35 days. Significant flea killing started at 4h after treatment, 8h after treatment all dogs had no fleas, and efficacy was ≥97.8% at 12h for 28 days. In the field study efficacy was 99.0% and 99.7% on Days 30 and 60, respectively. Clinical signs of FAD improved following treatment. Simparica Trio™ was well-tolerated in all studies. These studies demonstrated that Simparica Trio™ provided highly effective treatment and control of flea infestations on dogs.

Efficacy and speed of kill of a combination product containing sarolaner, moxidectin and pyrantel (Simparica Trio™) against five common tick species infesting dogs in the USA

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Ten dose confirmation studies and one speed of kill study evaluated the efficacy of a single, oral dose of Simparica Trio™ at 1.2 mg/kg sarolaner, 24 µg/kg moxidectin and 5 mg/kg pyrantel against five tick species that commonly infest dogs in the USA. Beagles or mixed-breed dogs (9-10 per group) were randomly allocated to treatment with placebo or Simparica Trio™ based on pre-treatment tick counts. In each of the ten studies, dogs were infested with ~50 unfed adults of either *Amblyomma americanum*, *A. maculatum*, *Dermacentor variabilis*, *Ixodes scapularis* or *Rhipicephalus sanguineus* on Days -2, 5, 12, 19, 26 and 33. Tick counts were conducted 48 hours post-treatment and after each subsequent re-infestation for *A. maculatum*, *D. variabilis*, *I. scapularis* and *R. sanguineus* studies and 48 hours (h) or 72 hours (h) post-treatment and after re-infestation in the first and second *A. americanum* studies, respectively. Against *A. maculatum*, *D. variabilis*, *I. scapularis* and *R. sanguineus*, a single dose of Simparica Trio™ provided ≥98.9% efficacy against existing infestations 48h post-treatment, and efficacy was ≥90% within 48h of re-infestation through at least Day 28. Against *A. americanum*, Simparica Trio™ provided ≥99.4% efficacy ≤72h after treatment, and ≥98.4% efficacy ≤72h after weekly re-infestation for 35 days. In the speed of kill study, dogs were infested with ~50 unfed adult *I. scapularis* on Days -2, 7, 14, 21, 28 and 35. Tick counts were conducted at 8, 12 and 24h after treatment on Day 0 and after each subsequent re-infestation. A single dose of the Simparica Trio™ started killing ticks 8h after treatment and provided 98.4% efficacy within 12h after treatment and ≥94.2% efficacy within 24h of re-infestation for 28 days. Simparica Trio™ was well-tolerated in all
studies. These studies demonstrated that Simparica Trio™ provided highly effective treatment and control of tick infestations on dogs.

80

**Seasonality of *Ixodes* species and stages infesting dogs and cats in the USA**
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To better define the seasonal activity of *Ixodes* spp. infesting dogs and cats in the United States, we examined 2,896 *Ixodes* spp. (2,364 female, 392 male, 140 immature) collected from 1,422 dogs and 422 cats in 43 states from March 2018 through February 2020. *Ixodes scapularis* was the most common tick identified (2,711/2,896; 93.6%); six other species, including *I. pacificus* (113; 3.9%), *I. cookei* (n=40), *I. angustus* (n=17), *I. affinis* (n=10), *I. haerlei* (tentative, n=4), and *I. texanus* (n=1), were also found. In the Northeast and Midwest, adult *I. scapularis* were more often collected October–November (1,133/2,004; 56.5%) and April–May (540/2,004; 26.9%); in the South, adult *I. scapularis* were primarily found on pets October–January (481/595; 80.8%).

A gradual shift in seasonality of *I. scapularis* was evident in the South, with peak activity in November (93/177; 52.5%) in the lower South, January (103/233; 44.2%) in the middle South, and dual peaks in October–November (64/185; 34.6%) and January–May (89/185; 48.1%) in the upper South. Immature *I. scapularis* were almost entirely from pets in the Northeast and Midwest (97/102; 95.1%) and active primarily April–July (90/102; 88.2%). *Ixodes affinis* and *I. cookei* were collected from pets from April–September (48/50; 96.0%). In the West, adult *I. pacificus* predominated (113/134; 85.6%) and were primarily found December–May (91/113; 80.5%); immature *I. pacificus* were not submitted from pets. *Ixodes angustus* (n=17) and *I. haerlei* (tentative) (n=4) were collected from pets in the West July–October. Older pets that spend relatively little time outdoors may be more likely to be infested with *I. scapularis* compared to other common tick species. Variations in seasonal activity of *Ixodes* spp. are evident across the United States and can provide insight into relative pathogen transmission risk throughout the year.

81

**Geographic diversity of ticks collected from dogs and cats throughout the United States**
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To characterize the geographic variation of tick species and stages infesting pets across the United States, we evaluated 21,686 ticks submitted from 3,858 dogs and 751 cats in 49/50 states over a two-year period; an additional 2,129 ticks in aggregate tick collections from 24 veterinary practices were also identified. Pets were most commonly infested with *Ixodes scapularis* (1,674/4,609; 36.3%), *Dermacentor variabilis* (1,588/4,609; 34.5%), *Amblyomma americanum* (999/4,609; 21.7%), and *Rhipicephalus sanguineus* sensu lato (318/4,609; 6.9%). Less common species were also found (292/4,609; 6.3%), and many pets were co-infested (240/4,609; 5.2%). Geographic distribution of tick species was determined for the four major regions of the United States, with Fisher’s exact test used to evaluate regional differences in species and stages (α = 0.05). Pets infested with *I. scapularis* were most common in the Northeast and Midwest (1,369/1,674; 81.8%); the majority of pets with *D. variabilis* were from the Midwest (828/1,588;
52.1%); and most pets with *Amblyomma maculatum* (109/135; 80.7%), *R. sanguineus* (192/318; 60.4%), and *A. americanum* (582/999; 58.3%) were from the South. Infestations with immature *I. scapularis* (61/1674; 3.6%) were primarily (55/61; 90.2%) on pets in the Northeast and Midwest; immature *A. americanum* (247/999; 24.7%) were identified on pets in the South, Midwest, and Northeast; and immature *R. sanguineus* (52/318; 16.4%) found on pets in the South and West (51/52; 98.1%). Aggregate tick collections revealed similar regional differences with the majority of *I. scapularis* (263/471; 55.8%) identified in collections from the Northeast; *A. americanum* (861/886; 97.2%), *R. sanguineus* (195/288; 67.7%), and *A. maculatum* (9/9; 100%) in collections from the South; and *D. variabilis* in collections from the South (43.7%; 199/455), Northeast (148/455; 32.5%), and Midwest (108/455; 23.7%). These data provide a large-scale overview of the geographic differences among tick species and stages infesting pets and highlight important regional foci of tick species.

82

**Has COVID-19 stay-at-home orders changed the risk of tick exposure for dogs?**

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The COVID-19 pandemic is causing rapid and massive social and behavioral changes globally. In most US states there are/have been stay-at-home orders aimed at minimizing contact between people and transmission of SARS-CoV-2. However, these orders generally allow individuals to continue physical activities outdoors as long as they maintain social distancing. Therefore, because people are looking for safe ways to get out of the household, we reasoned that they may be spending more time outdoors, and taking their dogs with them. On April 15, 2020, we launched an ongoing anonymous survey online, and asked respondents to document if there have been changes in the time they, their children, or their pets spend outdoors. Historical outdoor activity and tick encounters are requested to determine if change in behavior, and therefore risk, exists. Finally, detailed questions related to where they spent time outdoors and tick prevention measures are asked. To date, 2,896 individuals have completed the survey, and 1,849 owned one or more dogs. Many participants (45%) noted that their dogs have spent more time outdoors since COVID-19 restrictions, although no difference was noted in where they spent that time (e.g., paved areas, lawns, wooded areas, etc.). Since March 2020, 30% of people have found ticks on their dogs and 18.7% believe tick numbers are higher this year compared to last year. A small percentage of people (4.9%) noted that they had changed preventative use during COVID-19 restrictions, and of those, 28.7% stopped or used preventatives less frequently, mainly due to lack of funds or reluctance to travel. Our ongoing analyses indicate that during the COVID-19 pandemic more dogs are spending time outdoors, and exposure to ticks has been high. Decreased use of preventative measures, unfortunately, corresponds to the expected seasonal increase in the activity of ticks in many parts of the US.
Serologic evidence of select vector-borne pathogens in unowned dogs in the Southeastern United States
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In the United States vector-borne pathogens (VBP) are of concern for animal and human health, however there are many gaps in the surveillance and reporting data. The purpose of this study was to determine the presence of vector-borne pathogens in free-roaming/unowned dogs in Alabama (AL) and Georgia (GA). Residual anticoagulated blood samples were available for opportunistic retrieval and analysis for this study from dogs admitted to the spay and neuter program at the Auburn University’s College of Veterinary Medicine. Patient-side serology (SNAP® 4Dx® Plus, IDEXX Laboratories, Inc.), blood smear evaluation, and/or PCR were utilized for detection of Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia spp., and/or Anaplasma spp. Ectoparasites present at admission were documented, and if possible, collected for species identification. From May–October 2019, 114 samples were available for opportunistic retrieval. Forty (35.1%) were seropositive for one or more VBP: D. immitis (20.2%) and Ehrlichia spp. antibodies (20.2%) with 15% of the VBD-positive dogs (6/40) testing positive for both D. immitis and Ehrlichia spp. Neither B. burgdorferi nor Anaplasma spp. antibodies were detected in any dog. Microfilariae of D. immitis were present in blood smears of 7/23 D. immitis-positive dogs. Morulae of Ehrlichia or Anaplasma spp. were not identified in any blood smear. DNA of Ehrlichia or Anaplasma spp. was not detected. Fleas were present in 19.3% (22/114) and ticks in 9.6% (11/114) of dogs at admission. Over 35% of the dogs evaluated in this study tested seropositive for one or more VBP (higher than the 2019 data available through the Companion Animal Parasite Council website for D. immitis [AL=4.06%; GA=2.16%] and Ehrlichia spp. [AL=3.66%; GA=3.52%]). Our data indicate that VBP risk for dogs in this region may be higher than reported, and that the reservoir potential of domestic animals, especially free-roaming animals, warrants further investigation.

Do blacklegged ticks collected from domestic cats feed on feline blood and transmit Lyme disease
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Lyme disease is one of the most common vector-borne diseases in North America. It is caused by the spirochete species Borrelia burgdorferi and affects domestic animals and humans. Cats rarely exhibit clinical signs of the disease outside laboratory settings even though domestic cats are occasionally infested with ticks. This observation raises a question of whether cats are natural hosts for tick and if ticks found on cats are agents of Lyme disease? To address this question, we investigated if Ixodes ticks, vectors for Borrelia burgdorferi, naturally attached to cats. Fifty-four ticks were collected from domestic cats through the elective participation of their owners through a citizen science project with Cornell’s Feline Health Center (FHC). DNA was extracted
from the ticks; then specific primers were used to amplify 5.8S to 28S rDNA and fla (flagellin) genes for respective identification of *Ixodes* and *B. burgdorferi*. Fifty-two samples yielded specific bands for identifying *Ixodes scapularis* and detecting the presence of *B. burgdorferi* in some of them. Subsequently, nested PCR was performed to amplify the mitochondrially encoded 12S rRNA gene; a molecular marker for blood, to identify the host source from blood meal remnants found within the tick samples. Samples yielding inconclusive results were further investigated using restriction digestion and low-throughput DNA sequencing. All samples tested yielded amplification products, and 35 out of the 52 samples tested had distinct banding pattern identical to cat blood when treated with a single restriction enzyme (MseI). The remaining samples were sequenced and conclusively had 90-100% match to the mitochondrially encoded 12S rRNA gene of *Felis catus*. Our findings demonstrate that *I. scapularis* carrying *B. burgdorferi* can feed on domestic cat blood and therefore cats can act as vectors of Lyme disease.

