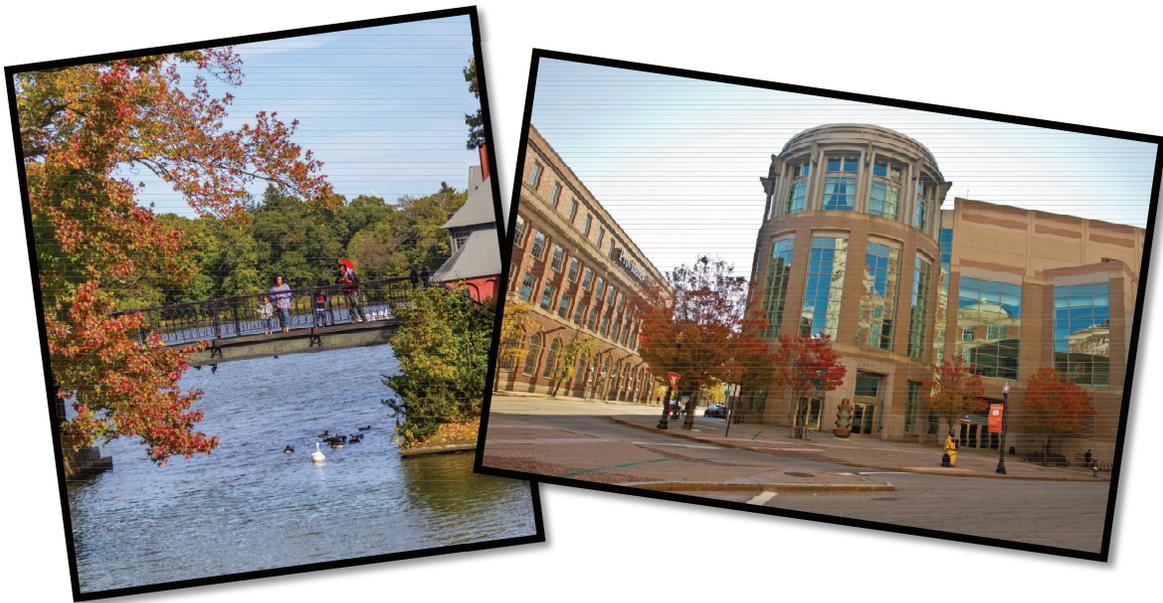


Proceedings of the
American Association of



Veterinary Laboratory Diagnosticians



58th Annual Conference

Rhode Island Convention Center

Providence Rhode Island

October 22-28, 2015



AAVLD Strategic Plan
Adopted July 31, 2014

Vision

The AAVLD is a world leader in advancing the discipline of veterinary diagnostic laboratory science to promote global animal health and One Health.

Mission

The AAVLD promotes continuous improvement and public awareness of veterinary diagnostic laboratories by advancing the discipline of veterinary diagnostic laboratory science. The AAVLD provides avenues for education, communication, peer-reviewed publication, collaboration, outreach, and laboratory accreditation.

Motto: Advancing veterinary diagnostic laboratory science

Core values

The AAVLD is committed to these core values:

- Continuous improvement
- Engagement of members
- Effective communication
- Collaboration
- Support of One Health

Goals

1. Advocate for the role of veterinary diagnostic laboratories in global health.
2. Foster the continuous improvement of diagnostic laboratory techniques and processes, personnel qualifications, and facilities.
3. Promote the continued professional growth of members.
4. Disseminate information concerning the diagnosis/monitoring of animal health and disease surveillance.
5. Provide a formal accreditation process for veterinary medical diagnostic laboratories.

American Association of Veterinary Laboratory Diagnosticians

The American Association of Veterinary Laboratory Diagnosticians (AAVLD) is a not-for-profit professional organization which seeks to:

- Disseminate information relating to the diagnosis of animal diseases.
- Coordinate diagnostic activities of regulatory, research and service laboratories.
- Establish uniform diagnostic techniques.
- Improve existing diagnostic techniques.
- Develop new diagnostic techniques.
- Establish accepted guidelines for the improvement of diagnostic laboratory organizations relative to personnel qualifications and facilities.
- Act as a consultant to the United States Animal Health Association on uniform diagnostic criteria involved in regulatory animal disease programs.

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Acknowledgments

The success of a meeting is a function of both presenters and attendees - a special thank you to all who present their data and findings, to all exhibitors and sponsors, and to everyone who attends our meeting. We would also like to give a special thank you to all of our invited speakers and moderators for the AAVLD Plenary Session and the USAHA-AAVLD Plenary Session.

Program Committee members, listed below, deserve special acknowledgement for their hard work, organization, review and editing of the abstracts, as do moderators of our scientific sessions. Kim Grant, from Thomson Reuters, helped us navigate the ScholarOne software. Jackie Cassarly coordinated all meeting room and exhibitor booth arrangements, sponsor agreements, breaks, and all of the many other details that go into making this meeting a success.

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AWARDS

2015 Trainee Travel Awardees

Erica Noland	Michigan State University
Lorelei Clarke	University of Georgia
Jenny Pope	University of Tennessee
	Pathology Committee Travel Awardee
Shahan Azeem	Iowa State University
Nandita Mirajkar	University of Minnesota
Jennifer Dill	University of Georgia
Elizabeth Elsmo	University of Georgia
Courtney Smith	Texas A&M University
Boying Liang	University of Kentucky
Shubhada Chothe	Pennsylvania State University
Oi Chen	Iowa State University
Camila Abreu	Federal University of Lavras

2015 Staff Travel Awardees

Susan Martin	University of Missouri
Marc Schwabenlander	University of Minnesota
Emily Collin	Kansas State University
John Loy	University of Nebraska-Lincoln
Eric Lee	Cornell University
Daniel Corrales	Texas A&M University

2015 ACVP/AAVLD Award

Courtney Schott
University of Guelph

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 Saturday, October 24, 2015
 556

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556

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Sunday, October 25, 2015
556

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 Saturday, October 24, 2015
 555

Moderators: Kelli Almes and Jamie Henningson

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 Sunday, October 25, 2015
 555

Moderators: Chanran Ganta and Danielle Nelson

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Sunday, October 25, 2015
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Serology; QA/QC
Sunday, October 25, 2015
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Moderators: Devi Patnayak and Pat Lukens

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 Sunday, October 25, 2015
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552

Moderators: Larry Thompson and Karyn Bischoff

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Virology 1
 Saturday, October 24, 2015
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Moderators: Julia Ridpath and Christie Mayo

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 Sunday, October 25, 2015
 551

Moderators: Jianqiang Zhang and Douglas Marthaler

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 Sunday, October 25, 2015
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POSTER SESSION

Posters

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ABSTRACTS

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 Saturday, October 24, 2015
 Ballroom D-E

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The 2015 avian influenza outbreak: phylogenetic analysis of the H5N2 influenza virus

Mia Kim Torchetti

Diagnostic Virology Laboratory, USDA-APHIS-NVSL, Ames, IA

Narrative (400 words): HPAI virus (H5N8 clade 2.3.4.4) originating from Eurasia (EA) spread rapidly along wild bird migratory pathways in the Eastern Hemisphere during 2014. Introduction of this virus into the Pacific Flyway of North America sometime during 2014 allowed mixing with North American (AM) origin low pathogenicity avian influenza A viruses generating new (novel) combinations with genes from both EA and AM lineages (so called “reassortant” H5Nx viruses). To date, the H5Nx viruses have been detected in the Pacific, Central, and Mississippi Flyways. These findings are not unexpected as the H5Nx viruses continue to circulate. The USDA APHIS National Veterinary Services Laboratories (NVSL) collaborated with the USDA ARS Southeast Poultry Research Laboratory (SEPRL) and the Influenza Division of the Centers for Disease Control and Prevention (CDC) to generate the analyses for this report. Consensus data from whole genome sequence is used to monitor the virus evolution and assess risk to veterinary or public health based upon presence/absence of specific amino acid substitutions or protein motifs. All viruses analyzed to date are highly similar, have an HA gene derived from the EA H5 clade 2.3.4.4, and are highly pathogenic in poultry. Both H5N2 and H5N8 were implicated in recent poultry outbreaks. Where there is molecular evidence that independent introductions as well as “common source” exposures are occurring concurrently further field epidemiologic investigation is warranted. Poultry events in Pacific Flyway appear to be largely due to point source/independent introductions as were early Midwest events based upon network analysis and available epidemiologic data. Data for later Midwest events suggest point source as well as “common source” exposures occurring concurrently. States affected last appear to be largely due to common source/human activity. Presently the risk to human health remains low; molecular markers associated with antiviral resistance or increased virulence and transmission in mammals have not been detected; however, virus monitoring continues with CDC. This analysis includes samples collected between December 2014 to early June 2015 from 17 states (>240 viruses). While these viruses remain highly similar overall (>99% similar to the index viruses within subtype, as well as to the nearest Asian isolate (A/crane/Kagoshima/KU1/2014(H5N8)), analytical tools that identify substitutions along the hemagglutinin (HA), neuraminidase (NA) and internal proteins can improve our understanding of the virologic, antigenic, and epidemiologic features of the virus (refer to section on Diagnostics and Characterization for H5Nx viruses).

Speaker Biography: Dr. Mia Kim Torchetti joined NVSL in 2013 as the Avian Viruses Section Head in the Diagnostic Virology Laboratory at the NVSL. She has a broad background in laboratory networking, research and diagnostics, epidemiology, and biosecurity; earning her veterinary degree and master’s in epidemiology at Colorado State University, and subsequently joining ARS in Athens, Georgia for her PhD and postdoctoral work largely focused on microbiology. While at the Southeast Poultry Research Laboratory, her initial focus was on rapid diagnostics for Newcastle disease, moving to influenza as the issues of highly pathogenic avian influenza emerged. Prior to the NVSL, she served as the deputy coordinator and South/Southeast Asia laboratory liaison for the Food and Agriculture Organization of the United Nations, Emergency Prevention Systems Lab Unit based in Rome, Italy. In that role, she worked closely with national veterinary authorities, and coordinated laboratory networking and capacity building primarily in Southeast Asia to address testing needs for control of H5N1 highly pathogenic avian influenza and other priority diseases. During her first year at the NVSL, incursion of a novel subtype causing illness in humans (H7N9 influenza) in China resulted in international collaboration across veterinary and public health. Dr. Torchetti and her team were included in a USDA Secretary’s Honor Award submitted by colleagues from USDA’s Agricultural Research Service (ARS) for their role in the coordinated ARS/APHIS response to the Chinese H7N9 Avian Influenza outbreak, providing critical scientific data necessary for control and diagnosis of the virus nationally and internationally.

The University of Minnesota Veterinary Diagnostic Laboratory: Responding to Highly Pathogenic Avian Influenza

Ann M. Fitzpatrick, Mary Thurn, Elizabeth Wiedenman, Sunil Kumar, Robert Porter, Kurt D. Rossow

Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN

Narrative: The incursion of Highly Pathogenic Avian Influenza (HPAI) into the United States during 2015 resulted in an unprecedented foreign animal disease event. The USDA has reported 191 positive HPAI PCR results in 15 states affecting over 43.5 million birds as of this writing. Minnesota is the largest turkey producing state in the nation, accounting for 18% of the U.S turkey production or about 45 million birds. The areas with concentrated number of turkeys in Minnesota were the epicenter of the AI outbreak in the spring of 2015. The first case of HPAI in Minnesota poultry was presumptively diagnosed at the end of February 2015 at the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL) and confirmed as HPAI H5N2 at the National Veterinary Services Laboratory on March 4, 2015. Since then Minnesota has seen 97 farms affected by HPAI H5N2 and to-date the UMN VDL has run 11,500 tests for avian influenza. We will demonstrate the progression of cases presumptively diagnosed as positive throughout the state over time as well as the massive increase in the amount of AI testing conducted at the UMN VDL. Current data from the UMN VDL Laboratory Information Management System will be presented as charts, maps and/or interactive video. A visual understanding of disease progression may help identify specific risk factors associated with disease spread. A graphic representation of the number of PCR tests conducted will help other diagnostic labs plan for the number of samples they may see submitted in an AI outbreak. This will also illustrate the important role of a veterinary diagnostic laboratory in a foreign animal disease event in the United States.

Second verse, but not quite the same as the first: Tips on preparing your lab for HPAIV ◇

*Rodger Main, Sarah Abate, David Baum, Phillip Gauger, Patrick G. Halbur, Karen Harmon, Yuko Sato,
Wendy R. Stensland, Katie Woodard, Kyoung-Jin Yoon, Jianqiang Zhang*

Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

Narrative: The Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) has been on the forefront of two emerging disease crises of national significance in as many years. While the core elements of preparedness identified during the PEDV pandemic of 2013-2014 remain the same (i.e., need for validated assays, high-throughput testing platforms, user-friendly and effective sample types for herd/flock level diagnosis, premises identification numbers (PINs) on submission forms, and electronic messaging of results); there have been many additional complexities realized throughout the HPAIV epidemic of 2015. Our objective is to share some of these experiences and actions taken during the HPAIV response in efforts to help the broader state of preparedness amongst the VDL community. The additional complexities appreciated during the HPAIV response included: a more time and equipment intensive testing algorithm; multiple batches of testing per day; confirmatory testing at NVSL; real-time communication with state and federal animal health officials; limited veterinary involvement in the diagnostic sample collection and submission process; requirements to quarantine and depopulate premises upon a positive test result; select agent based limitations of using clinical material for research purposes; continuity of business testing to allow permitted movements; extended work hours and weekend testing at VDL; and responding to the myriad of inquiries from the media. Noteworthy capabilities and modifications of practice that have proven useful during the HPAIV outbreak included: affiliating all AIV case submissions with the state and federal veterinary medical officials (agencies); creating a singular e-mail distribution list inclusive of the individuals needing to receive a copy of the AIV PCR presumptive positive case reports; providing additional compensation for VDL staff working extended hours and weekend shifts; establishing a well-defined and sustainable testing schedule that provides same-day results, seven days a week; instituting new protocols aiming to mitigate biosecurity risks associated with dropping samples off at the VDL; developing automated algorithms for generating the list of samples needing to be forwarded to NVSL; streamlining flow of NVSL case reports as not to clutter the e-mail of VDL case coordinators; appointing a qualified staff member whose primary responsibilities are to coordinate and manage program disease work and reporting; assigning a subject matter expert (faculty) to manage media inquiries; establishing systems that electronically incorporate PINS into VDL records; and ensuring ISU LIMS is capable of capturing and reporting the full complement of submitter, animal owner, and farm site (premises) level identifiers. In summary, the 2015 HPAIV outbreak has brought forth another wave of learning opportunities and system improvements that will be useful in preparing VDLs for future program disease response activities.

◇ USAHA Paper

Highly Pathogenic Avian Influenza Outbreak: epidemiologic findings and what they tell us about prevention and control

Brian McCluskey

USDA, APHIS, Fort Collins, CO

Narrative (400 words): Since the expansion of HPAI viruses into commercial poultry occurred in January 2015, APHIS Veterinary Services (VS) has initiated a number of epidemiologic and laboratory based investigations to better understand the factors associated with HPAI virus transmission. These investigations include: Field-based observational studies with data collected through surveys and site visits; Geospatial analyses; On-farm sampling efforts; and Phylogenetic investigations. With the data from these reports, APHIS concludes that there is not substantial or significant enough evidence to point to a specific pathway or pathways for the current spread of the virus. This is further supported by the molecular analysis of the virus. In a case series investigating 81 turkey farms across the Midwestern United States, we found turkey farms typically follow biosecurity protocols, which are established by the company with which they work. Common procedures include spraying vehicle tires with disinfectant at the farm entrance, requiring visitors and employees to wear coveralls and disposable boot covers (or dedicated footwear) before entering the barns, using disinfectant footbaths at barn entrances, using rodent control, and caring for younger birds before caring for older birds. The objective is to establish a clean-dirty line where outside contaminants are not carried into the barn. While most of the 81 farms surveyed had biosecurity protocols in place, only 43% of case farms reported that biosecurity audits or assessments were conducted on the farm by the company or a third party. Farms can decrease their HPAI risk by verifying that biosecurity procedures are being followed properly. We conducted a case-control study focused on egg layer flocks in Iowa and Nebraska. At the farm level, being located in an existing control zone was highly associated with farm status. Rendering dead birds was a risk factor. Having visitors change clothing was protective. Visits in the past 14 by a company service person were associated with farm status. At the barn level, having a hard-surface barn entry pad that was cleaned and disinfected was protective when compared with all other levels combined. Dead bird disposal within 30 yards of a barn remained a statistically significant risk factor. Findings from these and other studies form the basis for recommendations on prevention strategies at the farm and barn level.

Speaker Biography: Brian J. McCluskey is Chief Epidemiologist at USDA-APHIS Veterinary Services, a position he has held since 2011. He holds an affiliate faculty position at Colorado State University in the Department of Clinical Sciences and has published frequently on infectious disease epidemiologic investigations and animal health surveillance. He joined APHIS in 1990 and was stationed in Charleston, West Virginia as a section veterinary medical office, then was stationed at the Colorado Area Office as the Area Epidemiology Officer. During this time, he became a Diplomate in the American College of Veterinary Preventive Medicine. Brian then moved to the Center for Animal Health Monitoring in Fort Collins, Colorado as the Dairy Commodity Support Analyst. From 2003 to 2007, he was the Director of the USDA's National Surveillance Unit. He joined the Senior Executive Service in 2007 as the Director of Veterinary Services Western Region. Prior to joining APHIS, Dr. McCluskey was in large animal practice, primarily dairy, in western Washington. Dr. McCluskey received his PhD (2003) in epidemiology from Colorado State University, DVM (1987) from Washington State University, and master's degree from the University of Florida.

Avian influenza outbreak of 2015: regulatory effort and what was learned

John R. Clifford

Veterinary Services, USDA APHIS, Washington, DC

Narrative (400 words): The United States Department of Agriculture Animal and Plant Health Inspection Service (APHIS) identified Eurasian lineage H5 clade 2.3.4.4 highly pathogenic avian influenza (HPAI) in the United States in December 2014 in the Pacific Northwest. Since then, these HPAI viruses have been detected in commercial and backyard poultry flocks, wild birds, or captive wild birds in 21 states. A total of 15 states reported cases in commercial and backyard poultry, and control efforts resulted in the destruction of 7.5 million turkeys and 42.1 million egg-layer and pullet chickens. Warmer temperatures in conjunction with control and biosecurity efforts implemented in areas impacted by HPAI led to outbreak decline, with no new reported cases since June 17, 2015. The decline of HPAI detections provides an opportunity to review lessons learned, enhance prevention efforts, and prepare for additional cases that may occur in the fall when birds migrate south from their northern breeding grounds. The H5 clade 2.3.4.4 HPAI viruses have been identified in three of the four U.S. flyways since December 2014 and the potential for HPAI to enter the Atlantic flyway via migrating ducks is anticipated, should it not already be present but as yet undetected in the resident wild duck population. APHIS, along with its partners, has learned a great deal through responding to the largest animal health event in the country's history and has applied that experience to enhancing its response capabilities and processes. This presentation provides an overview of lessons learned and details initiatives the Agency has implemented, including working closely with state diagnostic laboratories participating in the National Animal Health Laboratory Network (NAHLN) to enhance and expedite nationwide capacity for detection and confirmation of HPAI. In addition to updating its protocols and guidance for diagnostic testing, APHIS has encouraged states to consider implementing barcoding of samples to ensure national premises identifications are up to date and available to the laboratories, and to consider options for surge testing and sample transport options. The Agency has worked with the NAHLN to evaluate staffing plans and surge capacity, the availability of equipment, sampling supplies and media, and proficiency-tested technicians. APHIS places great value on this partnership with the NAHLN and those with state, academic and industry partners and looks forward to continued collaboration to protect animal health in the U.S.

Speaker Biography: As the Chief Veterinary Officer of the United States, Dr. Clifford provides leadership for safeguarding U.S. animal health. Prior to becoming Deputy Administrator for the United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) in May 2004, Dr. Clifford served as VS' acting Deputy Administrator. He was the Associate Deputy Administrator National Animal Health and Policy program where he led VS' efforts to protect, sustain, and improve productivity, marketability, and health of the nation's animals, animal products, and biologics. Dr. Clifford served as VS' Assistant Deputy Administrator from 1997-2002. He also has extensive field experience. Since joining APHIS in 1985, Dr. Clifford has also served as the area veterinarian in charge in Ohio, West Virginia, Michigan, and Indiana; the National Health Monitoring System coordinator in Ohio; and the brucellosis epidemiologist and veterinary medical officer in Kentucky. Before beginning his work with APHIS, Dr. Clifford was a private veterinarian in a mixed practice. He received his DVM and BS degrees in animal science from the University of Missouri. A native of Kentucky, Dr. Clifford currently resides in Virginia with his wife, Sara.

Bacteriology 1
 Saturday, October 24, 2015
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Moderators: Lalitha Peddireddi and Kenitra Hammac

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1:15 PM	<i>Streptococcus bovis</i> Septicemia in a Group of Calves following Intramuscular Vaccination # † + <i>Lorelei L. Clarke, Robert L. Fathke, Susan Sanchez, James B. Stanton</i>	36
1:30 PM	A multiplex real-time PCR panel for detection of major bacterial and viral agents causing abortion in small ruminants # † <i>Shahan Azeem, Sara Ghorbani-Nezami, Ahmad Allam, Vickie L Cooper, Eric Burrough, Kent Schwartz, Paul Plummer, Paulo Arruda, Phillip Gauger, Joann M. Kinyon, Darin Madson, Orhan Sahin, Gregory Stevenson, Kyoung-Jin Yoon</i>	37
1:45 PM	Genetic characterization of novel pathogen “<i>Brachyspira hamptonii</i>” reveals associations of diverse clades, regions, host species and relationships with other swine <i>Brachyspira</i> species # † <i>Nandita Mirajkar, Aschalew Bekele, Yogesh Chander, Connie Gebhart</i>	38
2:00 PM	A diagnostic tool for the veterinary clinician and pathologist: panfungal polymerase chain reaction (PCR) on formalin-fixed paraffin embedded (FFPE) tissues to classify fungal organisms found histopathologically # † <i>Courtney M. Smith, Caitlin Older, Laura Bryan, Sara Lawhon, Jan Suchodolski, Joanne Mansell, Aline Rodrigues-Hoffmann</i>	39
2:15 PM	Application of MIQE guidelines for quantitative detection of <i>Lawsonia intracellularis</i> by PCR <i>Kent Doolittle, Wayne Chittick, Allison Spitz, Greg Cline, Jack Gallup</i>	40
2:30 PM	Comparative Assessment of Multiplex PCR Assays to Culture and Susceptibility Testing Methods for the Detection of Bovine Bacterial Respiratory Pathogens and Macrolide Antimicrobial Resistance Determinants § <i>Laura Pike, John D. Loy</i>	41
2:45 PM	Clinical evaluation of Vetericyc Plus™ Pinkeye Spray in calves with experimentally induced Infectious Bovine Keratoconjunctivitis ◇ <i>Julie Gard, Debra R. Taylor, Rachel Maloney, Megan Schnuelle, Sue Duran, Will Justus, Paul Walz, Annette O'Connor</i>	42

Symbols at the end of titles indicate the following designations:

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| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Avian *Coxiella*-like infection in a hyacinth macaw † +

Melissa Macias Rioseco²¹, Margaret A. Highland³¹, Rocio Crespo²⁴

¹VMP, Washington State University, Pullman, WA; ²Washington Animal Disease Diagnostic Laboratory, Pullman, WA; ³USDA-ARS ADRU, Pullman, WA; ⁴Avian Health and Food Safety Lab, Puyallup, WA

Narrative: A 550.0 g, 17-year-old, female hyacinth macaw (*Anodorhynchus hyacinthinus*) with a two week history of weight loss, vomiting, poor appetite, and lethargy was presented for necropsy. On macroscopic examination the caudal coelom contained a 14.0 cm diameter, soft, dark red, encapsulated mass. The mass was freely movable aside from attachment to the cranial aspect of the left kidney by a 2.0 cm long, thin strand of connective tissue. The left adrenal gland and left ovary were not identified. The spleen, and liver were identified grossly in the expected anatomic location and also examined microscopically. The coelomic mass shared histological features consistent with splenic tissue (encapsulated, blood filled tissue with fibrous connective tissue bands, and lymphoid aggregates adjacent to small arterioles). Throughout the coelomic mass and spleen were numerous, often aggregated, macrophages that contained variably discernible intracytoplasmic, finely granular to amorphous lightly basophilic intracytoplasmic vacuoles/inclusions. Numerous lymphocytes and plasma cells, often with pyknotic or karyorrhectic nuclei, accompanied the histiocytic inflammation. The bird also had moderate to severe histiocytic and lymphoplasmacytic hepatitis and mural proventriculitis. Lymphoplasmacytic perivascular cuffing and focal periventricular encephalitis was also identified. Intracytoplasmic microorganisms were identified in macrophages within the adrenal gland, liver, and proventricular wall. The intracytoplasmic microorganisms were Fite's acid fast positive, variably positive with Kinyoun's acid fast, exhibited no to light staining with Gimenez stain, and were slightly Gram-negative (Brown and Hopps). Microorganisms did not stain with Giemsa. Real-time PCR performed on DNA isolated from spleen was negative for *Chlamydophila* spp. Real-time PCR performed on DNA isolated from the liver and coelomic mass was positive for *Coxiella avium* ("*Coxiella*-like" species). Avian *Coxiella*-like infections have been described recently in other psitticine species and a toucan ^{1,2}. Presentation of infection in this case was unusual in that there was a large caudal coelomic mass that was histologically compatible with splenic tissue, yet on gross necropsy a spleen was identified in the normal location. Regardless of the tissue of origin, the large, blood-filled, inflammatory mass was space occupying and compression of the surrounding organs, including the intestinal tract, likely contributed to clinical symptoms of weight loss and vomiting. Avian *Coxiella*-like infections should be considered a differential diagnosis for lymphoplasmacytic and histiocytic splenitis and hepatitis.

1. Shivaprasad HL, Cadenas MB, Diab SS, *et al.* 2008: "Coxiella-like infection in psittacines and a toucan" Avian Dis. 52(3):426-32. 2. Vapniarsky N, Barr BC, Murphy B. 2012: "Systemic Coxiella-like infection with myocarditis and hepatitis in an eclectus parrot (*Eclectus roratus*)". Vet Pathol. 9(4):717-22

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

***Streptococcus bovis* Septicemia in a Group of Calves following Intramuscular Vaccination # † +**

Lorelei L. Clarke¹, Robert L. Fathke², Susan Sanchez³, James B. Stanton¹

¹Department of Pathology, University of Georgia, Athens, GA; ²Virginia Maryland College of Veterinary Medicine, Blacksburg, VA; ³Department of Infectious Diseases, University of Georgia, College of Veterinary Medicine, Athens, GA

Narrative: *Streptococcus bovis* is a normal component of the ruminal flora and faeces, but it can be an opportunistic pathogen in cases of ruminal acidosis and mastitis. In this case study, a cluster of calf deaths occurred within hours of intermuscular vaccination and was associated with *Streptococcus bovis*. Within 12 hours of vaccination of 46 calves at a cow/calf operation, four calves had died, three calves were ill, and one unvaccinated cow was dead. Clinically affected animals presented with fevers of 108°F and rectal bleeding. Necropsies were performed on the cow, two dead calves, and one affected surviving calf, which was euthanized approximately 24 hours after vaccine administration. Animals had similar gross and microscopic lesions, including subcutaneous and intramuscular dark hemorrhage on the caudal neck, occasionally extending into the brisket and shoulder regions. There was also multi-organ ecchymosis and petechiation, and a fibrinonecrotizing pneumonitis/alveolitis with intravascular Gram-positive diplococci. Skeletal muscle at the injection site was disrupted by a neutrophilic myositis and vasculitis with intravascular Gram-positive diplococci. Type D streptococcal organisms were cultured from the skeletal muscle and lung, as well as from the vaccines used on these animals. Isolates were speciated as *S. bovis*. These findings suggest a streptococcal septicemia and toxemia, likely resulting from contamination of the multi-dose vaccine vials. *S. bovis* has been associated in humans with the development of colonic adenocarcinoma and endocarditis, but these syndromes have not been described in cattle. This case highlights the necessity of vaccination protocols and the investigation and reporting of adverse vaccine reactions.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

A multiplex real-time PCR panel for detection of major bacterial and viral agents causing abortion in small ruminants # †

Shahan Azeem¹, Sara Ghorbani-Nezami¹, Ahmad Allam², Vickie L Cooper¹, Eric Burrough¹, Kent Schwartz¹, Paul Plummer¹, Paulo Arruda¹, Phillip Gauger¹, Joann M. Kinyon¹, Darin Madson¹, Orhan Sahin¹, Gregory Stevenson¹, Kyoung-Jin Yoon¹

¹College of Veterinary Medicine, Iowa State University, Ames, IA; ²Veterinary Research Division, National Research Centre, Cairo, Egypt

Narrative: Reproductive failure causes significant economic losses and consternation to small ruminant producers. Abortion in sheep and goats can be caused by myriad etiologies, which makes conventional laboratory testing laborious and time-consuming. Furthermore, many times etiological diagnosis cannot be established due to the suboptimal quality of samples received. The objective of this study was to develop and evaluate a multiplex polymerase chain reaction (PCR) panel (hereafter referred to as “small ruminant abortion PCR panel”) to detect various bacterial and viral agents commonly implicated in sheep and goat abortion. The small ruminant abortion PCR panel consisted of two one-step multiplex real-time reverse transcription-polymerase chain reactions (RT-PCRs): one for bacteria (*Campylobacter* spp., *Chlamydophila abortus* and *Coxiella burnetii*) and the other for viruses [border disease virus (BDV), Cache Valley virus (CVV) and caprine herpesvirus type 1 (CpHV-1)]. Once primer and probe design for each of the target agents and running conditions of multiplex assay were optimized, the panel was applied to caprine and ovine cases submitted to Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) with clinical history of abortion. A total of 23 submitted abortion cases during 2012-2015 lambing season were selected and tested using the small ruminant abortion PCR panel. The diagnostic performance of the panel was compared to results of conventional and/or molecular assays which had been routinely performed at ISUVDL for the selected bacterial and viral agents. For PCR testing, tissues (including placenta) or stomach contents from aborted or stillborn fetuses were homogenized in Earle’s balanced salt solution. Both DNA and RNA were simultaneously extracted from each sample using a commercial kit (MagMAX™ Total Nucleic Acid Isolation kit, Applied Biosystems, Carlsbad, CA, USA). PCR was done using the standard protocol established in ISUVDL. Of the tested cases, the prevalence of each target agent was as follows: *Campylobacter* spp. (47.8%), *Chlamydophila abortus* (4.3%), *Coxiella burnetii* (4.3%), CpHV-1 (4.3%). In comparison to conventional *Campylobacter* culture on the same cases, the PCR panel detected 4 more positive cases. These positive results were in agreement with histological assessment by diagnostic pathologists on the cases. Similarly, the small ruminant abortion PCR panel was able to detect *Chlamydophila abortus* in a case that was negative by immunohistochemistry. Neither BDV nor CVV was detected in any of the tested cases. All cases were tested negative for bovine viral diarrhea virus by a separate RT-PCR. Although the current study used samples from a small set of abortion cases, the findings to date suggest the small ruminant abortion PCR panel can be a useful tool for simultaneous and rapid detection of common bacterial and viral agents involved in sheep or goat abortion cases, improving the etiological diagnosis.

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† Graduate Student Oral Presentation Award Applicant

Genetic characterization of novel pathogen “*Brachyspira hampsonii*” reveals associations of diverse clades, regions, host species and relationships with other swine *Brachyspira* species # †

Nandita Mirajkar¹, Aschalew Bekele², Yogesh Chander², Connie Gebhart^{1,2}

¹Veterinary and Biomedical Sciences, University of Minnesota, Falcon heights, MN; ²Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, MN

Narrative: Outbreaks of bloody diarrhea in swine herds in the late 2000s signaled the re-emergence of swine dysentery in the U.S. Diagnostic investigations confirmed the emergence of a novel pathogen “*Brachyspira hampsonii*” with two distinct genetic clades circulating in the North American swine population, and subsequent research has reproduced clinical disease in swine experimentally infected with both clades. Since then, “*B. hampsonii*” has been detected in swine and migratory birds in North America and Europe, however the molecular epidemiology of this pathogen remains largely unknown. The objective of this study was to develop and implement a multilocus sequence typing (MLST) scheme in order to molecularly characterize “*B. hampsonii*” and to elucidate the diversity, distribution, population structure and genetic relationships of this pathogen from diverse epidemiological sources globally, as well as to evaluate the genetic relationships of “*B. hampsonii*” with other *Brachyspira* species. Using our newly developed MLST scheme, we genetically characterized 81 “*B. hampsonii*” isolates originating from diverse epidemiological sources (different farms, swine production systems, countries and host species) and identified a total of 20 genotypes known as sequence types (11 from the U.S. and nine from other countries). “*B. hampsonii*” exhibited a heterogeneous population structure with microevolution detected locally within swine production systems. Within the U.S., clade I was more frequently isolated/widespread and less diverse than clade II. The clustering patterns of “*B. hampsonii*” displayed the association of genotypes and their country or swine system of origin. Isolates from migratory birds represented distinct yet closely related genotypes when compared with swine origin isolates. The comparative multilocus sequence analysis of 430 isolates representing pathogenic and commensal *Brachyspira* species from 19 countries and 10 host species depicted clustering by bacterial species. It revealed a close clustering of all “*B. hampsonii*” irrespective of their genetic clade, thus providing support for both clades to be considered a single species. Interestingly, it also revealed the close genetic relatedness of “*B. hampsonii*” with commensals *B. murdochii* and *B. innocens*, rather than other pathogens *B. hyodysenteriae* and “*B. suanatina*”, known to cause mucohemorrhagic diarrhea in swine. In conclusion, this is the first study to establish a multilocus sequence typing scheme for “*B. hampsonii*”, to characterize its genotypes, and elucidate its population structure. It highlights the role of MLST in routine surveillance/monitoring and the importance of strict biosecurity measures in preventing the spread of pathogens such as “*B. hampsonii*” between swine herds and also potentially between migratory birds and swine. It also suggests that “*B. hampsonii*” represents a diverse but single bacterial species that has close genetic associations with commensal *Brachyspira* species.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

A diagnostic tool for the veterinary clinician and pathologist: panfungal polymerase chain reaction (PCR) on formalin-fixed paraffin embedded (FFPE) tissues to classify fungal organisms found histopathologically # †

*Courtney M. Smith^{2,1}, Caitlin Older², Laura Bryan¹, Sara Lawhon¹, Jan Suchodolski³, Joanne Mansell²,
Aline Rodrigues-Hoffmann^{2,1}*

¹VTPB, Texas A&M University, College Station, TX; ²Dermatopathology Service, Texas A&M University, College Station, TX; ³VSCS, Texas A&M University, College Station, TX

Narrative: Classification of fungal organisms often poses a problem for pathologists since fungal organisms can have very similar morphologies histologically. Furthermore, fungal culture can take long periods of time, or fresh tissues might not have been collected and submitted for culture. The purpose of this study is to validate the use of panfungal primers via PCR to classify fungal organisms on FFPE tissues. Samples from 77 cases where fungal organisms were observed histologically were selected and included in this study. These included tissues from canine, feline, equine and bovine hosts with cutaneous, nasal, and pulmonary fungal infections. DNA was extracted and isolated from FFPE tissues using the BiOstic FFPE Tissue DNA isolation kit (MoBio Laboratories, Carlsbad, CA). PCR was performed using ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers targeting the Internal Transcribed Spacer (ITS) region (found in all eukaryotes). Positive bands were purified, sequenced and classified at >97% identity match using the basic local alignment search tool (BLAST) and the NCBI database of ITS sequences. Of the 77 cases, 52 (68%) were PCR positive, 32 confirmed the histologic diagnosis to the species level, ten cases were classified to the genus level, and the remaining ten cases were belonging to the Phaeohyphomycoses and unable to classify the genus.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

Application of MIQE guidelines for quantitative detection of *Lawsonia intracellularis* by PCR

Kent Doolittle¹, Wayne Chittick¹, Allison Spitz¹, Greg Cline¹, Jack Gallup²

¹Boehringer-Ingelheim Vetmedica, Inc., Ames, IA; ²Iowa State University, Ames, IA

Narrative: Introduction and Objectives Publications by Pederson¹ and Collins² demonstrate the feasibility and benefits of quantitative PCR for detection of *Lawsonia intracellularis*. Building on work by Sylvia et al.⁴, the objective of this study was to implement a quantitative PCR for *Lawsonia intracellularis* from fecal samples using guidelines from the work of Bustin, including the Minimum Information for publication of Quantitative real-time PCR Experiments guidelines (MIQE). **Materials and Methods** Plasmid standards and PCR kits were provided by Life Technologies Inc. Plasmids were linearized by a Hind III digest (Promega corp., Madison WI) and diluted in a series of 1:10 dilutions using carrier RNA to create qPCR standards and a standard curve calibrator. A standard curve along with assay limit of detection (LOD) and quantification (LOQ) were established. The standard curve was saved and used for further analysis to standardize the results calculations using a calibrator. An inhibition study was conducted to assess the impact of sample type on the reported quantity³. A BLAST search using the assay target genes was performed to verify the target genes existed only once per genome. Results were calculated and reported as a number of bacteria per gram feces by taking into consideration all processing steps including sample preparation, extraction, and PCR setup. **Results** The lower limit of quantification is defined as $1.4E+03 \log_{10}$ bacteria per gram feces (Ct 35.5). The limit of detection is defined at $2.25E+02 \log_{10}$ bacteria per gram feces with a measurement of uncertainty (To Be Determined). **Discussion and Conclusions** This test represents a new tool to evaluate *Lawsonia* antigen load in animals to better understand disease severity. Now that an accurate measurement tool is established, future work in the field can be done to determine the level of clinical relevance (LOC) or the load of bacteria that causes clinical and subclinical disease. Future work will be necessary to assess quantitation in sample types such as oral fluid and environmental swabs. **References** 1. Pedersen et. al. Association between fecal load of *Lawsonia intracellularis* and pathological findings of proliferative enteropathy in pigs with diarrhea. BMC Veterinary Research 2012, 8:198 2. Collins et al. The critical threshold of *Lawsonia intracellularis* in pig feces that causes reduced average daily weight gains in experimentally challenged pigs. Veterinary Microbiology 2014. 3. Tuomi et. al. Bias in the Cq value observed with hydrolysis probe based quantitative PCR can be corrected with the estimated PCR efficiency value. Methods 2010. 4. Sylvia et.al. Application of MIQE guidelines for PCV2 qPCR. 2014 AAVLD proceedings

Comparative Assessment of Multiplex PCR Assays to Culture and Susceptibility Testing Methods for the Detection of Bovine Bacterial Respiratory Pathogens and Macrolide Antimicrobial Resistance Determinants §

Laura Pike, John D. Loy

University of Nebraska-Lincoln, Lincoln, NE

Narrative: Bovine Respiratory Disease (BRD) is the most common and costly disease affecting beef cattle. The most frequent bacterial pathogens associated with BRD include; *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. Detection of pathogen nucleic acid using PCR methodology can be advantageous as diagnostic submissions frequently originate from animals treated with antibiotics or have been mishandled during shipment, thus impeding recovery of live organisms. Disadvantages include lack of a clinical isolate for antimicrobial susceptibility testing. The primary objective of this study was to develop and compare Multiplex Real Time PCR methods with classic bacterial culture for the detection of pathogens associated with BRD in a variety of diagnostic matrices including nasopharyngeal swabs and lung tissue. A secondary objective was to determine if macrolide resistance determinants could be detected and associated with a resistant organism. A multiplex PCR was developed and target validation was performed with reference isolates for each pathogen and closely related organisms (*Bibersteinia trehalosi*, *Gallibacterium* sp., etc) A combination of 137 samples, including nasal swabs (67) and lung tissues (70) were tested in parallel using PCR and standard aerobic bacterial culture. *Mycoplasma bovis* was subjected to a validated conventional PCR for comparison. A total of 75 samples were found positive for any one or more of the pathogens. Samples with detected agents were screened for the presence of the following genes which confer macrolide-resistance; *mph*, *msr*, and *erm* by PCR. Isolates recovered from culture were subjected to a broth microdilution assay and MIC values for bovine drugs were determined. After comparing the recovery rates between PCR and culture the following data was observed; *M. haemolytica* was recovered 15(culture)/18(PCR) in lung tissue, 83% agreement and 10/23 in nasal swabs, 38% agreement. *P. multocida* was recovered 8/19 in lung tissue, 42% agreement and 13/9 in nasal swabs, 57% agreement. *H. somni* was isolated via nasal swabs 0/11 times, 0% agreement and 8/5 times in lung tissue, 71% agreement. *M. bovis* was detected 2 (conv. PCR)/4 (RT PCR) via nasal swabs, 50% agreement and 10/14 in lung, 71% agreement. After screening pathogen positive samples for macrolide-resistance genes 24 samples came up positive for both *mph* and *msr*, 16 for *erm*, and 1 for *mph* only. In conclusion, Real-Time PCR methods appear advantageous in the detection of BRD associated pathogens over culture in nasal swabs. In lungs, culture and PCR appear equivalent except for *H. somni*. Real-Time PCR detected more positive *M. bovis* samples than conventional PCR. Additionally, macrolide-resistant genes were detected by PCR testing but more data is needed to determine relationships between the presence of these genes and *in vitro* susceptibility profiles.