85

**Diversity of Rickettsia spp. in Dermacentor spp. from across the United States**

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Within the United States, *Dermacentor* spp. are known to be the primary vector for *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever. More recently, other work has demonstrated the wide diversity of *Rickettsia* spp. in *Dermacentor variabilis*. To better define this diversity, 17kDa and ompA sequences were used to identify *Rickettsia* spp. in 628 *Dermacentor* spp. ticks, including *D. variabilis* (n=607), *D. andersoni* (n=12), and *D. albipictus* (n=9), submitted from 359 dogs (n=577 ticks) and 39 cats (n=51 ticks) from veterinary practices in 44/50 states in 2018–2019. *Rickettsia* spp. were found in 11.0% (69/628) of the ticks tested with the majority of positive ticks originating from the Midwest (n= 38 ticks). Species detected include *R. montanensis* (n=33 ticks), *R. bellii* (n=15 ticks), *R. rhipicephali* (n=10 ticks), *R. peacockii* (n=8 ticks), *R. cooleyi* (n=1 tick), and novel *Rickettsia* sp. (n=2 ticks). The majority of *R. montanensis* was detected in ticks from the northern United States while *R. rhipicephali* was primarily detected in ticks in the southern United States. Even though *D. variabilis* was submitted from Rocky Mountain states (n=117), *R. peacockii* was found only in *D. andersoni* from that region (n=8/12) and no other *Rickettsia* spp. were detected in the submitted *D. andersoni*. Because most ticks had fed on dogs or cats prior to submission, these findings do not necessarily implicate a given *Dermacentor* sp. as a primary vector of these bacterial agents, but the data do support with other published work showing *D. variabilis*, the predominant *Dermacentor* spp. parasitizing dogs and cats in North America, likely harbor a diversity of *Rickettsia* species. These other *Rickettsia* spp. may have significance as mild or unrecognized veterinary or human pathogens and could influence results of serologic assays.
Sequential histologic comparisons of naïve and subsequent Amblyomma americanum bite lesions from induced infestations on dogs and cats
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*Amblyomma americanum*, lone star tick, is a generalist and will readily take a blood meal from dogs, cats, and a variety of other mammalian hosts. The purpose of our study was to characterize the dermal inflammatory cell response in dogs and cats due to naïve and subsequent infestation with *Amblyomma americanum* adults. We collected skin punch biopsies of tick bite lesions from dogs and cats during induced, naïve and subsequent tick infestations. Punch biopsies were processed for histopathology, sectioned, and stained with hematoxylin and eosin. Immune cells quantified were neutrophils, eosinophils, mast cells, lymphocytes/plasma cells, and macrophages. Overall, the number of immune cells in the tick bite lesion increased over the duration of infestation compared to baseline tissue collected immediately prior to infestation with *A. americanum*. Significantly more neutrophils, eosinophils, lymphocytes, and macrophages were counted at 192 hours post-infestation (hpi; 8 days), mostly during naïve infestation, in dogs. Whereas, significantly more eosinophils, lymphocytes, and macrophages were noted 192 hpi in cats whether during naïve or subsequent infestations. Overall, infestation with *A. americanum* produced robust inflammatory responses at bite sites that are not likely appreciated based on the gross, innocuous appearance of attached ticks on dogs and cats.

Horse Ticks/Mites/Insects

Initiation of the National Equine Tick Survey as a novel method for tracking ticks on horses in the US
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Currently, there is no persistent, highly effective tick prevention commercially available for horses, leaving them vulnerable to disease and discomfort. Studying ticks on horses via the National Equine Tick Survey reveals patterns in data about tick species’ geographic distribution, seasonal prevalence, and other species characteristics as they relate to horses. Since October 2018, 49 veterinarians and owners were enrolled in the study across 22 states. In total, 1204 ticks were found on 226 horses and sent to the lab to be categorized by species, sex, and life stage. Information about the infested horses was collected as well, including geographic location, time spent in pasture, and tick location on the body. This data was then analyzed using Excel and MapViewer. Tick species collected included *Dermacentor albipictus, Dermacentor variabilis, Dermacentor occidentalis, Amblyomma maculatum, Amblyomma americanum, Ixodes scapularis* and *Ototius megnini*, with *I. scapularis* and *A. americanum* representing the two most prevalent species collected year round (36% and 22%, respectively). The majority of *I. scapularis* ticks were collected during the fall months in the Northeast, while the majority of *A. americanum* ticks were collected during the spring months in the Southeast. Data also showed that the ventral head/neck area was the most common site of attachment by *I. scapularis*, while the inguinal area was the most common site for *A. americanum*. By documenting the burden and diversity of
equine ticks, the results of this survey highlight the significance of ticks as a medical problem for horses.

**Wildlife Ticks/Mites/Insects**

88

**Studies on sarcoptic mange in black bears (Ursus americanus) in Pennsylvania**

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There have been increasing reports of black bears (*Ursus americanus*) with severe skin disease in the Eastern and mid-Western United States over the last three decades. Diagnostic evaluations determined the majority of cases were due to *Sarcoptes scabiei*. We investigated if exposure to several pathogens commonly infecting black bears (canine distemper virus, canine parvovirus, canine adenovirus-1, *Toxoplasma gondii*, and *Trichinella* sp.) was a potential risk factor for clinical mange; no associations were noted. We also used a serological approach to determine the extent of exposure in bears without clinical mange to gain a better appreciation for which populations of bears are exposed to mites. We validated a commercially-available ELISA, designed for dogs, for use in black bears. To further examine assay performance, serial serum samples from black bears with confirmed sarcoptic mange were collected posttreatment to determine the persistence of antibodies. Antibodies waned to below the detection limit between 4-14 wks, suggesting that serology studies might underestimate the number of exposed black bears. State-wide serosurveys in Pennsylvania showed a significant difference in seroprevalence between regions with high occurrence of mange (mean seroprevalence 6.7%) and low occurrence of mange (no seropositive black bears were detected), potentially suggesting that subclinical exposure to mites is rare, even in regions with high mange). We also determined the ability of mites to survive off the live host to investigate indirect transmission of mites between black bears. Temperature significantly affected mite survival, shortest at 0 °C (mostly ≤ 4 h) and longest at 4 °C (up to 13 days). No mites survived beyond 8 days at 18 °C or 6 days at 30 °C. Collectively, our data advance our understanding of sarcoptic mange in wildlife and also can be used to drive management decisions or lay the groundwork for future research of this disease in bears.
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Acanthocephala

89
Oncicola canis (Acanthocephala) in a rescued dog (Texas to Pennsylvania).
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American Pit Bull Terrier was presented at the University of Pennsylvania Veterinary Hospital for evaluation for the surgical repair of a fractured pelvis. The dog (male, 18 kg, 2 years old) had been found along a road in Texas and brought back to Pennsylvania by a rescue group. The dog had been diagnosed with heartworm by a local veterinarian and had a heart murmur prompting the owners to seek an evaluation prior to surgery. A comprehensive tick-borne disease panel (NCSU) showed the dog was infected with Bartonella spp., Babesia canis, Dirofilaria immitis, and Hemoplasma haematoparvum. The dog had a severe systemic inflammation after being given Heartgard while in the hospital and, given his poor prognosis, was euthanized. During necropsy several acanthocephalans were recovered from the jejunum and ileum. These were identified as Oncicola canis based on their size, the spines on the proboscis, egg size (recovered from a female). This acanthocephalan uses the armadillo as an intermediate host and is common in coyotes in Texas, but only rarely reported from dogs. This case should serve as a warning to always evaluate and treat a potential rescue dog before moving it from the locality where it was original found!

Cestodes

90
Echinococcus multilocularis infections in domestic dogs and implications for wildlife and public health
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In Alberta and globally, human population expansion causes wildlife and domesticated animals to increasingly share altered habitats. As well, parasites relying on both wild and domestic hosts are increasingly shared in these habitats. Echinococcus multilocularis (Em) is a parasitic helminth cycling through definitive hosts (coyotes, wolves, foxes, dogs) and intermediate hosts (rodents) in a predator-prey system. Humans can be infected, often lethally, possibly through interactions with domestic dogs that are shedding Em eggs through their feces. In Alberta, an increase of human infections has been documented since 2013 and dog ownership has been considered a risk factor for infection. Domestic dogs provide a possible conduit for infection between wildlife and humans. We therefore used qPCR to detect Em in an existing sample of 696 dog feces which were paired with surveys outlining various risk factors for infection. We found that 2.31% (95% CI: 1.38-3.84%) of domestic dogs living near Calgary city parks were infected with Em intestinal infections. Dogs, which co-occur (often at high densities) with wildlife in urban and suburban areas therefore are becoming important hosts and likely an additional source of Em infection. Our findings suggest that areas of wildlife and domestic
animal overlap such as city parks could enhance the spread of diseases. However, more focus must be placed on determining activities and behaviours of dogs (ie. being walked off-leash in city parks, predation of rodents) which may increase their risk of infection with Em. Overall, Em presence in domestic dogs in Calgary, Alberta and its implications for public health warrant further exploration into both prevalence and risk factors due to the proximity of humans and wildlife enhanced by urbanization and human expansion.

91

Polyomics of the neglected equine tapeworm Anoplocephala perfoliata
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Anoplocephala perfoliata is the principle tapeworm species commonly infecting horses worldwide. Clusters of A. perfoliata are found attached close to the ileocaecal valve and likely cause significant gastrointestinal tract damage and dysfunction at the site of attachment. Furthermore, infection has been linked to colic. At present, there is limited evidence and understanding of A. perfoliata infection and its interaction with the digestive physiology and health of its host horse. Given the relative neglect into A. perfoliata infections, the current work aimed to generate molecular tools using a polyomic approach, supported with bioinformatics, to provide an increased knowledge of A. perfoliata. We have performed the first de novo assembled transcriptome for adult A. perfoliata and identify key RNA sequences likely expressed as proteins potentially involved in host-parasite interactions. Furthermore, we present the first analysis into the excretory/secretory (ES) products released by A. perfoliata. To this end, the extracellular vesicles (EVs) have been purified using size exclusion chromatography and characterised using proteomics. Know EV biomarkers were identified including annexins and tetraspanins. Our development of a discovery transcriptome and proteome datasets for EVs and EV depleted ES products provides a basis for the further study into the host-parasite interaction and host microbiome-parasite interactions. Furthermore, these datasets support investigation into diagnostic biomarkers, intelligent treatment strategies and novel vaccine candidates.

KEYWORDS: Anoplocephala perfoliata, Transcriptome, Proteomics, Extracellular Vesicles

92

Cattle Nematodes

Gastrointestinal nematode fecal egg shedding intensity, prevalence, and predominant species in western Canadian cow-calf operations.
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Emerging evidence for anthelmintic resistance in Canadian beef operations emphasizes the urgent need for understanding the current epidemiology of gastrointestinal nematode (GIN) infections, which is crucial for evidence-based targeted parasite control. A cross-sectional study was conducted to determine the GIN fecal egg shedding intensity, prevalence, and species diversity of calves from cow-calf operations across western Canada. Fecal samples were collected from 844 randomly selected calves in 43 herds from November 2016 to February 2017. Strongyle-type, Nematodirus spp., and Trichuris spp. fecal egg counts were obtained by a
modified Wisconsin sugar floatation method and expressed as eggs per gram of feces (EPG). Third stage larvae were harvested from coprocultures, pooled by the herd, and characterized by nemabiome sequencing. Predicted mean fecal egg counts and prevalence with 95% confidence intervals (CI) were estimated using generalized estimating equation models and herd-level clustering. Beta diversity comparisons determined the provincial difference of relative GIN species abundance. The mean strongyle-type fecal egg count and prevalence were 18.6 EPG (CI 14.3-22.8) and 92.3% (CI 86.4-98.9), respectively. Although the mean fecal egg count was meager (<1 EPG), the prevalence of Nematodirus and Trichuris spp. were 31.1 (CI 25.2-37.0) and 34.3% (CI 27.3-41.4), respectively. Ostertagia ostertagi was the predominant GIN species in most herds, while Cooperia oncophora was the second most abundant species. The relative abundance of Cooperia punctata was distinctly greater in Manitoba compared to Alberta and Saskatchewan. Fecal egg count intensity and prevalence of calves in this study were broadly comparable to more recent and historical studies in western Canada. However, the high regional abundance of C. punctata was unexpected in northern latitudes. Cooperia punctata is traditionally predominant in more southern, tropical regions and has the potential for significant production impacts; therefore, potential reasons for its’ high abundance should be explored further.

93
Assessing the production impacts of gastrointestinal nematode parasites in stocker cattle in Western Canada

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Gastrointestinal nematodes (GIN) have been associated with economic losses in beef cattle in many different regions of the world, with members of the family Trichostrongyloidea being the most significant. However, there have been few studies in western Canada. We investigated the production impacts of GIN on stocker cattle in Saskatchewan in terms of body weight and average daily gain (ADG). The study design involved treating a subset of calves in each herd with anthelmintics whilst the rest of the co-grazing herd was left untreated to act as controls exposed to the same level of pasture contamination. Anthelmintic treatments comprised of oral fenbendazole (Safeguard®, Merck, Canada) and parenteral extended-release eprinomectin (LongRange®, Boehringer Ingelheim, Canada). The study comprised seventeen independent cohorts of yearlings on 13 beef farming operations, each of which was visited three times during the grazing season (spring, summer, and fall). Animals were individually weighed, and rectal fecal samples obtained for pooled fecal egg count (FEC) by treatment group at the spring and fall visits, with a subset of fecal samples obtained during the summer. A total of 867 cattle was enrolled (n = 446 controls; n = 421 treatments). Overall, across all cohorts, there was a difference between treatment and control FEC (p < 0.001) and final weight (p < 0.01). There was no difference (p = 0.41) in overall ADG. However, there were statistically significant ADG differences in five cohorts in which the mean difference in ADG between groups was 0.3 lbs while it was 0.05 lbs in the overall analysis. The variance of the effects of GIN on ADG is likely due to differences in environmental conditions (rain, temperature, soil) and husbandry factors among the cohorts. This project provides contemporary information about the parasite burdens and production impacts in western Canada relevant to sustainable parasitic roundworm control.
Dog/Cat Nematodes

94

Updates on serological diagnosis in heartworm infection in dogs
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The present study aimed to evaluate the detection of *D. immitis* antigen in dogs co-infected with *Leishmania infantum* and *Babesia* spp. applying heat treatment of serum samples. The study was carried out on 51 dogs referred to the Veterinary Hospitals of Naples (Italy) and Iasi (Romania). Specifically, blood and serum samples were collected from 22 dogs (Italy) clinically suspected (then confirmed by IFAT) of leishmaniosis and from 29 dogs (Romania) with clinical signs compatible with babesiosis (then confirmed by blood smears and semi-nested Polymerase Chain Reaction). To exclude other co-infections, all the samples were submitted to molecular and/or serological analyses, in order to identify: *Dirofilaria* spp., *Acanthocheilonema reconditum*, *Ehrlichia/Anaplasma* spp., *Angiostrongylus* spp. The serum samples collected from three positive dogs of *D. immitis* that died of heartworm caval syndrome were used as positive control (cDi). Briefly, the serum samples of the positive dogs for *L. infantum* (pL) and *Babesia* spp. (pB) were mixed with cDi and analyzed pre and post heating by Petcheck Heartworm Canine ELISA. Of the 22 pL dogs, antigens of *D. immitis* were found in 5 (22.7%, 95%CI=8.7-45.8) not heated samples and in 22 (100%; 95%CI=81.5-99.6) heated samples. Of the 29 pB dogs, antigens of *D. immitis* were found in 8 (27.6%; 95%CI=13.5-47.5) not heated samples and in 29 (100%; 95%CI=85.4-99.7) heated samples. All the dogs were negative for the other pathogens tested. The outcome of the present study showed false negative results by the antigenic test of *D. immitis*, when dogs were co-infected with *L. infantum* or *Babesia* spp. These findings could have important implications for epidemiological studies in areas where these vector-borne infections co-exist.

95

Evaluation of canine fecal samples using a *Toxocara* species-specific real-time PCR
Todd Bezold*, Phyllis Tyrrell, Rita Hanna, David Elsemore. IDEXX Laboratories, Inc.

Parasite eggs are present in the environment. Contaminated soil, water, and fur as well as outright coprophagy are potential sources for ingestion of eggs by dogs. Spurious eggs are eggs that are either non-infective to the host animal or in a non-infectious stage such that they may pass through the host and be detected in the feces. Identification of *Toxocara cati* eggs in dog samples provides an avenue to explore the impact of spurious eggs and previous studies have estimated rates of 30-49% of *T. cati* eggs in dog fecal samples. In this study, 27 fecal samples positive for *Toxocara* eggs were selected for PCR analysis using a *Toxocara* species specific PCR on DNA extracted from floated eggs. Of the 27 samples, 20 were *T. canis* positive (74%) and 7 were *T. cati* positive (26%). Three samples were positive for both species. This survey indicates that the rate of spurious eggs in pet dog samples from the US are similar to the previous observations.
The difference of *Dirofilaria immitis* microfilaria concentrations in blood collected from the jugular and cephalic veins of dogs.

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The recommended in-clinic diagnosis of canine heartworm involves both antigen testing and microfilaria testing. Although microfilariae are present throughout the peripheral venous blood, it is not known whether their concentration differs between the two most common sites of sampling: the jugular and cephalic veins. This study aimed to determine the difference in microfilaremia between these sites and whether a morning or afternoon sampling affected this. Whole blood was collected from six beagles with patent infections of *Dirofilaria immitis* once a week by route of both the jugular and cephalic veins at 9:30am and 3:30pm over seven weeks. Microfilaria concentrations were obtained by thick blood smear. Concentrations in jugular vein and cephalic vein samples were compared, as were concentrations in blood collected in the morning and afternoon. Findings revealed that microfilaremia is higher (p < 0.001) in the cephalic vein (mean = 39,400 MF/mL; 95% CI, 33,700 to 45,100) than the jugular vein (mean = 33,800 MF/mL; 95% CI, 29,000 to 38,600). No differences were observed (p = 0.545) between blood collected at 9:30am (mean = 36,600 MF/mL; 95% CI, 31,400 - 41,900) and 3:30pm (mean of 36,600 MF/mL; 95% CI, 31,300 – 42,000). The data suggest that microfilariae are found in higher concentrations in blood collected from the cephalic vein of a dog.

The prevalence of *Ancylostoma* spp. in dogs in the Caribbean

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Caribbean islands, with their subtropical climates, provide ideal environments for *Ancylostoma* infections in dogs. Not only are these parasites a health hazard for dogs, but they also pose a zoonotic concern. While studies of cutaneous larval migrans infections in people in the Caribbean are limited, there are reports of infections in visitors to the area. Parasite control in this canine population also is important since many organizations rehome dogs from the region to North America and Europe. In this study a review of the published literature (Sceilo, Scopus, and PubMed databases) and grey literature (e.g., student theses, conference presentations) was performed to obtain a better understanding of the current situation with *Ancylostoma* in dogs in the Caribbean. Search terms used were *Ancylostoma*, hookworms, the names of the Caribbean islands, and regions in the Caribbean (e.g., West Indies, Antilles). Fifteen reports were found, dating from the 1950s to 2018, representing 12 of 22 islands included in the study. Methods of assessing infection ranged in sensitivity, with some using simple qualitative or quantitative (McMaster) flotation methods, and others using necropsy or centrifugation with Sheather’s sugar flotation solution. Dog populations sampled included stray, owned free-roaming, and owned contained. Parasite prevalence ranged from 2.3% to 95%, with these highest and lowest values from studies with small sample sizes. Due to the small number of data sources and the differences in methodologies, no statistical analysis was performed. However, there were no obvious differences between owned and unowned dogs or free-roaming and contained dogs. There also was no indication that hookworm prevalence has changed over time with data from
the last 5 years reporting prevalence of 18-68.8%. There is a clear need to expand the available data for the region and improve control programs for *Ancylostoma* infections to protect canine and human health.

98

**P-glycoproteins are potential drugable targets in Toxocara canis**

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P-glycoproteins (P-gp) are efflux transporters capable of transporting a wide range of compounds including macrocyclic lactones (ML) from cells. Somatic larvae of *Toxocara canis* in canids and other hosts are tolerant to the ML class of anthelmintics. The identity and expression levels of different P-gp genes was previously studied in adults, hatched larvae and somatic larvae of *T. canis*. In this study, we compare the pharmacological interaction of *T. canis* Pgp-11 with a panel of drugs known to interact with mammalian P-gps. This interaction was studied in a heterologous canine cell line system expressing no endogenous mammalian P-gp. The pharmacological profile of nematode P-gp is unique and different from mammalian P-gps. Differential pharmacology suggests that nematode P-gps could be exploited as potential druggable targets.

**Horse Nematodes**

99

**Analyst variability at the counting step for McMaster, Wisconsin, and automated equine fecal egg count methods**

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Fecal egg counts (FEC) are the keystone for a good equine parasite control program, informing equine caretakers which horses need to be given an anthelmintic treatment to control parasite burden on pasture and providing information regarding product efficacy. While it is assumed that methods used to perform FECs have inherent variability due to factors such as egg distribution in the feces, egg loss during preparation, and analyst training, few studies have been done specifically addressing variability at the analyst level. The goal of this study was to compare variability at the counting step between analysts for four different fecal egg count methods both before and after training. Three analysts with no prior experience were recruited to perform ten FECs per level for five different levels (negative, > 0 - 200 eggs per gram (EPG), 201 - 500 EPG, 501 - 1000 EPG, and 1000+ EPG) with McMaster (MM), modified Wisconsin (MW), and automated techniques using an algorithm (PS) and machine learning (ML) for analysis. After the first portion of the study, analysts went through a standard FEC training and repeated the protocol. The training led to the coefficient of variation (CV) being halved for MM, and had a significant decrease for MW ($p = 0.012$). Training did not have a significant effect on CV for the automated method. Prior to training, CV rankings from highest to lowest were MW, MM, ML, and PS, and after training rankings were PS, ML, MM, and CW. The results of this study suggest that with proper training, analysts perform just as well as automated systems at the counting step of FECs.
Pooled fecal samples for diagnosis of *Strongylus vulgaris*

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*Strongylus vulgaris* is a large strongyle that can be found in the large intestine of equines. It has clinical importance due to the pathogenicity of the migrating larvae in the mesenteric blood vessels. This can lead to non-strangulating infarctions, which are associated with a guarded prognosis for survival. Infections are typically diagnosed by coproculture, but a PCR test is available in some countries. While it is ideal to test animals individually, many clients wish to pool samples to reduce workload and cost of the diagnostic method. The purpose of this study was to determine if pooling of fecal samples would negatively impact diagnostic performance of the coproculture and the PCR for determination of *S. vulgaris* infection in naturally infected horses. Ten horses with strongylid eggs per gram (EPG) >500 and either *S. vulgaris*-positive or -negative were included in the study. Pools of five horses were created representing a range of ratios between positive and negative horses and 20 subsamples were set up of each. All samples were then analyzed with coproculture or PCR methods. Pools that contained 50% or greater *S. vulgaris*-positive animals were detected with both PCR and coproculture. In pools with less than 50% *S. vulgaris* positive feces, the coproculture detected 7/10 and PCR 6/10 of subsamples positive. These results indicate that diagnosing *S. vulgaris* on pooled samples is only reliable, when the prevalence in the test population is at least 50%. Since this is rarely the case in managed horses, pooled sample screening for *S. vulgaris* may not be very reliable.

Spatial Variation of Cyathostomin Mucosal Larval Counts

Avery Martin*, Martin Nielsen, Ashley Steuer, Jessica Scare. M.H. Gluck Equine Research Center

Cyathostominis are pervasive parasites of equids, of which the larval stages encyst in the intestinal walls of the cecum (CEC), ventral and dorsal colon (VC, DC). Larvae can induce a large inflammatory response leading to larval cyathostominosis, a life-threatening generalized typhlocolitis. Mucosal digests is the only gold standard procedure for identifying and quantifying all larval stages. There is a lack of standardization of this technique and several aspects are ambiguous, such as specification of location and number of replicates of mucosa collected. This present study evaluated spatial variation of encysted cyathostomin counts within an organ and precision of the mucosal digest technique. In this IACUC approved study (2018-3134), six naturally infected horses were euthanized as part of an anthelmintic efficacy study, and the cecum, ventral and dorsal colon were collected. Each organ was rinsed, weighed, and visually separated into 3 equal sized sections: the orad, middle, and aborad. Two 5% tissue samples were taken from each section, a total of six replicates per organ. The mucosa was digested, and 2% evaluated for presence of early third stage larvae (EL3) and late third/fourth stage larvae (LL3/LL4). There was no statistically significant differences between the three locations within organs (p=0.1166), but the dorsal colon had significantly lower counts than the other two organs (p<0.0001).Coefficients of Variation (CV) were high ranging from 87.71% for ventral colon LL3/LL4s to 243.87% for dorsal colon EL3s. Overall, precision was highest in the Cecum and lowest in the Dorsal Colon. The CV estimates did not change by increasing the number of
replicates, but the width of the confidence interval shrunk by up to 50%. In conclusion, mucosal larval cyathostomin counts are highly variable, complicating their use for treatment efficacy estimation. Increasing the number of replicates can improve estimation, but comes with a considerably increased workload.

According to mitochondrial DNA evidence, *Triodontophorus* species belongs to the Cyathostominae

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Strongylidae nematodes are the most prevalent nematode pathogens described in equine, which inhabit in the large intestine with a high prevalence in all ages horses. In the present study, ten complete mitochondrial genome sequences of strongylidae species were determined, namely Strongylinae: *Strongylus equinus*, *Triodontophorus brevicauda*, *Triodontophorus serratus*, *Triodontophorus nipponicus*, Cyathostominae: *Cylicocyclus insigne*, *Cylicocyclus ashworthi*, *Cyathostomum catinatum*, *Cyathostomum pateratum*, Poteriostomum imparidentatum and *Cylicostephanus minutus*. The mt genomes of ten strongylidae nematodes are all circular molecules with 13,701–14,545 bp in size. These circular mt genomes all encode 36 genes, including 12 protein-coding genes, two rRNA genes and 22 tRNA genes. All of these genes are transcribed in the same direction. Comparative analyses of mt genome organization for Strongylidae nematodes sequenced to date revealed that 5.4–22.3% diversity in nucleotide sequences. The mt genome nucleotide sequences of *Triodontophorus* species had relatively high identity with Cyathostominae nematodes, rather than Strongylus species of the same subfamily (Strongylinae). Phylogenetic analyses using mtDNA data indicated that the *Triodontophorus* species clustered with Cyathostominae species instead of Strongylus species. Thus, sequence comparison and phylogenetic analyses based on mtDNA sequences supported the hypothesis that *Triodontophorus* belongs to Cyathostominae. Our results have provided novel genetic markers for further studies of Strongylidae taxonomy, population genetics, and systematics. *Corresponding author e-mail: chunrenwang@sohu.com (C.R. Wang), songmx@neau.edu.cn (M.X. Song) This work was supported by grant from the National Key Research and Development Program of China (2017YFD0501300), the National Parasitic Resources Center (NPRC-2019-194-30).