§ AAVLD Laboratory Staff Travel Awardee

Clinical evaluation of Vetericyn Plus™ Pinkeye Spray in calves with experimentally induced Infectious Bovine Keratoconjunctivitis ◇

Julie Gard¹, Debra R. Taylor¹, Rachel Maloney¹, Megan Schnuelle¹, Sue Duran¹, Will Justus¹, Paul Walz², Annette O'Connor³

¹Clinical Sciences, Auburn University of College of Veterinary Medicine, Auburn, AL; ²Pathology, Auburn University College of Veterinary Medicine, Auburn, AL; ³VDPAM, Iowa State, Ames, IA

Narrative: Infectious Bovine Keratoconjunctivitis (IBK), commonly called “pink eye”, is a painful condition affecting beef and dairy cattle worldwide. The bacterium, *Moraxella bovis* is known to be responsible for this condition. It has been estimated that annual losses associated with only decreased weight gain from infected cattle exceeds 150 million dollars. Hence, this study evaluated Vetericyn Plus™ Pinkeye Spray as an aid in the reduction of pain, infection and healing. Thirty dairy bull calves having determined to have normal ophthalmic examinations and who were culture negative for *M. bovis* were randomly assigned to 3 groups for a single eye block randomized blinded challenge study. Calves were housed in pairs according to their respective group in an approved isolation facility. A 0.6 mm corneal lesion was made on the center of the left corneas of Groups 1 and 2 utilizing n-heptanol. Immediately following lesion formation, 1.0×10^7 of *Moraxella bovis* (strain Epp63-300; origin: NADC) was administered topically to the left central corneas of Groups 1 and 2. The calves in Group 3 (Control group) received topical corneal administration of *M. bovis* to their left eyes but nothing further. In Group 1, two mLs of Vetericyn Plus™ Pinkeye Spray was administered topically to each calves’ cornea twice daily for 10 days. In Group 2, two mLs of 0.9% Saline was administered topically to each calves’ cornea twice daily for 10 days. Each animal was given a pain score twice daily (based on blepharospasm, ocular discharge and tearing) utilizing a scale of one to four. All eyes were cultured on day -7, 0, 1-5, and day 10. Additionally, serum and plasma samples were drawn from all calves on days 0, 1, 10, 11, and 17 and evaluated for changes in sodium and chloride levels. All calves in group 1 and 2 developed lesions in the left eye as determined by fluorescein staining. All calves in group 2 developed lesions consistent with IBK in the left eyes. Calves in group 2 only were determined to be culture positive for *M. bovis* during the study period. Between Days 1 and 2, Group 1 had significantly, $P < 0.05$, decreased pain scores when compared to controls. On average there was a reduction in pain score by 79.1% by day 2 and an 83.7% reduction in pain by day 10 when compared to controls. Group 2 had an average reduction in pain score of 18.3%, and 67.9% by day 2 and by day 10, respectively, when compared to controls. There was no significant difference in sodium and chloride levels in the plasma and serum among all three groups at any of the sampling time points. The results of this study indicates that Vetericyn Plus™ Pinkeye Spray can be utilized as an aid in the reduction of pain and infection due to IBK in cattle. Acknowledgments: Innovacyn, Merial Summer Scholars Program

◇ USAHA Paper

Bacteriology 2a
Sunday, October 25, 2015
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Moderators: Akhilesh Ramachandran and Dubraska V. Diaz-Campos

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8:15 AM	Evaluation of a Molecular Serotyping Assay for <i>Salmonella enterica</i> <i>Shelley C. C. Rankin, D S. Munro</i>	46
8:30 AM	Improved <i>Streptococcus suis</i> isolate selection method for autogenous vaccines <i>Srivishnupriya Anbalagan</i>	47
8:45 AM	Intergenic Sequence Ribotyping (ISR) Method for <i>Salmonella</i> Serotyping in Veterinary Diagnostics <i>Roxana Sanchez Ingunza, Melissa Madsen, Jean Guard, John El-Attrache</i>	48

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| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
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| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Development and Implementation of a verification system for Bacterial identification using MALDI-TOF Biotyper §

Eric W. Lee, Loretta Miller, Bridget Randall, Rebecca Franklin-Guild, Melissa Aprea, Christie Williams, Craig Altier, ANIL J. THACHIL

AHDC Bacteriology, Cornell University, Ithaca, NY

Narrative: Eric Lee*, Lorrie Miller, Bridget Randall, Rebecca Franklin-Guild, Melissa S Aprea, Christie Williams, Craig Altier and Anil Thachil Advanced diagnostic technologies are becoming more prevalent in today's diagnostic laboratories and over the period of years diagnostic bacteriology has transitioned from traditional biochemical or automated biochemical identification systems and 16s rDNA sequencing to an era of rapid identification using MALDI-TOF Biotyper. Accuracy of test results for any new technique we use in our diagnostics is extremely important to improve the standards of accredited laboratories in compliance with AAVLD and ISO 17025 requirements. A systematic and robust verification process was developed at Cornell University Animal health Diagnostic center (AHDC) for MALDI-TOF Biotyper method of bacterial identification with proper documentation and data management for each species of bacteria as part of our quality system. Using the guidelines laid out in Cumitech 31A Verification procedure, confidence was achieved by compiling results from several methods performed including traditional biochemicals, automated biochemical identification system, 16s rDNA sequencing or rpoB sequencing which are compared with MALDI-TOF identification scores of 2.0 or greater. We analyzed data from at least 20 isolates of each bacterial species from different sources and species of origin. Only bacterial isolates having more than 90% agreement were qualified to be verified under this system. We have so far verified more than 60 organisms successfully at the species level and 10 of them failed to qualify in this process. Choosing a test system for each bacterial species is also critical in the verification process. Cornell AHDC MALDI-TOF verification system could easily be adapted by other veterinary diagnostic laboratories for improving their in-house verification process.

§ AAVLD Laboratory Staff Travel Awardee

Evaluation of a Molecular Serotyping Assay for *Salmonella enterica*

Shelley C. C. Rankin, D S. Munro

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Narrative: Serotyping using the Kauffmann-White scheme has been of great value to understand the epidemiology of *Salmonella* and investigate disease outbreaks. However, production and quality control of the hundreds of antisera required for serotyping the more than 2500 serovars identified to date, is difficult and time-consuming and is limited to a few specialized reference laboratories worldwide. Results from published work performed at CDC led to the development of the xMAP *Salmonella* Serotyping Assay. This assay is a microsphere based multiplex nucleic acid assay that detects genes involved in the expression of serotype-specific antigens. The *Salmonella* O-group-specific assay detects the six most common serogroups in the United States (B, C1, C2, D, E, and O13) plus serotype Paratyphi A. The *Salmonella* H-antigen assay detects 15 H antigens and 16 complex secondary antigens. The University of Pennsylvania, School of Veterinary Medicine, *Salmonella* Reference Center has serotyped more than 50,000 *Salmonella* isolates since 1997. 326 *Salmonella* isolates, all obtained from veterinary sources, were selected from the collection and these were tested with the xMap *Salmonella* Serotyping Assay. The isolates tested were selected to represent a range of diverse animal species, temporal variation (1997-2011) and in the case of Enteritidis and Typhimurium a selection of different phage types. 58 Enteritidis, 44 Heidelberg, 41 Kentucky, 48 Newport, 49 Typhimurium and 86 “Other” serovars from groups B, C1, C2, D, E, and O13 were selected to determine whether the xMAP assay could accurately identify the most common serotypes obtained in our laboratory. The assay showed a 100% correlation with the traditional Kauffman White serotype that was previously obtained at the *Salmonella* Reference Center and no discrepant results were observed.

Improved *Streptococcus suis* isolate selection method for autogenous vaccines

Srivishnupriya Anbalagan

Diagnostics, Newport Laboratories, Inc., Worthington, SD

Narrative: *Streptococcus suis* is an important pathogen causing meningitis, septicemia, arthritis, pneumonia, and endocarditis in pigs. Thirty three serotypes (type 1 through 31, 33, and 1/2) of *S. suis* have been identified. Serotyping is one of the most important diagnostic tools for differentiating *S. suis* isolates and continues being a widely used method to understand the epidemiology of a particular outbreak, monitor serotype prevalence, and guide in vaccine development. Since serotyping is laborious, time consuming, and relies only on capsule, molecular identification based on virulence factors involved in adherence, internalization, immune response evasion and dissemination could be an attractive alternative to conventional typing methods. In this study a virulence associated multi locus sequence typing (MLST) method was developed. Four capsule polysaccharide biosynthesis genes *cps1J* (serotype 1), *cps2J* (serotype 2), *cps7H* (serotype 7), and *cps9H* (serotype 9), arginine deiminase (*arc*), glutamate dehydrogenase (*gdh*), suilysin (*sly*), muramidase-released protein (*mrp*), and extracellular factor (*ef*) were picked. Multiple sequence alignment of up to 50 sequences from GenBankSM was conducted using MegAlignTM (DNASTar, Madison, WI). Primers were designed to amplify either the entire gene or the most variable portion of the gene. Each primer pair was checked to see if they amplified the desired product, which was also confirmed by DNA sequencing of the PCR products. Two multiplex PCR (mPCR) assays were then designed. Assay 1 amplified *cps1J*, *cps7H*, *cps9H*, *gdh*, and *ef*; assay 2 amplified *cps2J*, *arc*, *mrp*, and *sly*. Amplified products were separated on a 1% agarose gel and purified. DNA was quantitated and sequenced in Ion Personal Genome Machine[®] (PGMTM) sequencing platform (Life Technologies, Grand Island, NY) by standard methods. Sequence reads were assembled into contigs using SeqMan NGenTM program (DNASTar) and compared to an in house reference database created with *S. suis* sequences from GenBankSM. Sequences in the reference database were given a sequence type number. Individual sequences from the isolates that are $\geq 99\%$ identical to the reference sequence received the corresponding sequence type and the rest were assigned a new sequence type. The results are represented in a table with the isolate number and the list of genes identified with their corresponding sequence types. The virulence based MLST allowed for a clear differentiation of all the isolates, indicating its applicability in molecular typing. Methods similar to the one described here will improve pathogen typing in the genomics era and will be a valuable tool in selecting isolates for vaccine production.

Intergenic Sequence Ribotyping (ISR) Method for *Salmonella* Serotyping in Veterinary Diagnostics

Roxana Sanchez Ingunza¹, Melissa Madsen¹, Jean Guard², John El-Attrache¹

¹ISD SSIU, Ceva Biomune, Lenexa, KS; ²ESQRU, ARS, USDA, Athens, GA

Narrative: *Salmonella* serotyping represents an important step in the epidemiological study of salmonellosis. Rapid and accurate identification of *Salmonella* serotypes lead to an efficient and fast implementation of control interventions in Food Safety and in Animal Production. Following isolation, *Salmonella* is traditionally serotyped by the White-Kauffman-Le Minor scheme. More recently, molecular methods have been presented as alternative methodologies for *Salmonella* serotyping including a variety of approaches such as Check&Trace AOAC approved microarrays, multiplex PCRs, and genome sequencing based on flagella and LPS associated genes. Even though these methods are very reliable, their implementation as routine tools in veterinary diagnostic laboratories is still very limited. Therefore, we evaluated an Intergenic Sequence Ribotyping (ISR) method targeting the *dkgB* gene linked-ribosomal DNA sequences for its simplicity, accuracy, speed in generating reports and low cost in the monitoring of *Salmonella* contamination of poultry and poultry environments. *Salmonella* survey results generated between 2013 and 2015 are discussed. The ISR method to identify *Salmonella* serotypes requires no complex implementation procedures, highly specific training or acquisition of expensive laboratory equipment. Instead, the method significantly reduces processing time and increases accuracy in the identification of *Salmonella* isolates including rough, non-motile and very closely related *Salmonella* serotypes at relatively low cost. Vaccination strategies, prevalence studies, detection of unusual *Salmonella* isolates such as attenuated vaccine strains, detection of multiple serotypes, and organization of *Salmonella* freezer stored libraries were all facilitated by the utilization of ISR in *Salmonella* serotyping.

Bacteriology 2b
 Sunday, October 25, 2015
 556

Moderators: Akhilesh Ramachandran and Dubraska V. Diaz-Campos

10:00 AM	Novel <i>netB</i>-like toxin gene identified in isolates of <i>Clostridium perfringens</i> from canine necrohemorrhagic enteritis † + ◇ <i>Neha Mishra, Joan Smyth</i>	51
10:15 AM	Methicillin-Resistant <i>Staphylococcus</i> spp. Isolated from Companion Animals in The United States from 2003 to 2014 <i>Stephen D. Cole, Shelley C. C. Rankin</i>	52
10:30 AM	Characteristics of extended beta-lactamase (ESBL) producing <i>Escherichia coli</i> isolated from horses <i>Artem Rogovskyy, Chris Gillis</i>	53
10:45 AM	Improved real-time PCR detection of eight bovine respiratory disease pathogens using the Qiagen Rotor-Gene and QuantiTect Virus Kit <i>Laura Bradner, Karen Harmon, Phillip Gauger</i>	54
11:00 AM	Identification of hemotrophic mycoplasma infections in Michigan dairy cattle including dual infections with <i>Mycoplasma wenyonii</i> and ‘<i>Candidatus Mycoplasma haemobos</i>’ <i>Dodd G. Sledge, Ailam Lim, Steven R. Bolin</i>	55

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| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Novel *netB*-like toxin gene identified in isolates of *Clostridium perfringens* from canine necrohemorrhagic enteritis † + ◇

Neha Mishra, Joan Smyth

Pathobiology & Veterinary Science, University of Connecticut, Storrs, CT

Narrative: *Clostridium perfringens* is a well-recognized cause of enterotoxemia and/or necrotizing enteritis in cattle, sheep, pigs and rabbits, and of necrotic enteritis in poultry. *C. perfringens* produces an array of extracellular toxins. Differential production of the four major toxins (alpha, beta, epsilon and iota) is used to classify the organism as types A, B, C, D or E. NetB (necrotic enteritis toxin B-like) is a pore-forming toxin produced by *C. perfringens* type A, that has been reported as the major virulence factor for necrotic enteritis in poultry and, with one exception, has only been identified in isolates from poultry. The role of *C. perfringens* in hemorrhagic gastroenteritis in dogs is not well-characterized. To better understand the significance of *C. perfringens* in the canine intestine, we swabbed the jejunum and cecum of 121 dogs. 66% of dogs carried *C. perfringens* in intestine and there was little difference in carriage rate between dogs with or without enteritis. Toxinotyping revealed that 99% of the isolates were *C. perfringens* Type A, and of these isolates 15% and 5% were also positive for *beta-2* toxin and *cpe* respectively. 1% of the isolates were Type B. A *netB* like gene was found in 16 % of *C. perfringens* isolates from dogs which had enteritis. Histopathology revealed severe necrohemorrhagic enteritis in the *netB*-like positive dogs. The *netB*-like gene was not found in dogs that did not have enteritis. Sequencing of *netB* amplicons in both directions revealed 88% and 89% identity match with *netB* by BLASTN and BLASTX respectively. These canine strains were not toxic to Leghorn male hepatoma (LMH) cells. Sequencing of the full *netB* like gene shows that it encodes a protein related to the pore-forming Leukocidin/Hemolysin Superfamily.

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

◇ USAHA Paper

Methicillin-Resistant *Staphylococcus* spp. Isolated from Companion Animals in The United States from 2003 to 2014

Stephen D. Cole, Shelley C. C. Rankin

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

Narrative: *Staphylococcus* spp. are the most common pathogens associated with skin and soft tissue infections (SSTI) of humans and animals, in hospitals and in the community, world-wide. An increase in the prevalence of methicillin-resistant (MR) *Staphylococcus aureus* (MRSA) strains over the past four decades has led to higher morbidity, mortality, and hospital expenditures associated with human *S. aureus* infections. *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* are the primary staphylococcal pathogen of animals, of which the most common clinical manifestations are superficial bacterial folliculitis (pyoderma) and otitis externa. More than 90% of canine pyoderma is caused by *S. pseudintermedius* and until recently the organism had traditionally maintained a very predictable antimicrobial susceptibility spectrum, veterinarians typically treat these infections empirically. The aim of this study was to analyze antimicrobial susceptibility data to determine the prevalence of methicillin-resistance in clinical isolates from companion animals. Between 2003 and 2014, the Clinical Microbiology Laboratory at the Matthew J Ryan Veterinary Hospital at the University of Pennsylvania, School of Veterinary Medicine isolated 18, 515 *Staphylococcus* isolates from clinical samples submitted from a variety of animal species. These isolates were identified, speciated and had antimicrobial susceptibility profiles determined using a MicroScan Walkaway 40 PC20 gram-positive combo-panel 40 (Dade Behring, Siemens Healthcare Diagnostics, Deerfield, IL). Results were interpreted using Clinical and Laboratory Standards Institute (CLSI) breakpoints. Fifteen distinct species (not including subspecies) were identified and 8,347 (45%) of the isolates were resistant to oxacillin. Oxacillin is the antimicrobial tested to define an organism as “methicillin resistant.” In 2003, 153/608 (25%) of all *Staphylococcus* species isolated were shown to be methicillin-resistant (MR), whereas in 2014, 1173/2287 (51%) of isolates were classified as MR. Overall, *S. pseudintermedius* was the predominant species isolated (64%), followed by *S. schleiferi* (19%) and *S. aureus* (5%). *Staphylococcus schleiferi* isolates showed the highest prevalence of methicillin resistance (2179/3464, 63%), 4520/11380 (40%) of *S. pseudintermedius* isolates were MR and 244/1018 (24%) of *Staphylococcus aureus* isolates were found to be MR. No significant differences in MR rates were noted between isolates from canine, feline or “other species” (40-46%). Skin isolates were more commonly MR than isolates from any other body sites (50%). The increase in methicillin resistance since 2003 suggests the need for continued passive surveillance, such as this study, as well as supports the need for practitioners to submit clinical samples to diagnostic laboratories for improved patient care and antibiotic stewardship.

Characteristics of extended beta-lactamase (ESBL) producing *Escherichia coli* isolated from horses

Artem Rogovsky^{1,2}, Chris Gillis¹

¹Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX; ²Clinical Microbiology Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

Narrative: Antimicrobial resistance in bacteria has emerged as a major problem in human and veterinary medicine. Third and fourth generation cephalosporins are considered of critical importance by The World Health Organization. Resistance to these drugs is usually mediated by extended-spectrum beta-lactamases (ESBLs). ESBL-producing *Enterobacteriaceae* have been increasingly reported and, to date, three main ESBLs, TEM, SHV, and CTX-M are recognized. Despite ESBL genes are commonly identified in bacteria recovered from various animal species, there are very few reports on the frequency and distribution of ESBL types in horses. Thus, the objective of the current study is to examine the occurrence and genetic background of ESBL-producing *E. coli* in horses. ESBL-positive *E. coli* were detected in 11 equine patients that had been admitted to the Texas A&M Veterinary Teaching Hospital from 2009 to 2014. Both phylogroup analysis and Multi-Locus Sequence Typing (MLST) were applied in order to molecularly characterize these isolates. The results show that 36.3% and 27.3% of the isolates represent phylotype B1 and D, respectively; whereas phylotype A and B2 each constituted 18.2%. Furthermore, TEM and CTX-M genes were detected in 7 and 8 out of 11 equine isolates, respectively. In contrast, only 3 *E. coli* isolates contained SHV lactamase gene. Finally, in addition to previously reported ST types, MLST analysis revealed that some equine isolates represented *E. coli* with novel ST types.

Improved real-time PCR detection of eight bovine respiratory disease pathogens using the Qiagen Rotor-Gene and QuantiTect Virus Kit

Laura Bradner, Karen Harmon, Phillip Gauger

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Narrative: Bovine respiratory disease has extensive economic impact due to morbidity, mortality, treatment and prevention costs, loss of production and reduced carcass value. Since there are a large number of causative agents, correct identification is crucial for timely and cost effective treatment. Agents which are part of this disease complex include but are not limited to bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea virus (BVDV), bovine coronavirus (BCV), bovine herpesvirus-1 (BHV-1), *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. The Iowa State University Veterinary Diagnostic Laboratory offers a bovine respiratory panel polymerase chain reaction assay (BRP-PCR) to target these eight pathogens. This panel is run as two separate 4-target multiplexed PCR assays – one including the viral and the other the bacterial agents. Our goal has been to include an internal control (IC) in this assay to be able to monitor the presence of substances inhibiting the PCR but that has not been possible with the current assay format. This assay has historically been run using a 96-well plate-based, 5 channel real-time thermal cycler. Each assay includes 4 targets, and the instrument manufacturer recommends including a passive reference dye for signal normalization across the plate, which utilizes the one remaining fluorescent channel. High background signal was frequently observed which masked a weak fluorescent target signal causing potential for false negative results. Hence, an alternate real-time thermal cycler and real-time master mix were evaluated to be able to incorporate an IC and attempt to improve sensitivity and specificity of the assay. The two instruments that were compared were the Qiagen Rotor-Gene 5-plex + HRM (RGQ) and the Life Technologies 7500-Fast system. Likewise, the QuantiTect Virus Kit from Qiagen was compared to the Life Technologies Path-ID Multiplex One-Step RT-PCR kit for the viral agents and VetMAX-Plus qPCR master mix reagents for the bacterial agents. In general, the Qiagen equipment and master mix combination improved the cycles to threshold (Ct) value by 3-5 Ct's for the viral agents and 10-15 Ct's for the bacterial agents. Bacterial culture results correlated with the improved sensitivity observed with the Qiagen instrument and mastermix. The Quantitect Virus master mix run on the 7500 system gave equal or 3-4 Ct's weaker than when run on the RGQ. This combination also produced non-specific amplification. The rotor-based design and optics of the RGQ circumvent the need for a passive reference dye, thus allowing five channels to be used for target detection instead of four on the 7500 system, permitting the incorporation of an internal positive control. In conclusion, the RGQ plus the QuantiTect Virus kit greatly improved sensitivity and specificity for the highly multiplexed BRP-PCR assay allowing for better diagnostics of bovine respiratory disease.

Identification of hemotrophic mycoplasma infections in Michigan dairy cattle including dual infections with *Mycoplasma wenyonii* and ‘*Candidatus Mycoplasma haemobos*’

Dodd G. Sledge, Ailam Lim, Steven R. Bolin

Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI

Narrative: Hemotrophic mycoplasmas affect a wide variety of veterinary species. In general, the majority infected animals have subclinical presentations, but clinical signs can be severe, especially when animals have concurrent infections, or are stressed or immunosuppressed. Hemotrophic *Mycoplasma* spp. reported to affect cattle include *Mycoplasma wenyonii* and ‘*Candidatus Mycoplasma haemobos*’. Herein we report the identification of hemotrophic *Mycoplasma* spp. in Michigan dairy herds. Screening for hemotrophic *Mycoplasma* spp. was elected on diagnostic cases that presented to the Michigan State University, Diagnostic Center for Population and Animal Health from 2008-2015 that had clinical histories of unexplained edema, clinical or histologic evidence of hemolysis, and/or splenomegaly. Based on availability of samples for each case, DNA was extracted from either whole blood, fresh spleen, or formalin fixed spleen. Initial screening employed a 16s ribosomal RNA gene-based PCR assay previously described for detection of hemotrophic *Mycoplasma* spp. in feline, canine, bovine, and ovine species. Amplicons of 170 and 193 bp were generated in positive cases, which through nuclei acid sequencing of 597 or 617 bp regions of the same gene were respectively shown to be consistent with ‘*Candidatus M. haemobos*’ and *M. wenyonii*. Of the 27 total animals tested, 16 cattle from 8 separate herds were positive for one or both mycoplasmas with 11 being positive for only *M. wenyonii*, 3 being positive for only ‘*Candidatus M. haemobos*’, and 3 having dual infections. Affected animals ranged from an aborted fetus and a calf <24 hours of age to cows >8 years of age. In animals with reported edema, the severity of edema varied from mild to severe and, in descending order of frequency, affected the udder, rear limbs, or extensive portions of the body. One cow primarily presented with hematuria and hemolyzed serum. Other clinical signs in positive animals included fever, nasal congestion and conjunctival reddening, recumbency, and increased rates of abortion. In addition to subcutaneous edema, gross and histopathologic examination of 5 of the positive animals revealed pulmonary edema, splenomegaly, increased splenic erythrophagocytosis, and marked intrahepatic and extrahepatic cholestasis in individual animals. Overall, this series demonstrates the wide range of ages and presentations of cattle that can be affected by hemotrophic *Mycoplasma* spp., and serves to draw awareness to what are likely an under recognized contributors to disease in cattle. Further, to our knowledge, this represents the first formal report of ‘*Candidatus M. haemobos*’ in the United States.

Epidemiology 1
 Saturday, October 24, 2015
 553

Moderators: E. Jane Kelly and Gregg Hanzlicek

1:00 PM	Managing CWD in Farmed Cervids ◊ <i>Nicholas J. Haley</i>	59
1:15 PM	Developing sampling guidelines for PEDV surveillance † <i>Marisa Rotolo, Luis G. Gimenez-Lirola, Sarah Abate, Marlin Hoogland, Chong Wang, David Baum, Phillip Gauger, Karen Harmon, Rodger Main, Alex Ramirez, Jeff Zimmerman</i>	60
1:30 PM	Tracking of antimicrobial resistance in food-borne pathogens in small poultry production sectors, Options for action. ◊ <i>Mohamed A. El Bably</i>	61
1:45 PM	Diagnoses of cause of mortality in farmed mink in the Intermountain West <i>David J. Wilson, Thomas J. Baldwin, Chelsea H. Whitehouse</i>	62
2:00 PM	Bovine Viral Diarrhea detection in bovine samples and necropsy specimens - comparison between diagnostic test methods and age, sex, and breed <i>David J. Wilson, Thomas J. Baldwin, Jane Kelly, Arnaud Van Wettere, Gordon A. Hullinger, Jennifer Bunnell</i>	63
2:15 PM	Salmonella prevalence in small scale semi-commercial and backyard egg production flocks <i>Kristy Pabilonia, Sarah Millonig, Kyran J. Cadmus</i>	64
2:30 PM	Retrospective study of bovine neurological diseases in Georgia between 2001 and 2015 † + <i>Lorelei L. Clarke, Dan R. Rissi</i>	65
2:45 PM	Application of ORF5 sequencing in PRRS management <i>Jianfa Bai, Steven Henry, Elizabeth G. Poulsen, Lisa Tokach, Megan Potter, Richard Hesse, Gary Anderson</i>	66

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Managing CWD in Farmed Cervids ◇

Nicholas J. Haley

Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS

Narrative: Chronic wasting disease (CWD) is an efficiently transmitted spongiform encephalopathy of cervids (e.g. deer, elk, and moose), and is the only known prion disease affecting both free-ranging wildlife and captive animals. The management of CWD in farmed cervids will require three avenues of research: 1) the development of a sensitive live animal test, 2) the discovery and implementation of a safe and effective vaccine strategy, and 3) with or without a vaccine, the identification and cultivation of CWD-resistant cervids. The antemortem detection of CWD and other prion diseases has proven difficult, due in part to difficulties in identifying an appropriate peripheral tissue specimen and complications with conventional test sensitivity. At present, biopsies of the recto-anal mucosal-associated lymphoid tissues (RAMALT) have shown promising sensitivity in various assays and are not impractical to collect in live animals. Nasal brush collections have likewise proven both sensitive and practical for identification of prion infections in humans, though in cervids both rectal biopsy and nasal brush collection sensitivity is critically dependent on stage of infection and genetic background. A blood test would be ideal; however rudimentary assays currently in development have yet to be evaluated blindly on naturally occurring populations or on a large scale. Vaccine development is currently underway at several institutions, though an effectively protective strategy has yet to be identified. Ultimately, genetic resistance to CWD may be a critical corner piece in the management of CWD in farmed cervids – an approach which has been used effectively to reduce the incidence of scrapie in sheep worldwide. By exploiting resistant *PrP* alleles in currently available white-tail and elk genetic pools, and searching various isolated populations for evidence of additional resistance mechanisms, a suitable approach to improving CWD resistance in farmed cervids may be identified. Our research has specifically sought to develop an antemortem test for CWD using amplification-based assays on collections from recent CWD depopulations, while additionally using these assays to model CWD resistance in cervid populations. Our findings from this research represent the early stages in the management and ultimately eradication of CWD in farmed deer and elk.

◇ USAHA Paper

Developing sampling guidelines for PEDV surveillance †

Marisa Rotolo¹, Luis G. Gimenez-Lirola², Sarah Abate², Marlin Hoogland³, Chong Wang¹, David Baum¹, Phillip Gauger¹, Karen Harmon¹, Rodger Main^{1,2}, Alex Ramirez¹, Jeff Zimmerman¹

¹Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; ²Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA; ³Murphy-Brown LLC, Orange City, IA

Narrative: Oral fluids are a useful surveillance specimen because they are easily collected and can be tested for nucleic acids and/or antibodies to determine the infection status of the individuals contributing to the samples.¹ Assays for testing swine oral fluid specimens have been available at veterinary diagnostic laboratories since 2010 and pen-based oral fluid sampling has become common practice for monitoring a variety of endemic pathogens of swine, e.g., PEDV, PRRSV, PCV2, IAV and others. PEDV was identified in the U.S. in April 2013 by diagnosticians at the ISU-VDL.² Shortly thereafter, a PEDV RT-PCR became available for routine testing and ~39,000 oral fluid samples and 47,000 fecal samples were tested between May 2013 - November 2014. Development of an IgG serum indirect ELISA for PEDV began in September 2013 and the assay became available for routine testing in September 2014.³ This ELISA is also available on request for the detection of IgA and/or IgG in oral fluids, colostrum, and milk. The purpose of the study reported herein was to describe the spatiotemporal pattern of PEDV circulation in the field and to develop sampling guidelines. Oral fluid samples were collected from 36 pens (~25 pigs per pen) in 3 wean-to-finish barns on 3 sites for 8 weeks beginning one week post placement. ~2,916 individual oral fluid samples (108 pens per site x 9 sampling points x 3 sites) were tested for virus (RT-PCR) and antibody (IgG, IgA). Analyses performed to date confirm the utility of oral fluid in surveillance and suggest a high degree of variability in the circulation of PEDV within and between sites, i.e., the distribution of positive pens and disease progression was unique to each individual barn. Mapping the viral movement on the study sites supports the conclusion that all barns must be sampled in order to establish disease status of a site. References 1. Prickett JR, Zimmerman JJ. 2010. The development of oral fluid-based diagnostics and applications in veterinary medicine. *Anim Health Res Rev* 11:207-216. 2. Stevenson GW, Hoang H, Schwartz KJ, et al. 2013. Emergence of *PEDV* in the United States: clinical signs, lesions, and viral genomic sequences. *J Vet Diagn Invest.* 25:649–54. 3. Gimenez-Lirola LG, Baum D, Bower L, et al. Nov 2014. PEDV: antibody-based test development. The 22nd Annual Swine Disease Conference for Swine Practitioners, Ames, Iowa, p. 34-37.

† Graduate Student Oral Presentation Award Applicant

Tracking of antimicrobial resistance in food-borne pathogens in small poultry production sectors, Options for action. ◇

Mohamed A. El Bably

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Narrative: Background. Antibiotic resistance and the probable transmission to human bacteria through poultry food-borne pathogens have led to increased public concern and scientific interest regarding the administration of therapeutic and subtherapeutic antimicrobials to animals. Surveillance of antimicrobial resistance at targeted intervals constitutes a critical part of animal health and food safety strategies. **Objectives.** To investigate the occurrence and frequency of antimicrobial resistance and associated resistance genes in food-borne pathogens isolated from poultry and their environment in small commercial production sectors and to use these data to reduce the transfer of antimicrobial resistant bacteria from animals to humans. **Method.** A cross sectional study targeted poultry at different production sectors (backyard and small commercial farms) and types (broiler & layers) for tracking of different pathways of transmission of antimicrobial resistance bacteria such as, E.coli, Salmonella and Enterococcus species through poultry production chain. Data on the current pattern and determinant of antibiotic use and resistant were collected through the administration of questionnaire at veterinarian and stakeholder meetings. A representative samples were collected from poultry at backyards and small commercial farms (cloaca swabs and eggs), environment (feed, water, flies and fresh manure) ; from slaughtered birds at live-bird markets using stratified sampling technique. A standardized laboratory methodology for isolation and identification of pathogens of zoonotic importance was done. Identified bacteria tested against eighteen antimicrobial agents based on a disc diffusion method. Genetic characterization of resistant isolates involved plasmid analysis, detection of gene cassettes associated with integrons and investigation of multi-drug resistant efflux pumps. The obtained data were recorded and analyzed. **Results .** It revealed high levels of antimicrobial resistance in bacteria isolated from poultry and their environment. Multi-drug resistance to three or more antimicrobials was observed in (93.6%) of all the isolates. The highest percentage of antibacterial resistance were found in bacteria isolated from layer's farms and their environment (91.7 5 & 94.5 % resp.,) then slaughtered poultry followed by isolates from broiler farms (74.3 % & 81.3 % resp.,) while the least percentage of antimicrobial resistance was recorded in isolates from poultry raised at backyards. **Conclusion.** Poultry and their environment particularly layer's farms represent potential reservoirs of resistant bacterial strains and AMR genes that may spread from poultry farms to human populations via poultry meat. Reducing antimicrobial usage requires collaboration between farming, veterinary and public health communities

◇ USAHA Paper

Diagnoses of cause of mortality in farmed mink in the Intermountain West

David J. Wilson, Thomas J. Baldwin, Chelsea H. Whitehouse

Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT

Narrative: Postmortem examination of 373 farmed mink (*Neovison vison*; syn. *Mustela vison*) from 2009 through 2014 at the Utah Veterinary Diagnostic Laboratory resulted in diagnosis of primary causes of mortality in 339 mink (90.9%). Mink were farm raised in Utah or Idaho except for one submission of mink from Wisconsin. Mink were presented in groups of 1–10 with a mean of 3.6 animals per submission. Cause of death was established based on reported clinical signs, pathology, and results of ancillary tests. Tests included bacteriology, molecular diagnostics, histology, and vitamin and mineral analyses including inductively coupled plasma–mass spectroscopy. When not diagnosed by PCR, Aleutian mink disease was diagnosed by lateral flow immunoassay on heart blood for virus-specific immunoglobulin G detection. In the 339 mink where cause(s) of death were established, 311 (91.7%) died from a single disease or condition, whereas 28 (8.3%) had 2 diseases or conditions contributing to death. Eleven diseases accounted for 321 (94.7%) of the diagnoses: bacterial pneumonia (67, 18.8%), Aleutian mink disease (61, 17.7%), mink viral enteritis (56, 16.2%), hepatic lipidosis (28, 8.1%), nutritional myopathy (24, 7%), bacterial enterocolitis (17, 4.9%), bacterial septicemia (16, 4.6%), starvation (15, 4.3%), epizootic catarrhal gastroenteritis of mink (14, 4.1%), pancreatitis (13, 3.8%), and bacterial metritis (10, 2.9%). In an additional 16 (4.3%) of the mink, botulism was suspected from clinical history but could not be confirmed by laboratory testing. For 34 (9.1%) animals, no cause of death was evident. Juveniles were defined as mink with body weight ≤ 0.9 kg (approximately 0 to 4 months of age), intermediate aged were defined as mink with body weight 0.91 to 1.19 kg (approximately 4 to 10 months of age), and adults were defined as mink with body weight ≥ 1.2 kg (approximately ≥ 10 months of age). Mink with no indication of age or body weight recorded were defined as of unknown age. Sex of the mink was not routinely recorded. Most common causes of mortality varied somewhat among juvenile, intermediate, or adult aged mink. However, 4 diseases caused most of the death loss within all age groups: bacterial pneumonia (primarily caused by *Pseudomonas aeruginosa*), Aleutian mink disease, mink viral enteritis, and hepatic lipidosis. Control measures for the most common causes of death in farmed mink include vaccination (*P. aeruginosa* pneumonia, mink viral enteritis), testing and removal of positive animals (Aleutian mink disease), avoidance of obesity (hepatic lipidosis), and environmental management, including maintaining clean water cups, floors, feed handling equipment, cages, truck tires, workers' clothing including leather mink handling gloves, and dining areas for farm personnel.