The prevalence of cyathostomin anthelmintic resistance on horse farms in Prince Edward Island, Canada

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The majority of adult equines carry a parasite burden consisting mainly of small strongyles (cyathostomins). Currently there are three classes of anthelmintics to treat strongyle infections. These anthelmintics were initially efficacious in controlling cyathostomins; however, due to their widespread overuse there has been increasing anthelmintic resistance (AHR) to the classes benzimidazoles and pyrimidines, while the macrocyclic lactones display signs of early
resistance. Detection of early resistance is investigated using egg reappearance periods (ERP) with shortening of this period overtime being indicative of AHR. The objective of this study was to investigate the prevalence of cyathostomin pyrantel pamoate and ivermectin resistance, and to determine ERP on horse farms in Prince Edward Island (PEI), Canada. One hundred and one horses on 14 horse farms across PEI, Canada were enrolled in this study. Fecal egg counts (FEC) were performed on 270 horses. Horses shedding >200 eggs per gram (epg) were treated with 6.6 mg/kg PO of pyrantel pamoate (n = 101), and FEC were conducted every two week post treatment. Once FEC were over 200 epg, horses were dewormed with 0.2 mg/kg PO of ivermectin (n = 78), and FEC were performed every 2-3 weeks. Fecal egg count reduction tests (FECRT) and ERP were used to detect anthelmintic resistance.

Fecal egg count reduction tests detected pyrantel pamoate resistance on 2/14 farms. No resistance to ivermectin was detected using the FECRT; however, a shortened ERP of 5 weeks was detected after ivermectin administration on 2/12 farms. The prevalence of pyrantel pamoate cyathostomin resistance was less on PEI than other studies in North America, while the shortened ERP of 5 weeks for ivermectin was comparable to findings of early resistance in other parts of the world. These findings will allow us to inform horse owners on appropriate anthelmintic protocols in PEI, and can be used as a baseline for monitoring anthelmintic resistance in this region.

Other (Molecular) Nematodes

Using C. elegans to identify genes that affect ivermectin sensitivity of the filarial worm B. malayi

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In 2016, nearly 800 million tablets of ivermectin were distributed to countries for use in elimination programs for human filarial diseases. Despite its widespread use, the mode of action of ivermectin against filarial nematodes is not well understood, and its in vivo potency cannot be replicated in vitro. To better understand how ivermectin affects filarial worms, our lab previously performed a transcriptomics study to identify differently expressed genes (DEG) in *Brugia malayi* adults and microfilariae after treatment of infected gerbils. Forty-four of these DEG had *C. elegans* orthologs available as mutant strains through the *C. elegans* Genetics Center. We have assayed these mutant strains for differential sensitivity to ivermectin by measuring three phenotypes affected by ivermectin: egg production, development, and motility. We have identified several resistant and hypersensitive strains of *C. elegans* as well as differences between responses to the three assays. Mutations conferring resistance included those in che-12 (e1812), a gene involved in chemotaxis, cilium assembly, and hyperosmotic response; and inx-14 (ag17), which is predicted to have gap junction hemi-channel activity and is expressed in the muscular, nervous, and reproductive systems. The che-12 mutants are additionally resistant to ivermectin’s effect on pharyngeal pumping, while inx-14 mutants are not different from control. Overall, twenty-three genes, with eleven strong candidate genes, have been identified as altering ivermectin sensitivity in at least one assay, supporting the validity of the overall approach. These may give insight into how ivermectin acts against filarial parasites; we are currently testing the effect of ivermectin on these genes in *B. malayi* using RNAi to confirm our findings with *C. elegans*. 
Parasitic nematode beta-tubulin alleles cause benzimidazole resistance and affect organismal fitness
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Parasitic nematodes are major burdens on livestock production around the world. Anthelmintic drugs are the first tool to fight these infections, and resistance to these drugs continues to increase worldwide. To fight this resistance, we must thoroughly understand the genetics and mechanisms of resistance. The commonly used benzimidazoles (BZ) represent the most well understood, however, we still do not know the mechanisms of resistance and all of the genes involved. Three well known parasite beta-tubulin alleles (F200Y, E198A, F167Y) in a nematode-specific beta-tubulin gene have long been identified in resistant parasite populations. Recent advancements in sequencing technologies have allowed the identification of a number of new parasite beta-tubulin alleles, two of which we have included in our study, E198V and E198L. We independently introduced all five of these alleles into the ben-1 gene of the BZ-susceptible free-living nematode Caenorhabditis elegans. The genome-edited strains were exposed to either albendazole (ABZ) or fenbendazole (FBZ) in high-throughput assays that measure nematode responses to the BZ compounds. We performed these assays across a range of drug concentrations to quantitatively measure BZ resistance. All five alleles convey a similar level of ABZ and FBZ resistance as found in a deletion of the entire ben-1 gene. Another essential aspect of resistance control, is understanding the long term fitness effects of these alleles. We found that the E198V allele was resistant to BZs but was less fit in control conditions. Our results validate that the identified alleles in parasite beta-tubulin genes confer resistance. Additionally, we found that rare alleles in parasite species confer fitness consequences in comparison to other resistance alleles.

Small Ruminant Nematodes
Carvone modulates in vitro and in vivo the kinetic behaviour and efficacy of abamectin
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The search of novel strategies to control gastrointestinal nematodes in ruminants is a concern considering the increasing of anthelmintic resistance. Bioactive phytochemicals may contribute to improve parasite control by enhancing the effect of existing anthelmintic drugs. This work assessed the in-vitro and in-vivo pharmacological interaction and the in-vivo efficacy of abamectin (ABM) combined with the plant-derived compounds carvone (CNE), in lambs naturally infected with resistant gastrointestinal nematodes. At first, the modulation of P-glycoprotein (P-gp) by CNE was assessed using the intestinal explant model. Rhodamine-123 (Rho123) and ABM were used as substrates to measure their accumulation in cattle ileum in
For the in vivo assay, twenty-eight (28) lambs were allocated into three (3) experimental groups. Each group was treated orally with either ABM (0.2 mg/kg), ABM in combination with CNE (100 mg/kg, four doses every 24 h) or remained as untreated control. Blood samples were collected between 0 and 168 h post-treatment and plasma levels of both compounds were determined by HPLC. Individual fecal samples were collected on days -1 and 14 post-treatment to perform the fecal eggs count reduction test. The presence of CNE increased significantly (P<0.05) Rho123 and ABM accumulation in the intestinal explants. CNE coadministration prolonged ABM absorption in lambs. ABM T½ ab. were 1.57-fold longer (P<0.05) in the co-administered group. Concentrations of CNE between 420 and 2593 ng/mL were detected in the bloodstream between 1 and 48 h post-treatment. The in-vivo efficacy of ABM against gastrointestinal nematodes increased from 94.9% to 99.8 in the presence of CNE. In-vitro / in-vivo pharmaco-parasitological studies are relevant to corroborate the interactions and the efficacy of bioactive natural products combined with synthetic anthelmintics.

107
Developing the nemabiome as an alternative to fecal egg counting: Absolute quantitation of parasitic nematode DNA in fecal samples
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Routine helminth diagnostics in parasitology laboratories largely relies on microscopy methods that are labor-intensive, lack sensitivity and are difficult to standardize. We recently developed “Nemabiome” sequencing which determines the relative quantitation of parasite species using eggs or larvae harvested from fecal samples. We are now developing absolute DNA quantitation of parasite species in fecal stool samples using the sheep GI nematodes as a model to develop this approach and provide proof of concept for other host-parasite systems. The basic approach is to apply ITS-2 rDNA nemabiome sequencing to fecal samples spiked, prior to genomic DNA extraction, with accurate quantities of synthetic DNA comprising 500 bp of random sequence with terminal sequence tags complementary to primers used for parasite ITS-2 rDNA amplification. Parasite-derived Illumina read counts are then normalized to the synthetic DNA internal standard-derived DNA counts. The first step will be to use purified Haemonchus contortus eggs to establish a spike-in concentration that provides an appropriate ratio of H. contortus ITS-2 rDNA reads: spike-in synthetic DNA reads. We will then undertake experiments to determine the quantitative relationship of the normalized H. contortus nemabiome read count with egg numbers. Once optimized, the method will then be applied to genomic DNA directly prepared from fecal samples from sheep experimentally infected with H. contortus to test and validate its use directly on “stool DNA” samples. Finally, it will be tested on fecal samples from sheep in the field that contain mixed species infections with known fecal egg counts to further validate the technique and relate “DNA content” values to the more commonly used fecal egg count data. If, successful, this assay will allow quantitation of parasite infection intensities using molecular biology work flows without the need for microscopy-based fecal egg counting.
Detection of levamisole resistance in *Haemonchus contortus* populations from the United States

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*Haemonchus contortus* is the most prevalent trichostrongylid nematode in tropical and subtropical regions that can cause significant losses in the production system of small ruminants. One of the anthelmintics used to control this parasite is the imidazothiazole derivative, levamisole. In *H. contortus*, the mechanism of resistance to levamisole is not entirely clear, but has previously been associated with a 63bp indel in the *Hco-acr-8* gene that encodes a subunit for a nicotinic acetylcholine receptor. This study aimed at the molecular characterization of levamisole resistance in *H. contortus* populations from small ruminants in the United States. Samples of *H. contortus* populations were obtained from 16 farms in the US, and phenotypically characterized for levamisole resistance using the DrenchRite larval development assay (LDA). DNA was isolated from those same samples and real-time PCR (qPCR) targeting the *Hco-acr-8* indel was used to determine the allelic frequencies in the studied populations. Levamisole EC50 values varied from 0.25 to 20.53 μM and were considered adequate to differentiate between populations. Quantitative PCR results showed resistance allele frequencies ranged from 22.8 to 90.7%. These allelic frequency data are consistent with LDA in most of the studied populations, diverging in only five of them. In this sense, it is possible that other polymorphisms or indels in this or other genes are at play. This is supported by other recent evidence suggesting that levamisole resistance may be influenced by other mutations in the *Hco-acr-8* as well as mutations in other genes. Finally, while the real-time PCR (qPCR) assay used here appears to have some usefulness, it is clear that the *Hco-acr-8* indel alone does not sufficiently explain levamisole resistance. More studies are needed to further elucidate this issue.

17 years of the FAMACHA© program in the United States

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The current United States sheep and goat inventory is 7.86 million head, representing an attractive option for small farmers due to a relatively low initial investment when compared to other livestock systems. However, a major limiting factor for small ruminant health and production is infection with *Haemonchus contortus*, and high levels of anthelmintic resistance is magnifying this problem. The FAMACHA© system, first developed in South Africa in the late 1990’s has proven highly successful as a low-cost technology that reduces the development of anthelmintic resistance by permitting the implementation of targeted selective anthelmintic treatments. The American Consortium for Small Ruminant Parasite Control (ACSRPC) first validated FAMACHA© for use on both sheep and goat farms in the southern USA, and then beginning in June 2003, began conducting training workshops about the use of the FAMACHA© system within the context of education programs covering sustainable integrated parasite management (sIPM). Since then, under the auspices of the ACSRPC, the Kaplan Lab at University of Georgia has managed the FAMACHA© program for North America.
start of the program in 2003 through 2019, a total of 44,503 FAMACHA© cards have been
distributed in 49 US states and Canada. The five states with the most cards sold are North
Carolina, Georgia, Virginia, Tennessee and Oklahoma. New Mexico is the only state with no
registered purchases. Interestingly, the demand for FAMACHA© cards has remained quite stable
over time, averaging 2510/year, and ranging from 1520-3665/year. States in the southern region
represent 57% of total sales; this concentration is likely due to both the geographic focus of the
ACSRPC, and the regional severity of *H. contortus*. In conclusion, the adoption of
FAMACHA© and sIPM by sheep and goat farmers in the US has been fostered with a
remarkably successful program led and administered by the ACSRPC.

**Wildlife Nematodes**

110
*Naturally acquired Dracunculus infection in a Virginia opossum (Didelphis virginiana)*
from upstate New York.
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Manigandan Lejeune, Dwight Bowman. Cornell University College of Veterinary Medicine

A juvenile male Virginia opossum (*Didelphis virginiana*) presented to the Janet L. Swanson
Wildlife Hospital in May 2019 after being found stumbling on a porch with what appeared to be
an injured nose. Upon physical examination, the animal had evidence of severe trauma (acute
and chronic), with soft tissue injury and bone exposure at the nasal planum, a puncture wound on
the caudal right stifle, several missing digits, distal tail necrosis, a mildly prolapsed rectum, and
suspected chronic rib fractures. The opossum was in respiratory distress with suspected partial
obstruction due to nasal injuries. Due to the severity of the injuries, the animal was euthanized.
Exploration of the wound on the caudal right stifle revealed a white nematode subcutaneously
within the lesion. The nematode was identified as a *Dracunculus* species based on morphological
and morphometric characters of the adult female and characteristic first-stage larvae. There are
few reports of *Dracunculus* infection in opossums in North America, both prior to the broad use
of molecular tools. Molecular characterization using a nematode-specific 18S rRNA marker
confirmed the specimen to be a *Dracunculus* species with 99.36% sequence similarity to both,*
*Dracunculus lutrae* from an otter and *Dracunculus insignis* from a raccoon. As the resolution of
this marker is known to be inadequate for specific species confirmation we are currently in the
process of characterizing the CO1 gene for this purpose. The results of molecular
characterization and other parasitologic findings in this animal will be discussed.

**Cattle Protozoa**

111
*Compounds with in vitro activity against Tritrichomonas foetus*
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Katelyn Long, Kylie Thompson, Matthew T. Brewer. Iowa State University

*Tritrichomonas foetus* is a sexually transmitted protozoan parasite and the cause of bovine
trichomonosis. Infection with *T. foetus* results in early embryonic death, causing significant
economic losses due to culling and decreased calf crops. There are currently no approved
treatments for bovine trichomonosis in the United States. Development of a treatment for bovine trichomonosis will greatly reduce the economic impact of the disease. Our lab identified 16 compounds of interest through high throughput screening of compounds from an open access chemical library. Of these 16 compounds, four were found to have lethal effects against \textit{T. foetus} trophozoites in the micromolar range. These findings set the stage for further investigation of the four lethal compounds, as well as related compounds, to determine their utility as chemotherapeutic treatments for bovine trichomonosis. We also examined the effects of three common disinfectants on \textit{T. foetus} growth and viability. Bleach, ethanol, and acetic acid solutions at concentrations below 5\% were lethal to trophozoites. \textit{In vitro} studies are the first step in identifying compounds that may have the potential to be used as treatments of bovine trichomonosis. Future studies will evaluate \textit{in vivo} efficacy and safety of these compounds in cattle.

112

\textbf{Long read metabarcoding of bovine Eimeria, Neobalantidium and Buxtonella communities.}

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Members of the genera \textit{Eimeria}, \textit{Cryptosporidium}, \textit{Entamoeba}, \textit{Giardia}, \textit{Neobalantidium} and \textit{Buxtonella} are common constituents of protozoal communities inhabiting the bovine gastrointestinal tract. \textit{Eimeria} species such as \textit{E. bovis} and \textit{E. zuernii} can cause large intestinal diarrhoea and control is largely dependent on routine chemoprophylaxis, predominantly with ionophores. However, the pathogenicity of \textit{Neobalantidium coli} and \textit{Buxtonella sulcata} is still poorly understood. We are investigating gastrointestinal protozoa community diversity in cattle and the association of specific protozoan species and genetic variants with disease and drug resistance using next-generation amplicon sequencing. We have previously found that short read amplicon sequencing of a number of markers within the 18S rDNA locus provided poor discrimination of \textit{Eimeria} due to low interspecies diversity between several bovine \textit{Eimeria} species. Consequently, we are exploring the use of SMRT (PacBio) and Oxford Nanopore long read amplicon sequencing, of the full length 18S rDNA and Cox-3 mtDNA loci. We have collected and morphologically characterized a variety of \textit{Eimeria} and \textit{Neobalantidium/Buxtonella} populations from beef and dairy cattle, to validate the sequencing data. We have archived protozoal parasite populations from 150 beef cattle sourced from auction markets (overall prevalence of \textit{Eimeria} of 78.6 \%, \textit{Neobalantidium coli}/\textit{Buxtonella sulcata} 28.6 \%) and 40 pooled samples from cattle of 11 dairy farms as well as a number of populations from clinical cases. We will present a comparison of 18s rDNA long-read amplicon SMRT sequencing data with the morphological data on the cattle parasite communities that have been conducted to date.
Research progress of Bovine Neospora caninum in China
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Bovine Neospora caninum is a protozoan parasitic disease that is widespread throughout the world. Bovine Neospora caninum infection is considered to be one of the most common causes of livestock infection and abortion in the world. Significant Neospora caninum losses in dairy products and feed and the beef cattle industry. Because there is no effective treatment or vaccine for Neospora caninum, the diagnosis is very important for Neospora caninum. This article provides a comprehensive overview of the clinical etiology, epidemiological taxonomy, hazards, and specific diagnosis and treatment of Neospora caninum, with a view to adopting effective methods and measures to prevent the occurrence of Neospora caninum for rapid and accurate diagnosis and control. Provide evidence. *This work was supported by grants: Special funds for the Guidance of Central Government on local Science and Technology development (Grant No.ZY17C08).

Keywords: Neospora caninum; vertical transmission; diagnosis; research progress

Horse Protozoa

A molecular comparison of North American and South American Klossiella equi samples: One species spans the Americas?
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Klossiella equi (Apicomplexa, Adeleorina) is a coccidian parasite that infects the kidneys of equids including zebras and donkeys. When detected incidentally during routine necropsy, K. equi has been associated with gross and histological lesions. Detection of K. equi antemortem is rare because it relies on finding sporocysts shed in the urine. Detecting sporocysts is difficult because normal flotation and centrifugation methods can destroy sporocysts. Originally described from Hungarian horses by Baumann in 1946, K. equi has been reported globally (e.g. Iran, Australia, Canada, U.S.A and Kenya). Despite cosmopolitan distribution, mitochondrial genome (MH203050.1) and nuclear 18S rDNA (MH211602.1) sequences were obtained recently from a Southern Ontario K. equi isolate. Targeted PCR amplification of K. equi in mixed parasite-host samples is now practical. Sporocysts in urine of an Argentinian horse provided DNA to compare this South American isolate with Canadian isolates. Using K. equi-specific PCR-primers and amplicon sequencing, mitochondrial (mt) cytochrome c oxidase subunit III (mtCOIII) sequences from the Argentinian isolate was compared to Canadian K. equi. The Argentinian K. equi showed 100% pairwise sequence identity when compared to the Southern Ontario K. equi mt genome over 950 bp that included the majority of the COIII gene. For most coccidia, the mtCOIII gene is highly discriminatory for species delimitation. Although these observations reflect only two geographic regions and clearly more regions will need sampling for
confirmation, possibly only one species, *Klossiella equi*, is responsible for kidney infections in equids globally; these sequence data will permit comparison of *K. equi* to *Klossiella* species in other hosts such as rodents and marsupials. A PCR-based test for detecting *K. equi* in tissue samples and easily obtainable antemortem samples (i.e. equine urine, blood) is under development so global samples can be screened readily for presence and genetic diversity, if any, of this parasite of equids.

**Other Protozoa**

115

**An in vitro evaluation of ozonated water on cyst viability of the protozoon *Giardia duodenalis***

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*Giardia duodenalis* is a very common waterborne zoonotic protozoon, which infects most mammalian species, including livestock as water buffaloes, with negative impacts on their health, welfare and production. The sanitation of drinking water for livestock animals could be an useful strategy to control the diffusion of this protozoo in intensive farming. The aim of this study was to evaluate the *in vitro* effect of ozonated water on *G. duodenalis* cyst viability. A total of 54,000 *Giardia* cysts were isolated from faeces collected from naturally infected water buffaloes, using four sieves of different size and a sucrose flotation. Cysts were divided into 54 glass vials (1000 cysts per vial) to obtain 9 groups of 6 replicates each. Eight groups were treated with ozonated water at four concentrations (0.1, 0.3, 0.5 and 1 mg/l) and two times of contact (one and two minutes). One group was exposed to non-treated water (negative control). To evaluate sanitation kinetics the concentration-time concept was applied, i.e. ozone concentration (C) in mg/l was multiplied by contact-time (t) in minutes (C*t). *Giardia* cyst viability was evaluated by non-fluorogenic dye exclusion method with trypan blue. The percentage of non-viable cysts was calculated using the formula: [1- (total number of viable cysts per ml of aliquot /total number of cysts per ml of aliquot) ]× 100. Negative control group showed 99% of intact cysts. The best results were obtained by using ozonated water at Ct of 1 (0.5 mg/l*2 minutes) with 96.3%; and Ct of 2 (1mg/l*2 minutes) with 95% of destroyed cysts, respectively. These findings suggested that ozone could be a promising eco-friendly tool to control *Giardia* infections in farms. However further *in vitro* and *in vivo* studies are required to verify these results.

116

**Mitochondrial mix-up: How mitochondrial genome organization reflects evolutionary relationships of eimeriorinid coccidia**

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Eimeriorinid coccidia (phylum: Apicomplexa) include several notorious pathogens (*Neospora caninum, Toxoplasma gondii, Eimeria tenella*), and many more lesser-known parasites including *Goussia, Acroeimeria,* and *Choleoeimeria* species. The overwhelming majority of apicomplexan
research ignores the later group, both due to limited opportunities to encounter these species, and to their presumed minimal impact to host health. The major bias towards human and agricultural pathogens in the research of biodiversity and phylogenetics of apicomplexan species, along with a tradition of describing species based on only limited morphological data, have contributed to numerous contradictory and imprecise taxonomic designations within the phylum. The molecular renaissance has shed new light on the problematic nature of apicomplexan taxonomy. Morphology of exogenous stages, historically a central tenant of taxonomic assignations, has been found to be more plastic than previously believed. Formal descriptions of several genera overlap with one another (e.g. *Eimeria*, *Isospora*, and *Goussia*). The content of eimeriorinid mitochondrial genomes is consistent across the group (and beyond); we have found, however, the organization of these small extranuclear genomes to vary. Clade-specific organizational trends have begun to emerge. We present the organization of several eimeriorinid mitochondrial genomes recently sequenced by our group. The potential use of mitochondrial genome organization in assessing phylogenetic placement and the possible mechanisms driving reorganization and stabilization of mitochondrial genome features are discussed. Several amendments to taxonomic descriptions are suggested.

**Wildlife Protozoa**

**Molecular and morphological characterization of an undescribed *Isospora* species infecting the introduced European starling (*Sturnus vulgaris*) in Ontario, Canada**

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European starlings (*Sturnus vulgaris*) were introduced to North America in the 1890s and populations spread rapidly across North America and now comprise one-third of the global population. This highly destructive species is responsible for damages to agriculture (over US$800 million), building structures and aircraft due to their adaptability to human environments. Yet there is little known about the parasites infecting this prevalent host. About 150 species of *Isospora* (Apicomplexa: Eimeriidae) have been reported infecting numerous passerine birds. The majority of these parasite species descriptions are based solely on exogenous oocysts from feces. DNA barcoding techniques have proven useful for differentiating *Isospora* species and to better understand these parasites’ prevalence and pathogenicity. The purpose of this study was to characterize an *Isospora* species that infects the European starling in North America using morphometrics and sequence-based genotyping. Oocysts were collected from European starlings in Guelph, Ontario Canada from carcasses and observed droppings from nesting sites. The presence of *Isospora* oocysts in feces was determined using a modified gravity vial fecal flotation method and isolated using a double flotation technique. Morphometric description was accomplished using microscopy and digital imaging (n=50 oocysts per sample). Morphometric characteristics were compared to *Isospora* species infecting other passerine birds. Molecular characterization involved PCR and amplicon sequencing at two loci (nuclear 18S rDNA and mitochondrial (mt) whole genomes, including mtCOI and mtCOIII). Resulting sequences were compared to those from *Isospora* species described infecting birds or reptiles, and combined to infer phylogenetic relationships among these parasites using Bayesian inference. There is sufficient evidence to support this *Isospora* species as undescribed; a formal species description is required for this unnamed parasite. Molecular and morphometric data to
provide reliable identification of this new species will assist in understanding disease prevalence and pathogenicity of this coccidium infecting the widespread and abundant European starling.

**Dog/Cat Ticks/Mites/Insects**

118

**Acaricidal efficacy of dinotefuran-pyriproxyfen-permethrin (Vectra® 3D)-treated dog hair against adult *Ixodes scapularis* and *Ixodes ricinus* ticks using an *in vitro* feeding assay**

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Vectra® 3D is a spot-on solution combining three active ingredients: dinotefuran, pyriproxyfen and permethrin (DPP) used as an ectoparasiticide for dogs. Here we aimed to assess the efficacy of DPP against *I. scapularis* and *I. ricinus* ticks by using an *in vitro* feeding system with DPP treated dog hair. An artificial feeding system wherein ticks were fed in feeding units fitted with silicone membranes was set up. Each feeding unit (n= 4) was seeded with 40 adults of *I. scapularis* or *I. ricinus*. Bovine blood was added into each feeding unit and changed every 12 h for 4 days. Hair collected from an untreated dog was added to the feeding unit as an attractant. Moreover, hair was collected on day 2, 7, 14, 21 and on day 28 from a dog treated with DPP on day 0. Mortality of ticks was assessed 1h after exposure to the treated hair. One additional hour of incubation was added for moribund specimens. Finally, the rate of tick engorgement was recorded at 96h. Tick engorgement for *I. ricinus* and *I. scapularis* exposed to untreated hair was 45% and 72.5%, respectively, compared to 0% for both species in units with the DPP treated hair. Mortality of *I. ricinus* was 100% at all time-points except on day 28 (90%). The 1h mortality of *L.scapularis* was 100% at all time-points except when exposed to hair collected 21 days after treatment (96.6%) and on day 28 (83.3%) . The cumulative mortality, assessed at 2 hours of incubation, was 100% for both species at each time point. An artificial feeding system for adult *I. ricinus* and *I. scapularis* ticks was successfully utilized to assess the acaricidal efficacy of DPP, which provided a fast acaricidal action against both *I. ricinus* and *I. scapularis* ticks, with 100% mortality after only 2h.