Bovine Viral Diarrhea detection in bovine samples and necropsy specimens - comparison between diagnostic test methods and age, sex, and breed

David J. Wilson, Thomas J. Baldwin, Jane Kelly, Arnaud Van Wettere, Gordon A. Hullinger, Jennifer Bunnell

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Narrative: Bovine Viral Diarrhea (BVD) is an important cause of respiratory, gastrointestinal and reproductive disease in cattle. Estimates of prevalence of BVD in the U.S. primarily focus on Persistently Infected (PI) cattle and range from 0.1% to 0.13%. Prevalence of BVD (“detected” test results) was calculated among all bovine samples tested for BVD at the Utah Veterinary Diagnostic Laboratory from 2009 - 2013. Blood, serum, ear notch or skin biopsy samples were usually submitted in large groups, often for antigen capture or serum ELISA required for export or interstate shipment of animals and next most commonly to test for PI animals within commercial herds. Carcasses and aborted fetuses were submitted for general diagnosis; BVD in these animals was only reported if it was considered contributory to death. Most necropsied animals were tested by antigen capture ELISA, but fetuses were usually tested by real-time reverse transcriptase PCR. Cattle originated primarily from Utah, but also from surrounding states in the western U.S. Results were compared by age, sex, or breed of cattle and among BVD diagnostic methods. Although not a planned experiment, Chi-square was used to test for significant differences in BVD prevalence between age, sex, breed and test methods. Bovine Viral Diarrhea was detected in 105/8975 samples (1.2%), including 22/180 necropsies (12.2%). Detection of BVD by each test method follows: antigen capture ELISA 79/7692 (1.0%); serum ELISA 19/1195 (1.6%); PCR 7/88 (8.0%). Detection of BVD by age, sex, breed (when provided): male 5/215 (2.3%); female 9/382 (2.4%); fetus 3/36 (8.3%); calf (1 - 200 days old) 29/579 (5.0%); immature (201 - 729 days old) 4/183 (2.2%); adult (\geq 730 days old) 4/75 (5.3%); dairy 25/750 (3.3%); beef 26/1600 (1.6%). There were no significant differences in BVD detection by age or sex. Necropsied animals, those tested with PCR and dairy breeds ($P = 0.07$), were significantly more likely to be detected with BVD. Most common dairy breeds were Holstein (95%) and Jersey (4%); most common beef breeds were Angus (60%), “Beef” (23%) and Hereford (6%). The overall prevalence of BVD, especially the prevalence in necropsied animals and aborted fetuses demonstrates that despite the low reported prevalence of PI cattle, BVD remains an important bovine disease.

***Salmonella* prevalence in small scale semi-commercial and backyard egg production flocks**

Kristy Pabilonia, Sarah Millonig, Kyran J. Cadmus

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Narrative: Between 2000 and 2008, *Salmonella* spp. bacteria were responsible for 11% of all cases of foodborne illness in the US, and caused the highest proportion of hospitalizations (35%) and deaths (28%) among all types of foodborne illnesses. The most common source of infection has been attributed to consumption of raw or undercooked eggs. Over the past five years, backyard poultry flocks have been associated with a number of human outbreaks of *Salmonella*, primarily linked to direct contact with baby poultry. The background prevalence of *Salmonella enterica* in small semi-commercial and backyard egg production flocks is poorly understood. The objective of this study was to assess the prevalence of *Salmonella enterica* in the growing number of small egg production flocks, in addition to evaluating common production practices among these flocks. Environmental samples (n=487) were collected from poultry houses and egg processing areas from 56 semi-commercial and backyard poultry egg production flocks located in Colorado during the fall of 2014. Additionally, eggs were collected from 38 of the flocks and pooled for testing (n=43). Environmental samples were collected and cultured following the USDA National Poultry Improvement Plan Provisions, detailed in 9 CFR 147.12. All egg samples were tested by real-time polymerase chain reaction (qPCR), using a commercially available kit in accordance with the Food and Drug Administration (FDA) Egg Safety Rule. *Salmonella* was isolated from 11 (20%) of the sampled flocks. *Salmonella* was cultured from 29 (6%) environmental samples and one (2%) egg pool sample tested positive for *Salmonella* spp. by qPCR. Eleven *Salmonella* serotypes were identified, including *Salmonella* Infantis which could potentially be related to the 2014 multistate outbreak of human *Salmonella*, which was linked to contact with live poultry. Most of the *Salmonella* serotypes isolated during this study are potentially zoonotic and have been associated with human illness in previous outbreaks, including Enteritidis, Oranienburg, Typhimurium, Mbandaka, Montevideo, and Bredeney. This study indicates *Salmonella* spp. are recovered from small semi-commercial and backyard egg production flocks. As these types of flocks expand in numbers and geographical distribution, the potential for public health risks may increase as well. Data from this study will help investigators focus *Salmonella* reduction interventions and educational programs for small producers to reduce the public health burden of *Salmonella* and further increase the food safety of eggs originating from these sources.

Retrospective study of bovine neurological diseases in Georgia between 2001 and 2015 # † +

Lorelei L. Clarke¹, Dan R. Rissi^{1,2}

¹Department of Pathology, University of Georgia, Athens, GA; ²Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA

Narrative: There are multiple direct and indirect pathological processes that can cause neurological disease in cattle, including congenital or hereditary conditions, infectious diseases, nutritional or metabolic disturbances, toxicities, and neoplasia. Many of these processes lead to severe and debilitating disease that may result in death or euthanasia of affected animals, creating economic losses to the beef or milk industry. Furthermore, these neurological diseases frequently need to be differentiated from rabies because of public health concerns. A review of archived bovine necropsy cases submitted to the Athens Veterinary Diagnostic Laboratory from 2001 to 2015 yielded 92 cases with evidence of neurological disease related to cerebral lesions. The most commonly affected breeds were Aberdeen Angus (25 cases), Holstein-Friesian (13 cases), mixed-breed beef cattle (10 cases), Red or Black Angus (6 cases), Hereford (5 cases), and Jersey (4 cases). Younger animals (2 to 12 months of age) represented 38% of the cases, whereas animals older than 1 year represented 35% of cases. Cases consisted of bacterial meningoencephalitis (28 cases), polioencephalomalacia (19 cases), hydrocephalus (9 reportedly congenital and 5 occurring secondary to other changes), rabies (8 cases), hepatic encephalopathy (4 cases), malignant catarrhal fever (3 cases), pituitary or cerebral abscesses (3 cases), neosporosis (2 cases), cerebral lymphoma (2 cases), and physical trauma with extensive hemorrhage (2 cases). Gross lesions were reportedly observed in 34 cases in this study, including those with bacterial meningoencephalitis, polioencephalomalacia, and hydrocephalus. Twelve cases with bacterial infection had concurrent lesions including pneumonia, gastroenteritis, and omphalitis. Hydrocephalus was the most common secondary lesion seen in the brain of affected individuals (4 cases associated with bacterial meningoencephalitis and 1 associated with lymphoma). In addition, there were 15 cases of lymphoplasmacytic or lymphohistioplasmacytic meningoencephalitis of unknown cause. These 15 cases were tested for rabies and listeriosis by fluorescent antibody testing, but no further diagnostic work up was attempted, and lesions were attributed to a possible viral cause. The results of the present study highlight the prevalence of bovine neurological diseases in the Southeastern United States and illustrate the need for additional routine diagnostic assays to identify potential causative agents in cases of meningoencephalitis of unknown etiology.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Application of ORF5 sequencing in PRRS management

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¹Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; ²Abilene Animal Hospital, Abilene, KS

Narrative: Traditional PRRS genotyping has been done with PCR-RFLP on ORF5 of PRRSV to generate RFLP types using a combination of three different type II endonucleases. ORF5 encoding the structural protein GP5 is RT-PCR amplified, and digested with *Mlu*I, *Hin*CI and *Sac*II. A numerical 3-digit RFLP type is given based on each enzyme recognition sites. RFLP typing for PRRSV can be informative, but may not represent the real genotype of a given strain, as a single base difference in the enzyme recognition site will generate a different RFLP type. Our data indicate that different genotypes can have the same RFLP type; and nearly identical strains can belong to different RFLP types. Sequencing is a more informative way of classifying PRRSV genotypes in PRRS management. Abilene Animal Hospital has been working with KSVDL to generate ORF5 sequencing information for PRRS management. More than 250 PRRSV strains have been sequenced at KSVDL and used for decision-making process in PRRS management. Phylogenetic analysis indicated that they belong to several different sub-groups with nucleic acid identity ranging from 81% to 100%. Interestingly, some strains have identity of greater than 99% to three prototypes of the four commonly used North American type PRRSV vaccine strains, although Abilene Animal Hospital has not used any of the MLVs since 1997. Phylogenetic analysis also suggests that the genetic background of the four vaccine strains is somehow narrow, as they grouped together in a sub-cluster in one side of the un-rooted tree, and are distantly related to the majority of other field strains including contemporary strains collected in recent years. As a routine practice on an infected farm, once a predominant genotype is identified by sequencing, a “Load, Close and Expose” strategy is deployed, from which high concentration virus is collected from <10 day-old infected piglets, and sprayed onto the nose of all females. The farm is then closed down without animal trafficking. The virus is cleared in 18-26 weeks in many cases. Sequencing was also used for tracking geographic and temporal distributions of the viruses. An outbreak strain in newly purchased weaned pigs on a farm was identified to be distinct from an endemic strain of the sow farm from which the pigs were purchased. In a temporal tracking case, four PRRS strains were dominating a swine farm during 2007 and 2013, each occupies an overlapping period. Two of the four strains were more similar, with 97-99% identity to each other, and overlapped for 10 months indicating that they evolved very slowly, and no major change had occurred. Other two strains are very different from each other, with only 83-84% identity at nucleic acid level. They both are distinct from the two similar strains mentioned earlier. The ORF5 sequencing information generated at KSVDL has been critically used for decision making in PRRS outbreak management at Abilene Animal Hospital.

Epidemiology 2; Parasitology
 Sunday, October 25, 2015
 553

Moderators: Lindsey Holmstrom and David Wilson

8:00 AM	The missing piece: Utilizing a common database for disease outbreak investigations \diamond <i>Kerry Sondgeroth</i>	69
8:15 AM	Diagnostic records in a changing world: Enhancing preparedness and meeting the demands of today's progressive food animal production systems <i>Katie Woodard, Shiva Adhikari, Daniel Patanroi, Bret Crim, Kate Mueller, Randy Berghefer, Rodger Main</i>	70
8:30 AM	Progress made on developing tools that link veterinary diagnostic laboratory submissions, corresponding test results, and an interpreted premise-level health status to spatiotemporal mapping tools in support of area regional disease control and monitoring efforts in US swine <i>Kate Mueller, Jordan Bjustrom, Bret Crim, Rodger Main, Dale Polson, Zack Whedbee, Erin Lowe, Enrique Mondaca, Andres Perez, Beatriz Martinez-Lopez.</i>	71
8:45 AM	Non-fatal injury occurrence in Southern California Thoroughbred racehorses 2009-2010 <i>Ashley Hill, Jeff A. Blea, Rick M. Arthur, C. W. McIlwraith</i>	72
9:00 AM	Defining the Long-term Duration of Parasitemia and Antibody Response in Cattle Infected with Variable Strains and Doses of <i>Babesia bovis</i> and Evaluating Sero-diagnostic Tools <i>Chungwon J. Chung</i>	73
9:15 AM	Comparison of an alternative diagnostic sampling technique for <i>Tritrichomonas foetus</i> in cattle <i>Grant Dewell, Karen Harmon, Tyler Dohlman, Patrick Phillips, Phillip Gauger</i>	74

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| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | \diamond USAHA Paper |

The missing piece: Utilizing a common database for disease outbreak investigations ◇

Kerry Sondgeroth

Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, WY

Narrative: Pulse-field electrophoresis (PFGE) is a tool for genotyping bacterial strains. Currently, there is no common database for strains isolated from both animals and humans. The genotyping data from pathogens isolated from humans is maintained in PulseNet, while most veterinary diagnostic laboratories utilize the National Veterinary Services Laboratory to provide their genotyping information for *Salmonella* strains. *Salmonella* and *Campylobacter* isolated from animal specimens at the Wyoming State Veterinary Laboratory are genotyped by the Wyoming Public Health Laboratory and uploaded into the PulseNet database. Isolates with matching PFGE patterns can be identified, and clusters evaluated for a common source. A case study from Montana isolates will demonstrate that the interface of animal and human isolates in a single database allows for more robust disease investigations.

◇ USAHA Paper

Diagnostic records in a changing world: Enhancing preparedness and meeting the demands of today's progressive food animal production systems

Katie Woodard, Shiva Adhikari, Daniel Patanroi, Bret Crim, Kate Mueller, Randy Berghefer, Rodger Main

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Narrative: The emergence of PEDV and HPAIV in the US has brought preparedness to the forefront of the VDL community. These experiences coupled with ever increasing demands from those most progressive production systems re-enforced the need to elevate VDL data by improving the quality, consistency, completeness, and traceability of veterinary diagnostic records. The ISU VDL recently completed a significant overhaul of its diagnostic submission forms, laboratory information management system (LIMS), web-based diagnostic reporting, and web-submission tools. These updates aim to simplify the submission process and provide a more flexible, user-friendly, and complete platform for VDL submitters so that they may better utilize the information provided with submissions. This also serves to ease the burden on those VDL personnel responsible for interpreting client requests. These improvements enhance the clientele's ability to more readily monitor and/or query diagnostic information from the same owners, farm sites, sources (flows), or groups (lots/flocks) of animals over time. Updated tools also provide VDL submitters a number of user-friendly options for electronic incorporation of the premises identification number (PIN) into the diagnostic record, which is essential for linking diagnostic results back to the correct premises (farm site) of origin. These much needed improvements serve to enhance preparedness through traceability, increase the value of the diagnostic records, and augment the use of diagnostic data by better meeting the demands of the next generation of food animal production systems, which will unquestionably include the seamless transfer of records to and from diagnostic and animal health information databases.

Progress made on developing tools that link veterinary diagnostic laboratory submissions, corresponding test results, and an interpreted premise-level health status to spatiotemporal mapping tools in support of area regional disease control and monitoring efforts in US swine

Kate Mueller¹, Jordan Bjustrom¹, Bret Crim¹, Rodger Main¹, Dale Polson², Zack Whedbee³, Erin Lowe², Enrique Mondaca², Andres Perez⁴, Beatriz Martinez-Lopez³

¹Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA; ²Boehringer-Ingelheim, St. Joseph, MO;

³University of California-Davis, Davis, CA; ⁴University of Minnesota, Minneapolis, MN

Narrative: Highly collaborative development efforts are currently underway to provide swine veterinarians and pork producers with an effective disease management tool (Animal Health Information Management Network) that streamlines VDL submissions and corresponding interpreted test results to spatiotemporal mapping tools (Disease BioPortal™, UC Davis) for use in area regional disease management applications. A novel, web-based database application (Secure Animal Health Diagnostic Database, SAHDD) has been developed to upload premises (farm-site) information; receive diagnostic submission information and test results via HL7 messaging from any number of VDLs in the NAHLN; interpret diagnostic results; assign an appropriate health status and/or classification to farm sites; and transmit site information, test results and interpreted health status/classification information to Disease BioPortal™ for data summary, visual display, and further bioinformatic analytical purposes at different levels (i.e. genome-to-phenome and population level). These highly capable area regional disease management tools enable permissioned users to monitor the health status of farm sites and/or the movement of particular pathogens (or strains) within a given region, production system, or veterinary practice area in near-real-time. While the three pilot projects currently in progress have focused on PEDV and PRRSV in swine, the tools and systems being developed are designed to be scalable and readily adapted across a broader set of pathogens and animal species.

Non-fatal injury occurrence in Southern California Thoroughbred racehorses 2009-2010

Ashley Hill¹, Jeff A. Blea², Rick M. Arthur³, C. W. McIlwraith⁴

¹CAHFS - UC Davis, Davis, CA; ²Southern California Equine Foundation, Arcadia, CA; ³School of Veterinary Medicine, University of California, Davis, CA; ⁴Dept. of Clinical Sciences, Colorado State University, Fort Collins, CO

Narrative: Among racing and training Thoroughbreds, musculoskeletal injuries are the most common cause of death or euthanasia, the most common cause of exiting training, and are also a leading cause of wastage. The primary objective of the current study was to describe the incidence, anatomic distribution and characteristics of non-fatal injuries in Thoroughbred racehorses as diagnosed by private practice veterinarians in Southern California. Participating veterinarians recorded non-fatal injuries (definitive diagnosis of a musculoskeletal condition resulting in lameness, injury or loss of training > five days) incurred by Thoroughbred racehorses in their care. Secondary objectives were comparison of this private-practice recording system with an existing regulatory system, and comparison of fatal and non-fatal injury rates. Between May 1st 2009 and April 30th 2010, non-fatal injuries were recorded by seven veterinarians in four practices. Non-fatal injuries were diagnosed in 2-4% of horses in race training per month. The majority of injuries were acute rather than chronic. Stress fractures, superficial digital flexor tendonitis, and suspensory ligament desmitis were common. Agreement between non-fatal injuries recorded in the current study and those recorded via an existing regulatory system was poor, with neither system capturing all injuries. Non-fatal injuries occurred much more often than fatal injuries. Non-fatal musculoskeletal injury remains an ongoing issue for Thoroughbred racehorses, and an accurate, comprehensive system for recording these injuries is needed.

Defining the Long-term Duration of Parasitemia and Antibody Response in Cattle Infected with Variable Strains and Doses of *Babesia bovis* and Evaluating Sero-diagnostic Tools

Chungwon J. Chung

R&D, VMRD Inc, Pullman, WA; Washington State University, Pullman, WA

Narrative: Defining long-term duration of *B. bovis* parasitemia and antibody response in cattle infected with diverse *B. bovis* strains and doses is crucial for developing effective control measures for transmission of this important trans-boundary disease. In this study, parasitemia and *B. bovis*-specific antibody response were defined in calves infected with high and low doses of three *B. bovis* strains including two attenuated and one pathogenic. All six experimentally infected cattle had recurrent parasitemia lasting more than 10 months post-infection even those infected with a low dose of attenuated *B. bovis* strains, indicating persistent infection capacity of all three *B. bovis* strains regardless of pathogenicity and challenge dose. Low frequency of detectable parasitemia in calves challenged with attenuated strains, Mo7 and Tf-137-4, and inconsistent parasitemia in the calf challenged with even pathogenic strain, T2Bo, suggest lack of reliability of parasitemia detection-based diagnosis due to a narrow window of detection, particularly in carrier stage. In contrast, all six calves maintained robust *B. bovis* antibody responses during all 12 months of the monitoring period following initial detection at 14 to 15 days post-inoculation when analyzed by a low throughput indirect immunofluorescence assay (IFA). Persistence of *B. bovis*-specific antibody responses at all tested days after the first appearance, even at days with no detectable parasitemia, indicates the relative advantage of antibody-based diagnostics over antigen detection assays. A previously reported cELISA based on an epitope in rhoptry-associated protein-1 was not reliably antibody positive after 8 months post-inoculation when parasitemia was still detectable by PCR. Moreover, the initial positive detections by the cELISA in low-dose challenged-animals were approximately 6 days delayed compared to IFA. The diagnostic specificity of the cELISA against negative sera collected in Texas using IFA as the reference assay was 90.4%. Additionally, diagnostic sensitivity of the cELISA was 60% against samples collected in several areas of Mexico against reference IFA. It is posited that development of a high-throughput sero-diagnostic assay with better diagnostic sensitivity/specificity (>98%) against sera from global bovine herds and its use may be pivotal in preventing the spread of *Babesia bovis* from endemic to non-endemic areas.

Comparison of an alternative diagnostic sampling technique for *Tritrichomonas foetus* in cattle

Grant Dewell, Karen Harmon, Tyler Dohlman, Patrick Phillips, Phillip Gauger

VDPAM, Iowa State University, Ames, IA

Narrative: Bovine trichomoniasis is emerging as a major concern in the beef industry. Recent advancements in PCR diagnostics have increased the ability to detect the disease in asymptomatic bulls. However, the greatest limitation is proper collection of an adequate sample. Furthermore, the low repeatability of most sample collection techniques can cause confusion and misdiagnosis. The aim of the study was to validate a sample collection technique that increased sensitivity and was easier and safer to collect than preputial scraping. Eighty commercial bulls of unknown infection status, were sampled for detection of *Tritrichomonas foetus* using two different collection methods: 1) traditional preputial scraping with a dry insemination pipette (TPS) and 2) penile preputial swabbing (PPS). TPS samples were taken by vigorously scraping preputial/penile mucosa using a rigid insemination pipette while applying negative pressure. PPS samples were obtained by briskly swabbing the penile and preputial mucosa with gauze sponge during full extension of the penis. All samples were processed using InPouch™ TF media and submitted under similar conditions for PCR testing at ISU Veterinary Diagnostic Laboratory. Positive PCR results were observed in 28/80 (35%) bulls using TPS technique, however 31/80 (39%) were positive using PPS technique. Sensitivity was determined with web based application utilizing R software. The Newton-Raphson algorithm predicted the sensitivity of the TPS method was 0.897 (CI 0.637-0.978) and the sensitivity of the PPS was 0.962 (CI 0.774-0.995). This data indicates that the PPS technique is a reliable alternative to the TPS method.

Pathology 1
 Saturday, October 24, 2015
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Moderators: Kelli Almes and Jamie Henningson

1:00 PM	Breed-specific reference intervals for assessing thyroid function in seven dog breeds <i>Rebecca L. Davies, Sheila M. Torres, Leslie C. Sharkey, Sarah Gresch, Claudia A. Munoz-Zanzi, Peter R. Davies</i>	77
1:15 PM	Nephropathogenic Infectious Bronchitis Virus in Backyard Chickens <i>Santiago Diab, Patrick Pong, Karen Sverlow, Beate Crossley</i>	78
1:30 PM	Herd Outbreak of Bovine tuberculosis Illustrates that the Route of Infection Correlates with Anatomic Distribution of Lesions <i>Scott D. Fitzgerald, Charlotte Hollinger, Thomas P. Mullaney, Colleen S. Bruning-Fann, John Tilden, Rick Smith, James Averill, John B. Kaneene</i>	79
1:45 PM	Histopathologic Findings in the Livers of Broilers with Naturally-Occurring Inclusion Body Hepatitis <i>Keith L. Bailey, Steve W. Breeding, Robin Gilbert</i>	80
2:00 PM	Natural History of Pulmonary Hypertension: Adaptive versus Maladaptive Physiologic Responses in Beef Cattle Exposed to Chronic Hypoxia and the Role of Inflammation in the Progression of Pulmonary Hypertension † <i>Greta M. Krafzur, Dale Brown, Joseph Neary, Timothy Holt, Daniel Gould, Suzette Riddle, Angela Canovas, Juan Medrano, Mark Enns, Milton Thomas, Kurt Stenmark</i>	84
2:15 PM	Not So High Mountain Disease: Inflammation, Pulmonary Hypertension and Right Heart Failure in High Plains Fattening Beef Cattle † <i>Greta M. Krafzur, Dale Brown, Suzette Riddle, Maria Frid, Jens Poth, Daniel Gould, Joe Neary, Tim Holt, Kurt Stenmark</i>	82
2:30 PM	Hemochromatosis and equine degenerative myelopathy in a Shetland pony † + <i>Ida L. Phillips</i>	83
2:45 PM	Isolation of <i>Helcococcus ovis</i> from an aborted calf with pathology ◇ <i>Yan Zhang, Jing Cui, Jeffrey R. Hayes, Mary B. Weisner, Beverly Byrum</i>	84

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Breed-specific reference intervals for assessing thyroid function in seven dog breeds

*Rebecca L. Davies¹, Sheila M. Torres², Leslie C. Sharkey², Sarah Gresch¹, Claudia A. Munoz-Zanzi³,
Peter R. Davies¹*

¹Veterinary Population Medicine, University of Minnesota College of Veterinary Medicine, St. Paul, MN; ²Clinical Sciences, University of Minnesota College of Veterinary Medicine, St. Paul, MN; ³Division of Epidemiology and Community Health; School of Public Health, University of Minnesota, Minneapolis, MN

Narrative: Thyroxine, (T4), free T4 (FT4), and thyrotropin (TSH) concentrations were measured in serum from healthy representatives from 7 breeds to determine whether breed differences occur, and whether breed-specific reference intervals (RI) are warranted. Between 96 and 102 Alaskan Malamute (AM), Collie (C), English Setter (ES), Golden Retriever (GR), Keeshond (K), Samoyed (SAM) or Siberian Husky (SH) dogs participated after meeting the study inclusion criteria. A veterinarian reviewed the health history, performed a physical examination and approved laboratory screening data for enrolled dogs. Mean concentrations of T4, FT4, and TSH varied significantly among breeds. The range of mean concentration of T4 (0.53 µg/dl in ES to 2.25 µg/dl in K) and FT4 (0.98 ng/dl in ES to 1.57 ng/dl in SAM) was considerable. Median TSH values by breed ranged from 0.07 ng/ml (AM and GR) to 0.26 ng/ml (C). Mean T4 and FT4 concentration were higher in females than males. TSH concentration did not differ by sex. Increasing age was associated with decreasing concentration of T4 and FT4, and increasing concentration of TSH. The substantial ranges across breeds of measures of central tendency (mean, median) for all hormones, indicate that breed specific RIs are warranted. Reference intervals encompassing the central 95% of reference values for all breeds combined, and for individual breeds, were calculated using non-parametric (TSH) and robust methods (T4, FT4). Use of breed-specific RIs in combination with careful attention to the potential for preanalytical and analytical variability in test results will improve thyroid function assessment in these breeds. This research was funded by the American Kennel Club Canine Health Foundation Grant 372: Determination of breed-specific reference intervals for assessing thyroid function in several breeds), The Collie Foundation, and the Orthopedic Foundation for Animals.

Nephropathogenic Infectious Bronchitis Virus in Backyard Chickens

Santiago Diab¹, Patrick Pong¹, Karen Sverlow¹, Beate Crossley²

¹Pathology, University of California, Davis. California Animal Health and Food Safety Laboratory, Davis, CA;

²Virology/Molecular Biology, University of California. California Animal Health and Food Safety Laboratory, Davis, CA

Narrative: Infectious bronchitis virus (IBV) is a highly contagious disease of chickens that may manifest with respiratory signs, nephritis, airsacculitis, reduced egg production, poor egg quality and/or high mortality. We describe a case of high mortality in backyard chicks due to a nephropathogenic strain of infectious bronchitis virus. Approximately 100 out of 240, 3-week-old, backyard chicks died within a 2-week period without showing any clinical signs of disease. These chicks were purchased without a vaccination history at day 1 or 2 of life and were not vaccinated by the owner after purchase. On necropsy, 7 out of 7 chicks showed swollen, pale kidneys and urate-filled, distended ureters (renal gout); additionally, 1 out of 7 chicks had widespread deposition of urates on the pericardium, hepatic surface and intestinal serosa (visceral gout). Histopathology confirmed the presence of gouty nephrosis in all chickens and, additionally, showed moderate, multifocal, lymphoplasmacytic, interstitial nephritis in all of the chicks. No significant histological lesions were observed in other organs, including trachea and lung. Immunohistochemistry for IBV was strongly positive in the kidneys of all chickens and only weakly positive in the respiratory tract of a few chicks, showing a marked nephrotropism for this particular viral strain. IBV was further confirmed by virus isolation from an organ pool and qRT-PCR. IBV sequencing results showed this strain to be 97% identical to the IBV isolate GA/11323/2011 and 95% identical to the IBV isolate CA/1737/04. Other ancillary tests, all with negative results, included Salmonella PCR (liver), avian influenza qRT-PCR (oropharyngeal swab pool), and aerobic bacterial cultures (livers). In California, the Cal99 variant of IBV has been associated with respiratory disease and increased condemnation since 1999, but only recently with nephritis and nephrosis. Since there are multiple serotypes of IBV and immunization with only one antigenic type produces little or no protection against the other unrelated serotypes, genotyping of IBV field isolates is important to not only monitor the emergence of new variants, but also to evaluate the role and extent of protection of the commercial vaccines currently available. This is yet another example of the importance of monitoring for highly contagious infectious diseases in backyard chicken flocks, as these pathogens may spread quickly and pose a risk to the public, the large commercial poultry industry and the local, state, and national economies.

Herd Outbreak of Bovine tuberculosis Illustrates that the Route of Infection Correlates with Anatomic Distribution of Lesions

Scott D. Fitzgerald¹, Charlotte Hollinger², Thomas P. Mullaney¹, Colleen S. Bruning-Fann³, John Tilden⁴, Rick Smith⁵, James Averill⁵, John B. Kaneene⁶

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Narrative: A recent outbreak of bovine tuberculosis in a Michigan dairy herd resulted in quarantine, depopulation, pathologic, and epidemiologic investigations. This herd, compared to other recent bovine tuberculosis infected herds in Michigan, was unusual in several respects, including: the length of time since its last herd tuberculosis test, limited culling of dairy cows, and long-term feeding of waste milk to its replacement calves. The herd had 80 cattle with suspicious results on caudal tail-fold or gamma interferon testing. Necropsy revealed striking variation in the anatomic distribution of gross lesions, microscopic lesions, and culture positive lymph nodes between the adult cattle, the calves, and the domestic cats present on the farm. Adult cattle had greater than 90% of lesions and culture positive lymph nodes located within the thoracic lymph nodes while both calves and cats had 50% or more of their lesions and culture positive lymph nodes located within their abdomens. This difference in anatomic distribution correlated with the likely routes of infection, which are believed to be by direct airborne transmission in adult cattle and indirect ingestion of contaminated milk in both calves and cats. One unusual aspect of this farm's management was that calves were continually fed waste milk throughout their early months, instead of switching over to milk replacer as many farms currently practice. While bovine tuberculosis literature over the last 100-plus years states that the route of infection may manifest itself in differences in lesion anatomic distribution, our team has been working with bovine tuberculosis for over 20 years, and we have never encountered such striking variation between different groups of animals on the same farm. This case illustrates the critical role that pasteurization of milk plays in controlling the spread of bovine tuberculosis. It also illustrates how the pathologic and epidemiologic study of cattle herds infected with bovine tuberculosis can provide important information on the routes of disease transmission and lead to improved control of bovine tuberculosis even in countries with advanced progress in eliminating this zoonotic disease.

Histopathologic Findings in the Livers of Broilers with Naturally-Occurring Inclusion Body Hepatitis

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Narrative: In recent years, inclusion body hepatitis (IBH) has been recognized as a re-emerging disease of economic importance in commercial poultry. IBH occurs primarily in 19- to 32-day-old broilers and is due to adenoviral infection. Affected flocks exhibit an abrupt spike in daily mortality. Elevated mortality typically persists for 7-10 days, resulting in an overall flock mortality rate of 3-8%. Experimental lesions of IBH are well characterized; however, little information is available detailing the progression of microscopic findings in the livers of birds with naturally-occurring disease in the United States. Over an approximately 18-month period from 2013-2015, formalin-fixed livers from affected broilers were submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for microscopic evaluation. A spectrum of histopathologic changes was identified, ranging from intranuclear inclusion bodies in acute infections to extensive reparative processes in subacute to chronic lesions. Recognition of the continuum of microscopic lesions will facilitate timely diagnosis of disease and differentiate disease from other conditions in commercial poultry. Additional investigations into the epidemiology and viral types involved in naturally-occurring disease are ongoing.

Natural History of Pulmonary Hypertension: Adaptive versus Maladaptive Physiologic Responses in Beef Cattle Exposed to Chronic Hypoxia and the Role of Inflammation in the Progression of Pulmonary Hypertension †

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Narrative: Humans with group 3 pulmonary hypertension characterized by alveolar hypoxia exhibit diversity in disease progression outcomes. Similarly certain breeds and individuals among beef cattle, including the American Angus demonstrate variable susceptibility to hypoxia-induced pulmonary hypertension (PH) and right heart failure (HF). Despite selection of breeding stock with low pulmonary hypertension susceptibility, failure to thrive and death loss associated with PH and right HF remain problematic for unknown reasons. We therefore tested the hypothesis that inflammatory activation influences PH progression and right HF. Right heart catheterization was used to obtain the mean pulmonary arterial pressure (mPAP) in 200 Angus steers born and raised at the Rouse Ranch, Saratoga, Wyoming (7100 ft/2185 m). Steers (N = 10 each) were selected at weaning (age 6 mos) with low mPAP (≤ 38 mm Hg) or high mPAP (≥ 47 mm Hg). Mean PAP values were re-tested at 12-13 mos. age and animals were slaughtered at 15 mos. Three distinct phenotypes were identified at slaughter based upon mPAP values and clinical signs: (i) Non-hypertensive (n=10), mPAP range 36-66, median 42 mm Hg; (ii) PH without signs of RV dysfunction (n=7), mPAP 72-111, median 94 mm Hg; (iii) PH with signs of RV failure (n=3), mPAP 79-87 mm Hg. Average RV:body weight ratio was significantly increased for all PH animals compared to non-hypertensive, whereas LV: body weight was unchanged. Pulmonary histopathology confirmed remodeling along the length of the pulmonary vascular tree. Gross and microscopic cardiopulmonary lesions offer evidence that inflammation plays a significant role in the pathogenesis of pulmonary vascular remodeling, PH and right HF. Steers with the most severe pulmonary vascular remodeling, PH and right HF had evidence of pulmonary veno-occlusive disease, alveolar inflammation and fibrosis. Transcriptomic analysis of right ventricular gene expression by RNAsequencing reveals right HF is characterized by a striking pro-inflammatory signature of gene expression suggesting persistent activation of innate immunity and pro-inflammatory signaling pathways. Cardiac lesions in high mPAP steers included myocyte hypertrophy, myofibrillar disarray, and fibrosis. In conclusion, beef cattle reared in chronic hypoxic environments provide a natural model of Group 3 PH. Inflammatory processes appear to contribute to disease severity and outcomes, and may provide novel diagnostic and therapeutic targets for the treatment of PH in humans and beef cattle.

† Graduate Student Oral Presentation Award Applicant

Not So High Mountain Disease: Inflammation, Pulmonary Hypertension and Right Heart Failure in High Plains Fattening Beef Cattle †

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Narrative: Heart failure in beef cattle is historically associated with chronic exposure to hypoxic environments in the mountain West, leading to pulmonary vascular remodeling, pulmonary hypertension (PH) and right heart failure (HF). Long considered a problem of cattle reared at high altitude (2130 m/7000 ft), anecdotal evidence suggests PH-HF is occurring in fattening beef cattle at altitudes that encompass the Great Plains (300 to 1600 m) with no history of high altitude exposure. Failure to thrive and death loss occur late in the feeding period, amplifying economic losses. Moreover, PH-HF commonly occurs in fed cattle with a history of bovine respiratory disease. Histopathology and cardiac right ventricular gene expression in animals with PH-HF due to high elevation grazing reveal striking activation of innate immunity and inflammation. We hypothesize that in fattening cattle at low elevation, systemic hypoxemia resulting not from environmental hypoxia but instead from the interaction of increased metabolic demand and obesity during the finishing period, combined with a persistent pro-inflammatory state, drives progression to PH and HF. We investigated this hypothesis in two cases of low elevation PH-HF, as follows. In February 2014, an 11-month-old Angus steer from a feedlot in Akron, Colorado (elevation 1400 m) presented with a three-month history of respiratory dyspnea, severe brisket edema, bilateral jugular distention and muffled heart sounds. Echocardiogram revealed right atrial enlargement, dilation of the right ventricle, septal deviation to the left, reduction of the left ventricle and dilation of the main pulmonary artery. The PAP was between 85-90 mm Hg. In February 2015, another fattening Angus steer (12 mos) from the same Akron facility presented with history of respiratory disease and similar clinical signs of PH-HF. Based on poor clinical prognosis, the steers were euthanized and submitted for postmortem examination. Macroscopic changes consistent with PH-HF included pericardial, pleural and peritoneal effusions, severe dilation of the pulmonary trunk and right atrium, increased thickness of the right ventricular free wall and chronic passive liver congestion. Microscopic evidence of remodeling was apparent throughout the pulmonary arterial and venous tree. Hypertrophied cardiomyocytes, myofibrillar disarray, and myocardial fibrosis were evident in both steers. Pulmonary lesions indicative of chronic inflammation included bronchiolitis fibrosis obliterans (Steer 1), prominent lymphoplasmacytic infiltrates, and bronchus-associated lymphoid tissue (BALT) hyperplasia (Steer 2). In conclusion, better understanding of inflammatory processes may provide important insights into diagnosis and therapy of PH and HF in fattening cattle, and adverse interactions of PH with BRD.

† Graduate Student Oral Presentation Award Applicant

Hemochromatosis and equine degenerative myelopathy in a Shetland pony † +

Ida L. Phillips

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Narrative: A 16 year old Shetland pony with a history of hind end staggering, inappetence, and difficulty maintaining weight presented with an acute onset of respiratory distress. Endoscopy revealed bilaterally closed arytenoids on inspiration. Bloodwork showed marked hepatic derangements. The animal was euthanized and the body was submitted for necropsy. The main gross finding was an enlarged liver with swollen, rounded edges, and an irregularly nodular surface which bulged when cut and had a prominent micronodular pattern. Microscopic examination of the liver revealed severe, diffuse hepatocellular degeneration and loss, with bridging portal fibrosis, marked iron accumulation, and mild biliary hyperplasia. Prussian blue stains revealed abundant iron accumulation in the thyroid, kidney, pituitary gland, salivary gland, spleen, adrenal gland, and colonic epithelial cells. All white matter tracts of the spinal cord had variable amounts of digestion chambers with myelin sheath disruption and swollen axons. Liver iron and blood ferritin levels were 27,048ug/g (30x reference ranges) and 46,539ug/g (100x reference ranges), respectively. Hemochromatosis, was diagnosed and histologic findings will be compared to the disease in other species. The level of hepatic selenium was low and we speculate that this may have contributed to the degenerative myelopathy. Clinical signs of equine hepatic failure will be reviewed.

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Isolation of *Helcococcus ovis* from an aborted calf with pathology ◇

Yan Zhang, Jing Cui, Jeffrey R. Hayes, Mary B. Weisner, Beverly Byrum

Ohio Department of Agriculture, ADDL, Reynoldsburg, OH

Narrative: *Helcococcus ovis* is a Gram-positive, facultative anaerobic coccus. It was originally isolated in 1999 from sheep in different geographical locations. It is now considered to be an emerging veterinary pathogen and has been reported as the causative agent of bovine valvular endocarditis and metritis, pulmonary abscesses in a horse, and pleuritis and bronchopneumonia in sheep. *H. ovis* was also recently isolated in the United Kingdom from the stomach contents of an aborted bovine fetus, suggesting this agent as a potential causal pathogen for the abortion. However, pathology from the aborted fetus or placenta was not reported. Here, we report the finding of moderate to heavy growth of *H. ovis* from the placenta as well as the lung and stomach contents of a Holstein fetus, reported to have aborted at 115 days in gestation. The identity of the bacterium was confirmed by MALDI-TOF and 16S RNA sequencing. This was the fourth abortion in the herd over an 8 month period. Microscopic examination of the allantochorion revealed severe necrosuppurative placentitis with thrombosis, vasculitis and intralesional cocci. Lesions in fetal tissues included moderate suppurative bronchopneumonia with intralesional cocci, mild lymphohistiocytic myocarditis, mild lymphocytic interstitial nephritis and also moderate neutrophilic rumenitis. Other tests performed did not detect additional pathogenic agents. Based on microscopic lesions in multiple tissues, recovery of pure growth of *H. ovis* from two of those tissues as well as from fetal stomach contents, and the exclusion of other pathogens, a diagnosis of bacterial abortion associated with *Helcococcus ovis* was made. To our knowledge, this is the first report of bovine abortion associated with *Helcococcus ovis* in the United States.

◇ USAHA Paper

Pathology 2a
 Sunday, October 25, 2015
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Moderators: Chanran Ganta and Danielle Nelson

8:00 AM	Spontaneous Uterine Tumors in Guinea Pigs * † + <i>Tamara Veiga-Parga, Shelley Newman</i>	87
8:15 AM	Acute, fibrinohemorrhagic, interstitial pneumonia and suppurative myocarditis associated with <i>Bartonella henselae</i> in three Florida panthers (<i>Puma concolor coryi</i>). # † + <i>Elizabeth J. Elsmo, Heather M. Fenton, Michael J. Yabsley, Mark Cunningham, Elizabeth W. Howerth</i>	88
8:30 AM	Immunophenotyping and comparison of normal canine corneal endothelium and canine preiridal cellular membranes † + <i>Linda Huang, Matti Kiupel, Dodd G. Sledge</i>	89
8:45 AM	Pathology of blackleg in cattle # † + <i>Camila C. Abreu, Patricia C. Blanchard, John Adaska, Robert B. Moeller, Mark L. Anderson, Santiago Diab, Francisco A. Uzal</i>	90
9:00 AM	Pneumonic Plague in a Domestic Dog <i>Gary L. Mason, Kristy Pabilonia</i>	91
9:15 AM	Immunohistochemical staining in tissues from non-PI, seropositive, congenitally BVDV infected calves with varying degrees of neurological malformation <i>Sasha R. Lanyon, Michael P. Reichel</i>	92

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| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Spontaneous Uterine Tumors in Guinea Pigs † +

Tamara Veiga-Parga, Shelley Newman

BDS, University of Tennessee, Knoxville, TN

Narrative: Reproductive tumors in female domestic guinea pigs are under-reported. To provide a comprehensive review of uterine neoplasia in guinea pigs, a retrospective study of the pathology archives of the University of Tennessee College of Veterinary Medicine was performed. Uterine cases were selected, characterized by histology and 12 of 25 were neoplastic (48%). The most common uterine tumors were adenomas, representing 33.3% of guinea pig neoplasms (4/12), followed by leiomyomas (25%; 3/12), and leiomyosarcomas (16.6%; 2/10). Two animals had other anaplastic neoplasms with features of choriocarcinoma. Multiple animals with uterine tumors also had cystic rete ovarii. Immunohistochemistry for estrogen receptor and progesterone receptor indicated that some of these uterine tumors are hormone responsive. Immunohistochemistry for smooth muscle actin in combination with histology confirmed the diagnosis of leiomyoma and leiomyosarcoma. In the case of the anaplastic neoplasms, a panel of vimentin and cytokeratin failed to confirm trophoblast origin. To our knowledge, this is the largest case series of uterine tumors in guinea pigs.

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Acute, fibrinohemorrhagic, interstitial pneumonia and suppurative myocarditis associated with *Bartonella henselae* in three Florida panthers (*Puma concolor coryi*). # † +

Elizabeth J. Elsmo^{1,2}, Heather M. Fenton¹, Michael J. Yabsley^{4,1}, Mark Cunningham³, Elizabeth W. Howerth²

¹University of Georgia, Southeastern Cooperative Wildlife Disease Study, Athens, GA; ²Department of Pathology, University of Georgia, Athens, GA; ³Florida Fish and Wildlife Conservation Commission, Gainesville, FL; ⁴Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

Narrative: A case of acute, fibrinohemorrhagic, interstitial pneumonia and suppurative myocarditis associated with *Bartonella henselae* infection was diagnosed in an adult male Florida panther in 2014. The presence of *B. henselae* in samples of fresh lung from this panther was confirmed by polymerase chain reaction and sequence analysis of 16S-23S spacer region, pap31, and rpoB genes. Immunohistochemical staining for *B. henselae* identified positively staining organisms in sections of lung, heart, and lymph node, and morphologically consistent organisms were identified in intravascular histiocytes in the lung with transmission electron microscopy. A retrospective review of Florida panther mortalities identified an additional two cases with similar lesions of undetermined cause. Positive immunohistochemical staining for *B. henselae* was identified in sections of lung from both of these panthers. Sections of lung from three additional Florida panthers that died of vehicular trauma and did not have similar lung lesions were evaluated with *B. henselae* immunohistochemistry, and no positively staining organisms were identified. While serosurveys suggest that exposure to *B. henselae* is common in free-ranging panthers in the United States, it is presumed that infections are generally subclinical. However, this case series suggests that *B. henselae* is occasionally associated with a syndrome of fibrinohemorrhagic interstitial pneumonia and suppurative myocarditis in Florida panthers. Underlying causes of immune suppression were not identified in any of the three affected panthers. All three affected panthers did exhibit at least one of the congenital defects that have been described in association with poor genetic diversity in the Florida panther population, and an underlying congenital immunodeficiency cannot be ruled out.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Immunophenotyping and comparison of normal canine corneal endothelium and canine preiridal cellular membranes † +

Linda Huang, Matti Kiupel, Dodd G. Sledge

Michigan State University, Lansing, MI

Narrative: Preiridal membranes in canine eyes are of pathologic significance as they are often associated with peripheral anterior synechia and secondary glaucoma. Two morphologically distinct types of preiridal membrane have been described: fibrovascular membranes and cellular membranes. Fibrovascular membranes are associated with intraocular production of vascular growth factors and extend from the stroma of the anterior iris. The histogenesis and cause of cellular membranes is less clear. Morphologically, these membranes often resemble corneal endothelium, being composed of a single layer of attenuated to polygonal cells that have variably distinct cell borders, small amounts of lightly basophilic vesiculated cytoplasm, homogeneously basophilic oval nuclei, and occasional segmental basement membranes. The goals of the current study were to immunophenotype normal canine corneal endothelium and to compare immunomarker expression patterns between normal corneal endothelium and that of preiridal cellular membranes. Corneal endothelium is of neural crest origin, and normal human corneal endothelium reportedly expresses neuron specific enolase (NSE), S-100 protein, and vimentin. In addition, normal human corneal endothelium expresses an antigen recognized by anti-Tamm-Horsfall glycoprotein (THGP) antibodies. In this study, we evaluated immunoreactivity for NSE, S-100 protein, vimentin, and anti-THGP antibodies in 5 normal canine eyes and 5 canine eyes with previously diagnosed preiridal cellular membranes. Sections from cases with preiridal cellular membranes were concurrently stained with Alcian blue and Periodic Acid Schiff reaction (AB-PAS) to evaluate for basement membranes. Corneal endothelium in normal canine eyes had strong intracytoplasmic immunoreactivity for NSE, S-100, and vimentin and weak intracytoplasmic immunoreactivity for anti-THGP antibody. Preiridal cellular membranes had a similar pattern of immunoreactivity for all evaluated antibodies. In a number of cases, there was a PAS-positive basement membrane between basal segments of the preiridal cellular membranes and the anterior face of the iris. Overall, these results indicate that normal canine corneal endothelium is immunophenotypically similar to human corneal endothelium, and that preiridal cellular membranes are derived from corneal endothelium. In humans, proliferation and migration of abnormal endothelial cells over structures adjacent to the cornea is well described in iridocorneal endothelial (ICE) syndrome. While iridocorneal endothelialization has been suggested in conjunction with doubling of Descemet's membrane in dogs, iridocorneal endothelialization in dogs has been poorly described and is likely under recognized.

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Pathology of blackleg in cattle # † +

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Francisco A. Uzal⁴

¹Veterinary Pathology Laboratory, Federal University of Lavras, Lavras, Brazil; ²CAHFS, UC Davis, Tulare, CA;
³CAHFS, UC Davis, Davis, CA; ⁴CAHFS, UC Davis, San Bernardino, CA

Narrative: Blackleg is an infectious disease of cattle and rarely other ruminants, produced by *Clostridium chauvoei* and characterized by necrotizing myositis. The pathogenesis of the disease involves ingestion of spores, intestinal absorption, and distribution via bloodstream to multiple tissues, including skeletal and cardiac muscle. When the oxygen tension drops in areas of muscle where spores are present, they germinate and proliferate, producing toxins that are responsible for most clinical signs and lesions of blackleg. It is usually believed that in most cases of blackleg, the large muscles of the pectoral and pelvic girdles are affected, with other skeletal muscles and the heart being less frequently involved. However there is no published information on the specific distribution of blackleg lesions in cattle. A retrospective study was performed including 29 blackleg cases selected from the archives of the California Animal Health and Food Safety Laboratory. The cases were selected based on the presence of gross and microscopic lesions compatible with blackleg, plus positive result to one or more of the following *C. chauvoei* tests: fluorescent antibody test, immunohistochemistry and culture. The age of the affected animals ranged from 1 to 19 months and there was no history of treatment in any of the animals. Sixty six per cent of the animals had gross and/or microscopic lesions in both skeletal muscle and heart, 31% had lesions in the skeletal musculature alone and 3% in the heart alone. Gross and/or microscopic lesions in the skeletal musculature involved rear quarters (33%), front quarters (21%), neck (18%), lumbar region (11%), brisket (6%), diaphragm (5%), abdominal wall (2%), thoracic wall (2%) and tongue (2%). In most of the animals the lesions in skeletal musculature were observed in more than one muscular group: 32% in one group, 43% in two groups, 14% in three groups, 7% in four groups and 4% in five groups. Of the 20 animals that had lesions in the heart, 60% had pericarditis and myocarditis, 20% only myocarditis, 10% pericarditis, myocarditis and endocarditis and 10% only pericarditis. Because this was a retrospective study, we do not know how rigorous was the record keeping of muscle lesions. These results indicate that in blackleg, simultaneous lesions in skeletal musculature and heart are relatively common, and the most affected skeletal muscles are those of the rear legs.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Pneumonic Plague in a Domestic Dog

Gary L. Mason, Kristy Pabilonia

Micro, Immunol. & Pathol; Vet Dx Lab, Colorado State University, Fort Collins, CO

Narrative: A 2 year old, neutered male, pit-bull cross canine presented with history of rigid jaw tone, right forelimb ataxia, drooling, pale mucous membranes and contusion of the right dorsal lung. Hemoptysis developed, leading to a decision to perform humane euthanasia. Client concerns included trauma from being kicked by a mule or diphacinone poisoning; a prairie dog town on the property had been eliminated with this anticoagulant several months earlier. Gross findings included a large quantity of blood in the stomach and intestine, a few small gastric ulcers and hemorrhage of the left intercostal muscles, thymic interstitium, left caudal thoracic soft tissue and diaphragm. The lungs were dark red in color and the right caudal lung lobe was firm with a homogenous dark red color on section. After consultation, the client elected rabies and anticoagulant testing, but declined histopathology or further testing. Approximately 10 days later, the client was hospitalized with an undiagnosed severe acute pulmonary disease. Formalin fixed tissues from the canine were processed for histopathology and frozen archived tissue samples were submitted for *Yersinia pestis* & *Francisella tularensis* real-time PCR (qPCR). All tissues tested, including lung, liver, kidney, intestine, stomach contents, brain and blood tested positive for *Y. pestis*. Histopathology revealed acute bronchopneumonia with hemorrhage and many intra-alveolar bacteria. Acute fibrinopurulent to suppurative inflammation of spleen, liver and meninges was also appreciated. Pathologic and laboratory findings were interpreted as consistent with pneumonic plague. The dog in this report is thought to have been the source for four human cases¹. Dogs are known to be susceptible to *Y. pestis* infection² although transmission of *Y. pestis* from domestic dogs to humans is rare or underreported. A case study from China implicated a domestic dog as the point source for a human outbreak involving twelve people³. Plague should be considered in the differential diagnosis of ill domestic dogs which have a history of access to wild rodents and rabbits in plague endemic areas. 1 Runfola, JK, House, J, Miller, L. et al. Outbreak of Human Pneumonic Plague with Dog-to-Human and Possible Human-to-Human Transmission — Colorado, June–July 2014. *MWR Weekly*. May 1, 2015 / 64(16);429-434 2 Nichols MC, Ettestad PJ, VinHatton ES, et al. *Yersinia pestis* infection in dogs: 62 cases (2003–2011). *J Am Vet Med Assoc* 2014;244:1176–80. 3 Wang H, Cui Y, Wang Z, et al. A dog-associated primary pneumonic plague in Qinghai Province, China. *Clin Infect Dis* 2011;52:185–90

Immunohistochemical staining in tissues from non-PI, seropositive, congenitally BVDV infected calves with varying degrees of neurological malformation

Sasha R. Lanyon, Michael P. Reichel

School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia

Narrative: Three calves were born displaying varying degrees of clinical neurological signs following fetal infection with bovine viral diarrhoea virus (BVDV) at day 90 gestation as part of a larger research trial. All three calves were seropositive for BVDV-specific antibodies at birth (prior to colostrum ingestion) indicative of immunocompetence prior to fetal infection. Clinical signs included recumbency, ataxia, wide-based stance, vision impairment, inability to stand or maintain standing position, weaving motions of the head and a tendency to low head carriage. Post-mortem examination revealed hydrocephalus and cerebellar hypoplasia, consistent with previous reports of congenital BVDV infection. Immunohistochemistry of two calves revealed the presence of BVDV antigen, with a distribution apparently limited to neural tissues. Immunohistochemistry results of the third calf and histological results for all three calves are pending at the time of abstract submission and will be presented in full. This unusual report demonstrates that despite immunocompetence at the time of in utero infection, tissues from congenitally infected calves may still harbour residual virus.

Pathology 2b
 Sunday, October 25, 2015
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Moderators: Chanran Ganta and Danielle Nelson

10:15 AM A proposed histopathological case definition of naturally-acquired *Salmonella enterica* serovar Dublin infection in Holstein cattle in the northeastern United States
Heidi L. Pecoraro, Belinda S. Thompson, Gerald E. Duhamel95

10:30 AM Catastrophic humeral fractures in race horses
Francisco A. Uzal, Meridith Rhea, Francisco R. Carvallo, Santiago Diab96

10:45 AM Lesion characterization and exploration of infectious etiologies in growing pigs with ear tip necrosis
Bailey L. Arruda, Eric Burrough, Kent Schwartz, Adam Krull, Paul Plummer, Jeff Blythe97

11:00 AM Systemic fibrinoid vasculitis and severe renal lesions in a free-ranging, young-of-the year raccoon (*Procyon lotor*) from Prince Edward Island, Canada associated with infection with a *Bartonella taylorii*-like bacterium
Heather M. Fenton, Michael J. Yabsley, Elizabeth J. Elsmo, Christopher Cleveland, Scott McBurney98

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A proposed histopathological case definition of naturally-acquired *Salmonella enterica* serovar Dublin infection in Holstein cattle in the northeastern United States

Heidi L. Pecoraro^{1,2}, Belinda S. Thompson^{2,1}, Gerald E. Duhamel^{2,1}

¹Biomedical Sciences, Cornell University, Ithaca, NY; ²New York Animal Health Diagnostic Center, Ithaca, NY

Narrative: *Salmonella enterica* subsp. *enterica* serovar Dublin (*Salmonella* Dublin) is a host-adapted bacterium that causes high morbidity in dairy cattle worldwide. Clinical signs range from asymptomatic to diarrhea to systemic infection. Previous experimental infection studies have elucidated the pathogenesis and outlined some of the histologic changes seen with *Salmonella* Dublin infection; however, there is limited information about naturally-acquired lesions and disease. The objective of the current study was to characterize histopathologic lesions in cattle naturally-infected with *Salmonella* Dublin. A retrospective search of archives at the New York Animal Health Diagnostic Center revealed 51 culture-confirmed *Salmonella* Dublin cases in which detailed tissue histology was performed. Database information included age, sex, breed, location, and results of ancillary testing. Tissues for histologic evaluation included sections of heart, lungs, liver, spleen, and lymph nodes. Of the 51 cases, all were from Holstein breed, 48 were female, 44 were under 6 months of age, and 47 were from NY or PA. There were 28 bacterial, 8 viral and 3 parasitic co-infections. Mild to severe myocarditis was found in over 70% (8/11) of hearts examined. Eighty-six percent (44/51) of lungs had moderate to severe pneumonia. In addition, moderate to severe inflammation was present in 55% (19/34) of livers, 50% (10/20) of spleens and 40% (14/35) of lymph nodes examined. Inflammation was primarily composed of neutrophils, with fewer lymphocytes and histiocytes, and was often accompanied by necrosis. Based on the histopathology, we propose a histopathological case definition of *Salmonella* Dublin in Holstein cattle less than 6 months of age which will assist in the development of improved protocols for the diagnosis of infectious diseases of dairy cattle.

Catastrophic humeral fractures in race horses

Francisco A. Uzal, Meridith Rhea, Francisco R. Carvalho, Santiago Diab
California Animal Health and Food Safety, UC Davis, San Bernardino, CA

Narrative: Catastrophic fractures are the most prevalent cause of death in race horses in California and elsewhere. Scientific evidence indicates that the great majority of catastrophic fractures in race horses are predisposed by pre-existing stress fractures, many of which are treatable, which makes catastrophic fractures preventable. The Post-Mortem Program of the California Horse Racing Board has been in place since 1990 and to date over 6,000 horses have been submitted for necropsy and diagnostic work up to CAHFS. To date, however, little information has been made public to the diagnostic laboratory community on musculoskeletal lesions of race horses. We reviewed the necropsy records of 91 horses with humeral fractures submitted to CAHFS between 2008 and 2014. Ninety four per cent of the horses were euthanized due to catastrophic humeral fractures, while 6% of the animals died spontaneously from severe trauma to the thorax and/or head. All horses had unilateral fractures of the humerus that affected the left (54%) or the right (46%) leg. All the fractures affected the shaft of the humerus and were complete, spiral, displaced and close. In 76% of the cases, the main line of the catastrophic fracture intersected an area of periosteal callus consistent with stress remodeling in the caudoproximal aspect of the humeral cortex. The periosteal callus was characterized by minimal periosteal elevation and red discoloration (mild lesions), moderate periosteal elevation with a soft, rough surface (moderate lesions) or marked periosteal elevation with a firm surface (severe lesions). Stress fractures can be diagnosed in the live animal and treated, avoiding thus the risk of catastrophic fractures. In this study more than two thirds of the catastrophic fractures could have been prevented.

Lesion characterization and exploration of infectious etiologies in growing pigs with ear tip necrosis

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Narrative: Ear tip necrosis is a fairly common yet enigmatic malady encountered in swine. Cases presented to the Iowa State University Veterinary Diagnostic Laboratory suggest that a majority of US herds have had affected pigs but with large variation in frequency and extent. In this investigation, 25-50% of piglets were commonly affected with one finisher group nearing 100% prevalence and having a projected mortality of >10%. Proposed etiologies of this condition are many and generally speculative; indeed, there may be multiple causes and risk factors that can manifest as ear tip necrosis. The mechanism of lesion development has not been characterized but perhaps offers a common feature amongst at least a portion of the cases. The objective of this disease investigation was to characterize the progression of lesions in conjunction with the presence of potential pathogens. Punch biopsies were obtained for histopathology, molecular diagnostics, and metagenomics from acutely affected, chronically affected, and unaffected pigs. Histologic examination of the epidermis and dermis of acutely affected pigs revealed deep dermal inflammation with minimal changes to the epidermis. Severe changes were present in chronically affected pigs and included ulceration, irregular acanthosis, granulation tissue formation, and deep dermal thrombosis. Histologic examination of the epidermis and dermis of unaffected pigs was unremarkable. Preliminary polymerase chain reaction results for the detection of *Staphylococcus hyicus* exfoliative toxin genes were negative. Metagenomic analysis of the epidermis and dermis using amplification of the V3-V4 hypervariable region of the bacterial 16S rRNA gene is underway. The presence of deep dermal inflammation with minimal epidermal changes is suggestive of an “inside-out” rather than “outside-in” process.

Systemic fibrinoid vasculitis and severe renal lesions in a free-ranging, young-of-the year raccoon (*Procyon lotor*) from Prince Edward Island, Canada associated with infection with a *Bartonella taylorii*-like bacterium

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Narrative: A young-of-the-year, female raccoon presented to the Veterinary Teaching Hospital of the Atlantic Veterinary College with clinical signs of weakness and tremors on September 20, 2012. The raccoon was euthanized and was subsequently submitted to the Canadian Wildlife Health Cooperative diagnostic service. At necropsy, the raccoon was found to be in poor body condition and had a diffuse lymphadenopathy. The kidneys were diffusely pale and firm with multifocal petechial hemorrhages present at the renal cortex. A tick removed from the medial canthus of the left eye was identified as *Ixodes muris*. Histologic lesions included systemic fibrinoid necrosis of blood vessels in the uvea, tongue, and lung as well as lymphoplasmacytic interstitial nephritis, fibrinosuppurative glomerulonephritis, and lymphoplasmacytic myocarditis. Metastatic mineralization was present within mesenteric lymph nodes, the adrenal gland, and the gastric wall. Ancillary testing on a section of kidney by polymerase chain reaction (PCR) for *Borrelia burgdorferi*, porcine circovirus-2 (PCV- 2), *Leptospira* sp. and feline coronavirus were all negative. Immunohistochemical testing for *Leptospira* sp. and PVC-2 were negative. Transmission electron microscopy was performed on sections of kidney, which revealed large numbers of bacterial rods surrounded by a trilaminar cell wall that measured approximately 1.3 μm by 0.35 μm . The bacteria were located within the glomeruli and were associated with aggregates of fibrin and vascular damage. Sequence analysis of the ITS-1 region of *Bartonella* DNA amplified from kidney tissue indicated that the organism was ~80% similar to numerous strains of *Bartonella taylorii*. This species of *Bartonella* is genetically diverse and is associated with a wide range of Eurasian rodent and flea species. To the authors' knowledge, it has not been previously reported in raccoons associated with lesions similar to those described in this case.

Serology; QA/QC
Sunday, October 25, 2015
553

Moderators: Devi Patnayak and Pat Lukens

10:15 AM	Evaluation of commercial porcine epidemic diarrhea virus (PEDV) serology tests using sera from experimentally infected pigs † <i>Kathleen A. Gibson, Dong Sun, Darin Madson, Luis G. Gimenez-Lirola, Leslie Bower, Hai T. Hoang, Mahesh Bhandari, Erin Kalkwarf, Ronaldo Magtoto, Kyoung-Jin Yoon</i>	101
10:30 AM	Comparison of three serological tests for the detection of antibodies against porcine epidemic diarrhea virus <i>Devi P. Patnayak, Albert Rovira, Sagar M. Goyal, James E. Collins</i>	102
10:45 AM	Pre-treatment of serum samples to reduce interference of colostrum-derived specific antibodies with detection of bovine viral diarrhoea virus antigen by ELISA in young calves <i>Sasha R. Lanyon, Michael P. Reichel</i>	103
11:00 AM	Raising the Bar – Evaluating the Quality of Root Cause Analyses § <i>Susan L. Martin, Thomas J. Reilly, Timothy Evans, Shuping Zhang</i>	104
11:15 AM	Improving Data Integrity and Reliability in the Research Phase of Diagnostic Test Development <i>Rebecca L. Davies, Katrina Laube, Brendan R. Davies, Audrey Tseng, Matilda Wagner, Kyra Martins, Suzanne Stone, Cheryl Dvorak, Carrie Wees, Michael Murtaugh</i>	105

Symbols at the end of titles indicate the following designations:

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|---|--|
| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Evaluation of commercial porcine epidemic diarrhea virus (PEDV) serology tests using sera from experimentally infected pigs †

Kathleen A. Gibson, Dong Sun, Darin Madson, Luis G. Gimenez-Lirola, Leslie Bower, Hai T. Hoang, Mahesh Bhandari, Erin Kalkwarf, Ronaldo Magtoto, Kyoung-Jin Yoon

College of Veterinary Medicine, Iowa State University, Ames, IA

Narrative: Porcine epidemic diarrhea virus (PEDV) causes an acute infection of mature swine enterocytes clinically manifested by effusive diarrhea in pigs of all ages. In 2013, the first outbreak occurred in the United States causing significant economic loss due to 90% pre-weaning mortality. Enhanced biosecurity and 'feedback' practice have become crucial tools for prevention and control of PED. Serological assays have been developed to assist in surveillance efforts to prevent the spread of PEDV. Anti-PEDV antibodies can be detected by indirect fluorescent antibody (IFA) test, ELISA, and serum-virus neutralization tests. Of these tests, the IFA assay and ELISA have become commercially available in North America. This study was completed to evaluate the diagnostic performance of the commercial PEDV serological tests. Two commercial test kits were selected and evaluated in this study: 1) Swinecheck® PED indirect ELISA (Biovet) and 2) Porcine epidemic diarrhea virus FA substrate slide (VMRD). The Biovet ELISA kit detects IgG against PEDV nucleoprotein, while the VMRD FA slide detects IgG against the whole virus. Serum samples collected periodically from weaned pigs (n=9) experimentally challenged with an US PEDV isolate were used to assess test performance. Commercial kits were compared to in-house IFA test and whole-virus based indirect ELISA (WV ELISA) offered by Iowa State University Veterinary Diagnostic Laboratory. Diagnostic specificity and sensitivity were calculated along with the test agreement. Both the commercial and in-house tests showed 100% specificity, when they were evaluated using known negative pig sera. The tests had varying degrees of sensitivity on sera from pigs with known infection status. Nonetheless, all tests detected positive results on sera collected on or after 2 weeks post inoculation. When comparing results of the Biovet ELISA kit to the in-house WV ELISA, both tests had 89% test agreement. The VMRD FA slide tested comparably with the in-house IFA test, resulting in 92% agreement between the tests. Based on these results, both commercial kits described here were in agreement with the standardized in-house tests, suggesting these kits are a viable option for the detection of PEDV-specific antibodies in porcine sera. Commercial kits may serve as a beneficial diagnostic tool due to their high quality control.

† Graduate Student Oral Presentation Award Applicant

Comparison of three serological tests for the detection of antibodies against porcine epidemic diarrhea virus

Devi P. Patnayak, Albert Rovira, Sagar M. Goyal, James E. Collins

Veterinary Population Medicine (Vet Diagnostic Lab), University of Minnesota, St. Paul, MN

Narrative: Porcine Epidemic Diarrhea (PED) is a highly contagious disease of pigs characterized by acute diarrhea, vomiting, dehydration and high mortality in neonatal piglets leading to huge economic losses. The disease is caused by porcine epidemic diarrhea virus (PEDV), a member of the family *Coronaviridae*. Historically, PED has been reported in European and Asian countries for the last 30 years. The United States was free from this virus until mid-April 2013, when first PEDV outbreak was reported in the Midwest area. Since then, various diagnostic tests have been developed for the detection of virus and virus-induced antibodies. This study describes the comparison of an in house ELISA based on recombinant nucleocapsid protein, a commercially available assay and an indirect immunofluorescence assay (IFA) for the detection of PEDV antibodies. Samples from different studies collected during early, mid and late infection with PEDV were used. Specificity was estimated as 88.2% (95% CI 80.8-93.0) for in house ELISA, 95.5% (95% CI 89.8-98.0) for Biovet ELISA and 100% (95% CI 96.6-100) for IFA. Sensitivity depended on the stage of infection. It ranged from 40%-70% during early infection (1st week), 90%-94% during mid infection (2-5 wks) and 40%-55% during late infection (8-9 wks). Biovet ELISA was the most sensitive test, followed by IFA and in house ELISA.

Pre-treatment of serum samples to reduce interference of colostrum-derived specific antibodies with detection of bovine viral diarrhoea virus antigen by ELISA in young calves

Sasha R. Lanyon, Michael P. Reichel

School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia

Narrative: The antigen ELISA is the preferred method for the diagnosis of persistently bovine viral diarrhoea virus infected (BVDV PI) individuals, however, colostrum-derived antibodies may interfere with antigen detection in serum from young PI calves. This proof-of-concept study aimed to assess serum pre-treatment methods for reducing such interference. The study consisted of three stages. First, serum from a BVDV PI animal was serially diluted in antibody-positive serum or (antibody-negative) diluent. The dilution series showed that antibody levels equivalent to those previously observed in colostrum-fed calves were able to eliminate all antigen signals in a serum sample when tested by antigen ELISA. Second, artificially created antibody- and antigen-positive samples that mimic the ELISA results observed in colostrum-fed PI calves were used to test and optimise varying sample treatment methods. When serum was treated with EDTA at pH 4.5, 5.5, 6.5 and 7.5, boiled, centrifuged and the supernatant recovered, BVD antibody was undetectable, and antigen signal improved. Antigen signal recovery was optimal, with >90% recovery, at pH 5 ± 0.5 . Third, the optimal treatment method was applied to field samples from three experimentally generated PI calves and their congenitally infected, but non-PI herdmates. When applied to samples from the three PI calves (which were negative in the antigen-capture ELISA without treatment), the antigen signal improved and gave a positive result in each case. Therefore, the interference of colostrum-derived antibodies in the detection of young PI calves by antigen ELISA appears to be overcome by first treating the sample with EDTA and heat. While further validation is necessary before practical uptake, this method may provide a major improvement in the diagnosis of young PI calves.

Raising the Bar – Evaluating the Quality of Root Cause Analyses §

Susan L. Martin¹, Thomas J. Reilly^{1,2}, Timothy Evans^{1,2}, Shuping Zhang^{1,2}

¹Veterinary Medical Diagnostic Lab, University of Missouri, Columbia, MO; ²Veterinary Pathobiology, University of Missouri, Columbia, MO

Narrative: Purpose: Root cause analysis (RCA) is a vital component of the investigation and resolution of incidents requiring corrective and preventive action. Ultimately, the effectiveness of a resolution and the ability to prevent a reoccurrence of the same incident is highly dependent upon actually determining the true root cause, or, in a manner of speaking, the quality of the root cause analysis. Therefore, we sought to develop a method to evaluate the quality of root cause analyses completed by faculty and staff writing corrective and preventive actions. **Hypothesis:** Review of corrective action and preventive action (CAPA) documents during the course of eight months suggested that the quality of the RCA within the CAPAs had improved. However, we believed empirical data was necessary to objectively demonstrate improvement. **Methods:** A Likert scale was initially considered as a tool to evaluate the root cause analyses. While a Likert scale could be used to rate RCAs, the results would be primarily opinion. The method was rejected as too subjective. The question became, “What criteria constitutes an effective root cause analysis?” The first criterion came from the method itself – asking why. Secondly, the resolution needed to create or modify a system to prevent re-occurrence of the problem. The third criterion originated from an observed problem in our RCAs; a tendency to restate the problem rather than analyze it. A rubric was designed to evaluate and score the RCAs; the higher the score, the better the RCA. RCAs from two distinct groups of CAPAs were evaluated using the rubric. The first group contained CAPAs written on or before October 24, 2014 and the second contained CAPAs completed on or before April 15, 2015. **Results:** Scores for the 2014 CAPAs ranged from 1 – 4, with 90% of the scores being a “1”. Scores for the 2015 CAPAs ranged from 0 – 6, with 60% of the scores being “4” or higher. For 2014, the average score was 1.3 compared to 2015’s average score of 3.5 for these two data sets. The paired data sets (year closed, composite scores) yielded a correlation coefficient of 0.97, a very high correlation that demonstrates an improvement of RCAs over time. **Conclusion:** The rubric will be a useful tool not only to evaluate and improve future RCAs but also to set consistent expectations and to educate staff members.

§ AAVLD Laboratory Staff Travel Awardee

Improving Data Integrity and Reliability in the Research Phase of Diagnostic Test Development

Rebecca L. Davies¹, Katrina Laube¹, Brendan R. Davies², Audrey Tseng², Matilda Wagner², Kyra Martins², Suzanne Stone², Cheryl Dvorak², Carrie Wees¹, Michael Murtaugh²

¹Veterinary Population Medicine, University of Minnesota College of Veterinary Medicine, St. Paul, MN;

²Department of Veterinary and Biomedical Sciences, University of Minnesota College of Veterinary Medicine, St. Paul, MN

Narrative: Veterinary Diagnostic Laboratories strive to improve their services by meeting the rigorous quality requirements of the American Association of Veterinary Laboratory Diagnosticians accreditation program. The program requires a quality assurance (QA) system to provide evidence that results are accurate and meet client needs. This program addresses the QA needs of the service laboratory, but does not serve the research mission (establish, improve and develop diagnostic techniques) of our diagnostic laboratories, nor does it contribute to the training of research scientists who are rarely introduced to QA principles in their current training programs. Research is critical to (1) ensure that laboratories gain access to new or enhanced tests that improve diagnostic strategies; and (2) create new diagnostic tools to respond to emerging threats to animal and human health. To meet these goals, the transfer of knowledge from the research to the service mission must be based on reproducible data. The National Institutes of Health has issued a call to address the ‘troubling frequency of published reports that claim a significant result, but fail to be reproducible,’ and they ask for help ‘to reset the self-corrective process of scientific inquiry.’ (Collins F, Tabak L. Policy: NIH plans to enhance reproducibility, Nature. 27 Jan 2014). Principles of QA, as applied in an AAVLD accredited service laboratory, are designed to preserve data integrity (consistency, reliability, repeatability) and can be applied to basic research intended to generate new diagnostic capabilities. Research and QA scientists collaborated to design a voluntary QA program (Good Research Practice, GRP) for research projects funded to improve immunodiagnostic testing for Porcine Epidemic Diarrhea Virus and Porcine Delta Coronavirus. Principles of QA were integrated to ensure that research methods were fit for purpose and that data integrity was preserved. A GRP checklist was created to define the quality requirements. Standard operating procedures (SOPs) were written and managed to ensure that (1) data were accurate, legible, contemporaneous, original and attributable; (2) method validation approaches were designed to meet pre-established acceptance criteria; and (3) laboratory procedures, equipment, notebooks, reagents and specimens were managed to improve data traceability. A GRP audit was performed and results showed that the data were accurate, reliable and a worthwhile investment for the swine industry. Knowledge transfer from basic research to service implementation was straightforward due to the ease of transfer of documentation supporting the reliability of the new method. Research and QA collaboration within AAVLD accredited labs will provide important training opportunities and hasten the translation of research findings into service outcomes resulting in enhanced laboratory capabilities. This project was funded by the National Pork Board (Projects 14-175, 14-176,13-262).

Toxicology 1
Saturday, October 24, 2015
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Moderators: Tim Evans and Tricia Talcott

1:00 PM	Proficiency Test to Detect and Quantify Melamine and Cyanuric Acid in Fish Tissues <i>Sarah Nemser, Yang Chen, Ramesh Yettella, Salvador Lopez, Andriy Tkachenko, Eric Evans, Jake Guag, Steve M. Ensley, Ravinder Reddy, Renate Reimschuessel</i>	<i>109</i>
1:15 PM	Inter-laboratory evaluation of a high performance liquid chromatography-fluorescence method for detection and quantification of aflatoxins M1 and B1 in animal liver tissues <i>Dahai Shao, Paula M. Imerman, Dwayne E. Schrunk, Steve M. Ensley, Wilson K. Rumbeiha</i>	<i>110</i>
1:30 PM	Analytical method for the determination of sulfide, sulfite, and thiosulfate in serum of mice exposed to hydrogen sulfide. <i>Paula M. Imerman, Poojya Anantharam, Wilson K. Rumbeiha</i>	<i>111</i>
1:45 PM	Biomarkers of Hydrogen Sulfide Poisoning <i>Poojya V. Anantharam, Elizabeth Whitley, Dahai Shao, Paula M. Imerman, Belinda Mahama, Wilson K. Rumbeiha</i>	<i>112</i>
2:00 PM	Development of sensitive quantitative diagnostic tests for novel nephrotoxic mushrooms. <i>Shusheng Tang, Dahai Shao, Paula M. Imerman, Dwayne E. Schrunk, Wilson K. Rumbeiha</i>	<i>113</i>
2:15 PM	Analysis of Microcystins in Liver, Hepatotoxicity in a Dog, Case Presentation <i>Paula M. Imerman, Steve M. Ensley, Joseph S. Haynes</i>	<i>114</i>
2:30 PM	Wildlife Poisonings Associated with Illegal Marijuana Grow Sites on Public and Tribal Lands in California <i>Mourad Gabriel, Robert H. Poppenga, Leslie W. Woods, Mark Higley, Greta Wengert, Michael Filigenzi.</i>	<i>115</i>
2:45 PM	Intra-laboratory development and evaluation of a urine-based quantitative diagnostic test for aflatoxicosis. <i>Dahai Shao, Paula M. Imerman, Dwayne E. Schrunk, Steve M. Ensley, Wilson K. Rumbeiha</i>	<i>116</i>

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Proficiency Test to Detect and Quantify Melamine and Cyanuric Acid in Fish Tissues

Sarah Nemser¹, Yang Chen², Ramesh Yettella², Salvador Lopez², Andriy Tkachenko¹, Eric Evans¹, Jake Guag¹, Steve M. Ensley³, Ravinder Reddy², Renate Reimschuessel¹

¹FDA CVM, Laurel, MD; ²Moffett Campus, Illinois Institute of Technology, Institute for Food Safety and Health, Bedford Park, IL; ³College of Vet Medicine, Iowa State University, Ames, IA

Narrative: Human and animal food can be adulterated with melamine and cyanuric acid for economically motivated reasons. These compounds can increase the apparent protein content by increasing nitrogen content. Melamine and cyanuric acid can crystallize in kidney causing illnesses or even death. Previous food adulteration events in the U.S and China determined a need to establish laboratory capability to detect these triazines in foods. The purpose of the proficiency test (PT) is to evaluate competency of six laboratories in detection and quantitation of melamine and cyanuric acid in fish muscles at levels close to the level of concern (2.5 mg/kg). An interlaboratory study was performed using incurred fish muscles. Catfish were dosed with melamine (10 or 20 mg/kg) and/or cyanuric acid (10, 20, or 40 mg/kg) for 1 or 3 days. Residues of melamine and cyanuric acid in fish were pre-determined by study organizers using LC-MS/MS method and ranged at concentrations from non-detected to 12.3 and 1.2 mg/kg respectively. Selected fish were used to prepare the proficiency test (PT) samples. Fish filet was pre-cut into slices and homogenized with dry ice in a blender. The resulting powder was subdivided into twelve blind coded test samples (15 g each) and shipped to six participating laboratories. The assigned values for PT samples were determined based on consensus and “fit-for-purposes” approaches. The data were processed according to ISO 13528:2005, Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons. The established assigned values for PT samples were in agreement with the values pre-determined by organizers. Fifty eight out of sixty results reported by five laboratories regarding melamine residues were concluded as satisfactory. All sixty results reported by five laboratories regarding cyanuric acid residues were concluded as satisfactory. The study revealed that five participating laboratories are proficient to determine melamine and cyanuric acid in fish muscles at levels close to the level of concern.