119

**Seasonality of questing ticks in Claiborne County, TN: understudied risks associated with Ixodid ticks in rural Appalachia**

Barbara Shock*, Emily Burke, Matilda Tate, Elizabeth Maggard, Joey Morgan, Vina Faulkner. Lincoln Memorial University

Tick-transmitted diseases are increasing in incidence, and tick spatial distributions and abundances are also shifting. Data on the seasonality of tick questing behavior are sparse, especially in rural Appalachia. The purpose of this project was to investigate the phenology of questing of Ixodid ticks in Claiborne County, Tennessee. Ticks were opportunistically collected from two humans and a domestic dog after daily or weekly walks in early successional habitat from May 2019 until May 2020. The domestic dog was and continues to be on a preventative regimen of Bravecto®. To date, 247 ticks have been identified to three species, *Dermacentor variabilis* (n=172), *Ixodes scapularis* (n=17), and *Amblyomma americanum* (n=58).
Identification of ticks collected from December 2019-May 2020 were delayed due to the Covid-19 pandemic; however, collection was ongoing, and identification will occur in May 2020 upon microscope access. Current data suggests seasonality of tick behavior, with *Ixodes scapularis* adult questing peaking in December. *Dermacentor variabilis* were the most frequently identified tick to date. Ticks will be screened for tick-transmitted pathogens. These findings, although limited, suggest that the average risk of tick encounter for humans and domestic animals in this area warrants further study. These data, along with an ongoing larger study of Ixodid ticks, will also help us to understand the natural history of ticks in the Cumberland Gap Area as well as allow us to determine changes in seasonality or shifts in species composition, i.e., *Haemaphysalis longicornis*.

120 *Flea-Borne Bacterial Pathogens in Fleas and Tissues from Free Roaming Domestic Cats*
Charlotte Manvell*1, Erin Lashnits1, Hanna Berman2, Kelli Ferris3, Benjamin Callahan2, Edward Breitschwerdt1, 1Intracellular Pathogens Research Laboratory, North Carolina State University, 2The Callahan Lab, North Carolina State University, 3College of Veterinary Medicine, North Carolina State University

Free-roaming domestic cats (FRDC) live at an interface between humans, companion animals, wildlife and parasites creating opportunities for cross-species pathogen transmission including zoonotic *Bartonella*, hemotropic *Mycoplasma*, and *Rickettsia* species transmitted by *Ctenocephalides felis*, the most common ectoparasite of FRDC. The goal of this study was to characterize the prevalence of pathogenic bacterial species via targeted real-time PCR (qPCR) and 16S rRNA next generation sequencing (NGS). Fleas were collected from FRDC at clinics in California, Louisiana, the United Kingdom, and North Carolina. In North Carolina and Virginia, eartips and reproductive tissues were concurrently collected. qPCR was performed on DNA extracted from whole washed fleas and tissues, with primers for *Bartonella* spp. and hemotropic *Mycoplasma* spp. targeting the ssrA and 16S regions, respectively. In addition, the microbial communities of 45 fleas were characterized using NGS. By qPCR, 19% of *C. felis* contained *Bartonella* spp. (37/197), and no fleas contained hemotropic *Mycoplasma* spp.. The largest proportion of *Bartonella* spp. positive fleas were from North Carolina (30%) and Louisiana (27%), while few fleas were *Bartonella* PCR positive from California (3%) or the UK (0%). Of cat tissues tested, 19% of eartips (13/68) and 18% of reproductive samples (31/173) were *Bartonella* spp. positive and 16% of eartips (11/68) and 7.5% of reproductive tracts (13/173) were hemotropic *Mycoplasma* spp. positive. NGS showed that the most common pathogenic bacteria were those of the genera *Bartonella* and *Rickettsia*. The sensitivity and specificity of 16S-NGS (qPCR reference) for *Bartonella* spp. was 76% and 92% respectively. Our findings document geographic differences among *Bartonella* infection in fleas, as well as a lack of hemotropic *Mycoplasma* spp. in fleas, despite its presence in the tissue of their feline hosts. Further regional investigations into coinfection dynamics and geographic distribution are necessary to develop a clearer understanding of the transmission dynamics of these pathogens.
**Horse Ticks/Mites/Insects**

121

**Equine tick infestation in fall and winter in northeastern Oklahoma: diversity, seasonality, and attachment site preferences**
Kellee Sundstrom*, Megan Lineberry, Michelle Ientile, Amber Grant, Susan Little. College of Veterinary Medicine, Oklahoma State University

Ticks are common on horses, but publications documenting equine tick infestation risk, particularly in the winter months, are lacking. To further understand the seasonality, species, stages, and attachment site preferences of ticks on horses in northeastern Oklahoma, 84 horses from eight farms were evaluated every two weeks (September 2019–March 2020) by systematically inspecting each horse beginning at the head and moving caudally to the tail. When found, attachment site was noted and then all ticks were removed and identified to species and stage using standard keys. Horses (26 male and 58 female) enrolled in the study ranged in age from 1 to 23 years (mean=12.1, 95% CI 10.9-13.3). Over this seven-month period, 345 ticks were collected from the study population, and more than half (53.6%; 45/84) of the horses were infested (median=2 ticks) at one or more examinations. Tick species found included *Ixodes scapularis* (62.3%; 215/345), *Amblyomma americanum* (22.3%; 77/345), and *Dermacentor albipictus* brown variant (15.4%; 53/345). A majority of ticks were adults, but nymphs of both *A. americanum* (36/77; 46.8%) and *D. albipictus* (6/53; 11.3%) were also identified. Over fall, winter, and early spring, the largest number of ticks (138/345; 40.0%) were collected in November (*P*<0.0001). *Amblyomma americanum* was the only tick recovered in September, *I. scapularis* and *D. albipictus* predominated November through February, and both *A. americanum* and *I. scapularis* were common in March. *Amblyomma americanum* and *D. albipictus* were most often attached to the inguinal area and *I. scapularis* was most commonly found on the chest and axillary region (*P*<0.0001). This research confirms that ticks infest horses in Oklahoma throughout the year, including during the winter, although more data are needed to fully understand the risk these infestations pose to equine health.

**Wildlife Ticks/Mites/Insects**

122

**Tracking an invader: Wildlife Surveillance for *Haemaphysalis longicornis* in the Eastern U.S.**

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*Haemaphysalis longicornis*, also known as the Asian longhorned tick, is native to eastern Asia, but it has become invasive in several countries including, Australia, New Zealand, and now the U.S. Since its 2017 discovery in New Jersey, the presence of *H. longicornis* has been confirmed
in 12 states in the eastern U.S., on a variety of domestic animals, wild and feral carnivores, cervids, rodents, and avian species. Archived specimens previously identified as *H. leporispalustris*, the native rabbit tick, were recently re-examined and determined to be the first detection of *H. longicornis* free-ranging wildlife in the U.S. (white-tailed deer [WTD] in 2010). To better assess the current geographic distribution of *H. longicornis* and to identify wild mammal and avian host species, we conducted active wildlife surveillance at two sites in New Jersey and Virginia with known infestations. In addition, we conducted a passive regional survey in collaboration with wildlife biologists and rehabilitation centers in the southeastern U.S. For rehabilitation centers, all wildlife species were sampled, whereas regional surveillance targeted cervids and bears. Collectively, our surveillance detected *H. longicornis* infestations on 40 individual cervids (WTD and elk) from six states, 50 mesomammals (raccoon, Virginia opossum, striped skunk, red fox, and gray fox) from three states, 2 woodchuck from two states, 4 domestic dogs, 2 coyotes, 1 black bear, 1 eastern cottontail, and 1 red-tailed hawk, from one state each. This surveillance effort resulted in numerous new host, state, and county records for *H. longicornis*. Although infestation prevalence was high for several wildlife species, the availability of cervid samples (specifically WTD), through vehicle strike, rehabilitation, depredation removals, and seasonal hunting, suggests they are potentially efficient sentinels to determine geographic distribution of *H. longicornis*.

Identification and molecular analysis of Ixodid ticks (Acari: Ixodidae) infesting wild boars (*Sus scrofa*) and tick-borne pathogens at the Meihua mountain of southwestern Fujian, China

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Wildlife is essential to biodiversity of the Meihua mountain, southwestern Fujian province, China. However, there have been few surveys of the distribution of ixodid ticks (Acari: Ixodidae) and tick-borne pathogens affecting wild animals at these locations. In this study, 1,197 adult ixodid ticks infesting wild boars were collected from 10 sampling sites during 2019. Ticks were identified to species based on morphology, and the identification was confirmed based on mitochondrial 16S, ITS1 and ITS2 rRNA sequences. Eight tick species belonging to 2 genera were identified, including *H. longicornis* (n=373, 31.1%), *H. flava* (n = 265, 22.1%), *D. auratus* (n=153, 12.8%), *H. hystricis* (n=119, 9.9%), *D. silvarum* (n = 116, 9.7%), *H. bispinosa* (n = 114, 9.5%), *D. atrosignatus* (n=33, 2.8%), and *D. taiwanensis* (n = 24, 2.0%). DNA sequences of *Rickettsia* spp. (spotted fever group) and *Babesia* spp. were detected in these ticks. Phylogenetic analyses revealed possible existence of *Candidatus Rickettsia laoensis* and *Rickettsia raoultii*. This study illustrates potential threat to wild animals and humans from tick-borne pathogens.
Clonorchiasis, caused by *Clonorchis sinensis*, is an important zoonoses disease in the world. The excretory secretory products (ESPs) of *C. sinensis* are causative agents and play important roles in host–parasite interactions. In this study, rabbits were artificially infected by *C. sinensis*, and the serum was collected at different periods, including 14d, 35d and 77d. Adult worms were collected and cultured for preparation of ESPs. Using Co-IP assay to pull down three kinds of serum, the serum of *C. sinensis*, *Fasciola hepatica*, and *Schistosoma japonicum*, respectively. Shotgun LC–MS/MS analysis was used to identify the proteins specific to *C. sinensis*. Many proteins were identified, for the annotated proteins, 10, 36, and 63 proteins were pulled down by the infected serum different periods, respectively, which *C. sinensis* own specifically. And 9 proteins were detected in all three periods. Among them, including two hypothetical proteins (H2KQ80, A0A3R7G318), two proteins belong to cathepsin family (G7YHP4, H2KPM6), one protein related to glutathione S-transferase (A0A3R7BYZ0). Other proteins related to catalytic activity and cellular process. This research could provide new eyesight on further studies about the interaction between *C. sinensis* and host. It also provides theoretical basis for screening potential antigens on clinical diagnosis of Clonorchiasis. Keywords: *Clonorchis sinensis*, ESPs, Co-IP, Mass spectrometry

Ethics statement:
All Japanese white rabbits used in the study were handled according the recommendations of the national guidelines for animal caring: 2017 Revision "Regulation on the Administration of Laboratory Animals". This study was approved by the National Institute of Animal Health Animal Care and Use Committee at Heilongjiang Bayi Agricultural University (approval number 2016-015). * This work was supported by grant from the National Key Research and Development Program of China (2017YFD0501300), the National Natural Science Foundation of China (Grant no. 31972703) and the Natural Science Foundation of Heilongjiang Province of China (C2017048). Corresponding author email: chunrenwang@sohu.com (C. R. Wang)

Prevalence of *Clonorchis sinensis* infections in Southeast Asian fish: a systematic review and meta-analysis from 1976 to 2020
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*Clonorchis sinensis*, an important fishborne zoonotic trematode (FZT), is widely distributed in Southeast Asia, especially in China. Infections from human and animal reservoir hosts occur due to the consumption of raw or undercooked fish with *C. sinensis* metacercariae. This study aimed
to evaluate the prevalence of *C. sinensis* metacercariae in Southeast Asian fish via systematic review and meta-analysis. We searched PubMed, ScienceDirect, China National Knowledge Infrastructure (CNKI), Wanfang, and Chongqing VIP databases for studies published between 1976 and 2020 that related to the prevalence of *C. sinensis* metacercariae in fish. Studies were screened with keywords based on inclusion and exclusion criteria. Seventy-one eligible articles were identified, covering three countries: China, Korea, and Vietnam. The pooled prevalence of *C. sinensis* metacercariae in fish from Southeast Asia was 30.5%, with 36.0% in China, 26.9% in Korea, and 8.4% in Vietnam. In subgroup analyses of climate, season, water source and publication date, the highest prevalence was identified in the Dwb climate type (43.3%), summer (70.2%), rivers (35.5%), and pre-2009 (36.4%), respectively. In comparison, the lowest prevalence was found in the Dfa climate type (14.5%), winter (19.5%), lakes (8.0%), and post-2009 publications (22.6%). Meta-regression results indicated that country (*P* = 0.009) and water source subgroups (*P* = 0.003) may be the source of heterogeneity. Overall, our study indicates that a high-prevalence of *C. sinensis* infections occurs in fish in China, Korea, and Vietnam, illuminating a significant public health concern in these countries. * These two authors contributed equally to this work and are equal first authors. ** Corresponding author email: chunrenwang@sohu.com (C.R. Wang)

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126

**Complete mitochondrial genome of *Prosthogonimus cuneatus* (Trematoda: Prosthogonimidae), as the first representative from the Superfamily Microphalloidea**

Xiaoxiao Ma, Xinru Guo*, Tingting Wu, Zhuo Lan, Yan Jiang, Junfeng Gao, Qiaocheng Chang, Chunren Wang. Heilongjiang Bayi Agricultural University

*Prosthogonimus cuneatus* (Trematoda: Prosthogonimidae) is a common fluke of birds and poultry, which causing serious losses in poultry industry. Many methods were used in the identification, however, taxonomic identification of *P. cuneatus* is still controversial and confused. In some reports, the mitochondrial (mt) genome provided the genetic marker for the identification of closely related species. Thus, in the present study, the mt genome of *P. cuneatus* was firstly determined, and phylogenetic relationship with other trematodes was also reconstructed. The results showed that the mt genome of *P. cuneatus* was 14,829 bp in length, containing 12 protein-coding genes (*cox1-3, nad1-6, nad4L, cyt b and atp6*), 22 transfer RNA genes (tRNA), two ribosomal RNA genes (rRNA) and one non-coding region (NCR). All *P.cuneatus* genes transcribed in the same direction and arranged in the same gene order with *Fasciola hepatica, Clinostomum complanatum* and some Opisthorchidae species (*Opisthorchis felineus, Metorchis orientalis, Clonorchis sinensis*). The A+T content of *P. cuneatus* mt genome was 64.47%. ATG, GTG, TAG and TAA are the most common initiation and termination codons, respectively. Phylogenetic analysis of the concatenated amino acid sequences of 12 protein-coding genes showed that *P. cuneatus* was the closest to the *Dicrocoelium chinensis* and *Eurytrema pancreaticum* (Dicrocoeliidae). This is the first report of the mt genome of *P. cuneatus*, moreover, this is also first time to reveal the mt genome of the member of superfamily Microphalloidea and family Prosthogonimidae. These data provide a significant resource of
molecular markers for further studies of Microphalloidea taxonomy, population genetics, and systematics.