Inter-laboratory evaluation of a high performance liquid chromatography-fluorescence method for detection and quantification of aflatoxins M1 and B1 in animal liver tissues

Dahai Shao, Paula M. Imerman, Dwayne E. Schrunk, Steve M. Ensley, Wilson K. Rumbleha

Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

Narrative: Aflatoxins are a group of potent mycotoxins produced by various fungal species including *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi grow on a wide variety of animal feeds and feed ingredients including corn, peanuts, nuts, cottonseeds, cheese, etc. Aflatoxin B1 and M1 are the two most potent toxins in this group, which can cause severe acute poisoning. They are also strong carcinogens, mutagens and are immunosuppressive. A recurrent challenge in the diagnosis of aflatoxicosis is a lack of a fully validated methods for the etiological diagnosis and confirmation of aflatoxicosis. Working with Veterinary Laboratory Investigation and Response Network (Vet-LIRN), FDA, we have extended an intra-laboratory validated method to an inter-laboratory level validation. An inter-laboratory validation study consisted of three participating laboratories. Control liver samples were fortified with aflatoxin M1 at three levels of 0.2, 1.0, and 4.0 ng g⁻¹, or with aflatoxin B1 at 0.2, 1.0, and 7.0 ng g⁻¹. Three replicates of each level (a total of nine samples) were distributed to each participating laboratory. Preliminary results indicate satisfactory results for the upper two fortification levels for both B1 and M1 among all three laboratories. Inter-lab reproducibilities were 28.5% and 19.7% for aflatoxin M1 at 1.0 and 4.0 ng g⁻¹, respectively; 25.8% and 27.3% for aflatoxin B1 at 1.0 and 7.0 ng g⁻¹, respectively. Inter-lab accuracies were 95.5% and 87.6% for aflatoxin M1 at 1.0 and 4.0 ng g⁻¹, respectively; 100% and 97.0% for aflatoxin B1 at 1.0 and 7.0 ng g⁻¹, respectively. Results of the lowest level 0.2 ng g⁻¹ were not satisfactory, indicating more research effort is needed to improve the assay performance at this level. Completion of this study will result in a fully validated tissue-based method for the diagnosis of aflatoxicosis in animals. This work was funded by FDA grant number 1U18FD005006-01.

Analytical method for the determination of sulfide, sulfite, and thiosulfate in serum of mice exposed to hydrogen sulfide.

Paula M. Imerman, Poojya Anantharam, Wilson K. Rumbeiha

VDPAM, Iowa State University, Ames, IA, IA

Narrative: Hydrogen sulfide (H₂S) is a colorless, flammable, water-soluble gas. Exposure to this gas can cause central nervous system induced anoxia and death. This gas can be produced in lagoon situations and manure pit gas confinements causing livestock deaths. A mouse animal model for exposure to this gas was used to investigate biological markers. An analytical sensitive method was employed to see if sulfide, sulfite or thiosulfate could be used as a biomarker for exposure. Mice were exposed to approximately 315 ppm H₂S in a closed chamber enclosed in a fume hood for up to 40 minutes during a week time period. Serum was collected at various time points and used for analysis of sulfide, sulfite, and thiosulfate. Sulfide, sulfite, and thiosulfate were measure by reaction with monobromobimane to form a fluorescent derivatives which were separated by HPLC and measured at Ex 396 Em 476 with a fluorescence detector. By this method LOD for sulfide, sulfite, and thiosulfate was 10 ppb and LOQ was 50 ppb. Measurement of sulfide, sulfite, and thiosulfate showed that thiosulfate proved to be a suitable biomarker for H₂S exposure in the mouse model.

Biomarkers of Hydrogen Sulfide Poisoning

Poojya V. Anantharam¹, Elizabeth Whitley², Dahai Shao¹, Paula M. Imerman¹, Belinda Mahama¹,
Wilson K. Rumbleha¹

¹Iowa State University, Ames, IA; ²Pathogenesis, LLC, Gainesville, FL

Narrative: Hydrogen sulfide (H₂S) is a colorless, highly neurotoxic gas with a rotten egg odor. It is an environmental pollutant, mainly associated with the oil and gas industry. H₂S is also a byproduct of organic matter decomposition, a common source of hazard in swine industry. In ruminants, repeated sub-acute consumption of feeds containing high sulfur has resulted in polioencephalomalacia, a neurodegenerative disease of cattle. Depending on the route of exposure, clinical signs of H₂S poisoning can vary from eye irritation, dyspnea, acute collapse, neurodegeneration, and even death. Tissue-based etiological diagnosis of H₂S poisoning in livestock has been a challenge. Current diagnostic approaches rely on history of potential exposure, or in case of ruminants, analysis of feeds and water. The objective of this study was to develop biomarkers of H₂S exposure using a rodent model. C57 Black mice were exposed to 310 ppm H₂S by whole body inhalation for 40 minutes on day 0, followed by 15 minutes/day exposures for 6 consecutive days. Control mice were exposed to breathing air. Serum was collected for measurement of H₂S metabolites using HPLC fluorescence. Fresh, brain tissue was collected for neurotransmitter analysis, biochemical enzyme activity studies and for ATP quantification. Formalin fixed tissue was routinely processed for histopathology. H₂S-exposed mice exhibited seizures, dyspnea, and knockdown. H₂S-exposed mice showed significant increase in inferior colliculus (IC) dopamine concentration compared to controls (p<0.05). In the same region, GABA and glutamate concentrations showed a significant, time dependent decrease. H₂S significantly suppressed cytochrome c oxidase activity (p<0.01) in the IC. Histopathology revealed neurodegenerative lesions in the central nucleus of the IC, the most sensitive brain region to H₂S. Of the different metabolites measured in serum, thiosulfate was the only biomarker which was elevated following H₂S exposure and the most promising biomarker of H₂S exposure. Thus, a novel approach using a combination of neurochemical, biochemical and morphological biomarkers identified in this study may be useful in the etiological diagnosis of H₂S poisoning in livestock.

Development of sensitive quantitative diagnostic tests for novel nephrotoxic mushrooms.

Shusheng Tang^{1,2}, Dahai Shao², Paula M. Imerman², Dwayne E. Schrunk², Wilson K. Rumbelha²

¹College of Veterinary Medicine, China Agricultural University, Beijing, China; ²Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

Narrative: Mushroom poisoning is frequently encountered, especially in dogs. *Cortinarius* mushrooms which grow though out North America and in Europe contain orellanine, a highly toxic novel nephrotoxin. Although clinical cases of orellanine poisoning in animals are yet to be reported, numerous human cases have been reported. Orellanine poisoning should be considered in the differential diagnosis of acute renal failure in animals. Lack of a suitable diagnostic test is a bottleneck in the etiological diagnosis of orellanine poisoning in animals. We have developed sensitive HPLC UV-vis and LC-MS methods for detection and quantitation of orellanine in mushrooms. Both methods used a solid phase extraction procedure. The HPLC UV-vis method involves extraction using 3M hydrochloric acid. The limit of quantitation (LOQ) of the HPLC UV-vis procedure was 62.5 ng. For the LC-MS procedure, solid phase extracts were diluted 1:10 in methanol and run using PRP-1 10 μ m 250x4.1mm Hamilton column with mobile phase 97:3, acetonitrile:0.1% formic acid, isocratic and ESI in positive mode. The LOQ was 20 ng. Using these methods, we investigated eight different species of *Cortinarius* mushrooms growing in North America. We positively identified and quantified orellanine in a novel *Cortinarius armillatus* species. By HPLC, the novel *Cortinarius armillatus* mushroom contained, on average, 113.5 μ g g⁻¹ orellanine Vs 130 μ g g⁻¹ by LC-MS. *C. rubellus*, a positive control mushroom, contained on average of 18,551 μ g g⁻¹ orellanine by HPLC UV-vis Vs 25,507 μ g g⁻¹ by LC-MS. Results of HPLC and LCMS were comparable from a diagnostic perspective. Thus we developed two highly sensitive, reproducible diagnostic methods for detection and quantification of a novel nephrotoxin orellanine toxin in mushrooms suspected of causing acute renal failure in animals.

Analysis of Microcystins in Liver, Hepatotoxicity in a Dog, Case Presentation

Paula M. Imerman¹, Steve M. Ensley¹, Joseph S. Haynes²

¹VDPAM, Iowa State University, Ames, IA, IA; ²Veterinary Pathology, Iowa State University, Ames, IA

Narrative: Microcystins are cyclic heptapeptides produced by blue green algae including *Anabaena*, *Microcystis*, and *Planktothrix*. The algae grow in freshwater ponds and lakes. These toxins are detrimental to humans, livestock, and pets. Microcystins are hepatotoxins since the primary target is the liver. These toxins also are considered potent tumor promoters by inhibiting protein phosphatases 1 and 2 A. The detection of Microcystins LA, LR, RR, and YR in liver was developed using SPE and LCMS detection. The LOD is 0.5 ppb and the LOQ is 5 ppb for Microcystins in liver. Grossly the dog liver had hepatic damage and the liver was sent to the diagnostic lab for Microcystin testing to delineate exposure. This showed approximately 21ppb Microcystin LA after correction for recovery. This evidence along with the histopathology indicates Microcystin poisoning in a dog. Confirmation of Microcystin in liver is a valuable finding when investigating cases of hepatotoxicity in animals.

Wildlife Poisonings Associated with Illegal Marijuana Grow Sites on Public and Tribal Lands in California

Mourad Gabriel², Robert H. Poppenga¹, Leslie W. Woods¹, Mark Higley³, Greta Wengert², Michael Filigenzi¹

¹California Animal Health and Food Safety Laboratory System, Davis, CA; ²Integral Ecology Research Center, Blue Lake, CA; ³Hoopa Tribal Forestry, Wildlife Department, Hoopa, CA

Narrative: Illegal marijuana grow sites on public and tribal lands in California are having a significant ecological impact on both flora and fauna. The indiscriminant use of a variety of fertilizers and pesticides on grow sites has been documented. Exposure of the endangered Pacific fisher to multiple AR used on and around grow sites in California is common and in some cases has been determined to be the cause of death based upon necropsy findings and detection of AR in liver samples. The impact of non-AR pesticides on wildlife has not been as well documented. We report two cases of wildlife mortality believed to have been caused by intentional use of cholinesterase-inhibiting insecticides to kill animals. A male Pacific fisher was found dead approximately 10 meters from the edge of an illegal grow site. Large fishing treble hooks attached to high strength line were strung from several trees around the perimeter of the grow site. Hotdogs had been placed on the hooks. The fisher carcass was transferred to wildlife biologists and then frozen pending submission to CAHFS for postmortem examination. Samples of the hotdogs and treble hooks were also collected and submitted to CAHFS for analysis. The carcass was judged to be in good postmortem condition. Gross and histopathologic examinations were generally unremarkable, although a piece of material believed to be part of a hotdog was found in the distal esophagus. In addition, excessive amounts of saliva around the muzzle were noted. Methomyl, a highly toxic carbamate, was detected in stomach contents, esophageal contents, and a hot dog bait sample by liquid chromatography-mass spectrometry (LC-MS-MS). Brain cholinesterase activity was determined to be 1.2 micromoles/gram/minute. The measured activity was interpreted as moderately depressed. The second generation AR, brodifacoum, was detected in the liver at 0.11 ppm. This was considered to be an incidental finding in the case. A diagnosis of methomyl intoxication was made. In a second case, the remains of a black bear were found near a grow site along with a tin can containing what was believed to be a bait material. The carcass was too decomposed for postmortem evaluation, but swabs were taken from the interior of the skull and submitted for toxicologic analysis along with the tin can. Trace amounts of the carbamate insecticide carbofuran were detected on the swabs and material in the tin can by LC-MS-MS. Given the toxicity of carbofuran, the confirmation of exposure, anecdotal evidence of pesticides placed in food material to discourage marijuana plant damage, and the proximity of the carcass to the tin, a presumptive diagnosis of carbofuran toxicosis was made. Based upon these two cases and other photographic and video evidence of dead wildlife on and near illegal marijuana grow sites, it is believed that many wildlife species are being intentionally or accidentally poisoned through such activity.

Intra-laboratory development and evaluation of a urine-based quantitative diagnostic test for aflatoxicosis.

Dahai Shao, Paula M. Imerman, Dwayne E. Schrunk, Steve M. Ensley, Wilson K. Rumbleha

Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

Narrative: Aflatoxins B1 (AFB1) is a widely present mycotoxin produced by various fungal species including *Aspergillus flavus* and *Aspergillus parasiticus* as a secondary metabolite. It has been found in a wide variety of produce used as raw materials in manufacture of animal feeds. It is the most potent aflatoxin. Aflatoxicosis is characterized by acute hepatic injury and immunosuppression, among other effects. Aflatoxin contamination is also common cause of pet food recall. Diagnosis of aflatoxicosis in live pets can be challenging, primarily because of a lack of tissue-based assays for etiological confirmation of exposure. We have developed a urine-based HPLC fluorescence method for detection and quantitation of AFB1 and its metabolite aflatoxin M1 (AFM1) using canine, feline, and porcine urine samples. For canine urine, limits of detection for AFB1 and AFM1 are 0.31 and 0.63 pg respectively. Lower limits of quantification are 5 ng mL⁻¹ for both AFB1 and AFM1, indicating high sensitivity. Inter-day repeatability was 9% and 7% for AFB1 and AFM1 at 5 ng mL⁻¹. Recoveries were 73%, 74% for AFB1 and AFM1 respectively. Recoveries were also determined for feline and porcine urine, and were comparable to those of canine urine. These results indicate that the method is suitable for the detection and quantification of AFB1 and AFM1 in animal urine. This noninvasive method will be very helpful in confirming aflatoxin exposure in live animals, which is currently lacking. The next step is inter-laboratory evaluation of the method before it can be implemented for the routine analysis. This work was funded by FDA grant number 1U18FD005006-01.

Toxicology 2a
Sunday, October 25, 2015
552

Moderators: Larry Thompson and Karyn Bischoff

8:00 AM	Accidental, or intentional, xylitol poisoning in canines as a result of ingesting xylitol-laced baits used to control predators (e.g., wolves) <i>Patricia Talcott, Michael Filigenzi, Robert H. Poppenga</i>	119
8:15 AM	Quantitation of Eight Anticoagulant Rodenticides in Animal Liver by LC-MS/MS with a d-SPE Clean-up Method # <i>Boying Liang, Lori Smith, Cynthia Gaskill</i>	120
8:30 AM	A Rapid Method for the Detection of Bromethalin Using MALDI-TOF Mass Spectrometry <i>Christina Wilson, Mary Mengel, Jonathan Butz, Kendal Weger</i>	121
8:45 AM	Semen as an effective monitor of trace mineral status in male bovine specimens and the effect of trace minerals on the motility and morphology of semen † <i>Dwayne E. Schrunk</i>	122
9:00 AM	MALDI-TOF MS Detection of Coniine in Poison Hemlock <i>Christina Wilson, Elexa Baron, Jonathan Butz, Mary Mengel</i>	123
9:15 AM	High hepatic selenium concentrations associated with acute hepatocellular necrosis and mortality in calves # † + <i>Erica Noland, Thomas Herdt, Dodd G. Sledge</i>	124

Symbols at the end of titles indicate the following designations:

- | | |
|---|--|
| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Accidental, or intentional, xylitol poisoning in canines as a result of ingesting xylitol-laced baits used to control predators (e.g., wolves)

Patricia Talcott¹, Michael Filigenzi², Robert H. Poppenga²

¹WADDL, WSU College of Veterinary Medicine, Pullman, WA; ²School of Veterinary Medicine, University of California, California Animal Health and Food Safety Laboratory System, Davis, CA

Narrative: Xylitol, a simple five carbon sugar alcohol, is naturally found in many fruits and vegetables. It is a common and popular sugar substitute found in many human products, such as gum, candy, and baked goods. Because of its anticariogenic properties, xylitol is increasingly being found in dental hygiene products, sometimes present in concentrations as high as 100%. Within the last few years, xylitol is becoming an increasingly popular ingredient in over-the-counter products and prescription drugs, including nasal sprays, sleep aids, multivitamins, antacids, stool softeners, and sedatives. Overconsumption of xylitol is uniquely problematic to dogs, where it can lead to hypoglycemia and liver disease. These changes can be fatal if left unrecognized or untreated. Because of the canine species being uniquely sensitive to the effects of xylitol, it is becoming apparent that individuals are using xylitol laced baits to target wolves and coyotes. Four cases of xylitol poisoning, involving a total of 4 dogs, one coyote, and three wolves, are reported where exposures apparently occurred in rural or forested areas where typical xylitol-containing materials would not be found. Diagnoses were made based on a combination of exposure history, clinical evidence of hypoglycemia and/or acute hepatic necrosis, and confirmation of the presence of xylitol in source material, stomach contents, or liver. Case 1 was a dead coyote found dead atop a pile of freshly deposited butchered beef bones located off a dirt road in a rural, forested area of northeastern WA. Case 2 was a dog that died of acute liver failure 3 days after seen ingesting ‘chunks of meat’ found in piles while running in a forest in southeastern ID. Case 3 involved three dead wolves that were submitted to Idaho Fish and Game, where xylitol was found in stomach contents of one of the 3 wolves. And xylitol was confirmed in liver tissue collected from 1 of 3 dogs that became acutely ill during a hike with their owners in a rural area of southern CA. Liver, GI content and source material samples were analyzed for xylitol by extraction with water, cleanup using solid phase extraction, derivatization with bis(trimethylsilyl)trifluoroacetamide (BSTFA), and detection of the penta(trimethylsilyl) derivative by gas chromatography-mass spectrometry. These cases are good reminders to clinicians and diagnosticians that xylitol should be considered on the differential list when presented with free roaming canines with evidence of hypoglycemia and/or liver disease, or found dead, in geographical areas where anti-wolf sentiment runs high.

Quantitation of Eight Anticoagulant Rodenticides in Animal Liver by LC-MS/MS with a d-SPE Clean-up Method #

Boying Liang, Lori Smith, Cynthia Gaskill

Toxicology, University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY

Narrative: Anticoagulant rodenticides (ACRs) have been widely used to control a variety of rodents for many years internationally. Ingestion of ACRs can cause fatal coagulopathy in the animal. As the quantity of the poisoned animal tissues is limited and the amount of ACRs in the tissues is usually very low, multi-residue methods with high specificity and sensitivity are desired for quantitation. A selective and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed to quantitate seven ACRs (warfarin, coumachlor, diphacinone, chlorophacinone, bromodiolone, brodifacoum and difethialone) and a naturally occurring AC (dicoumarol) in animal liver using dispersive solid phase extraction (d-SPE) method for sample clean-up. With d-SPE method, the total sample preparation time is within three hours. The minimum quantitation limits for liver prepared as described are 30 ppb for warfarin, coumachlor, dicoumarol and bromodiolone, 40 ppb for diphacinone and chlorophacinone, 20 ppb for brodifacoum and 50 ppb for difethialone. The calibration curves are linear ($R^2 > 0.99$) for all ACRs from 50 ppb to 4000 ppb with $1/x^2$ weighting. The percent recoveries of all ACRs at 50 ppb, 500 ppb and 2000 ppb spiked levels range from 50% to 75%, depending on analyte identity. Matrix effects are measured by comparing the ratios of peak areas for each ACR detected in liver fortified prior to sample extraction to liver extract fortified after sample preparation, including d-SPE clean up. The ratios are close to 100% (within 20% range), indicating the d-SPE clean-up method minimizes the matrix effects. The mean measured values of 50 ppb, 500 ppb and 2000 ppb spiked levels are within 20% of the nominal value. Coefficients of variation for replicate analyses determined at 50 ppb, 500 ppb and 2000 ppb spiked levels are less than 20%, indicating a good degree of precision across days. These results suggest that this method has passed the intra-laboratory validation for quantitation of ACRs extracted from liver.

AAVLD Trainee Travel Awardee

A Rapid Method for the Detection of Bromethalin Using MALDI-TOF Mass Spectrometry

Christina Wilson^{1,2}, Mary Mengel¹, Jonathan Butz¹, Kendal Weger¹

¹Indiana Animal Disease Diagnostic Lab, Purdue University, West Lafayette, IN; ²Comparative Pathobiology, Purdue University, West Lafayette, IN

Narrative: Bromethalin is a potent, neurotoxic rodenticide that is currently being marketed under the trade name Tomcat® in bait block packs and mole bait worm products at concentrations of 0.01% and 0.025% respectively. Bromethalin exerts neurotoxic effects in rodents and non-target species through uncoupling oxidative phosphorylation in mitochondria, ultimately disrupting the osmolar balance in the CNS causing cerebral and spinal cord edema. Due to the recent cancellation of second-generation anticoagulant rodenticide formulations, it is anticipated that exposure to bromethalin-containing rodenticide products in non-target species will increase. Therefore, development of a rapid, sensitive method to detect bromethalin in diagnostic toxicology cases is necessary. This study highlights the potential use of MALDI-TOF MS for the rapid detection of bromethalin in diagnostic toxicology. MALDI-TOF MS analysis for bromethalin briefly involves the following: 1) 10 µL of neat standard or sample extract is mixed with 10 µL of α -cyano-4-hydroxycinnamic acid matrix, 2) 1 µL of each standard/sample is pipetted onto a stainless steel MALDI target plate, 3) standard/samples are analyzed in reflector negative and positive mode with a reflector voltage set at 19.99 kV and a detector scan range of 0 to 1,200 Da, and 4) 1,000 laser shots are scanned and accumulated for all $[M+H]^+$ and $[M-H]^-$ ions. Using this method, MALDI-TOF MS was able to detect both unique $[M+H]^+$ and $[M-H]^-$ ions; however, analyzing the toxicant in negative mode produced optimal results. In negative mode, the diagnostic ions detected for bromethalin were 576/578 m/z $[M-H]^-$, 562/564 m/z $[M-CH_3]^-$, 497/499 m/z $[M-Br]^-$, and 452/454 m/z $[M-Br-NO_2]^-$. This method highlights the use of MALDI-TOF MS for the detection of bromethalin and some of its characteristic fragment ions when analyzed in negative mode. Use of this technique for diagnostic toxicology cases can afford a means for rapid detection of bromethalin in suspect cases of exposure.

Semen as an effective monitor of trace mineral status in male bovine specimens and the effect of trace minerals on the motility and morphology of semen †

Dwayne E. Schrunk

Toxicology and Nutrition, Iowa State University, Ames, IA

Narrative: Bull semen is routinely collected and used to assess breeding soundness in beef cattle operations. Traditionally bull soundness testing includes sperm motility and morphology. Preliminary data indicates that the same sperm sample can potentially be used to monitor the trace mineral status of the bull and thus provide insight into the health without using more invasive sampling techniques. Currently trace mineral status is determined on either jugular venipuncture blood/serum and/or liver biopsy. A novel approach was used to perform trace mineral analysis on bull semen. Trace mineral analysis including 14 elements (Na, Mg, P, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, and Co) was performed by ICP/MS following nitric acid digestion. Trace mineral analysis was performed on bull semen as received, bull semen acid digested, bull semen supernatant, and the bull semen solid material. Trace mineral values in the semen and semen products were compared to trace mineral values in serum, blood, and liver biopsies before and after treatment with a mineral supplement. The effect of trace mineral concentration on bull semen motility and morphology was determined.

† Graduate Student Oral Presentation Award Applicant

MALDI-TOF MS Detection of Coniine in Poison Hemlock

Christina Wilson^{1,2}, Elexa Baron³, Jonathan Butz¹, Mary Mengel¹

¹Indiana Animal Disease Diagnostic Lab, Purdue University, West Lafayette, IN; ²Comparative Pathobiology, Purdue University, West Lafayette, IN; ³College of Veterinary Medicine, Purdue University, West Lafayette, IN

Narrative: Toxicosis associated with ingestion of poison hemlock (*Conium maculatum*) has been reported in humans and livestock. Clinical signs of poisoning include nervousness, tremors, incoordination, mydriasis, muscle fasciculations, respiratory depression, and death. In some cases, birth defects such as arthrogryposis, scoliosis, torticollis, and cleft palate have been reported. The toxicity of poison hemlock is due to the presence of piperidine alkaloids, such as coniine. These alkaloids are nicotinic acetylcholine receptor agonists, exerting their toxicity through the direct over-stimulation and subsequent paralyzation of nicotinic receptors at the neuromuscular junction. Standard separation and detection of coniine for diagnostic cases has been predominantly performed using GC/MS analysis. Although GC/MS works well in separating and detecting coniine, the amount of time it takes to extract, chromatographically separate, and detect the toxin can take several hours. In this investigation, MALDI-TOF mass spectrometry is shown to be a more rapid detection method for coniine because it does not require chromatographic separation prior to MS analysis. Extraction of poison hemlock plants (stems, leaves, and flowers) included: 1) adding 20 mL ultrapure water to 10 grams of homogenized poison hemlock, 2) increasing the pH of the water/plant solution to 12 with 37% KOH, 3) extracting the pH adjusted sample with 50 mL toluene on a mechanical shaker for 10 minutes, 4) aliquotting the toluene layer and mixing it with 10 mL 10% HCl, 5) increasing the pH of the aliquotted 10% HCl layer to 12 with 37% KOH and then extracting back into 1 mL toluene. Ten μL of neat standard or sample extract was mixed with 10 μL of α -cyano-4-hydroxycinnamic acid matrix and 1 μL of each standard/sample was pipetted onto a target plate for MALDI-TOF MS analysis. Samples were analyzed in reflector positive mode and 1,000 laser shots were scanned and accumulated for all $[\text{M}+\text{H}]^+$ ions. Coniine neat standard was detected at 128 m/z $[\text{M}+\text{H}]^+$ with an instrument detection limit of 0.1 ppm. Coniine was detected at high concentrations in poison hemlock samples at 128 m/z $[\text{M}+\text{H}]^+$. Other $[\text{M}+\text{H}]^+$ ions detected in the plant extracts include 142 m/z and 157 m/z, suggesting the detection of the piperidine alkaloids N-methylconiine and N-methylpseudoconhydrine. This study is the first to report the rapid detection of coniine in poison hemlock using MALDI-TOF mass spectrometry.

High hepatic selenium concentrations associated with acute hepatocellular necrosis and mortality in calves # † +

Erica Noland, Thomas Herdt, Dodd G. Sledge

Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI

Narrative: In January of 2015, a cow-calf ranch in Louisiana experienced a sudden mortality event in 250-450lbs calves. During routine deworming and vaccination on January 27th, twelve calves received 2 to 5 times the recommended dosage of an injectable mineral supplement that contained selenium, copper, zinc, and manganese. Prior to this injection, these calves had free choice access to a mineral tub for 6-8 weeks which also contained selenium, copper, zinc, and manganese among other minerals. Within 72 hours of processing, 5 calves developed clinical signs including lethargy, hypersalivation, ataxia, and head pressing that progressed to recumbancy and death. A 6th calf went down, but this animal did not show neurologic signs and eventually recovered. On gross necropsy of calves that died, there were multifocal petechiae of the parietal pleura and peritoneum. Fresh and fixed liver and kidney from 3 of these calves along with additional fixed lung and heart specimens from 1 animal were submitted for histopathologic examination and trace nutrient analysis. The most significant lesion in all 3 was submassive to massive hepatocellular necrosis with variable sparing of periportal regions and bile ductular hyperplasia. This finding had all the hallmarks of acute hepatotoxicity. There were no noted algal blooms, feed contaminants, or toxic plants on the pasture. Interestingly, the hepatic concentrations of selenium were markedly elevated and ranged from 11.8 to 33ug/g on a dry matter basis (reference range: 0.7-2.5ug/g). Although not reported in cattle, excess selenium has been previously linked with centrilobular hepatocellular degeneration and necrosis in other species. In those cases, the degree of periportal sparing was dependent on the dosage of selenium. An additional histologic finding that appears consistent across multiple species is epicardial and myocardial hemorrhage. Although only one heart specimen was received for evaluation, that calf exhibited moderate epicardial hemorrhage that focally extended into the myocardium. Like reported in the dead calves from this ranch, gross findings of other species typically include multifocal petechiae of the body walls. Similarly, reported clinical signs in various veterinary species include acute onset of dullness, ataxia, inability to stand, hyperexcitability, sweating, tachycardia, dyspnea, pyrexia, frothing at the nares, to rapid death following high dosages of sodium selenite. Overall, the history, clinical signs, and laboratory data suggest an association of the histopathologic findings with excessive selenium. Although selenium is an essential part of animal health, its identification as a toxic element requires monitoring of sources of administration to prevent excessive supplementation.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Toxicology 2b
Sunday, October 25, 2015
552

Moderators: Larry Thompson and Karyn Bischoff

10:15 AM	An analytical method for the analysis of eight neonicotinoid insecticides in hummingbird remains <i>Michael Filigenzi, Lisa Tell, Robert H. Poppenga</i>	127
10:30 AM	Finding traces of rodenticide anticoagulant in the liver of race horses <i>Francisco R. Carvalho, Robert H. Poppenga, Hailu Kinde, Carol A. Nyaoke, Santiago Diab, Robert B. Moeller, Rick M. Arthur, Francisco A. Uzal</i>	128
10:45 AM	Quantitation of Fumonisin B₁ and B₂ in Feed Using FMO C Precolumn Derivatization and UPLC-Fluorescence <i>Lori Smith, Cynthia Gaskill</i>	129
11:00 AM	QuEChERS made easy for toxicants screening <i>Daljit Vudathala, Margie Cummings, Lisa Murphy</i>	130
11:15 AM	Acute Liver Necrosis with Massive Death Loss in a Herd of Beef Cows in Northern Colorado ◊ <i>Gene A. Niles</i>	131

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An analytical method for the analysis of eight neonicotinoid insecticides in hummingbird remains

Michael Filigenzi¹, Lisa Tell², Robert H. Poppenga¹

¹California Animal Health and Food Safety Laboratory, University of California at Davis, Davis, CA; ²Medicine and Epidemiology, School of Veterinary Medicine, University of California at Davis, Davis, CA

Narrative: Neonicotinoids are a class of insecticides structurally similar to nicotine which were developed in the 1970's and 1980's. They exhibit low mammalian toxicity but have proven to be highly effective in insect pest control and have become among the most commonly used insecticides in the world. Within the last ten years, evidence has accumulated linking declining numbers of insect pollinators, particularly bees, with exposure to neonicotinoids. This has resulted in numerous studies on exposure of bees to these insecticides and the potential effects of such exposure. Little work has been performed on the potential exposure of other pollinators to neonicotinoids. As part of a study with the goal of determining exposure of hummingbirds to eight neonicotinoids, an analytical method has been developed to measure their levels in whole hummingbird bodies. The neonicotinoids included in this method are imidacloprid, acetamiprid, clothianidin, dinotefuran, nitenpyram, sulfoxaflor, thiacloprid, and thiamethoxam. The hummingbirds were obtained from rehabilitation facilities where the birds either died or were euthanized. The hummingbird bodies are frozen in liquid nitrogen and ground using a Stein mill. The ground tissue is extracted using a modified QuEChERS protocol and analyzed using HPLC-MS/MS on a quadrupole-Orbitrap system. The mass spectrometer is set up to detect compounds using both full scan and selective ion monitoring-data dependent MS/MS (SIM-ddMS2). This allows for the detection of the neonicotinoids at 1 part per billion using SIM-DDMS2 while maintaining the ability to detect non-target compounds such as carbamate and organophosphorous insecticides in the range of 1 – 50 parts per billion by full scan. In this presentation, the development and validation of this method will be discussed.

Finding traces of rodenticide anticoagulant in the liver of race horses

Francisco R. Carvallo², Robert H. Poppenga¹, Hailu Kinde¹, Carol A. Nyaoke¹, Santiago Diab¹, Robert B. Moeller¹, Rick M. Arthur³, Francisco A. Uzal²

¹California Animal Health and Food safety Laboratory, University of California, Davis, CA; ²California Animal Health and Food Safety Laboratory, University of California, San Bernardino, CA; ³University of California, Davis, CA

Narrative: Eight horses originating from different California race tracks were submitted to the California Animal Health and Food Safety Laboratory for necropsy and diagnostic work up. Six of them had a history of sudden collapse and death during exercise, and two of them were euthanized and submitted for routine diagnostic work up after catastrophic bone fractures. In animals with sudden death (6/8), massive hemoperitoneum and hemorrhages in other cavities or organs were observed. In euthanized animals (2/8), one of them evidenced extensive petechial and ecchymotic hemorrhages in multiple organs and no lesions were detected in internal organs of the other horse. In all of them (8/8), traces of anticoagulant rodenticides (Brodifacoum, Diphacinone, Bromadiolone) were detected in the liver. In animals with hemorrhages and sudden death, we propose that increased blood pressure during strenuous exercise and other factors, such as drug interactions, played a role in this finding. The presence of anticoagulant rodenticides in animals euthanized due to catastrophic fractures make us theorize that this is an overlooked, subclinical finding in race horses.

Quantitation of Fumonisin B₁ and B₂ in Feed Using FMOc Precolumn Derivatization and UPLC-Fluorescence

Lori Smith, Cynthia Gaskill

Toxicology, University of Kentucky Veterinary Diagnostic Lab, Lexington, KY

Narrative: Fumonisin B₁ and B₂ are metabolites produced by the fungus *Fusarium moniliforme*, a common fungal contaminant in corn, and have been implicated in human and animal diseases at trace levels. Traditional methods of analysis using liquid chromatography with fluorescence detection employ precolumn derivatization with *o*-phthalaldehyde (OPA) to introduce a fluorescing moiety into the chemical structures of Fumonisin B₁ and B₂ and achieve sensitive detection. Disadvantages of this particular approach include relative instability of the resulting fluorescent derivatives. After adding the OPA reagent to a prepared sample extract, it is often recommended the derivatized solution be injected onto the liquid chromatography system within 3 minutes. The timing of injection for all calibrants and samples must be consistent because fluorescence of OPA-Fumonisin begins to decrease after approximately 2 minutes. A sensitive and stable alternate method for the quantitation of Fumonisin B₁ and B₂ in corn-based feed has been developed and validated. Briefly, liquid-solid extraction using an aqueous:acetonitrile:methanol (50:25:25) solution was performed. Matrix components were removed from the extract using immunoaffinity solid phase extraction columns. Extracted fumonisins were derivatized with 9-fluorenylmethyl chloroformate (FMOC-Cl) to yield highly stable, fluorescent reaction products in less than 10 minutes at room temperature. Calibration curves were obtained over a linear range of 0.04 to 2.50 µg / mL with correlation coefficients routinely better than 0.995. A single set of calibrants, analyzed daily for a period of seven days and stored at room temperature between analyses, produced consistent calibration curves. Method recoveries were determined using fortified commercial cornmeal at 0.1, 1.0, 15.0 and 30.0 µg / g. Recoveries ranged from 75.1% to 108.5% for Fumonisin B₁ and 96.3% to 114.3% for Fumonisin B₂. The greatest loss in recovery occurred at high concentrations and was determined to be caused by approaching the maximum loading capacity of the immunoaffinity clean-up columns. Inter-assay precision was good for both analytes and ranged from 1.5% to 15.8%. These results indicate this method is suitable for quantitation of Fumonisin B₁ and B₂ from corn-based feed and is an improvement over more traditional methods.

QuEChERS made easy for toxicants screening

Daljit Vudathala, Margie Cummings, Lisa Murphy

Pathobiology, University of Pennsylvania, Kennett Square, PA

Narrative: In veterinary toxicology, submitted samples are commonly screened for unknown toxicants using gas chromatography-mass spectrometry (GC/MS) equipped with a library. The samples are prepared by extraction with an organic solvent followed by a clean-up method before analysis. Application of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method, first introduced for pesticide analysis in fruits and vegetables, has found its usefulness in a variety of matrices for a wide range of analytes. Using a combination of sorbents, such as C18 and PSA (primary secondary amine), has been adapted to analyze samples submitted in suspected poisoning cases. Once the QuEChERS tubes are prepared with an appropriate amount of the sorbents, this sample clean-up method is quick and effective. Preparation of tubes containing different sorbents is streamlined by using commercially available standardized measurement spoons originally designed to accurately dispense different amounts of sodium chloride. The actual weights of sorbents dispensed by these spoons was found to be within a consistent and acceptable range. Using this simple technique to prepare QuEChERS tubes, clean-up of baits and stomach contents has been successfully accomplished for toxicant screening. The following three clinical cases are typical examples of cases solved by the use of this technique. In the first case, a dog became hyperactive and had diarrhea after consuming a sausage found in the neighborhood. Upon examination of the sausage, it was found to contain capsules containing an unknown substance. Analysis of the capsules by GC/MS with the QuEChERS clean-up method showed the presence of caffeine. In the second case, a dog died within 24 hours of consuming garbage. At necropsy, a large amount of coffee grounds was found in the GI tract; caffeine was the suspected cause of death. A screen of the stomach contents instead revealed not only caffeine but also fluoxetine, the active ingredient in the antidepressant Prozac®. In the final case, GI contents of a chicken suspected of consuming mothballs were analyzed and found to contain their active ingredient naphthalene. Preparation of QuEChERS tubes for clean-up of baits and stomach contents of varying nature using standardized spoons has simplified the detection of unknown toxicants.

Acute Liver Necrosis with Massive Death Loss in a Herd of Beef Cows in Northern Colorado ◇

Gene A. Niles

Colorado State University Veterinary Diagnostic Lab, Rocky Ford, CO

Narrative: A herd of beef cattle in northern Colorado experienced the loss of approximately one-third of its mature cows due to acute liver failure after they were fed alfalfa hay heavily contaminated with kochia. Cows were found dead in less than 24 hours after the initial exposure to the hay and deaths continued for several weeks. The majority of the deaths occurred within a week of exposure to the hay although the cows had access to the hay for less than 24 hours before it was removed from the pastures. Deaths occurred in two groups of cows which were fed this hay. Death loss did not occur in a group of bulls and cull cows which did not receive the hay. All groups of cattle drank from the same water sources and were given the same mineral mixture. The cattle did not receive any additional feeds or supplements. The liver damage was characterized as severe centrilobular necrosis. This presentation will outline the case history, clinical syndrome, treatments, pathology and diagnostic tests. A wide group of veterinarians, animal and plant scientists and laboratory diagnosticians from around the country have contributed in the effort to determine the toxic agent in this case, which has not been identified. Presentation of this case to the AAVLD toxicology committee will hopefully bring new insight in determining the cause of the acute liver lesions in these cows.

◇ USAHA Paper

Virology 1
 Saturday, October 24, 2015
 551

Moderators: Julia Ridpath and Christie Mayo

1:00 PM	WVDL Real-time RT-PCR Neuraminidase Typing Assay for Canine Influenza <i>Francine K. Cigel, Jennifer Cooper, Sandra Newbury, Keith P. Poulsen, Kathy L. Toohey-Kurth</i>	135
1:15 PM	Development and Evaluation of Two Singleplex Reverse Transcription-Insulated Isothermal PCR Tests and a Duplex Real-Time RT-PCR Test for the Detection of Porcine Epidemic Diarrhea Virus and Porcine Deltacoronavirus <i>Jianqiang Zhang, Yan Zhang, Qi Chen, Yun-Long Tsai, Cheng-Jen Chiang, Yu-Han Shen, Fu-Chun Li, Pei-Yu A. Lee, Hsiao Fen Grace Chang, Phillip Gauger, Karen Harmon, Hwa-Tang Thomas Wang</i>	136
1:30 PM	Novel strains of porcine reproductive and respiratory syndrome virus <i>Yan Zhang, Leyi Wang</i>	137
1:45 PM	Bovine respiratory disease model based on dual infections with infection with bovine viral diarrhoea virus and bovine corona virus <i>Julia F. Ridpath, Anthony W. Confer, Robert W. Fulton, Shollie M. Falkenberg, Fernando Bauermann</i>	138
2:00 PM	Solutions for rapid detection of pathogen nucleic acids in liquid animal samples – introducing QIAGEN’s new cadior MagBead Kit ♦ <i>Carsten Schroeder, Phoebe Loh</i>	139
2:15 PM	Swine influenza in 6 sows at a Michigan abattoir <i>Melinda Jenkins-Moore, Alicia Janas-Martindale, Colleen S. Bruning-Fann, Annette Olson, Mary L. Killian, Thomas Flynn, Linda Cox, Arach J. Wilson, Sabrina L. Swenson.</i>	140
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WVDL Real-time RT-PCR Neuraminidase Typing Assay for Canine Influenza

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Narrative: Influenza A affects many mammalian and avian species including humans, swine, seals, whales, ferrets, mink, canines and numerous orders of domesticated birds. Surveillance for influenza viruses in multiple species is critical for assessing risk and determining what measures are necessary to halt a pandemic. Surveillance for influenza A is accomplished by use of a broadly reactive real time PCR that detects a conserved gene such as matrix. The Wisconsin Veterinary Diagnostic Laboratory (WVDL) has an in-house developed matrix assay, designed to detect influenza A matrix from mammalian and avian species. Real-time RT-PCR positive influenza A samples are subsequently subtyped, which identifies the two major surface glycoproteins of the virus, hemagglutinin and neuraminidase. There are 16 H types and 9 N types for influenza A viruses. WVDL has developed a panel to facilitate subtyping neuraminidase, regardless of the species from which the virus originated. The WVDL panel, along with the sequence data from collaborators at Cornell University, was instrumental in identifying the new H3N2 canine virus, which is responsible for the current outbreak in the United States. As part of the current outbreak, the first diagnostic sample was received at the WVDL for testing on the canine respiratory PCR panel. The sample was negative for herpesvirus, distemper virus, parainfluenza virus, adenovirus, canine respiratory coronavirus and *Bordetella bronchiseptica*. Influenza A was identified using the WVDL matrix real-time PCR assay. Sub-typing was initiated to complement the work in progress at Cornell University. The WVDL neuraminidase assays, specifically N2 and N8, revealed that the positive influenza A sample was not N8 as in previous canine influenza outbreaks, but rather N2. The diagnosis of a new H3N2 strain was critical to explain the epidemic spread of respiratory disease in the Chicago area. A major component of disease involves animal shelters in the Chicago area, which are reporting significant morbidity and rapid spread. In collaboration with the Shelter Medicine program at the UW School of Veterinary Medicine, WVDL has tested nasal swab samples from dogs showing clinical signs or those with direct contact of ill animals. Of 339 samples tested to date, 163 are positive for the matrix assay. The neuraminidase subtyping was performed using the WVDL N2 and N8 singleplex panel and of the 47 typed so far, all have been subtype N2. Feasibility studies have been conducted to multiplex the N2, N8 and matrix assays with an internal control. Preliminary work indicates the absence of nonspecific reactions in the multiplex format. Additional validation work is in progress. Data presented shows that subtyping for neuraminidase is valuable for identifying emerging strains when combined with a broadly reactive matrix assay.

Development and Evaluation of Two Singleplex Reverse Transcription-Insulated Isothermal PCR Tests and a Duplex Real-Time RT-PCR Test for the Detection of Porcine Epidemic Diarrhea Virus and Porcine Deltacoronavirus

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Narrative: Recent outbreaks and wide-spread prevalence of porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) in multiple countries have caused significant economic losses and remain a serious challenge to the swine industry. Rapid diagnosis is critical for the implementation of efficient control strategies before and during PEDV and PDCoV outbreaks. Insulated isothermal PCR (iiPCR) on the portable POKKIT™ device is user friendly for on-site pathogen detection. In the present study, a singleplex PEDV RT-iiPCR, a singleplex PDCoV RT-iiPCR, and a duplex PEDV/PDCoV real-time RT-PCR (rRT-PCR) targeting the M gene were developed and compared to an N gene-based PEDV rRT-PCR and an M gene-based PDCoV rRT-PCR that were previously published and used as reference PCRs here. All five PCR assays were highly specific and did not cross react with the major porcine enteric viruses and bacteria. Analysis of viral RNA extracted from 10-fold serial dilutions of PEDV cell culture isolates (a US PEDV prototype strain and a US PEDV INDEL-variant strain) showed that the PEDV RT-iiPCR and the duplex PEDV/PDCoV rRT-PCR had similar analytical sensitivities but were 10-100 fold less sensitive compared to the reference PEDV rRT-PCR. The PDCoV RT-iiPCR and the duplex PEDV/PDCoV rRT-PCR were equal to and 10-fold less sensitive than the reference PDCoV rRT-PCR, respectively, when testing 10-fold serial dilutions of a PDCoV cell culture isolate. Performance of each PCR assay was further evaluated using 170 clinical samples (86 fecal swabs, 24 feces, 19 intestines, and 41 oral fluids). Among 170 samples, 88 were positive by the reference PEDV rRT-PCR with cycle threshold (Ct) ranges of 12.2-35.7; 82 were positive by the reference PDCoV rRT-PCR with Ct ranges of 15-37.1; 16 were positive for both PEDV and PDCoV. Compared to the reference PEDV rRT-PCR, the sensitivity, specificity and accuracy of the PEDV RT-iiPCR were 97.73%, 98.78%, and 98.24%, respectively, and those of the duplex PEDV/PDCoV rRT-PCR were 98.86%, 96.34%, and 97.65%, respectively. Compared to the reference PDCoV rRT-PCR, the sensitivity, specificity and accuracy of the PDCoV RT-iiPCR were 100%, 100%, and 100%, respectively, and those of the PEDV/PDCoV duplex rRT-PCR were 96.34%, 100%, and 98.24%, respectively. Overall, all three new PCR assays were comparable to the reference rRT-PCRs for detection of PEDV and/or PDCoV. PEDV and PDCoV RT-iiPCRs are potentially useful tools for on-site detection and the duplex PEDV/PDCoV rRT-PCR provides a convenient method to simultaneously detect the two viruses and differentiate PEDV from PDCoV.

Novel strains of porcine reproductive and respiratory syndrome virus

Yan Zhang, Leyi Wang

Ohio Department of Agriculture, ADDL, Reynoldsburg, OH

Narrative: Two sets of clinical samples from pigs experiencing severe respiratory diseases in an Ohio pig farm were submitted to the Animal Disease Diagnostic Laboratory of Ohio Department of Agriculture. All samples were tested positive for PRRSV using a real-time RT-PCR assay. Phylogenetic analysis of the whole genome sequence showed that both sets of samples (OH28372-2013 and OH155-2015) are closely related to one Chinese strain HENAN-XINX and two US strains XW001 and NADC30 in the North American lineage. Further analysis revealed that OH28372-2013 genome has a unique 13-nucleotide deletion in the 3' untranslated region (UTR) in comparison with those of North American strains whereas no nucleotide deletion was observed in 5' UTR. By contrast, OH155-2015 has two single nucleotide deletions at position 118 and 130 of the 5' UTR but no deletion in 3' UTR. In addition to the deletion in the 3' UTR, OH28372-2013 encodes a 124-amino acid-length nucleocapsid (N) protein that is one amino acid longer than that of other North American strains including OH155-2015. Moreover, the remaining genome of OH28372-2013 is more closely related to that of NADC30 strain of PRRS virus including a similar deletion pattern in the NSP2 (333, 3, 57nt) when compared with the prototype strain VR-2332. Different from that of OH28372-2013, NSP2 of OH155-2015 only has a 298-nt deletion in the same region of OH28372-2013 virus. A previous study reported that a deletion of either a single or two nucleotides was observed in both 5' and 3' UTRs in highly pathogenic PRRSV isolates from China. Intriguingly, a single nucleotide deletion was also observed in an overattenuated PRRSV strain derived from a highly pathogenic PRRSV strain by in vitro serial passage. OH28372-2013 strain has a unique deletion of 13 nucleotides in the 3' UTR, which has not been previously reported. According to the previous finding that the 40 nucleotides in the 5' end of 3'UTR were dispensable for the viability of type II PRRSV, it is possible that the 13-nt deletion in the OH28372-2013 virus was likely a result from the natural selection event and did not affect the viability of the virus. For OH155-2015 virus, two unique single nucleotide deletions observed in the 5' UTR are different from the single nucleotide deletion reported in the Chinese highly pathogenic strains and a continuous deletion of 2 nucleotides in the BJPG strain. The role of double deletions in 5' UTR for this virus remains unclear. Most previous studies of PRRSV genetic diversity have been focused on the ORF5 and ORF7 sequences of genotype 2 virus including the highly pathogenic strains of PRRS virus, this and other studies suggest that whole genome analysis are important to understand the genomic diversity of this virus.

Bovine respiratory disease model based on dual infections with infection with bovine viral diarrhea virus and bovine corona virus

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Narrative: Bovine respiratory disease complex (BRDC) is the leading cause of economic loss in the U.S. cattle industry. BRDC likely results from simultaneous or sequential infections with multiple pathogens including both viruses and bacteria. Bovine viral diarrhea virus (BVDV) and bovine corona virus (BoCV) are frequently isolated from cattle suffering from BRDC. However, animal models based on infection with either of these viruses alone have had limited success in reproducing clinical respiratory disease. Further, it has been reported that simultaneous infection of cattle with BVDV and BoCV did not result in BRDC. In this study seven infection protocols were compared. Colostrum deprived Holstein calves, at least two weeks of age were exposed to mock inoculum, BVDV alone, BoCV alone, BoCV followed 3 days later by BVDV and BVDV followed 3, 6 or 9 days later by BoCV. Day 3 post infection (pi) was chosen because this corresponds to first detection of viremia for either BVDV or BoCV. Days 6 and 9 pi with BVDV was chosen because the reduction in circulating lymphocytes, typically observed following infection with BVDV, is usually greatest between days 6 and 9. Body temperature was monitored using internal probes and blood samples and nasal swabs were collected, at two to three day intervals, to monitor viremia and changes in circulating immune cells. For calves, which underwent dual exposures, lungs were scored and samples of lung and immune tissue were collected 14 days after inoculation with the second virus. Short-term pyrexia was observed in all animals infected with BVDV. The only infection protocols that resulted in lung lesions consistent with respiratory disease were BVDV followed 6 or 9 days later by BoCV. These lesions were more severe and observed in a greater majority of calves in the BVDV followed 6 days later by BoCV group than in the BVDV followed 9 days later by BoCV group. The observed lung lesions are consistent with a mild to moderate interstitial pneumonia. Immunohistochemistry and virus isolation indicate that sequential infection with BVDV followed by BoCV resulted in prolonged replication of BoCV compared to infections of BoCV alone. These results indicate that while single infection with BVDV or BoCV do not result in respiratory disease, sequential infections of BVDV followed by BoCV can result in gross and microscopic lesions that are consistent with respiratory disease. The window of time in which BoCV infection is most likely to result in respiratory disease coincides with the immunosuppression peak caused by BVDV.

Solutions for rapid detection of pathogen nucleic acids in liquid animal samples – introducing QIAGEN's new *cador* MagBead Kit ◊

Carsten Schroeder, Phoebe Loh

QIAGEN GmbH, Leipzig, Germany

Narrative: An effective, robust sample preparation method ensuring the reliable purification of DNA and RNA is key to the successful identification of pathogen nucleic acids. Processing different samples in parallel – including multiple pathogens and starting materials – with the same protocol enables a streamlined workflow for veterinary pathogen detection, saving time and effort. Furthermore, expanded throughput options increase flexibility when processing a large number of samples. With the new MagAttract 96 *cador* Pathogen Kit (*cador* MagBead Kit) using magnetic bead technology and the *cador* Pathogen 96 QIAcube HT Kit using silica membrane-based spin-column technology, QIAGEN has developed two sample preparation kit solutions specially designed for fully automated use in a 96-well format. Both kits can be used for the parallel processing of different sample types and for the co-extraction of viral RNA and DNA and bacterial DNA. In an internal validation study, various sample material like whole blood, serum, oral fluid and homogenized tissue were tested for different RNA viruses such as bovine virus diarrhoea virus (BVDV), porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus, Schmallenberg virus (SBV) and classical swine fever virus (CSFV). Sample extraction was performed in parallel with the new *cador* MagBead Kit and the *cador* Pathogen 96 QIAcube HT Kit, and compared to proven manual extraction methods. Purified RNA was then analysed by real-time RT-PCR using *virotype* RT-PCR Kits (QIAGEN). The internal study revealed comparable real-time RT-PCR results for both automated sample preparation kits. Depending on the sample material and pathogen, the results varied from slightly lower mean Ct values to somewhat higher mean Ct values. For serum samples, an average Ct deviation ranging from 0.26 to 1.4 was measured using the *cador* MagBead Kit. Testing BVDV and PRRSV positive serum samples extracted with the *cador* MagBead Kit showed slightly better RT-PCR results compared to another commercially available magnetic bead kit. Furthermore, external testing was performed by using the new *cador* MagBead Kit in different veterinary diagnostic labs. The external validation study showed mostly better results when using the new *cador* MagBead Kit for the extraction of PRRSV, influenza A virus and bacteria positive samples. QIAGEN provides a new magnetic bead automated processing solution for rapid automated purification of both, viral RNA and DNA, as well as bacterial DNA from a variety of different animal samples. The new MagAttract 96 *cador* Pathogen Kit is all-inclusive, containing all the necessary plasticware. This allows fast and reliable processing of 96 samples per run using the same universal protocol. The *cador* Pathogen 96 QIAcube HT Kit can also be used for parallel processing of different sample types in one run. Both extraction kits enable robust, reproducible purification of nucleic acids ready for analysis by real-time PCR/RT-PCR.

◊ USAHA Paper

Swine influenza in 6 sows at a Michigan abattoir

Melinda Jenkins-Moore¹, Alicia Janas-Martindale¹, Colleen S. Bruning-Fann², Annette Olson¹, Mary L. Killian³, Thomas Flynn⁴, Linda Cox⁵, Arach J. Wilson⁶, Sabrina L. Swenson¹

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Narrative: On March 22, 2015, 6 sows arrived at a Michigan abattoir all appearing healthy. On March 23, 2015, 4 sows were dead, and 2 were very ill. The two ill sows and one of the dead sows were necropsied. In each case, the lungs were firm to the touch, approximately 90% dark purple with fibrin tags and foam in the bronchioles. There were no abscesses or other lesions. All other tissues, including pulmonary lymph nodes, were normal in appearance. Tissue samples were submitted to the National Veterinary Services Laboratories. Fixed tissue was processed routinely for histologic examination. Significant microscopic lesions consisted of severe, acute, multifocally extensive, fibrinopurulent, proliferative broncho-interstitial pneumonia with multifocal alveolar hemorrhage, with a significant number of alveolar and large airway lumina such as bronchioles and bronchi filled with lymphocytes, type II pneumocytes, and macrophages. Bacterial culture and virus isolation were conducted. Lung was plated on sheep blood agar with a Staph streak, Chocolate agar, and MacConkey agar. Liver was plated on sheep blood and MacConkey agars. *Haemophilus parasuis*, *Staphylococcus* sp. (2 different isolates), *Globicatella* sp., *Streptococcus* sp (not *S. suis*), *Psychrobacter* sp., and *Bergeyella zoohelcum* were isolated in the lung. *Rothia* sp., *Globicatella* sp., and *Psychrobacter* sp. were isolated in the liver. Isolates were identified using MALDI-TOF and/or 16S rDNA sequencing. Lung homogenate was run on a real time reverse transcriptase PCR (rRT-PCR) for the influenza A virus of swine (IAV-S) matrix gene as well as a subtyping PCR for the hemagglutinin (H1 and H3) and neuraminidase (N1 and N2) genes of IAV-S. The matrix rRT-PCR was positive, and the typing PCR was positive for H1, H3, and N2. Tonsil, spleen/kidney pool, lymph node pool, and lung tissue homogenates were inoculated onto the following cell cultures: swine testicle (ST) and swine kidney (3 different lines-pSK, PK-15, and SK6). Lung homogenate was also inoculated onto Madin-Darby canine kidney (MDCK), Marc 145, and swine alveolar macrophage cell cultures. Cytopathic effects were observed in the first passage of the MDCK, pSK, PK-15, ST, and SK-6 cultures for all inoculated tissues. The isolates were positive for IAV-S by an indirect immunofluorescence assay. The lung homogenate isolate on MDCK cells was run on the subtyping PCR and underwent whole genome sequencing. The subtyping PCR was positive for H1, H3, and N2, indicating a mixed infection. Sequence analysis shows high identity to IAV-S strains currently circulating in the swine population. This case is of interest because there were 6 seemingly healthy sows that were either dead or ill in less than 24 hours. A question to consider was the acute death in this case due to the 2 viruses the sows were infected with, the bacterial strains isolated, and/or the stress of transport. Animal inoculation studies could help determine the cause of the acute deaths.

Canine influenza virus (H3N2) in Illinois

Melinda Jenkins-Moore¹, Alicia Janas-Martindale¹, Natalie Marks², Katherine Mazingo¹, Mary L. Killian³, Leo Koster¹, David Barber⁴, Sabrina L. Swenson¹

¹BPA, USDA/NVSL, Ames, IA; ²Blum Animal Hospital, Chicago, IL; ³Avian, NVSL/USDA, Ames, IA; ⁴USDA/APHIS/VS, Springfield, IL

Narrative: Chicago, Illinois and its surrounding suburbs started seeing an increase in dogs with severe and persistent pneumonia at the end of March and beginning of April 2015. Initially, the increase thought to be due to bordetella or the H3N8 canine influenza virus (CIV). Subsequent testing determined that an avian lineage CIV strain, H3N2, most closely related to CIV found in Asia was circulating in the Chicago dog population. In an effort to make the virus readily available for research and development of tests and vaccines, the National Veterinary Services Laboratories (NVSL) received swab samples for analysis from Chicago. A total of 21 samples, 19 dog samples and two cat samples, were received. All samples were tested by three approaches: 1.) real-time reverse transcriptase PCR (rRT-PCR) for the influenza A virus (IAV) matrix gene, 2.) virus isolation (VI) in Madin-Darby canine kidney (MDCK) cell cultures with trypsin in the media for two passages, and 3.) VI in 9-11 day old embryonating chicken eggs (ECE) via the allantoic route for one passage. Fourteen of the twenty one submitted swab samples were positive by rRT-PCR for the IAV matrix gene. Virus was isolated from eight of the fourteen rRT-PCR positive samples in the ECE, and confirmed by rRT-PCR. In contrast, cytopathic effects (CPE) were not seen in any of the MDCK cell cultures that were directly inoculated with the swab samples for two passages. A pan hemagglutinin (HA) RT-PCR determined these isolates were of Eurasian H3 lineage. One of the egg isolates was inoculated onto MDCK cells, and the resulting cell culture isolate underwent whole genome sequencing on the ION Torrent. This isolate had a 99% identity to A/canine/Korea/S1/2012 (H3N2) and A/canine/Korea KRIBB01/2011 (H3N2), with the exception of the genes polymerase PB2 (PB2) and neuraminidase (NA), which had a 98% identity to the Korean isolates. This is an emergence of a novel introduced virus in a naive population and highlights the importance of using multiple test platforms to determine and isolate an emerging virus in the US.

***Pseudocowpox* viral epidermitis in a mountain goat: another zoonotic *Parapox* virus causing contagious ecthyma in wild ruminants**

Danielle D. Nelson^{1,2}, Juan Munoz^{3,4}, Dan Bradway^{1,2}, Peregrine L. Wolff⁵

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Narrative: Contagious ecthyma (aka Orf) is an important disease associated with proliferative epidermitis and cheilitis in wild and domestic ungulates. Lesions in affected animals can cause severe debilitation, and direct skin contact can cause similar lesions in humans. In mountain goats (*Oreamnos americanus*) and bighorn sheep (*Ovis canadensis*), the causative agent was thought to be *ovine parapoxvirus-1* (*Orf virus*) based on typical histological changes and transmission electron microscopy which was adequate for ruling out exotic *capripoxvirus* infection. However, in a single wild mountain goat from Nevada with typical gross and histological lesions of contagious ecthyma, PCR for *ovine parapoxvirus-1* was negative and PCR using universal *parapoxvirus* primers with subsequent sequencing identified *pseudocowpox virus*, a closely related virus in the *parapox* genus historically known to cause a similar disease in domestic cattle. To the authors' knowledge, this is the first report of *pseudocowpox virus* infection in mountain goats and the first report of infection in a wild ruminant in North America. In our laboratory, all the 8 cases in bighorn sheep confirmed by specific PCR have definitively proven to be *ovine parapoxvirus-1*. In cattle, camelids, and Finnish reindeer, *pseudocowpox* viruses behave similarly to *Orf virus* infection in small ruminants, and skin contact with humans can also result in zoonotic infection. It is possible that productive infection by each virus may be limited to specific species, but it is also possible that the host range for each virus is broad which would increase the transmission risk. Definitive diagnosis on future cases via PCR and sequencing will be necessary to explain the host range and interspecies transmission of the zoonotic parapoxviruses.

Virology 2a
Sunday, October 25, 2015
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Moderators: Jianqiang Zhang and Douglas Marthaler

8:00 AM	Sites of equine arteritis virus localization in the reproductive tract during long-term persistence in the stallion † <i>Mariano Carossino, Alan Loynachan, Juliana R. Campos, Bora Nam, Igor F. Canisso, Yun Young Go, Peter J. Timoney, Kathleen M. Shuck, Pamela Henney, Mats H. Troedsson, R. F. Cook, Thomas Swerczek, Edward L. Squires, Ernest Bailey, Udeni BR Balasuriya</i>	<i>145</i>
8:15 AM	Elimination of a centrifugation step in the NAHLN avian influenza RNA extraction protocol does not impact assay sensitivity <i>Beate Crossley, Richard Breitmeyer, Dan Bradway, Tim Baszler, Terry McElwain, Donna M. Mulrooney, Jerry R. Heidel, Sarah Tomlinson, Christie M. Loiacono, Janice C. Pedersen, Mia Kim Torchetti, Monica Reising</i>	<i>146</i>
8:30 AM	Novel Human-Like Swine H3N1 and H3N2 Influenza A Viruses Detected in Pigs <i>Phillip Gauger, Daniela Rajao, Tavis Anderson, Eugenio Abente, Jianqiang Zhang, Amy Vincent.</i>	<i>147</i>
8:45 AM	Discovery of a novel porcine pestivirus in pigs infected with porcine reproductive and respiratory syndrome virus § <i>Ben Hause, Emily Collin, Lalitha Peddireddi, Fangfen Yuan, Zhenhai Chen, Richard Hesse, Phillip Gauger, Travis Clement, Ying Fang, Gary A. Anderson.</i>	<i>148</i>
9:00 AM	Diagnosis of Rift Valley Fever Nucleocapsid Protein by Luminex-based Fluorescence Microsphere Immunoassay <i>Izabela Ragan, Rachel Palinski, Mohammad Hossain, William C. Wilson, D. Scott McVey, Raymond R. Rowland</i>	<i>149</i>

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Sites of equine arteritis virus localization in the reproductive tract during long-term persistence in the stallion †

Mariano Carossino¹, Alan Loynachan², Juliana R. Campos¹, Bora Nam¹, Igor F. Canisso¹, Yun Young Go¹, Peter J. Timoney¹, Kathleen M. Shuck¹, Pamela Henney¹, Mats H. Troedsson¹, R. F. Cook¹, Thomas Swerczek¹, Edward L. Squires¹, Ernest Bailey¹, Udeni BR Balasuriya¹

¹Department of Veterinary Science, University of Kentucky, Maxwell H. Gluck Equine Research Center, Lexington, KY; ²Department of Veterinary Science, University of Kentucky, Veterinary Diagnostic Laboratory, Lexington, KY

Narrative: Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory and reproductive disease of horses. EAV can establish persistent infection (carrier state) in 30-70% of sexually mature colts and stallions following natural infection. Carrier stallions continuously shed the virus in their semen for an extended time. Thus, the carrier stallion constitutes the natural reservoir of EAV and maintains and perpetuates the virus in horse populations between breeding seasons. However, the tropism of EAV to persist in the male reproductive tract and the mechanism(s) enabling persistence remain to be elucidated. The primary objective of this study was to identify tissue localization and EAV tropism in the male reproductive tract of experimentally infected, long-term carrier stallions. Tissues from the reproductive tract (testicle, epididymis, vas deferens, ampulla, and accessory sex glands [bulbourethral gland, prostate, and vesicular gland]) and regional lymphoid tissues from EAV carrier (n=3) and previously infected stallions that had stopped shedding (n=6) were collected at necropsy. Samples were processed for virus isolation, histopathology (data not shown), and detection of viral antigen by single and dual immunohistochemistry (IHC). EAV was isolated from the accessory sex glands of carrier stallions, with varying titers between tissues: ampulla $2 \times 10^2 - 1.7 \times 10^5$ PFU/g, vesicular glands $1 \times 10^2 - 1 \times 10^3$ PFU/g, and prostate and bulbourethral glands < 10 PFU/g. Isolates were further confirmed by a Taqman® real-time RT-PCR targeting ORF7. No infectious virus was isolated from regional lymphoid tissues. EAV antigen was detected in frozen tissue sections by IHC using a monoclonal antibody (3E2) specific for the nucleocapsid (N) protein of the virus. Intracytoplasmic viral antigen was localized in fibroblasts, lymphocytes, and other mononuclear cells in the accessory sex glands; the number of infected cells was quantified using Image J digital image analysis software (NIH). The ampulla had the largest number of cells expressing viral antigen (10 to > 25 positive cells/100X field). Dual IHC was performed to identify EAV infected lymphocytes; this showed that viral antigen localizes in CD2⁺, CD3⁺, CD5⁺, and CD8⁺ T lymphocytes and CD21⁺ B lymphocytes. This study unequivocally substantiates that EAV persists mainly in the ampulla and to a lesser extent in other accessory sex glands of the carrier stallion. Most interestingly, viral antigen was only present in fibroblastic and mononuclear cells (identified as specific T and B lymphocytes) in these tissues but not in glandular epithelial cells.

† Graduate Student Oral Presentation Award Applicant

Elimination of a centrifugation step in the NAHLN avian influenza RNA extraction protocol does not impact assay sensitivity

Beate Crossley⁶, Richard Breitmeyer⁶, Dan Bradway¹, Tim Baszler¹, Terry McElwain¹, Donna M. Mulrooney², Jerry R. Heidel³, Sarah Tomlinson³, Christie M. Loiacono³, Janice C. Pedersen⁴, Mia Kim Torchetti⁴, Monica Reising⁵

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Narrative: Surveillance and disease outbreak response are important missions of veterinary diagnostic laboratories nationally. Membership in the National Animal Health Laboratory Network (NAHLN) allows approved state laboratories to use standardized federal protocols and assays for the detection of avian influenza virus (AIV). Samples available from surveillance and outbreak testing in 2014/2015 AIV outbreak on the West Coast of the United States provided a unique opportunity to evaluate a lengthy sample preparation step using guidelines developed by the NAHLN Methods Technical Working Group. At the time this study was performed, highly pathogenic avian influenza virus (HPAI) was detected in wild birds of the Pacific flyway, backyard flocks in WA and OR and commercial flocks in CA. Wild birds are considered the most challenging for the testing protocol due to presence of increased organic matter related to sample type (cloacal swabs), diet, and environmental conditions. The NAHLN RT-PCR standard operating procedure for AIV requires centrifugation of the liquid media from swab samples for 25 min prior to nucleic acid extraction of the supernatant in order to remove organic matter which may contain substances inhibitory to molecular assays. In this study, results from molecular testing were compared for samples processed with and without centrifugation prior to nucleic acid extraction; the study included samples from wild birds, backyard birds and commercial birds. Three NAHLN and AAVLD accredited laboratories (WA, OR and CA) participated in the study. The procedure included evaluation of a commercial Type A kit that includes an internal control in addition to the existing influenza A NAHLN protocol. Method comparison was performed by testing a total of 339 samples (265 wild bird samples and 74 commercial samples) using the two Type A assays chemistries and positives were further tested by the H5/H7 subtyping assays (n= 2712). Based upon internal control values, no inhibitory substances were detected in either sample preparation group. Statistical evaluation of molecular assay results suggests that there was no difference in molecular assay performance between sample preparation groups with and without centrifugation or between the two mastermix chemistries used. There was insufficient data to draw statistical conclusions from H5 positive wild bird samples. The data from all other evaluations (wild bird and commercial bird influenza A, wild bird H7, and commercial bird H5 and H7) indicates that elimination of the centrifugation step in sample preparation does not negatively impact the subsequent sensitivity of molecular assays.

Novel Human-Like Swine H3N1 and H3N2 Influenza A Viruses Detected in Pigs

Phillip Gauger¹, Daniela Rajao², Tavis Anderson², Eugenio Abente², Jianqiang Zhang¹, Amy Vincent²

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Narrative: Swine are considered an important reservoir for the evolution of influenza A viruses (IAV) that occasionally result in spillover events from pigs to humans. However, it has become apparent from recent increases in surveillance and sequencing of swine IAV that humans frequently transmit IAVs to swine. A novel human-like swine H3N2 virus was detected in the USDA surveillance system in 2012 with surface glycoprotein genes hemagglutinin (HA) and neuraminidase (NA) that were genetically similar to contemporary human seasonal strains, with internal genes from the 2009 H1N1 pandemic viruses. Subsequent second generation reassortant IAV that maintained the human-like H3 gene but replaced the NA with the classical swine N1 lineage (H3N1) were repeatedly detected in swine since 2013, followed by a third generation reassortant detected with an N2 of 2002 human-seasonal lineage that is common in United States (US) swine IAV. The original human-like swine H3N2 virus and an H3N1 IAV from 2013 were additionally demonstrated to be antigenically distant from all swine H3 viruses that currently circulate in the US and demonstrated antigenic drift from human seasonal H3N2 strains. Human-like swine H3 viruses have continued to be detected in Iowa and Missouri swine from the same farms throughout 2013-2015 and the HA genes range in nucleotide homology from 98.4% to 97.5% to the index human-like H3. The pathogenicity and transmission of the swine human-like 2012 H3N2 and the reassortant H3N1 IAV from 2013 were evaluated in pigs in comparison to a human H3N2 with common HA ancestry. Both of the swine isolates of human-like H3 viruses infected and replicated in pigs, caused lung pathology, and transmitted to indirect contacts. These results indicate that these novel H3 viruses are fully virulent and can sustain onward transmission in pig populations. Importantly, their HA are distinct from currently circulating or vaccine IAV strains and could significantly impact the swine industry if they continue to be transmitted and cause more widespread outbreaks.

Discovery of a novel porcine pestivirus in pigs infected with porcine reproductive and respiratory syndrome virus §

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Narrative: Species in the genus Pestivirus, bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV), and border disease virus (BDV), are some of the most significant pathogens affecting ruminants and swine. Clinical disease can lead to high morbidity and mortality. Host immunosuppression and persistent viremia are hallmarks of pestivirus infections. In this study, metagenomic sequencing of a swine serum sample identified several contigs with similarity to pestiviruses. Further sequencing enabled assembly of an 11,276 base pair contig encoding a predicted 3,635 amino acid polyprotein with 68% identity to a recently partially characterized *Rhinolophus affinis* pestivirus (RaPV1) and approximately 40% identity to BDV, BVDV and CSFV. The virus was provisionally named as porcine pestivirus 1 (PpEV1). Interestingly, this sample was also positive for porcine reproductive and respiratory syndrome virus (PRRSV). Metagenomic sequencing of 182 PRRSV-positive sera samples identified four additional PpEV1-positive samples while molecular screening of a collection of 292 PRRSV-negative swine samples failed to identify PpEV1. Quantitative real time reverse transcription PCR (qRT-PCR) targeting the Erns and E2 regions of the genome further verified the PpEV1 positive samples. The samples originated from five states, suggesting widespread distribution of PpEV1 in the U.S. swine herd. Enzyme-linked immunosorbent assays using recombinant PpEV1 Erns found cross reactive antibodies in 94% of PRRSV qRT-PCR positive samples. The molecular and serological results suggest that PpEV1 is a novel, highly divergent porcine pestivirus widely distributed in U.S. pigs which might be involved in co-infection with PRRSV.

§ AAVLD Laboratory Staff Travel Awardee

Diagnosis of Rift Valley Fever Nucleocapsid Protein by Luminex-based Fluorescence Microsphere Immunoassay

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Narrative: Rift Valley Fever Virus (RVFV) is a zoonotic viral disease that infects ruminants including cattle, sheep, goats, camels and buffalo. A Luminex-based fluorescence microsphere immunoassay (FMIA) was developed for the detection of RVFV antibodies towards the immunodominant nucleocapsid (N) protein in cattle and sheep. The purpose of this study was to validate the FMIA for the detection of IgG antibodies against the N protein and apply it to the screening of animals for positive or negative disease status. Well-characterized experimentally infected sheep and calf sera from a MP12 vaccine study were used for the validation of the assay. Viral proteins were expressed using *E. coli* and baculovirus systems and then coupled to polystyrene magnetic bead sets for analysis using Luminex xMAP technology. A viral antigen coupled control bead set was added to account for non-specific binding of antibodies to the antigen-bead complex. Median Fluorescence Intensity (MFI) results were converted to Sample/Positive ratios to standardize test results. Results showed MFI values as high as 40,000 for the detection of IgG antibodies against the N protein in positive sheep and calf samples. In addition the FMIA correlated closely to serum neutralization assay results. The development of additional RVFV protein targets for the multiplex bead assay is currently under investigation. The results demonstrate that the FMIA provides a rapid and robust diagnostic screening tool for the detection of antibodies against RVFV. The work from this study will be applied to a multiplex assay that can simultaneously detect and screen several ruminant diseases.

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Symbols at the end of titles indicate the following designations:

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|---|--|
| § AAVLD Laboratory Staff Travel Awardee | * Graduate Student Poster Presentation Award Applicant |
| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | \diamond USAHA Paper |

To be PED, or not to be PED – that is the question! ◇

Yan Zhang, Jeffrey R. Hayes, Leyi Wang, Jing Cui, Beverly Byrum

Ohio Department of Agriculture, ADDL, Reynoldsburg, OH

Narrative: Four 14-day-old nursing piglets were submitted for investigation of pre-weaning diarrhea. The farm had a history of porcine epidemic diarrhea virus (PEDV) infection on the premises. At necropsy, all piglets had similar changes. The stomachs were filled with casein curds. The small intestines were thin walled, flaccid and contained fluid yellow ingesta. The colon and rectum of each pig contained fluid yellow feces. Sections of pancreas, duodenum, jejunum, ileum, spiral colon and lymph node were examined microscopically from each piglet. Small intestines of all 4 pigs exhibited mild to moderate segmental neutrophilic enteritis, with intralesional enteroadherent coccobacilli and intraluminal large bacterial rods. There was very mild multifocal villous atrophy in intestinal sections of only 1 pig. Sections of spiral colon of three pigs had mild to moderate segmental neutrophilic colitis, with intralesional enteroadherent coccobacilli. Many sections of both small and large intestines contained moderate to large numbers of large bacterial rods in the lumen. Small intestinal tissues and content of each pig were subjected to real time PCR for PEDV, transmissible gastroenteritis virus (TGEV), and porcine deltacoronavirus (PDCoV). All four pigs' samples were positive for PEDV, with Ct values ranging from 15 to 18. All samples were negative for TGEV and PDCoV. Next Generation Sequencing (NGS) indicated that the virus is the virulent strains of PEDV. NGS also confirmed the presence of type A *Clostridium perfringens* and *E. coli* DNA in the sample material. The Ct values for PEDV in intestinal content of each pig indicated a high viral load of the coronavirus. However, microscopic changes noted were primarily compatible with intestinal colibacillosis. Furthermore, the presence of large bacterial rods in the luminal contents also suggested a possible role of type A *Clostridium perfringens* infection. The lack of villous atrophy and attenuation of superficial villous enterocytes was surprising in these piglets. It is unknown if maternal antibody precluded the development of atrophic enteritis lesions in the piglet, or if other factors prevented the development of typical PED virus-induced mucosal lesions. Further pathogenesis studies of this PED virus isolate are warranted.