127

**Repurposing oxfendazole as a potential flukicidal compound**

Laura Ceballos¹, Candela Canton*¹, Paula Domínguez¹, Laura Moreno¹, Valeria Gallo², Carlos Lanusse¹, Luis Alvarez¹. ¹Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), Facultad de Ciencias Veterinarias, UNCPBA, ²Instituto DILAVE, ‘Miguel C. Rubino’

Oxfendazole (OFZ) is a nematodicidal drug without flukicidal activity at its recommended therapeutic dose in sheep (5 mg/kg). However, flukicidal activity has been described when OFZ was used at a higher dose (30 mg/kg) both in sheep and pigs. The goals of this study were to characterize the OFZ/metabolites plasma disposition kinetics after OFZ administration at either 5 (OFZ⁵) or 30 (OFZ³⁰) mg/kg dose to non-infected sheep (PK study), and to evaluate the dose-related pattern of *in vivo* accumulation of OFZ/metabolites into *F. hepatica* (Accumulation study). *Pk study*: sheep (n=12) were orally treated with OFZ at either 5 (OFZTD) or 30 (OFZ³⁰) mg/kg. Blood samples were collected over 96 h p.t. *Accumulation study*: *F. hepatica* infected animals (n=8) were orally treated with OFZ at either 5 or 30mg/kg. Animals were sacrificed by captive bolt in accordance with the Animal Welfare Policy (Act 087/02) of the Faculty of Veterinary Medicine, UNCPBA, Tandil, Argentina and internationally accepted animal welfare guidelines (AVMA, 2001). After sacrifice, samples of blood, bile, liver and adult liver flukes were obtained at different times. OFZ was the main analyte detected in plasma from OFZ treated sheep and its systemic exposure (AUC₀–LOQ) increased from 17.9 ± 3.71 (OFZ⁵) up to 85.4 ± 22.6 (OFZ³⁰) µg.h/mL. The Cmax value was 4-fold higher in the OFZ³⁰ group than that in OFZ⁵ group. These differences were also reflected in the pattern of OFZ accumulation into *F. hepatica*, which was 332 % higher in group OFZ³⁰ (4.28 µg/g) than OFZ⁵ (0.99 µg/g). The OFZ dose increment was clearly associated with a higher plasma drug exposure and accumulation into the *F. hepatica*, which help to explain the OFZ flukicidal efficacy observed after a dose of 30 mg/kg. The reported pharmacological data may contribute to assess OFZ repurposing for a new use as flukicidal.
2020 AUTHOR INDEX

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# Author Index

<table>
<thead>
<tr>
<th>Abstract Number</th>
<th>Abstract Number</th>
<th>Abstract Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aafink, Linde</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Abbas, Ghazanfar</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Abdu, Amira</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Abraham, Ambily</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Adams, Amanda</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Akbar, Haroon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfred, Jeffery</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Allen, Kelly</td>
<td>29, 73</td>
<td></td>
</tr>
<tr>
<td>Alvarez, Luis</td>
<td>14, 127</td>
<td></td>
</tr>
<tr>
<td>Andersen, Erik</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Aniz, Ana</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Aroian, Raffi</td>
<td>43, 49</td>
<td></td>
</tr>
<tr>
<td>Avramenko, Russell</td>
<td>15, 51, 52</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bader, Christopher</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Bae, Donghoon</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Bakshi, Mariam</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Ballent, Mariana</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Bangoura, Berit</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Barker, Virginia</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Barta, John</td>
<td>66, 67, 68, 69, 114, 116, 117</td>
<td></td>
</tr>
<tr>
<td>Bartley, Dave</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Bauquier, Jenni</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Beam, Rachel</td>
<td>28, 86</td>
<td></td>
</tr>
<tr>
<td>Beasley, Anne</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Beck, Mariah</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Becskei, Csilla</td>
<td>17, 78</td>
<td></td>
</tr>
<tr>
<td>Begoc, Noemie</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Bell, Julie</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Bellaw, Jennifer</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Benabed, Slimania</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Ben Fayala, Nour El Houda</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Berman, Hanna</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Bertran, Judith</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Beveridge, Ian</td>
<td>39, 55</td>
<td></td>
</tr>
<tr>
<td>Bevins, Sarah</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Bezold, Todd</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Bianchi, Riccardo</td>
<td>62, 63</td>
<td></td>
</tr>
<tr>
<td>Biliska-Zajac, Ewa</td>
<td>45, 46</td>
<td></td>
</tr>
<tr>
<td>Blagburn, Byron</td>
<td>22, 24, 26</td>
<td></td>
</tr>
<tr>
<td>Blalock, Cody</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Boltax, Ariana</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Bonar, Chris</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Bonigjorno, Giola</td>
<td>62, 63</td>
<td></td>
</tr>
<tr>
<td>Borges, Dyego</td>
<td>42, 44</td>
<td></td>
</tr>
<tr>
<td>Borges, Fernando</td>
<td>42, 44</td>
<td></td>
</tr>
<tr>
<td>Borst, Mindy</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Bosco, Antonio</td>
<td>62, 63, 115</td>
<td></td>
</tr>
<tr>
<td>Bourgoin, Gilles</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Bowersock, Laurel</td>
<td>17, 79</td>
<td></td>
</tr>
<tr>
<td>Bowles, Joy</td>
<td>24, 26</td>
<td></td>
</tr>
<tr>
<td>Bowman, Dwight</td>
<td>60, 84, 110</td>
<td></td>
</tr>
<tr>
<td>Brandão, João</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Breitschwerdt, Edward</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Bremer, Catherine</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Bremer, Cathy</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Bress, Todd</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Brewer, Matthew</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Brewer, Matthew T</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Brown, Justin</td>
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<td>Kerr, Moira.</td>
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<td>Ketjis, Jennifer</td>
<td>97</td>
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<tr>
<td>Khan, Matullah.</td>
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<tr>
<td>Khoo, Lester.</td>
<td>58</td>
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<td>Kim, Jenny.</td>
<td>97</td>
<td></td>
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<tr>
<td>Knoll, Stephane.</td>
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<td>Koch, Ryan.</td>
<td>54</td>
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<td>66, 67, 116</td>
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<td>17, 19, 20, 31, 78, 79</td>
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<td>Kulke, Daniel.</td>
<td>32</td>
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<td>Lahmers, Kevin</td>
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<tr>
<td>Lakritz, Jeffrey</td>
<td>. . . . INVITED</td>
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<tr>
<td>Lan, Zhuo</td>
<td>. . . . 126</td>
<td></td>
</tr>
<tr>
<td>Lane, Taylor</td>
<td>. . . . 66, 67</td>
<td></td>
</tr>
<tr>
<td>Lanusse, Carolos</td>
<td>. . . . 14, 106, 127</td>
<td></td>
</tr>
<tr>
<td>La Rosa, Giuseppe</td>
<td>. . . . 45, 54</td>
<td></td>
</tr>
<tr>
<td>Lashnts, Erin</td>
<td>. . . . 120</td>
<td></td>
</tr>
<tr>
<td>Leary, John</td>
<td>. . . . 58</td>
<td></td>
</tr>
<tr>
<td>Leathwick, Dave</td>
<td>. . . . 34, 35</td>
<td></td>
</tr>
<tr>
<td>Lee, Alice</td>
<td>. . . . 30</td>
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<tr>
<td>Lejeune, Manigandan</td>
<td>. . . . 25, 60, 110</td>
<td></td>
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<tr>
<td>Levy, Michel</td>
<td>. . . . 51</td>
<td></td>
</tr>
<tr>
<td>Lewis, Roy</td>
<td>. . . . 112</td>
<td></td>
</tr>
<tr>
<td>Li, Hanchen</td>
<td>. . . . 43</td>
<td></td>
</tr>
<tr>
<td>Li, Qi</td>
<td>. . . . 102</td>
<td></td>
</tr>
<tr>
<td>Lifschitz, Adrian</td>
<td>. . . . 14, 106</td>
<td></td>
</tr>
<tr>
<td>Lin, Kaixiong</td>
<td>. . . . 123</td>
<td></td>
</tr>
<tr>
<td>Lin, Weiming</td>
<td>. . . . 123</td>
<td></td>
</tr>
<tr>
<td>Lin, Xipan</td>
<td>. . . . 123</td>
<td></td>
</tr>
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<td>Lineberry, Megan</td>
<td>. . . . 29, 73, 86, 121</td>
<td></td>
</tr>
<tr>
<td>Liotta, Janice</td>
<td>. . . . 84</td>
<td></td>
</tr>
<tr>
<td>Little, Susan</td>
<td>. . . . 28, 29, 80, 81, 85, 121, INVITED, INVITED</td>
<td></td>
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<td>Lloberas, Mercedes</td>
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<td></td>
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<tr>
<td>Long, Katelyn</td>
<td>. . . . 111</td>
<td></td>
</tr>
<tr>
<td>Long, Li Jiang</td>
<td>. . . . 105</td>
<td></td>
</tr>
<tr>
<td>Long, Maureen</td>
<td>. . . . 22</td>
<td></td>
</tr>
<tr>
<td>Loutet, Bruno</td>
<td>. . . . 53</td>
<td></td>
</tr>
<tr>
<td>Lownachan, Alan</td>
<td>. . . . 40</td>
<td></td>
</tr>
<tr>
<td>Lucio-Forster, Araceli</td>
<td>. . . . 97, 110</td>
<td></td>
</tr>
<tr>
<td>Ludwig, Dariann</td>
<td>. . . . 77</td>
<td></td>
</tr>
<tr>
<td>Luque, Sonia</td>
<td>. . . . 106</td>
<td></td>
</tr>
<tr>
<td>Lv, Bo</td>
<td>. . . . 125</td>
<td></td>
</tr>
<tr>
<td>Lv, Qingbo</td>
<td>. . . . 65</td>
<td></td>
</tr>
<tr>
<td>Lyons, Eugene</td>
<td>. . . . 34</td>
<td></td>
</tr>
<tr>
<td>Ma, Xiao-Xiao</td>
<td>. . . . 124</td>
<td></td>
</tr>
<tr>
<td>Ma, Xiao Xio</td>
<td>. . . . 65, 126</td>
<td></td>
</tr>
<tr>
<td>MacKay, Evelyn</td>
<td>. . . . INVITED</td>
<td></td>
</tr>
<tr>
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<td>. . . . 19, 20, 31, 78</td>
<td></td>
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<td>Maggard, Elizabeth</td>
<td>. . . . 119</td>
<td></td>
</tr>
<tr>
<td>Mahabir, Sean</td>
<td>. . . . 17, 19, 20, 31, 78, 79</td>
<td></td>
</tr>
<tr>
<td>Mansour, Abdelmoneim</td>
<td>. . . . 32</td>
<td></td>
</tr>
<tr>
<td>Marwell, Charlotte</td>
<td>. . . . 120</td>
<td></td>
</tr>
<tr>
<td>Marcondes, Mary</td>
<td>. . . . INVITED</td>
<td></td>
</tr>
<tr>
<td>Marsh, Antoinette</td>
<td>. . . . 25, 33, 56, 57</td>
<td></td>
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<tr>
<td>Martin, Avery</td>
<td>. . . . 101</td>
<td></td>
</tr>
<tr>
<td>Martin, Katy A</td>
<td>. . . . 111</td>
<td></td>
</tr>
<tr>
<td>Martin, Richard</td>
<td>. . . . 47</td>
<td></td>
</tr>
<tr>
<td>Martinescu, Gabriela</td>
<td>. . . . 94</td>
<td></td>
</tr>
<tr>
<td>Massolo, Alessandro</td>
<td>. . . . 10, 13, 90</td>
<td></td>
</tr>
<tr>
<td>Mather, Thomas</td>
<td>. . . . 82</td>
<td></td>
</tr>
<tr>
<td>Matt, Crystal</td>
<td>. . . . 75</td>
<td></td>
</tr>
<tr>
<td>Maura, Daniela</td>
<td>. . . . 30</td>
<td></td>
</tr>
<tr>
<td>Maurrelli, Maria-Paola</td>
<td>. . . . 94</td>
<td></td>
</tr>
<tr>
<td>Maurrelli, Maria Paola</td>
<td>. . . . 115</td>
<td></td>
</tr>
<tr>
<td>McColl, John</td>
<td>. . . . 19, 31</td>
<td></td>
</tr>
<tr>
<td>McGrath, Patrick</td>
<td>. . . . 105</td>
<td></td>
</tr>
<tr>
<td>McHugh, Mark</td>
<td>. . . . 47</td>
<td></td>
</tr>
<tr>
<td>McLean, Nancy</td>
<td>. . . . 27</td>
<td></td>
</tr>
<tr>
<td>McTier, Tom</td>
<td>. . . . 19, 20, 31</td>
<td></td>
</tr>
<tr>
<td>Meinikoff, James</td>
<td>. . . . 73</td>
<td></td>
</tr>
<tr>
<td>Meloni, Luisa</td>
<td>. . . . 11</td>
<td></td>
</tr>
<tr>
<td>Melville, Lynsey</td>
<td>. . . . 52</td>
<td></td>
</tr>
<tr>
<td>Mercer, Nicola</td>
<td>. . . . 9, 21</td>
<td></td>
</tr>
<tr>
<td>Merchant, Daniel</td>
<td>. . . . 93</td>
<td></td>
</tr>
<tr>
<td>Mertins, James</td>
<td>. . . . 122</td>
<td></td>
</tr>
<tr>
<td>Meyer, Leon</td>
<td>. . . . 17, 78, 118</td>
<td></td>
</tr>
<tr>
<td>Miller, Brad</td>
<td>. . . . 12</td>
<td></td>
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<tr>
<td>Millward, Laurie</td>
<td>. . . . 33</td>
<td></td>
</tr>
<tr>
<td>Miron, Liviu</td>
<td>. . . . 94</td>
<td></td>
</tr>
<tr>
<td>Miró, Maria Victoria</td>
<td>. . . . 106</td>
<td></td>
</tr>
<tr>
<td>Mones, Alissa</td>
<td>. . . . 110</td>
<td></td>
</tr>
<tr>
<td>Monteiro, Jomar</td>
<td>. . . . 108</td>
<td></td>
</tr>
<tr>
<td>Moorhead, Andrew</td>
<td>. . . . 56, 96</td>
<td></td>
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<td>Morelli, Simone</td>
<td>. . . . 18</td>
<td></td>
</tr>
<tr>
<td>Moreno, Laura</td>
<td>. . . . 127</td>
<td></td>
</tr>
<tr>
<td>Morgan, Joey</td>
<td>. . . . 119</td>
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<tr>
<td>Morgoglione, Maria Elena</td>
<td>. . . . 115</td>
<td></td>
</tr>
<tr>
<td>Morosetti, Arianna</td>
<td>. . . . 15, 107</td>
<td></td>
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<tr>
<td>Morphew, Russell</td>
<td>. . . . 91</td>
<td></td>
</tr>
<tr>
<td>Moré, Gastin</td>
<td>. . . . 114</td>
<td></td>
</tr>
<tr>
<td>Mulcahy, Grace</td>
<td>. . . . 36</td>
<td></td>
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<td>Musiani, Marco</td>
<td>. . . . 90</td>
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<td>Mussiani, Marco</td>
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<td>Myers, Melanie</td>
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<td>Norris, Jamie</td>
<td>. . . . 41, 64</td>
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<td>Ohmes, Caramel</td>
<td>. . . . 86</td>
<td></td>
</tr>
<tr>
<td>Oliva, Gaetano</td>
<td>. . . . 62, 63</td>
<td></td>
</tr>
<tr>
<td>Ossiloff, Robert</td>
<td>. . . . 59</td>
<td></td>
</tr>
<tr>
<td>Ostroff, Gary</td>
<td>. . . . 43</td>
<td></td>
</tr>
<tr>
<td>Ott-Conn, Caitlin</td>
<td>. . . . 70</td>
<td></td>
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<td>Page, Lauren</td>
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</tr>
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<td>Paramasivan, Vijayapalani</td>
<td>. . . . 98</td>
<td></td>
</tr>
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<td>Pardonnet, Sylvia</td>
<td>. . . . 53</td>
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</tr>
<tr>
<td>Parimsetti, Venkateswara Rao</td>
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<td></td>
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<td>Park, Doyeon</td>
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<td>Parrish, Rebecca</td>
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<td>Pearl, David</td>
<td>. . . . 9, 21</td>
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<td>Pelletier, Sarah</td>
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<td>Peregrine, Andrew</td>
<td>. . . . 9, 21, INVITED</td>
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<td>Perregrino, Ali</td>
<td>. . . . 83</td>
<td></td>
</tr>
<tr>
<td>Peters, Kerri</td>
<td>. . . . 99</td>
<td></td>
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<td>Phan, Phan</td>
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<td>Piccione, Julie</td>
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<td>. . . . 45, 54</td>
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<td>Price, Elexis</td>
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<tr>
<td>Prior, Craig</td>
<td>. . . . 56</td>
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<td>Pullins, Aleah</td>
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<td>Qiu, Hongyu</td>
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<td>Qiu, Yang-Yuan</td>
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<td>Qiu, Yangyuan</td>
<td>. . . . 102</td>
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<td>Qiu, Yuan</td>
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<td>Queiroz, Camila</td>
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</tr>
<tr>
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</tr>
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<td>Raines, Janis</td>
<td>. . . . 73</td>
<td></td>
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<td>Ramsay, Edward</td>
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<td></td>
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<tr>
<td>Randall, Adam</td>
<td>. . . . 122</td>
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<td>Ranjan, Siva</td>
<td>. . . . 24</td>
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<tr>
<td>Rashid, Muhammad Imran</td>
<td>. . . . 145</td>
<td></td>
</tr>
</tbody>
</table>
American Association of Veterinary Parasitologists
65th Annual Meeting, June 20th – 23rd 2020, Virtual Meeting

<table>
<thead>
<tr>
<th>Number</th>
<th>Abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raue, Katharina</td>
<td>18</td>
</tr>
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<td>Rauf, Umber</td>
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<td>Reaves, Barbara</td>
<td>104</td>
</tr>
<tr>
<td>Reckziegel, Guilherme</td>
<td>42</td>
</tr>
<tr>
<td>Redman, Elissabeth</td>
<td>51</td>
</tr>
<tr>
<td>Redman, Elizabeth</td>
<td>15, 52, 92, 112</td>
</tr>
<tr>
<td>Redman, Libby</td>
<td>53</td>
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<td>Reichard, Mason</td>
<td>28, 54, 70, 72, 75, 86</td>
</tr>
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<td>Reinemeyer, Bree</td>
<td>16</td>
</tr>
<tr>
<td>Reinemeyer, Craig</td>
<td>16, 17</td>
</tr>
<tr>
<td>Rejman, Evelin</td>
<td>117</td>
</tr>
<tr>
<td>Revell, Sarah</td>
<td>10</td>
</tr>
<tr>
<td>Revo, Anshula</td>
<td>53</td>
</tr>
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<td>Rey, Benjamin</td>
<td>53</td>
</tr>
<tr>
<td>Rezansoff, Andrew</td>
<td>13</td>
</tr>
<tr>
<td>Riley, Jennifer</td>
<td>122</td>
</tr>
<tr>
<td>Rinaldi, Laura</td>
<td>62, 63, 94, 115</td>
</tr>
<tr>
<td>Riner, John</td>
<td>79</td>
</tr>
<tr>
<td>Robertson, Alan</td>
<td>47</td>
</tr>
<tr>
<td>Rodrigues, Vincius</td>
<td>42</td>
</tr>
<tr>
<td>Rohdich, Nadja</td>
<td>18</td>
</tr>
<tr>
<td>Roher, Amber</td>
<td>99</td>
</tr>
<tr>
<td>Roman, Constantin</td>
<td>94</td>
</tr>
<tr>
<td>Rosenthal, Benjamin</td>
<td>46</td>
</tr>
<tr>
<td>Rothenburger, Jamie</td>
<td>10</td>
</tr>
<tr>
<td>Rotolo, Jessica</td>
<td>68, 69</td>
</tr>
<tr>
<td>Ryon, Grant</td>
<td>33</td>
</tr>
<tr>
<td>Rozycki, Miroslaw</td>
<td>45</td>
</tr>
<tr>
<td>Ruckstuhl, Kathleen</td>
<td>13</td>
</tr>
<tr>
<td>Ruder, Mark</td>
<td>61, 122</td>
</tr>
<tr>
<td>Rudinsky, Adam</td>
<td>33</td>
</tr>
<tr>
<td>Rugg, Jady</td>
<td>20, 78</td>
</tr>
<tr>
<td>Rumschlag, Anthony</td>
<td>INVITED</td>
</tr>
<tr>
<td>Rus, Florentina</td>
<td>43</td>
</tr>
<tr>
<td>Ryan, Kathryn</td>
<td>77</td>
</tr>
<tr>
<td>Ryan, William</td>
<td>INVITED</td>
</tr>
<tr>
<td>Répérant, Jean-Michel</td>
<td>68</td>
</tr>
</tbody>
</table>

**S**

<table>
<thead>
<tr>
<th>Number</th>
<th>Abstract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saleh, Meriam</td>
<td>56, 80, 81, 85</td>
</tr>
<tr>
<td>Sampeck, Bridgette</td>
<td>77</td>
</tr>
<tr>
<td>Sanders, John</td>
<td>49</td>
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<tr>
<td>Sanders, Justin</td>
<td>74</td>
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<td>Sanders, Tiana</td>
<td>54</td>
</tr>
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<td>Santa, Maria</td>
<td>13</td>
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<td>Sauer, Christian</td>
<td>34</td>
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<tr>
<td>Scala, Antonio</td>
<td>11</td>
</tr>
<tr>
<td>Scaléa, Giulia</td>
<td>42, 44</td>
</tr>
<tr>
<td>Scare, Jessica</td>
<td>100, 101</td>
</tr>
<tr>
<td>Scare-Kenealy, Jessica</td>
<td>37</td>
</tr>
<tr>
<td>Scare Kenealy, Jessica</td>
<td>34</td>
</tr>
<tr>
<td>Schatz, Serena</td>
<td>84</td>
</tr>
<tr>
<td>Schunack, Bettina</td>
<td>86</td>
</tr>
<tr>
<td>Schurer, Janna</td>
<td>10</td>
</tr>
<tr>
<td>Scimeca, Ruth</td>
<td>70, 72, 75, 86</td>
</tr>
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<td>68, 69</td>
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**U**

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**V**

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<td>62, 63, 118</td>
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<td>Verma, Saurabh</td>
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<td>Verocai, Guilherme</td>
<td>23, 25, 27</td>
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**W**

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<td>Wang, Chunren</td>
<td>.65, 102, 126</td>
</tr>
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<td>Wang, Hao-Xian</td>
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<tr>
<td>Weldon, Morgan</td>
<td>30</td>
</tr>
</tbody>
</table>
Wellehan, Jr, James .................. 59
Wetherly, Patricia .................. 109
White, Holly ......................... 60
White, Seth ........................... 61, 122
Whitley, Derick ...................... 86
Wilkes, Edwina ...................... 39
Will, Edith ............................ 70, 72
Williams, Paul ....................... 47
Wilson, Georgette .................... 77
Wilson, Natalie ...................... 104
Wise, David ........................... 58
Wititkornkul, Boontarikaan ....... 91
Wolstenholme, Adrian ............. 104
Wonfor, Ruth ......................... 91
Woods, Debra ....................... 19

Wozniakiewicz, Magda ............ 17
Wright, Ian ........................... 126

Wu, Tingting .......................... INVITED

X
Xu, Wenwen ......................... 102

Y
Yabsley, Michael .................. 61, 71, 82, 88, 122
Yang, Fei .............................. 123
Yee, Heather .......................... 33
Young, David ......................... 78, 79
Young, Rebecca ...................... 110
Z
Zajac, Anne ......................... 25, 43, 49
Zarlenga, Dante ..................... 46, 50
Zeldenrust, Elizabeth .......... 114
Zhang, Xiao-Xuan .................. 113
Zhang, Ying ......................... 124, 125
Zhu, Nina ............................. 84
Zhu, Shawna ......................... 53
Zohdy, Sarah ....................... 26, 83
The 2020 Membership Directory contains all members who have paid the calendar year (January 1 - December 31, 2020) regular or student dues. Emeritus members are also included in the directory.
American Association of Veterinary Parasitologists
65th Annual Meeting, June 20th – 23rd 2020, Virtual Meeting

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65th Annual Meeting, June 20th – 23rd 2020, Virtual Meeting

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156
American Association of Veterinary Parasitologists
65th Annual Meeting, June 20th – 23rd 2020, Virtual Meeting

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Snowbird, UT: June 25–28, 2022