◇ USAHA Paper

Keeping up with the demand – are the PCR results ready-yet?

Wendy R. Stensland, Karen Harmon, Sarah Abate, Laura Bradner, Derek Dunn, Allison Kolker, Lacey Kurth, Chad Reinke, Leanne Schulz, Morgan Thomes

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Narrative: In the world of veterinary diagnostics, it is imperative to remain relevant. Client needs are always a concern for laboratory personnel as we strive to meet increasingly demanding expectations. Polymerase chain reaction (PCR) is an important tool which is utilized in the diagnosis of animal disease. The sensitivity, specificity and speed of PCR assays make them increasingly attractive to veterinarians, for the determination of individual and herd health. As the utility of PCR results increases, so does the demand for expedient results. From 2009 through 2014 PCR testing at Iowa State University's Veterinary Diagnostic Laboratory (ISU VDL) has increased threefold. For the majority of the real-time PCR testing, the ISU VDL provides same-day test results. The expectation of quality assured results and same day turn-around-time has remained the same, irrespective of the increasing testing volumes and staffing levels that have remained relatively flat. Because of this situation and our drive for continuous improvement, we began to investigate approaches to further streamline testing without adversely affecting the quality of test results. The evaluation presented here investigated the potential for decreasing the turnaround time of several high volume assays, without sacrificing sensitivity or specificity. A comparison of several commercially available real-time PCR master mix buffers was completed using well characterized positive controls, along with previously tested diagnostic samples. These controls and samples were evaluated in a side by side comparison of previously validated procedures against new protocols. A total of ten different assays were evaluated for this comparison, which comprised a majority of the agents for which PCR testing is requested at the ISU VDL and represented DNA- and RNA- viruses and bacteria. The original assays used several different master mix buffers, PCR reaction setups, and thermal cycling conditions. This evaluation showed that all assays included in the study could be performed with one single master mix buffer, PCR reaction setup and set of thermal cycling conditions. Overall, the average turnaround time of each assay was reduced by nearly one hour without sacrificing sensitivity or specificity. For some agents, a dramatic decrease in Cycle Threshold (Ct) was observed with the new master mix and cycling conditions. Additionally, a positive internal control reagent was identified that can be utilized for all agents at the time of nucleic acid extraction. The positive internal control provides a means for evaluating the efficacy of the PCR reaction and monitoring for the presence of potential PCR inhibitors, ensuring that the reaction was successful. Furthermore, the impact of utilizing a "universal PCR" platform for these assays has improved the overall efficiency of the laboratory's molecular diagnostic process by allowing for much more uniform and streamlined testing for a majority of our PCR assays.

Development of Porcine Deltacoronavirus (PDCoV) Diagnostic Tools and Investigation of Pathogenesis of a US PDCoV Cell Culture Isolate in 5-day-old Piglets # †

Qi Chen, Phillip Gauger, Molly Stafne, Joseph Thomas, Paulo Arruda, Eric Burrough, Darin Madson, Joseph Brodie, Drew Magstadt, Rachel Derscheid, Michael Welch, Jianqiang Zhang

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Narrative: Porcine deltacoronavirus (PDCoV) was first identified in Hong Kong in 2009-2010 and reported in US swine for the first time in February 2014. However, diagnostic tools other than PCR for PDCoV detection were lacking and Koch's postulates had not been fulfilled to confirm the pathogenic potential of PDCoV. The objectives of this study were to develop PDCoV-specific immunofluorescence and immunohistochemistry (IHC) assays and investigate the pathogenesis of PDCoV in 5-day-old piglets. The predicted antigenic peptides of PDCoV spike (S), membrane (M) and nucleocapsid (N) proteins were synthesized and rabbits were immunized to generate peptide-specific antisera. PDCoV M and S peptide antisera were successfully used to develop an immunofluorescence assay but M peptide-specific antisera had enhanced staining signals. An IHC assay based on the PDCoV M peptide antisera was also developed and validated. Twenty 5-day-old, PDCoV-negative piglets were randomly divided into two groups of 10 pigs each. Both groups were orogastrically inoculated with a plaque-purified PDCoV cell culture isolate (3×10^3 TCID₅₀/ml, 10ml per pig) or virus-negative culture medium. Over the 7-day study period, PDCoV-inoculated pigs did not experience apparent loss of appetite, lethargy, dehydration or mortality. Average weight gain was not significantly affected by PDCoV infection during the study period. Challenged pigs developed diarrhea 5 days post inoculation (DPI) with progression to watery diarrhea observed at 6 DPI, and decreasing severity of diarrhea at 7 DPI. Virus shedding was detected by PCR in 10%, 40%, 80% and 100% of rectal swabs at 2, 3, 4, and 5-7 DPI, respectively, with average virus titers gradually increasing from 2 to 7 DPI, indicating that PDCoV infection progressed slowly under the conditions of this study. PDCoV RNA was detected in sera of 100% of the challenged pigs at 4, 5, and 7 DPI. Gross lesions consistent with viral infection were observed in the small intestines of some PDCoV-inoculated pigs at both 4 and 7 DPI; however, histopathological lesions were only observed in small intestines. Histopathological lesions and IHC staining were mainly observed in the middle and distal jejunum and ileum, suggesting that middle and distal jejunum and ileum are better intestinal segments than duodenum and proximal jejunum for lesion and antigen evaluations. In summary, PDCoV peptide-specific rabbit antisera were generated for the development of PDCoV-specific immunofluorescence and IHC assay. The pathogenic potential of PDCoV was confirmed as naïve pigs developed diarrhea, shed virus in rectal swabs, and developed macroscopic and microscopic lesions in the small intestine with viral antigen confirmed within lesions by IHC staining.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

Canine distemper: Detection of a novel genotype causing disease in wildlife # † +

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¹Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN; ²Forestry, Wildlife & Fisheries, University of Tennessee, Knoxville, TN

Narrative: Canine distemper virus (CDV) is a common cause of a multi-systemic disease in both domestic dogs and wildlife species, including raccoons and foxes. Outbreaks of CDV in domestic dogs in eastern Tennessee have occurred since 2012 and our lab determined that these outbreaks resulted from a novel genotype of CDV. We hypothesize that this virus is also capable of infecting area wildlife, which may be a source of the virus for these outbreaks in dogs. Therefore, necropsies were performed and tissues were collected from raccoons (*Procyon lotor*; n=50) and gray foxes (*Urocyon cinereoergenteus*; n=8) from 2013-2014 for CDV testing. A real-time RT-PCR assay was used to document presence of CDV in the tissue samples, and a portion of the CDV matrix gene and matrix-fusion intergenic region was sequenced from positive samples for phylogenetic analysis. A high percentage of both clinically ill (89%) and clinically healthy (59%) wildlife in East Tennessee tested positive for CDV. The majority of all animals that tested positive for CDV (78%) had the novel genotype. Histologic findings from these animals were also consistent with canine distemper. Most commonly, lesions within the central nervous system revealed minimal inflammation as is typical of the acute neurologic manifestation of distemper. Inclusions in the central nervous system were most commonly observed intranuclear in astrocytes. However, inclusions overall were most often seen intracytoplasmic in urothelium. Syncytia were only observed in the lungs. Pneumonia and parasitism were common findings in CDV infected animals. Based on these results, CDV is prevalent in East Tennessee wildlife, and subclinical or clinically-recovered shedders are a potential source of this novel genotype for domestic dogs.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Applications of In Situ Hybridization for Novel Aquatic Viruses # † +

Jennifer Dill¹, Elizabeth W. Howerth¹, Terry Fe Fan Ng², Alvin Camus¹

¹Department of Pathology, University of Georgia, Athens, GA; ²Virology, Centers for Disease Control and Prevention, Atlanta, GA

Narrative: The rapid growth of aquaculture and international trade in live fish and fish products has led to the global emergence of multiple significant aquatic animal pathogens. To support commercial aquaculture and the aquarium trade, there is a need to increase disease surveillance and improve laboratory diagnostic methods, with an ultimate goal to more rapidly diagnose and assess the impacts of novel disease agents affecting both wild fish populations and aquarium collections. However, diagnostic investigations in fish are frequently hindered by a lack of cross reactivity with commonly used mammalian antibodies and in the case of suspected viral infections, suitable cell lines for virus isolation. When a quarantined giant guitarfish (*Rhynchobatus djiddensis*) developed viral induced skin lesions, next generation sequencing (Illumina MiSeq) and metagenomic analysis revealed two, currently unnamed, novel DNA viral genomes. An in situ hybridization test was successfully developed to facilitate visualization and detection of viral nucleic acid to augment other diagnostic modalities and ongoing research. Primers designed from the novel DNA genomes were designed to generate digoxigenin-labeled PCR products for both DNA viruses. Positive hybridization signals in formalin fixed paraffin embedded tissue sections were used to localize viral nucleic acids in the tissue of interest and to identify the viral genomic sequence responsible for the lesions. Results of the ISH in conjunction with histologic, electron microscopic and polymerase chain reaction findings have determined which virus contributed to the development of the skin lesions in this elasmobranch species.

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Senecavirus A vesicular disease outbreak in Midwest show and commercial pigs

Pablo E. Pineyro, Chris Rademacher, Rachel Derscheid, Baoqing Guo, Kyoung-Jin Yoon, Jianqiang Zhang, Karen Harmon, Drew Magstadt, Phillip Gauger, Qi Chen, Paulo Arruda, Gregory Stevenson, Eric Burroughs, Kent Schwartz, Vickie L Cooper, Bailey L. Arruda, David Baum, Jeff Zimmerman, Luis G. Gimenez-Lirola, Daniel Correia-Lima-Linhares, Patrick G. Halbur, Rodger Main, Darin Madson

VDPAM, ISU-DVL, Ames, IA

Narrative (400 words): Senecavirus-A (SV-A) is a non-enveloped, positive-sense, single-stranded RNA virus. Senecavirus-A lesions in swine include vesicles, erosions, and ulcers of the coronary band, oral cavity, and nasal planum. Gross lesions in pigs are clinically indistinguishable from foot-and-mouth disease (FMD), vesicular stomatitis (VS), swine vesicular disease (SVD), and vesicular exanthema of swine (VES). From July 21, 2015 through August 18, 2015 the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) received five independent cases of vesicular disease affecting show and commercial pigs. Animals from three different farms (**cases 1, 2, and 3**) were located in Southwest and Central Iowa and observed at two county fair exhibitions. Clinical signs included acute lameness, anorexia and pyrexia. Lesions included vesicles, erosions, and ulcers of the coronary band, mouth, and snout. **Case 4** occurred in a commercial farm with 1,200 market weight finisher pigs. Initially 30-40% of these pigs showed clinical signs and gross lesions similar to those previously described. Morbidity approached 95% within twenty days. Four show pigs (**case 5**), exhibited at the Iowa State Fair presented with fever and acute lameness with vesicular lesions on the coronary band. No mortality has been reported amongst these cases. From **cases 1, 2, and 3** hoof scrapings, vesicular fluid and nasal swabs were evaluated by gel-based PCR targeting the VP1 region and qPCR targeting the 5'UTR. Hoof scrapings and vesicular fluid were positive by both PCR methods (Ct 17.7-28.2). Nasal swabs were negative. Samples from **case 4** testing positive included hoof scrapes, vesicular fluid, and nasal scrapings (Ct 11.69 to 29.1). Serum samples were negative. Oral fluids from four pens tested positive (Ct 17.9-23.62). Five affected animals (**case 4**) were euthanized and necropsied. All pigs had severe erosions and ulcerations of the coronary bands and nasal planum. Inguinal lymph nodes (Ct 23.9-27.2) and tonsil (Ct 25.4-29.16) tested positive in four pigs. All 20 oral fluids from the pens adjacent to these pigs were positive (Ct 23.4-29.2). **Case 5**, vesicular swabs and nasal/hoof scrapings from three animals were positive (Ct 12.6-18.8). Virus isolation from positive cases yielded characteristic cytopathic effects in porcine kidney (PK15) and swine testicle (ST) cell lines. All cases tested negative for FMD, and samples from cases 1, 4 and 5 tested negative for SVD, VES, and VS. This is the first description of a SV-A outbreak in the USA. Early detection and etiological diagnosis of SV-A is necessary to differentiate from other vesicular diseases.

Speaker Biography: Academic Achievements Doctor of Philosophy in Biomedical and Veterinary Sciences (Phd): Novel approaches towards vaccine developments against PCV2 and PRRSV. 2015 Doctor in Veterinary Science (Phd): Swine influenza virus infection: seroepidemiological, anatomopatological and immunohistochemical studies. 2012 Master of Veterinary Science (MVSc): Development of two animal models to study the function of Vibrio parahaemolyticus type III secretion systems. 2010 Doctor in Veterinary Medicine (DVM): Facultad de Ciencias Veterinarias, Universidad Nacional de la Plata, Buenos Aires, Argentina. 1999 **Professional Experience** 2015-Present: Assistant professor, Veterinary Diagnostic and Production, Iowa State University. 2014-2015: Clinician/Veterinary Pathologist, Veterinary Diagnostic and Production, Iowa State University. 2010-2014: Teaching Assistant. Colleague of Veterinary Medicine. Virginia Tech. 2008-2010: Teaching Assistant. Colleague of Veterinary Medicine. Washington State University 2004-2010: Research Assistant, La Plata National University, College of Veterinary Medicine. La Plata, Argentina. **Selected recent refereed manuscripts published (from over 30 publication)** Piñeyro PE, Kenney SP, Giménez-Lirola LG, Heffron CL, Matzinger SR, Opriessnig T, Meng XJ. Expression of antigenic epitopes of porcine reproductive and respiratory syndrome virus (PRRSV) in a modified live-attenuated porcine circovirus type 2 (PCV2) vaccine virus (PCV1-2a) as a potential bivalent vaccine against both PCV2 and PRRSV. *Virus Res.* 2015 Jul 31;210:154-164 Subramaniam S., Piñeyro P.E., Tian D., Overend C., Yugo D.H., Matzinger S., Rogers A., Haac M.E.C., Cao Q., Heffron L., Catanzaro N., Kenney S., Huang Y., Meng X.J. In vivo targeting of Porcine Reproductive and Respiratory Syndrome Virus antigen through DC-SIGN to dendritic cells elicits antigen-specific CD4 T cell immunity. *Vaccine.* 2014 Nov 28;32 (50):6768-75. Huang YW, Dickerman AW, Piñeyro P, Li L, Fang L, Kiehne R, Opriessnig T, Meng XJ. 2013. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. *mBio* 4(5):00737-13.

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|---|--|
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| # AAVLD Trainee Travel Awardee | † Graduate Student Oral Presentation Award Applicant |
| + AAVLD/ACVP Pathology Award Applicant | ◇ USAHA Paper |

Poster 1

Evaluation of a real-time PCR assay for detection of *Salmonella* spp. in veterinary matrices

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Narrative: There is a large demand for rapid screening for enteric bacterial pathogens among veterinary hospitals and clinics. The MicroSEQ® *Salmonella* spp. Detection Kit detects *Salmonella* spp. in purified nucleic acid preparations using a lyophilized reagent format; it is AOAC-approved for a variety of human foods and dry animal feed and AFNOR validated for human foods, animal feeds, animal feces, and environmental samples from the primary production stage. We have conducted in-house validation tests on this kit for small and large animal tissues and established a workflow for efficiently and accurately performing molecular testing of all veterinary matrices and environmental swabs together. Overall, this assay was more sensitive than culture alone in detecting *Salmonella* spp. in tissues, but equally sensitive to culture for fecal samples. Guidelines for interpretation of Ct values in comparison to bacterial viability were also established. This study was funded and performed in collaboration with the Food and Drug Administration's Veterinary Laboratory Investigation and Response Network (Vet-LIRN).

Poster 2

Map-based serotyping of *Streptococcus suis* field isolates for vaccine formulation

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Narrative: *Streptococcus suis* is a gram positive pathogen that causes arthritis, septicemia, bronchopneumonia, and fatal meningitis in young piglets. Piglets are usually infected through exposure to asymptomatic adult carriers. Historically, 35 different serotypes of *S. suis* were identified, however, recent work has suggested that only 29 unique serotypes exist. Despite this, many isolates remain non-typeable using currently available methods. Of the 35 serotypes described to date, serotype 2 is most commonly associated with disease but serotypes 1, ½, 3, 4, 5, 7, 8, 9, and 16 are also commonly isolated from diseased swine. Currently, two methods are used for serotyping *S. suis* isolates. The first method relies on the use of a panel of antisera with specific reactivity to each serotype. A more recent development has been the advent of PCR based methods in which portions of the *cps* and *gdh* genes are amplified, yielding PCR products of unique size for each serotype. The need for continued maintenance of validated antisera to use in the serum based test is cumbersome, time consuming, and not reproducible across laboratories. However, this test remains the gold standard for serotyping of *S. suis* isolates. PCR based methods are convenient and can rapidly narrow the serotype of an isolate down to one or two different types, often to a specific serotype. Nonetheless, this method is unable to distinguish serotypes 2 from ½ and 1 from 14. Serotyping based on both the above methods has limitations and is often ambiguous when typing field isolates. Newport Laboratories has developed a comprehensive, unambiguous method of serotyping based on next generation sequencing and bioinformatics. Using this method isolated field strains are sequenced using an Illumina Miseq instrument. Once sequenced, various softwares are used to assemble the genome and map the isolate in question to GenBank reference genomes representing each *S. suis* serotype. The raw data, once mapped to the capsule locus of the reference genomes, can accurately and unambiguously identify the serotype. When field isolates appeared to be non-typeable by agglutination or PCR, we were able to accurately identify the serotypes and the culture status (mixed vs. pure) of each isolate. Furthermore, we found that the capsular locus of *S. suis* is amenable to horizontal gene transfer mediated by insertion sequence elements and transposases. The map-based serotyping approach described here will accurately identify serotypes and can be used as a powerful epidemiological tool for surveillance and ultimately for formulation of an efficacious herd specific vaccine.

Poster 3

Identification of *Streptococcus parasuis* using MALDI TOF MS

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Narrative: *Streptococcus suis* is a Gram-positive coccus and common pathogen in swine farms and is associated with a variety of diseases such as meningitis, arthritis, bronchopneumonia, and septicemia. Thirty-five serotypes of *S. suis* (serotypes 1–34 and serotype 1/2) have so far been described on the basis of their polysaccharide capsular antigens. However, in 2014 researchers proposed that *S. suis* serotypes 20, 22, and 26 should be reclassified as a novel species with the name *Streptococcus parasuis*. The reclassification was based in part on phylogenetic analysis of the gene encoding the recombination/repair protein (*recN*). In our laboratory, 5 strains of *Streptococcus* that had previously identified as *Streptococcus suis* with conventional identification methods and also identified as *Streptococcus orisratti* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) were tested using a described PCR method for detection of *S. suis*-specific *recN*. All 5 strains were PCR negative and subsequently added to a MALDI TOF MS user database. Dendrogram analysis of these spectral profiles showed that these isolates clustered together separately from *S. suis* with *S. orisratti* as the nearest neighbor. Afterwards 40 isolates representing various *S. suis* serotypes were identified using MALDI TOF MS using the manufacturer's database plus the *S. parasuis* user database additions. These isolates were also tested using the *S. suis*-specific *recN* PCR. *S. parasuis* was identified in 10 isolates of which 8 were positive for serotype 22. The other 2 isolates were positive for serotype 15. All isolates identified as *S. parasuis* were also negative using *S. suis*-specific *recN* PCR. An additional 5 isolates of various serotypes (2, 7, 10, 25, 28) were negative for *S. suis*-specific *recN* PCR. These results indicate that MALDI TOF MS may be useful to identify *S. parasuis* more consistently than a *S. suis*-specific *recN* PCR. They also indicate that *S. parasuis* may include serotypes other than what has been proposed. Additional investigation of *S. suis/parasuis* is needed to understand the relationships of these species to serotype designation and detection by various methods.

Poster 4

Evaluation of Bacterial Identification Systems for Species Identification of Bacterial Isolates from Milk # *

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Narrative: In our study we evaluated Aris®2x Sensititre (ARIS), API® (API) and Bruker MALDI-TOF MS (BMALDI) bacterial species identification systems using 132 diverse bacterial isolates from quarter or pooled milk samples received at our diagnostic laboratory. The results were compared with 16s rRNA gene sequence analysis which served as reference method for species identification. As compared to 16s rRNA gene sequencing data, the ARIS, API and BMALDI systems were able to identify 0%, 33.4% and 50% of species classified as gram positive rods (n=6 isolates belonging to genera *Arthrobacter*, *Bacillus*, *Brachybacterium*, *Brevibacterium*, *Corynebacterium*), respectively. With regard to catalase negative gram positive cocci (n=33; *Aerococcus*, *Enterococcus*, *Lactococcus*, *Streptococcus*), 57.5%, 78.7% and 96.9% of the isolates were correctly identified to their species by ARIS, API and BMALDI systems, respectively. While 22.3%, 80% and 95.5% of catalase positive gram positive cocci (n=45; *Kocuria*, *Staphylococcus*) were correctly identified to their species by ARIS, API and BMALDI systems, respectively. A total of 48 isolates (*Acinetobacter*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pantoea*, *Pasteurella*, *Providencia*, *Pseudomonas*, *Serratia*) of gram negative bacteria were examined of which 81.2, 89.5, and 97.9% of the isolates were correctly identified to their species by ARIS, API and BMALDI systems, respectively. In our laboratory the cost for supplies and consumables was the least for BMALDI (\$2.00), followed by Aris®2x (\$5.95), 16s rRNA (\$6.80), and API® systems (\$9.90-\$13.10) identification methods. Identification of bacterial species (primary plating to identification) was accomplished in 22-26h, 36-38h, 44-48h, and 48-64h using BMALDI, 16s rRNA, ARIS and API identification systems, respectively. The findings of the study suggest that BMALDI system accurately species identified most of the isolates from milk as compared to ARIS and API systems. Although only a few isolates (n=6) gram positive rods were examined, It is suggested that BMALDI database needs to include more profiles of gram positive rods, In conclusion, BMALDI is faster, cheaper, and accurate method for routine species identification of isolates from milk samples. It is anticipated that MALDI-TOF based technology will likely replace existing biochemical-based methods for bacterial species identification and be widely used in diagnostic laboratories.

AAVLD Trainee Travel Awardee

* Graduate Student Poster Presentation Award Applicant

Poster 5

Identification of strain-specific sequences that distinguish field isolates from *Mycoplasma gallisepticum* vaccine strain ts-11

Naola Ferguson-Noel

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Narrative: Despite attempts to control avian mycoplasmosis through management, vaccination, and surveillance, *Mycoplasma* continues to cause significant morbidity, mortality, and economic losses in poultry production. Live attenuated vaccines are commonly used in the poultry industry to control avian mycoplasmosis; unfortunately, some vaccines may revert to virulence and vaccine strains are generally difficult to distinguish from natural field isolates. In order to identify genome differences among revertants, vaccine, and field isolates, whole genome sequencing of the *M. gallisepticum* vaccine strain ts-11, and several “ts-11-like” strains isolated from commercial flocks was performed using Illumina and 454 pyrosequencing and compared to *M. gallisepticum* R_{low} reference genome. The collective contigs for each strain were annotated using fully annotated *Mycoplasma* reference genomes. The analysis revealed genetic differences among *vlhA* alleles and genes annotated as choline kinase, cell wall surface anchor protein, and a hypothetical protein unique to *M. gallisepticum* ts-11 strains. PCR was designed to target 5 sequences unique to ts-11 strains (alleles/genes: *vlhA* 3.04a, *vlhA* 3.04b, *vlhA* 3.05, *csa*, ts-11 *hp*). All ts-11-like isolates were positive for the five gene alleles tested by PCR, however 4-34% of field isolates were also positive for at least one of the alleles tested. A combination of PCRs for *vlhA* 3.04a, *vlhA* 3.05, and ts-11 *hp* was able to distinguish ts-11 from field strains. This method will further supplement current approaches to quickly identify *M. gallisepticum* ts-11 strains.

Poster 6

Optimal Swab Elution Media for Diagnostic Avian Mycoplasma Real-time PCR

Rachel Jude, Naola Ferguson-Noel

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Narrative: Mycoplasma infections are a source of substantial economic loss in commercial poultry production. Although advances in real-time PCR technology have resulted in extremely sensitive and fast tests that are affordable and amenable to high throughput testing, the added sensitivity of real-time PCR may be severely reduced due to inappropriate handling and processing of swabs. In poultry, swabs are often processed for multiple tests (e.g. respiratory viruses and MG and MS) and careful consideration needs to be made when choosing the appropriate media for swab transport and preparation for PCR. It has been shown that dry tracheal swabs are a convenient and appropriate sample for mycoplasma PCR, and the primary goal of this study was to compare five media that may be used to elute tracheal swab samples for molecular diagnostics. Brain heart infusion (BHI) broth, water, phosphate buffered saline (PBS), a modified PBS, and mycoplasma media (modified Frey's broth) were evaluated for the effect on sensitivity of mycoplasma real time PCR. This information will allow the poultry industry to maximize the benefits of these costly diagnostic assays and set scientifically based standards in different laboratories for sample handling for MG and MS real-time PCR.

Poster 7

Validation of Single and Pooled Manure Drag Swabs for the detection of *Salmonella* ser. Enteritidis in Commercial Poultry Houses: A collaborative Study

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Narrative: A single swab is currently used in the FDA official method for sampling the environment of commercial laying hens for the detection of *Salmonella enterica* ssp. serovar Enteritidis (*Salmonella* Enteritidis). The FDA has also granted provisional acceptance of the National Poultry Improvement Plan's (NPIP) *Salmonella* isolation and identification methodology for samples taken from table-egg layer flock environments. The NPIP method, as with the FDA method, requires single swab for the environmental sampling of laying houses for *Salmonella* Enteritidis. The main objective of this collaborative study was to compare the reference method (standard FDA) which requires testing a single swab with that of the alternative method (NPIP) using 4 pool swabs. Single and multi-laboratory testing of replicate manure drag swab sets ($n = 525$ and 672 , respectively) collected from a *Salmonella* Enteritidis free commercial poultry flock was performed by artificially contaminating swabs with either *Salmonella* Enteritidis phage type 4, 8, or 13a at one of two inoculation levels: low, $x = 2.5$ CFU (range 2.5 – 2.7), medium, $x = 10$. 1 CFU (range 9.6-10.5). For each replicate of single, sets of 2 (one inoculated and one uninoculated) or 4 swabs (one inoculated and three uninoculated), testing was conducted using the reference or alternative culture method. Swabs inoculated with phage type 8, the alternative method was more efficient ($P < 0.05$) for all swab sets at both inoculation levels than the reference method. The single swabs in the alternative method were significantly ($P < 0.05$) better than 4-pool swabs in detecting *Salmonella* Enteritidis at the lower inoculation level. In the collaborative study ($n = 13$ labs) using *Salmonella* Enteritidis phage type 13a inoculated swabs, there was no significant difference ($P < 0.05$) between the reference method (single swabs) and the alternative method (4-pool swabs). The study concludes that alternative method is equivalent to the reference method in detecting *Salmonella* Enteritidis in drag swabs in commercial poultry laying houses. Furthermore, the alternative method was more efficient and cost effective.

Poster 8

Comparison of the VersaTREK Automated Microbial Detection System and ESP Culture System II for Detection of *Mycobacterium avium* ssp. *paratuberculosis*

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Narrative: Johne's disease (paratuberculosis) is a chronic enteric disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis*. The disease continues to be of economic importance for livestock industry in many countries due to loss of production, culling, mortality and test costs. Antemortem laboratory diagnosis of Johne's disease can be achieved by detection of the causative agent from fecal samples or indirectly by demonstrating the presence of pathogen-specific antibodies in serum or milk samples. Several liquid and solid microbiological media and culture systems have been used to culture *M. avium* subsp. *paratuberculosis*, which is a very slowly growing bacterium. The liquid culturing systems facilitate faster diagnosis of Johne's disease compared to solid media. Previously, our laboratory adapted the ESP Culture System II for the detection of *Mycobacterium avium* subspecies *paratuberculosis* in fecal samples. Using a panel of samples, a semi-quantitative method was developed to categorize fecal samples as high, medium, and low shedding based on time to positive in the liquid culture that showed a strong correlation with the number of bacterial colonies on the HEY solid medium. The objective of the present study was to validate the new VersaTREK Automated Microbial Detection System for the detection of *Mycobacterium avium* ssp. *paratuberculosis* and compare it with ESPII. Both VersaTREK and ESP liquid culture systems detect microbial growth and metabolic activity by monitoring changes in the pressure inside the culture bottle and report results as time to positive (TTP) in days. Further confirmation of the microbial identification is achieved using acid fast staining and IS 900 PCR. We cultured 36 bovine fecal samples from our collection in parallel on ESP and VersaTrek for 42 days. Thirty-three samples yielded acid fast organisms and 3 samples were negative for acid fast organisms and PCR negative. Out of 33 positive samples, the growth was detected for 21 samples by the either liquid systems (TTP < 42 days). About 67% samples (14 out of 21) had less than 5 days difference in TTP and 33% samples (7 out of 21) had greater than 9 days difference in TTP. Additional studies using 26 NVSL proficiency test samples indicated that TTP were comparable for 5 high and 4 moderate shedding samples, suggesting a good agreement between the systems. Our study emphasizes the importance of verification and validation of diagnostic assays when any changes are implemented including instrumentation in the testing methods. Additionally, it is important to develop consistent standards for quantitative assays across diagnostic platforms and laboratories for meaningful interpretation of the results.

Poster 9

Adaptation of a published assay for serotyping of *Actinobacillus pleuropneumoniae* by multiplexed PCR for laboratory diagnostics

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Narrative: *Actinobacillus pleuropneumoniae* (APP) is a bacterial respiratory pathogen that causes morbidity and mortality in pigs globally. This disease is restricted to swine but is widely distributed in major swine producing countries. There are two biotypes of APP within which at least 15 serovars are recognized based on the surface carbohydrates of the organism. The classical “gold standard” for identifying APP serotypes/serovars is through antibody based diagnostics. However, cross reactions between various serovars have been reported. Therefore, multiplex PCR based assays have been developed to identify the different APP serovars. We evaluated a recently published assay (Bosse et al, J Clin Micro 52(7):2380-2385) describing a multiplexed PCR that was developed to detect and differentiate serovars 1, 2, 3, 5, 6, 7, 8, 10 and 12 of APP. Our goal was to establish a standardized test that could be offered to the clients of the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). As per the published assay a small amount of growth from a chocolate agar plate was resuspended in 200 microliters of molecular biology-grade water and boiled to extract the DNA. PCR was performed using the Qiagen Multiplex PCR Plus kit and products were analyzed on a Qiagen QIAxcel capillary electrophoresis system. We discerned after running the multiplexed assay that it was difficult to distinguish between serotypes APP 1, 5, and 8; APP 2, 10 and 12; and serotypes 3, 6 and 7 because their PCR amplified bands were too close in size to confidently discriminate among all the serovars in a single reaction. In order to circumvent this problem, we grouped APP 1, 2 and 3; APP 5, 7, and 10; and APP 6, 8 and 12 in a panel of three multiplexed assays. The assay was validated with 5 ATCC strains as well as 20 APP isolates from cases submitted to ISU VDL that had been serotyped at a reference laboratory at University of Montreal, that are serving as our positive controls. We also determined that column extracted DNA was much more stable compared to boil prep when used to prepare the above nine controls. However, we found that for the preparation of sample culture a simple boil prep was adequate when they were used within two days of extraction. These observations and alterations from the published assay have allowed us to offer a reliable test for determining the above mentioned serovars as a routine test from culture at the ISU VDL.

Poster 10

An unusual yeast urinary tract infection in a cat

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Narrative: *Cryptococcus neoformans* is the most common systemic mycosis in cats. The disease is generally subacute or chronic resulting in granulomatous lesions with a mucoid discharge and lymphadenopathy or localized lesions in the oral and/or nasal mucosa. Today we report a case of a urinary tract infection in a 4 year old male castrated domestic shorthair cat due to *Cryptococcus neoformans*_var_*grubii*. The cat had been reportedly licking his urogenital region due to a presumptive UTI, so he was treated with antibiotics, but did not improve. The referring veterinarian then submitted a urine sample for aerobic culture and susceptibility testing. The sample was plated following our normal protocol for aerobic urine culture, on Columbia blood agar, Columbia colistin nalidixic acid agar, and MacConkey agar and was incubated at 37°C in air. After 24 hours of incubation, very pinpoint growth was observed in numbers too numerous to count and budding yeast were seen on Gram stain. The Bruker MALDI biotyper was used to identify the yeast using the extended Direct Transfer (eDT) method, from the 24 hour culture plate, which resulted in an identification of *Cryptococcus neoformans*_var_*grubii* with a score of 2.019. The yeast was plated on HardyCHROM™ Candida agar, but no pigment production was observed. 18S rRNA sequencing was performed to verify the MALDI biotyper results. A nucleotide sequence in the forward direction of 509 base pairs had 100% homology with *Cryptococcus neoformans*_var_*grubii*, and a 538 base pair nucleotide sequence in the reverse direction showed 99% homology with *Cryptococcus neoformans*_var_*grubii*. A yeast susceptibility was performed on a YeastOne® YO9 plate from Trek Diagnostic Systems following the recommended method. This isolate was susceptible to the imidazoles and resistant to the echinocandins. The cat was placed on itraconazole, since the yeast was susceptible, and because of the once daily dosing (sid). It is suspected that this animal contracted the disease due to foot traffic into the home, since he is an indoor cat. Presumably his cleaning and licking resulted in an ascending UTI. The cat has no history of systemic disease, but has elevated BUN and creatinine. The cat was FIV and FLV negative when he was adopted 2 years ago.

Poster 11

Influence of Diet on Microbial Diversity of Intestinal Microbiota in Broiler Chicken and its Comparison with Microbiota of Wild Birds *

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Narrative: A study was conducted to determine the microbial diversity in the faeces of broiler chickens fed with three different diets including, conventional (antibiotic-supplemented), natural (antibiotic-free and probiotic-supplemented) and organic (antibiotic-free with standard organic probiotics) diets. A total of 30 samples (ten composite samples each from conventional, natural and organic broiler farms) were serially diluted and plated on 5% sheep blood agar. From each composite of 10 samples, 30 isolates were randomly taken and speciated using Matrix-assisted laser desorption/ionization technique. Bacteria identified in broiler faecal samples were; *Aerococcus viridians*, *Bacteroides fragilis*, *Brevibacterium ravenburgense*, *Corynebacterium* spp. (n=4 species), *Empedobacter brevis*, *Enterococcus* spp. (n=5 species) *Escherichia* spp. (n=2), *Globicatella sulfidifaciens*, *Lactobacillus* spp. (n=6), *Parabacteroides distasonis*, *Staphylococcus* spp. (n=5), and *Streptococcus* spp. (n=2). Broilers fed with natural diet had the highest Simpson's index of diversity (0.83) followed by broilers fed on organic diet (0.61) and conventional diet (0.31). Ten wild turkey faecal samples were examined as done with broiler faecal samples. Five to ten colonies from each sample was randomly selected and speciated. Bacteria identified in the samples were; *Bacillus* spp. (n=3 species), *Buttiauxella gaviniae*, *Clostridium* spp. (n=3), *Enterobacter* spp. (n=2), *Enterococcus* spp. (n=6), *Lactobacillus* spp. (n=4), *Paenibacillus* spp. (n=3), *Pantoea agglomerans*, *Raoultella ornithinolytica*, *Solibacillus silvestris*, and *Streptococcus pleomorphus*. A total of 52 bacterial species were identified of which only six species (*E. faecalis*, *E. faecium*, *E. hirae*, *E. coli*, *L. agilis*, and *L. salivarius*) were observed in both broiler and turkey faecal samples. Based on the preliminary findings of the study, it can be inferred that type of diet and environment could influence the diversity of gut microflora.

* Graduate Student Poster Presentation Award Applicant

Poster 12

Isolation and Identification of *Actinobacillus* sp. from the Granulomatous Tongue Lesion of a Four Year Old Cow

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Narrative: A four year old cow with a history of inappetence for three weeks and clinical diagnosis of wooden tongue was submitted for necropsy at Oklahoma Animal Disease Diagnostic Laboratory (OADDL). Grossly, more than 85% of the lingual muscle was replaced by multiple firm, pale tan nodules, that were often centrally caseous. Histopathology of the tongue revealed numerous multifocal pyogranulomas within the lingual muscle that were centered on large bacterial colonies and Splendore Hoeppli material, surrounded by fibrous connective tissue and adjacent myonecrosis and degeneration. *Actinobacillus ligniersii* is the etiological agent of wooden tongue in cattle. Bacterial culture of the tongue grew *Actinobacillus* sp. and *Trueperella pyogenes*. The automated biochemical based bacterial identification system, MALDI-TOF MS and 16S rDNA sequencing identified the *Actinobacillus* sp. as *Actinobacillus pleuropneumoniae*. The *Actinobacillus* sp. isolated grew on blood agar plates after 24 hr incubation. Other colony characteristics and biochemical reactions for the *Actinobacillus* sp. were as follows: non-hemolytic on 5% sheep blood agar, no growth on MacConkey agar, catalase, oxidase and urease positive, CAMP negative, fermented maltose and mannitol and lactose but did not ferment salicin, sorbitol and trehalose. *Actinobacillus ligniersii*, *Actinobacillus pleuropneumoniae* and *Actinobacillus equuli* are genetically very similar and cannot be distinguished by 16S rDNA sequencing alone. Based on the colony characteristics, biochemical reactions and the clinical lesion the isolate was identified as *Actinobacillus ligniersii*. This report highlights the limitations of automated systems, MALDI-TOF MS and 16SrDNA sequencing for the identification of *Actinobacillus ligniersii*.

Poster 13

Application of the Dutch Working Party on Infection Prevention Guideline to Define *E. coli* Isolates as Multi-drug Resistant

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Narrative: Multi-drug resistant (MDR) strains of *E. coli* are recognized as a serious threat to the treatment of infections in veterinary medicine, yet no guidelines exist that clearly define what exactly constitutes an MDR *E. coli*. In 2004, the Dutch Working Party on Infection Prevention issued a guideline on the prevention of nosocomial transmission of highly resistant microorganisms (HRMO). HRMO are defined as microorganisms which 1) are known to cause disease, 2) have acquired an antimicrobial resistance pattern that hampers (empirical) therapy, and 3) have the potential to spread if – in addition to standard precautions – no transmission-based precautions are taken. The designation HRMO is, therefore, dependent on the bacterial species and the antibacterial agent(s) against which it has acquired resistance. Using the Dutch Guidelines an *E. coli* isolate would be classified as an HRMO if 1) it is resistant to a carbapenem or 2) if it is resistant to a fluoroquinolone AND an aminoglycoside. The Matthew J Ryan Veterinary Hospital at the University of Pennsylvania, School of Veterinary Medicine (VHUP), has used these guidelines as part of its hospital infection control program since 2007 to manage hospital in-patients and prevent nosocomial infection. In order to determine the prevalence of MDR *E. coli* at our institution, this study examined the antimicrobial susceptibility data obtained from 5790 *E. coli* isolates obtained from companion animal specimens during the years 2003 to 2014. 4219 of these isolates were from VHUP patients and 1571 were specimens submitted to the laboratory from external veterinary practices. Overall, 1488/5790 (25.7%) were resistant to a fluoroquinolone and 582 (10%) were resistant to an aminoglycoside. 522 (9%) isolates were resistant to both classes and were therefore classified as an MDR *E. coli*. The prevalence of MDR isolates from the external practices was 10% (166/1571) and fluoroquinolone and aminoglycoside prevalence was 26.7% and 12%, respectively, indicating that the data were not skewed by the addition of these isolates into the overall dataset. Only 9 isolates were carbapenem resistant (0.0002%). These results show that 10% of *E. coli* isolates are classified as MDR and also show that the prevalence of carbapenem resistance is extremely low. 26% of all *E. coli* isolates were found to be fluoroquinolone resistant and this may have implications for empiric therapy at this institution. The application of these guidelines could be useful at other veterinary hospitals to inform infection control programs.

Poster 14

Extended-Spectrum Beta-Lactamase Resistant *E. coli* isolates from Companion Animals and Co-Resistance to Empiric Drug Classes

Gabriel Innes, Shelley C. C. Rankin

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Narrative: A substantial increase in the number of *E. coli* strains that produce ESBLs throughout the world has been most recently attributed to the rapid spread of plasmid-mediated CTX-M genes. In the first report to show the presence of ESBLs in companion animal *E. coli* isolates, we previously identified SHV-12, CTX-M-14, and CTX-M-15 genes. This study also found a high percentage of resistance to other antimicrobial drug classes, including aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole. This multidrug resistance (MDR) is important, as it highlights the crux of the clinical problem of how best to treat infections caused by ESBL-producing *E. coli*. This finding also contributes to the need to better understand the epidemiology and spread of ESBLs and resistance to other drug classes. The aim of this study was to analyze antimicrobial susceptibility data to determine the prevalence of co-resistance in *E. coli* isolates. From 2003 to 2014, 5970 *E. coli* isolates were obtained from companion animals at the Mathew J Ryan Veterinary Hospital of the University of Pennsylvania, School of Veterinary Medicine. MICs for all *E. coli* isolates were determined using a Negative Combo 31 panel on a MicroScan Walkaway 40 (Dade Behring, Siemens Healthcare Diagnostics, Deerfield, IL). Results were interpreted using Clinical and Laboratory Standards Institute (CLSI) breakpoints. ESBL confirmation was performed using the Etest method; cefotaxime/cefotaxime-clavulanic acid, ceftazidime/ceftazidime-clavulanic acid and cefepime/cefepime-clavulanic acid. One hundred and fifty one (2.5%) *E. coli* isolates were confirmed as ESBL producers; for each of these the ceftazidime MIC was susceptible. 132/151 (87%) were fluoroquinolone (FQ) resistant, 80/132 (61%) were resistant to a FQ and an aminoglycoside; 3 of which (2.3%) were resistant to amikacin. 85/151 (56%) ESBL's were resistant to trimethoprim-sulfamethoxazole (TMS) and 83/151 (54.9%) were also resistant to a FQ: 27/85 (32%) of these ESBL/FQ/TMS resistant isolates were from UTI's. In addition to the MDR strains, high levels of resistance were observed with amoxicillin-clavulanic acid (AUG) 1107/5970, 18.5%), FQ's (1488/5970, 25.7%), FQ/AUG (657/5970, 11%), TMS (1189/5970, 20%), TMS/FQ (948/5970, 16%), TMS/FQ/AUG (462/5970, 7.7%). Amikacin and carbapenem resistance was low at 0.4% and 0.002% respectively. While not labeled for all indications, cefpodoxime resistance was high (1486/5970, 25%). While a carbapenem may be the recommended drug of choice to treat infections caused by ESBL producing *E. coli*, the use of these drugs in veterinary medicine to treat "uncomplicated" infections remains controversial. Oral drug choices are frequently sought for MDR *E. coli* infections and the high rates of resistance observed in this data set should therefore be cause for concern. Accurate identification and confirmation of ESBL's is critical to the proper management of these patients and laboratories are encouraged to pursue this.

Poster 15

A Survey of Oregon Raptors for *Chlamydia psittaci* and the presence of a *Rhabdochlamydia* spp

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Narrative: The Phylum Chlamydiae is a group of obligate intracellular pathogens that infect many animals and humans. Different Chlamydiae also infect a variety of invertebrate species, including molluscs, insects, and protozoans. One zoonotic species within this group is *Chlamydia psittaci*, a pathogen that colonizes a variety of avian species. We used a PCR-based approach to examine captive raptors as a possible reservoir for *C. psittaci* in Oregon. Eighty-two individual raptors from Wildlife Rehabilitation Centers across Oregon were sampled by taking swabs of the choanal and cloacal regions. A set of 16S ribosomal primers were tested on these birds, followed by DNA sequence analysis of any positives to determine the infecting chlamydial species. Three of 82 birds were positive by PCR, with two of these positives leading to successful 16s sequence analysis. One of the animals was positive for *C. psittaci*, supporting the hypothesis that these animals can carry the pathogen. Surprisingly, sequence analysis of the other PCR amplicon demonstrated colonization by a member of the genus *Rhabdochlamydia*. We hypothesize that this bird was colonized by lice that carried a *Rhabdochlamydia* endosymbiont. The presence of large numbers of lice was noted during the clinical evaluation of the bird by the caretakers. This is the first recorded instance of *Rhabdochlamydia* spp. being identified within the United States. These findings are significant because colonization by this insect endosymbiont may possibly lead to false positive results in diagnostic tests for identification of Chlamydia-infected animals.

Poster 16

Prevalence study of *Tritrichomonas foetus* in commercial bulls in The State of Chihuahua Mexico Tested with MagMAX™ Sample Preparation System and VetMAX™-Gold Trich Detection Kit

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Narrative: Bovine trichomoniasis is sexually transmitted by *Tritrichomonas foetus* resulting in significant monetary losses to the cattle industry. *Tritrichomonas foetus* is a flagellated protozoan found in bovines that colonizes in the uterine, vaginal and preputial epithelium, resulting in early embryonic death, abortion, and infertility. Once a bull becomes infected, it remains asymptomatic and carries the disease for its entire life. There is no effective and approved treatment to clear the infection from bulls, and management relies on the elimination of infected bulls from the herd. In the state of Chihuahua Mexico, little or no information is available about the presence of *Tritrichomonas foetus*. Despite good nutrition programs and genetic improvement efforts, some of the ranches are still experiencing a calving rate of only 50-60%. The extremely low calving rates that the State of Chihuahua is experiencing may be due to the lack of proper diagnostic programs and management strategies to address health concerns specific to abortive infectious diseases. Most recently, increased interest in implementing molecular diagnostics by academia and government testing laboratories in Mexico has been increased. Thus the aim of this study was to perform a pilot study in collaboration with the Facultad de Zootectina from the Universidad Autonoma de Chihuahua Mexico with the implementation of the MagMAX™ sample preparation system and the USDA licensed VetMAX™-Gold Trich Detection Kit to investigate the presence of *T. foetus* in the State of Chihuahua. This study resulted in 450 smegma samples being collected from natural service bulls from multiple breeds across the entire state. Results showed a prevalence of 25% *T. foetus* positives. The extremely high prevalence seems to be in agreement with the lack of proper diagnostics for abortive diseases in the state of Chihuahua. Also the results correlate with a the 28% *T. foetus* positive rate reported in Argentina by Perez et al. (2006), where similar beef cattle management practices are performed.

Poster 17

Retrospective analysis of diagnostic virology test data from clinical samples associated with bovine respiratory disease complex

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Narrative: Bovine respiratory disease (BRD) is a major economically significant disease of cattle worldwide. In the U.S.A., BRD is estimated to cost the cattle industry over \$2 billion every year. The disease has a multi-factorial etiology that includes various bacterial and viral pathogens as well as other associated environmental and host risk factors. The major viral pathogens associated with BRD include bovine viral diarrhoea virus (BVDV), bovine herpesvirus 1 (BoHV-1), bovine parainfluenza type 3 virus (BPIV-3) and bovine respiratory syncytial virus (BRSV). In the present study we analyzed retrospective results of diagnostic tests for these four viruses performed on clinical samples submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) from 2009 to 2014. Samples tested for BRD included nasal swabs, respiratory tissues and serum samples submitted to TVMDL from cattle showing respiratory signs. Samples such as swabs and tissues were tested either by virus isolation on cell culture or by PCR. Serum samples were tested for antibodies to these viral agents by virus neutralization assay. Viruses isolated in cell culture from these cases included BVDV, BoHV-1 and BPI-3. The PCR assays detected BVDV, BoHV-1, BPI-3 and BRSV. Viral serology results indicated exposure to BVDV, BoHV-1, BPIV-3 and BRSV in about 50% or more of the animals tested. These data support virus-associated BRD as an ongoing economically significant problem in cattle.

Poster 18

Microbial entities associated with abortion in small ruminants: A retrospective evaluation of 58 cases with emphasis on detection of *Coxiella burnetii* by PCR §

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Narrative: Q fever is a zoonotic disease with abortigenic potential in ruminants. It is caused by the obligate intracellular, gram-negative, bacterium *Coxiella burnetii*. In the current study we performed a retrospective analysis with respect to the etiology of caprine and ovine abortions (and in particular to the diagnosis of *C. burnetii*) on clinical samples submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) from January 2014 to April 2015. Abortion specimens (placenta, fetal tissues, stomach contents and vaginal swabs) from fifty-eight cases (goat, n = 44; sheep, n = 14) submitted to TVMDL were tested by Taqman Real-time PCR targeting the transposase gene of *C. burnetii*. The *C. burnetii* DNA was detected in six caprine cases by PCR. Three of these six PCR positive cases were also confirmed to be positive upon histopathological examination. Besides *C. burnetii*, the clinical specimens from most of the cases were also screened for other abortion-specific microbial agents by the following methods: *Brucella* and *Campylobacter* using culture; *Leptospira* and *Chlamydia* using PCR testing; and viral agents using cell culture and/or PCR. Serology for multiple etiologies was also carried out in some of these cases. Interestingly, *Campylobacter jejuni* was isolated from stomach contents in two cases, and *Chlamydia* PCR was positive on specimens from two of the fifty-four cases. The testing on placental tissue submissions improved the possibility of bacterial diagnosis. No concurrent infection was identified in any of the cases. These data supports the use of PCR based detection as a valuable tool for definitive diagnosis of infectious etiologies associated with abortion, and *C. burnetii* to be the most common cause of abortion in small ruminants.

§ AAVLD Laboratory Staff Travel Awardee

Poster 19

***Ajellomyces capsulatus* (formally *Histoplasma capsulatum*) infection in a Bactrian camel (*Camelus bactrianus*) with granulomatous and ulcerative typhlocolitis**

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Narrative: A 4-year-old, female, Bactrian camel (*Camelus bactrianus*) was presented recumbent, cachexic and anorexic with anemia, hypoproteinemia, hypoalbuminemia, inflammatory leukogram and severe left shift. The patient did not respond to treatments and was euthanatized. Thickening of the cecum and the spiral colon were observed at postmortem examination, along with bicavitary effusion and systemic serous atrophy of fat. Cecal and colonic mucosae were dull and irregular with thick diphtheritic-like membranes and various-sized button ulcers. Microscopic examination revealed multifocal to coalescing, chronic active, granulomatous and ulcerative typhlocolitis. Lamina and submucosal propria were markedly expanded by sheets of epithelioid macrophages with numerous, 2-3 micrometer in diameter, globose, capsulated, yeast-like organisms in the cytoplasm of the macrophages. The organisms were positive for Grocott's methenamine silver stain, and identified as *Ajellomyces capsulatus* (formally *Histoplasma capsulatum*) by 18S ribosomal RNA sequencing. Occurrence of histoplasmosis in camels is rare. The only previous case was an Arabian camel (*Camelus dromedarius*) reported in 1994.

Poster 20

Cerebral oligodendroglioma mimicking intraventricular neoplasia in three dogs

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Narrative: Oligodendroglioma is one of the most common primary central nervous system neoplasms of dogs. It is often diagnosed in older, brachycephalic breeds, and although its typical clinical features and neuroanatomic location have been well described, less common presentations may hinder its diagnosis. We describe 3 cases of canine cerebral oligodendroglioma that clinically and grossly presented as intraventricular tumors. Histologic findings in all cases were typical of oligodendroglioma. Neoplastic cells were uniformly immunoreactive for Olig2 and negative for neuron-specific enolase, neurofilament, and glial fibrillary acidic protein. In addition to the immunopositivity for Olig2, a cluster of morphologically distinct neoplastic cells in one of the cases was immunoreactive for synaptophysin, and the case was diagnosed as an oligodendroglioma with neurocytic differentiation. Based on these findings, oligodendroglioma should be included as a differential diagnosis for intraventricular neoplasia in dogs. Furthermore, oligodendroglioma with ventricular involvement should be differentiated from central neurocytoma by immunohistochemistry.

Poster 21

The uses and limitations of special stains in tissue sections for the diagnosis of small ruminant bacterial abortion.

Alisha Curtis, Karen Sverlow, Santiago Diab

California Animal Health and Food Safety Laboratory, Davis, CA

Narrative: Special stains, such as Gimenez, Gram and Steiner are frequently used to aid in the characterization of bacteria associated with a diverse range of infections in diagnostic veterinary pathology. These three stains are particularly helpful in the diagnosis of sheep and goat bacterial abortion, including infections by *Coxiella burnetii* (Q fever), *Chlamydia* sp., *Brucella* sp., *Campylobacter* sp., and *Yersinia* sp., which are among the most common causes of infectious abortion in small ruminants and often pose a diagnostic challenge, as they can present with similar gross and microscopic lesions in the placenta. They all cause severe necrotizing placentitis that may have a few or abundant intralesional, intracellular small bacteria that cannot be differentiated based on the examination of histology slides stained routinely with hematoxylin and eosin. Gimenez stain is routinely used to highlight the presence of *Coxiella* and *Chlamydia*, but *Brucella*, *Campylobacter* and *Yersinia*, among other bacteria, can also stain positively; therefore bacterial morphology along with the use of Gram and Steiner stains are important to narrow down the possible etiology/ies. *Coxiella* should appear as small, pleomorphic, thin rod shaped structures, while chlamydial elementary bodies are uniformly small and round when stained with Gimenez. *Coxiella* and *Chlamydia* will not stain with Gram, and will variably stain with Steiner. Both *Coxiella* and *Chlamydia* are found mainly within trophoblasts but also extracellularly. *Brucella* is an intracellular, small, Gram negative coccobacilli found mainly within placental trophoblasts and will also stain with Gimenez and Steiner. *Campylobacter* sp. are curved Gram negative bacilli that are usually found extracellularly or within the lumen of small placental capillaries (bacterial emboli); Steiner and Gimenez stain are often preferred over a Gram stain for the detection of *Campylobacter* sp. in tissue sections, especially if microorganisms are sparse. *Yersinia* sp. are Gram negative coccobacilli that may be found within the cytoplasm of trophoblasts and also extracellularly and it will also stain with Steiner and Gimenez. It is important to note that, occasionally, even with the use of special stains, bacterial morphology may still not be appreciated clearly in tissue sections. *Coxiella* and *Chlamydia* cause lesions mainly in the placenta, whereas *Brucella*, *Campylobacter* and *Yersinia* can cause lesions in the placenta and fetal tissues. In summary, based on the staining properties of bacteria using a combination Gimenez, Gram and Steiner special stains, bacterial morphology and location within the placental lesion, it is possible to narrow down the list of differential diagnoses for small ruminant bacterial abortion and placentitis. However, confirmation of the specific etiology must rely on the use of more specific ancillary laboratory tests, such as bacterial cultures, immunohistochemistry, PCR, and/or direct fluorescent antibody tests.

Poster 22

Pleomorphic adenoma of the submandibular salivary gland in a horse

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Narrative: Pleomorphic adenoma (or “mixed tumor”) is the most common, benign, salivary gland tumor of humans, but is rarely diagnosed in animals. We describe a submandibular neoplasm in a 28-year-old, female Paint horse that was histologically compatible with pleomorphic adenoma of the salivary gland of humans. Information regarding the size and aspect of the mass in situ was unavailable. Thirty small and irregularly shaped, formalin-fixed pieces of tissue removed from a subcutaneous mass in the left caudal submandibular region were submitted. Grossly, most pieces were unencapsulated and mottled tan (firm)/dark red (soft and crumbly); a few pieces had small fragments of bony laminae and one piece had overlying intact haired skin. Twenty one sections were examined histologically. The neoplasm was composed of a variably dense population of neoplastic epithelial cells (epithelial component) supported by an abundant myxoid/mucoid, and occasionally hyalinized, stroma that was populated with a moderate number of spindloid cells (myoepithelial component). In some sections of the tumor, the epithelial component was predominant, whereas in other sections, the bulk of the mass was composed of stroma and myoepithelial cells. Neoplastic epithelial cells were closely packed, arranged in duct-like structures, nests, or sheets, were cuboidal or basaloid type and had moderate amount of poorly distinct, basophilic cytoplasm. Nuclei were round to oval, hyperchromatic, with finely stippled or condensed chromatin and none, one or two nucleoli. The myoepithelial cells within the stroma had moderate amount of distinct, basophilic cytoplasm; nuclei were large, oval, had stippled chromatin and one or two nucleoli. Anisocytosis and anisokaryosis in both cell populations was mild. Mitoses averaged approximately 1 per 10 hpf. No fibrous capsule was observed in any of the twenty one sections examined. Occasionally, thin, well differentiated, bony trabeculae were entrapped and focally invaded by the neoplasm. The epithelial component was diffusely strongly positive for pancytokeratin and negative for vimentin, smooth muscle actin, S-100 and GFAP immunohistochemistry. The myoepithelial component was diffusely strongly positive for vimentin and smooth muscle actin, only occasionally and weakly positive for S-100 and diffusely negative for pancytokeratin and GFAP. These findings were compatible with the pleomorphic adenoma with osseous differentiation of the salivary gland of humans. Although a benign tumor of people, pleomorphic adenoma can cause problems in clinical management due to its tendency to recur and a small risk of malignant transformation. In this horse, no follow-up information was available, for which the post-surgical clinical behaviour of this tumor is unknown.

Poster 23

Polyserositis, Bronchopneumonia, Colitis, Exudative Dermatitis and Cobalt Deficiency in a Piglet in Utah

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Narrative: Mixed infections involving 2 pathogens are not uncommon in domestic animal species. However, it is unusual to diagnose 4 infections caused by 4 different bacterial species in one animal, especially when it is on medicated feed. This type of finding could be suggestive of immune compromise and there are many areas in Utah in which livestock copper and/or selenium deficiencies exist. However, our detection of a cobalt deficiency in an animal is much less frequent. An 8.2 kg 8 week-old mixed-breed female piglet from a small commercial swine operation was submitted to the Central Utah Branch of the Utah Veterinary Diagnostic Lab (UVDL) for necropsy in the fall of 2014 with a 2 week history of weight loss. Gross lesions included widespread erythema, alopecia and crusting of the skin, fibrin deposition on the serosal surfaces of most abdominal organs, consolidation of the right and left cranial lung lobes, and reddening of the intestinal mucosa. Samples of lung, spleen, liver, colon, and skin were cultured aerobically. Histologic lesions included purulent bronchopneumonia, fibrinous peritonitis and epicarditis, necrotizing colitis, and purulent epidermitis/dermatitis. Bacterial isolates were *Streptococcus suis* (liver and spleen), *Pasteurella multocida* (lung), *Staphylococcus hyicus* (skin), and *Salmonella Anatum* (colon). In an effort to find an underlying cause of immunosuppression in this animal, a liver mineral analysis was performed. The only abnormality was a low cobalt, which can be indicative of cobalamine deficiency. Through the case investigation it was found that the pigs on the farm regularly ingest coal, which is high in carbon. It is possible that the coal acted similarly to activated charcoal and bound the cobalamine. The coal may have also impaired absorption of other water soluble vitamins or the antibiotics that were in the feed resulting in increased susceptibility to infection.

Poster 24

Bovine “winter dysentery” outbreak during the summer in southern California

Santiago Diab

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Narrative: Bovine coronavirus (BoCV), a *Betacoronavirus* 1, has been associated with acute, often hemorrhagic, diarrhea in beef and dairy cattle almost exclusively during the cold months of the year; therefore the disease is commonly known as “winter dysentery”. We report a large outbreak of “winter dysentery” in dairy cattle that occurred during early August in Southern California (San Diego County) with an average daily high temperature of 27° Celsius and average low temperature of 20° Celsius during this month. Fifty out of 500 Holstein cows presented with lethargy, decreased appetite and hemorrhagic diarrhea during a 2-week period. Four cows died and two were submitted for necropsy. Grossly, both cows had distended spiral and descending colon and rectum, which were filled with abundant hemorrhagic, finely chopped, moist digesta admixed with scant amount of clotted blood. The mucosa of the colon and rectum was diffusely dark red. Histologically, there was severe, hemorrhagic, necrotizing colitis and proctitis with crypt abscesses, necrosis of superficial epithelium, and crypt blunting and fusion. Colon and rectum samples from both cows were positive for BoCV by direct fluorescent antibody test, immunohistochemistry, rtPCR and Coronavirus-like viral particles were detected by direct electron microscopy in fecal samples. Tests for *Salmonella* (rtPCR on rectum and feces), bovine viral diarrhea virus (rtPCR on spleen and immunohistochemistry on rectum and colon sections), *Yersinia* (cold enrichment culture from feces), Rotavirus (antigen ELISA from feces) and anticoagulant screen (liver) were all negative and no parasite eggs were observed on fecal floats in both cows. “Winter dysentery” should be considered a differential diagnosis for hemorrhagic diarrhea in cattle even during the summer time. Additional studies are necessary to better characterize the viral strains of BoCV associated with “winter dysentery” during the warm, summer months and determine if there are any genetic differences among the BoCV strains associated with “winter dysentery” that may potentially make this a year-round disease of beef and dairy cattle.

Poster 25

Characterization of the local inflammatory response in the reproductive tract of the equine arteritis virus carrier stallion *

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Narrative: Equine arteritis virus (EAV) can establish persistent infection (carrier state) in 30-70% of sexually mature colts and stallions following natural infection. Persistently infected stallions will continuously shed the virus in their semen for a variable and frequently extended period of time. Thus, carrier stallions play a major epidemiological role and contribute to the maintenance of the virus in horse populations between breeding seasons. The pathogenesis and factors associated with persistent infection in the reproductive tract of the stallion are poorly understood. The objective of this study was to characterize the histopathological lesions and identify specific inflammatory infiltrates in the reproductive tract of long-term carrier stallions. Diverse tissues from the reproductive tract of long-term carrier (n=3) and previously infected stallions that had stopped shedding (n=3) were assessed for histopathological lesions and specific cellular infiltrates were identified immunohistochemically (IHC) using a panel of differentiation (CD) markers. Tissues analyzed included testis, epididymis, ductus deferens, and accessory sex glands (ampulla, vesicular glands, prostate, bulbourethral glands). Both carrier stallions and those that had stopped shedding exhibited similar histological lesions characterized by minimal to mild, focal to multifocal lymphoplasmacytic inflammation in all tissues examined, with perivascular cuffs and occasional siderophages. The ampulla exhibited the most severe histological lesions. Specific immunostaining was performed on frozen sections from the ampulla, and cellular infiltrates were semiquantitatively scored and normalized using two seronegative and age-matched stallions. Extensive CD3⁺ CD8⁺ T lymphocyte infiltration was identified in both carrier stallions and stallions that stopped shedding. In contrast, the inflammatory infiltrates in carrier stallions additionally comprised CD2⁺ (low to high), CD4⁺ (low to moderate), and CD5⁺ (moderate to high) T lymphocytes; CD25⁺ T regulatory lymphocytes (low to moderate); and CD21⁺ B lymphocytes (minimal to moderate). These findings were shown to be statistically significant (Kruskal-Wallis test, p-value<0.05). Even though the presence of activated macrophages in the inflammatory infiltrates was rare, there was minimal to moderate infiltration of CD83⁺ dendritic cells particularly in carrier stallions, but no statistically significant differences were observed between both groups. In conclusion, CD8⁺ T lymphocyte infiltration seems to be a common mediator of the anti-viral inflammatory response in reproductive tract tissues from infected stallions. Also, the presence of CD4⁺ T lymphocytes, CD25⁺ T regulatory lymphocytes, and CD83⁺ dendritic cells in long-term carrier stallions might be associated with the immunomodulatory mechanisms that contribute to maintenance of persistent infection in stallions.

* Graduate Student Poster Presentation Award Applicant

Poster 26

“Atypical” Chronic Wasting Disease in *PRNP* Genotype 225FF Mule Deer

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Narrative: We compared mule deer (*Odocoileus hemionus*) of two different *PRNP* genotypes (225SS, 225FF) for susceptibility to chronic wasting disease (CWD) in the face of environmental exposure to infectivity. All three 225SS deer had immunohistochemistry (IHC)-positive tonsil biopsies by 710 days post exposure (dpe), developed classical clinical signs 723-1,200 dpe, and showed postmortem gross and microscopic pathology, enzyme-linked immunosorbent assay (ELISA) results, and IHC staining typical of prion disease in mule deer. In contrast, although all three 225FF deer also became infected, the two individuals surviving >720 dpe were consistently biopsy negative, developed more subtle clinical signs of chronic wasting disease, and died 924 or 1,783 dpe. 225FF deer were ELISA “suspect” postmortem but showed negative or equivocal IHC staining of lymphoid tissues; both clinically-affected 225FF deer had spongiform encephalopathy in the absence of IHC staining in brain tissue. The experimental cases resembled three cases encountered among five additional captive 225FF deer that were not part of our experiment but also succumbed to chronic wasting disease. In all of these cases of CWD in 225FF mule deer, clinical presentation was atypical, as were the IHC staining properties of the associated PRP^{CWD}. Our findings suggest that the current gold standard of IHC testing for diagnosis of CWD is insensitive for detection of disease in 225FF deer.

Poster 27

Organizing pneumonia and *Pneumocystis murina* in an experimentally naive *Rag1*-deficient mouse *

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Narrative: Organizing pneumonia(OP) occurs as the result of a final common pathway resulting from various types of lung injury. In cases where no underlying etiology can be detected, OP is referred to as Cryptogenic Organizing Pneumonia (COP). OP is characterized by buds of organizing granulation tissue within distal pulmonary airways. Herein we report a case of an experimentally naive female *Rag1*-deficient mouse that developed organizing pneumonia in combination with *Pneumocystis murina* infection. Histologic examination of the lungs demonstrated multifocal granulomatous inflammation combined with frequent *Pneumocystis murina* organisms. In addition, nascent and organizing fibroplasia with plump fibroblasts and scant matrix frequently formed polypoid projections into the lumina of distal respiratory bronchioles and alveoli. Organizing pneumonia has been reported in people in combination with *Pneumocystis jirovecii* infection. This is the first reported case of organizing pneumonia in a mouse with *Pneumocystis murina*.

* Graduate Student Poster Presentation Award Applicant

Poster 28

Histological techniques used to identify amyloid beta plaques in the cerebrum of a Ring-Tailed Lemur

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Narrative: Alzheimer disease findings in animals are similar to humans. In this case a 35 year old, captive Ring-tailed Lemur, who was recently observed to be sluggish, inappetent, and progressively losing weight, was found in sternal recumbency one morning with dull mentation. Due to poor quality of life, the lemur was euthanized and sent to CAHFS for necropsy. Gross post-mortem examination was fairly unremarkable, with obvious loss of subcutaneous and cardiac adipose tissue, and presence of variably amounts of bile in the stomach and proximal small intestine. Portions of all organs were submitted for routine hematoxylin and eosin Histopathologic evaluation. The primary lesions were discovered in the cerebrum, especially the cortex. Variably-sized, pale eosinophilic, acellular plaques were scattered throughout the cortex and into the white matter, and were primarily associated with eosinophilic matrix within and radiating from small blood vessels. To better determine the distribution and microarchitecture of the plaques, a Sevier-Munger Modification of Bielschowsky Method or “Alzheimer stain” was performed. Additionally, Congo Red for amyloid, and immunohistochemistry for beta amyloid were performed to further characterize the plaques. Using a polarizing microscope, the amyloid fibrils show an apple green birefringence. Only some of the plaques (vessel-associated mainly) were congophilic, however all plaques were strongly positive by beta amyloid immunohistochemistry stain. Beta amyloid plaques are a key feature in the brains of people with Alzheimer disease. The Alzheimer stain, which uses an ammoniacal silver solution, was more successful. The Alzheimer stain best demonstrated the full distribution of the plaques and provided a better view of their structure. Electron microscopy was also used to better characterize the variation in amyloid fibrils within various plaques, and provided insight as to why there was variable congo red staining.

Poster 29

Equine *Leptospira*-associated abortions in Northern California

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Narrative: *Leptospira interrogans* is a highly contagious bacterium often leading to infections in geographic regions with heavy rainfall and flooding. In horses, *L. interrogans* is often implicated as a cause of recurrent uveitis. In December, 2014 through February, 2015, two late-term aborted equine fetuses and one neonate from three different farms were submitted to the California Animal Health and Food Safety Laboratory System (CAHFS), University of California, Davis. One farm had two previous cases of late-term abortion but necropsies were not performed in those cases. Animals had mild to moderately enlarged, mottled livers, and the neonate demonstrated subcutaneous edema of the thorax and elbow regions, possibly associated with dystocia. *Leptospira*-like organisms were detected by immunofluorescent antibody testing on fresh kidney tissue impression smears as well as Steiner silver staining and immunohistochemical staining of formalin-fixed kidney sections. A PCR assay based on the *lipL32* gene was utilized to confirm the presence of pathogenic *Leptospira sp.* and was strongly positive in all three cases. Serology on fetal fluid demonstrated a titer of >1:3200 to *L. Interrogans* serovar Pomona in each of the foals as well as the dam of one animal. *Leptospira* culture was attempted from kidney tissues of two of the submitted fetuses; however, no viable organisms could be recovered for further molecular characterization. *Leptospira*-associated abortion is a rarely diagnosed condition in horses of this region. A review of cases from the previous eight years indicated that no *Leptospira sp.*-associated abortions had been identified in equine fetuses. During the first three weeks of December 2014, the region received 11-18 inches of rain, resulting in localized flooding. Each of these farms was located near water systems in which severe flooding was reported, which may have presented a route of exposure in these animals. Abortion in horses has been associated with *L. Interrogans* serovar Pomona type kennewicki, which has also been identified in a variety of wildlife species including skunk, raccoon, deer, and opossum. Even in non-endemic regions, *L. interrogans* represents a zoonotic health concern and cases such as these highlight the need for enhanced surveillance.

Poster 30

Spontaneous Necrotizing Esophagitis in a Sprague Dawley Rat *

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Narrative: Spontaneous esophageal lesions in rats are rare, with most esophageal pathology associated with oral gavage procedures. A 2 year old female Sprague Dawley rat with no history of oral gavage presented for dyspnea, lethargy and a hunched posture. Due to poor prognosis, the animal was euthanized and a diagnostic necropsy was performed. On gross exam, the esophageal wall was thickened but no perforation was present. Histologic examination revealed severe focally extensive transmural esophageal necrosis, aspiration pneumonia and fibrinosuppurative mediastinitis. The root cause of morbidity could not be determined on gross or histologic evaluation. Therefore, the possibility of spontaneous disease must be considered. A rare syndrome of acute esophageal necrosis has been described in people, however, this case represents the first report of spontaneous esophageal necrosis in a rat.

* Graduate Student Poster Presentation Award Applicant

Poster 31

Evaluation of Commercial Rapid PED, TGE, and Rota Ag Test Kits for Detecting Virus Pathogens in Porcine Feces

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Narrative: Porcine rotavirus, porcine epidemic diarrhea virus (PEDV), and transmissible gastroenteritis virus (TGEV) are major viral pathogens causing diarrhea in swine. A rapid and easy-to-use immunochromatographic assay to detect PEDV, TGEV and rotavirus on swine farms can help in the timely management of an outbreak and to restrict the spread of disease and reduce losses. In this study, three commercial rapid Ag Test Kits respectively for PED, Rota, and TGE (BioNote, Inc., South Korea) were evaluated in comparison to the virus-specific real-time RT-PCRs (rRT-PCR) for detecting the respective viral pathogens. Cell culture isolates of PEDV, TGEV, porcine rotavirus A, and porcine deltacoronavirus (PDCoV), and clinical fecal samples respectively positive for PEDV, TGEV, rotavirus A, rotavirus B, rotavirus C, or PDCoV as confirmed by virus-specific rRT-PCR, were tested by three Ag Test Kits. The PED, TGE and Rota Ag Test Kits were highly specific and did not cross react with other viral pathogens evaluated in this study. The PED Ag Test Kit detected both US prototype and INDEL-variant PEDV strains. The Rota Ag Test Kit specifically detected rotavirus A, but not rotavirus B, rotavirus C, PEDV, TGEV or PDCoV. The PEDV, TGEV, and rotavirus A cell culture isolates with known infectious titers were 10-fold serially diluted in liquid fecal sample and tested by three Ag Test Kits. The limits of detection of the Ag Test Kits were 10^7 TCID₅₀/ml of PEDV, 10^7 TCID₅₀/ml of TGEV, and 10^6 TCID₅₀/ml of Rotavirus A, respectively. Diagnostic performance of PED, TGE, and Rota Ag Test Kits was further evaluated on clinical fecal samples and compared to the virus-specific rRT-PCR results. Based on testing 100 feces, the overall sensitivity, specificity and accuracy of the PED Ag Test Kit were 68.2%, 100% and 72%, respectively, compared to the N gene-based PEDV rRT-PCR. The sensitivity of PED Ag Test Kit was 100% for feces with PEDV rRT-PCR cycle threshold (Ct) <21 and 78.6% for feces with Ct 21-24. Based on testing 105 feces, the sensitivity, specificity and accuracy of the Rota Ag Test Kit were 73.2%, 82.6% and 75.2%, respectively, in comparison to the rotavirus A rRT-PCR. The sensitivity of Rota Ag Test Kit was 95.8% for feces with rotavirus A rRT-PCR Ct <24 and 85% for feces with Ct 24-28. Rotavirus B and rotavirus C infection status did not affect the relationship between rotavirus A Ct values and Rota Ag Test results. There were only 10 TGEV positive fecal samples accessible to us with Ct value range from 17.3 to 33.6. Among them, 80% were positive by the TGE Ag Test Kit. While the differences in sensitivity between the rRT-PCRs and the Ag Test technologies were evident in this study, overall, the PED, TGE and Rota Ag Test Kits provided comparable results in samples where higher concentrations of the virus were present. In addition, these Ag Test assays are rapid (10-15 min), easy-to-perform, and can be used on farm without a need for special equipment or facility.

AAVLD Trainee Travel Awardee

Poster 32

Evaluation of a rapid immunochromatographic antigen test for bovine pathogens in feces §

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Narrative: A calf enteric, rapid immunochromatographic, antigen test (Anigen Rapid BoviD-4 Ag Test Kit; BioNote, INC) is available for the detection of four common calf fecal pathogens: *Cryptosporidium* sp. (Crypto), Bovine Coronavirus (BCV), Bovine Rotavirus (Rota) and *Escherichia coli* K99. The cassette based test can be conducted in 10 minutes. Fecal samples from diagnostic cases submitted to the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL) were used to determine the sensitivity, specificity, and level of detection (LoD) of the rapid test. To determine the LoD, ten laboratory fecal samples with a range of pathogen concentrations were collected for each pathogen except for *E. coli* K99 as no samples were available. Serial dilutions in PBS at 1:10, 1:100, 1:1000, and 1:10,000 were created from each of the above fecal samples. For each pathogen, the 10 fecal samples and associated dilutions were tested by the rapid, calf enteric test kit and the applicable laboratory reference tests. Reference methods were polymerase chain reaction (PCR) with a cut-off value of 40 cycles (CT) for Rota and BCV and Fluorescent Antibody (FA) for Crypto. The rapid test was able to detect antigens in the samples with higher viral load (lower CT) for Rota and Corona but could not detect antigens in the more diluted samples. The LoD was approximately CT 20 for Rota and CT 25 for BCV. Similarly, 100% of strongly positive samples for Crypto by FA were positive with the calf enteric kit, while 41% of weakly positive samples by FA were positive using the calf enteric kit. To evaluate the relative diagnostic sensitivity and specificity of the rapid calf enteric kit, 60 known positives and 60 known negatives for each pathogen were used. The diagnostic sensitivity was 41%, 37% and 81% for Rota, BCV and Crypto, respectively, while the corresponding diagnostic specificity was 98%, 99% and 87%. Sensitivity for Rota and BCV appeared low until the LoD information and the clinical relevance of samples (i.e. lower CTs) was taken into account. Rota's LoD was around CT 20. If we consider the sample CTs ≤ 21.39 (N=33), Rota sensitivity was 90.9%. Similarly, BCV's LoD was around CT 25. In samples with CTs ≤ 25.84 (N=22), BCV sensitivity was 100%. The calf enteric kit performed well for detection of Rota, BCV and Crypto antigens in acutely infected, clinically relevant cases. This test can be a useful tool to quickly diagnose acute calf diarrhea in the field. Reference tests are appropriate if a lower amount of pathogen is expected such as monitoring shedding in healthy cattle or in the environment.

§ AAVLD Laboratory Staff Travel Awardee

Poster 33

Evaluation of Three In-clinic Serological Tests for Specific Detection of FeLV Antigen in Cats

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Narrative: Feline leukemia virus (FeLV) is a highly contagious virus that is spread primarily via saliva among cats in casual close contact such as sharing food and water as well as mutual grooming. FeLV infection can be a life-threatening condition with approximately one-third of cats developing progressive infections resulting in FeLV-related disease syndromes and death within 3 years. During the initial stage of infection, the concentration of FeLV antigen in blood is approximately twenty-fold lower than in later stages. Test sensitivity is a key factor during the initial stage of FeLV infection. Use of the tests with the highest sensitivity will allow identification of cats early in the course of infection so supportive care and strategies to limit transmission of the virus to casual-contact naïve cats can be implemented. Random samples collected from IDEXX reference laboratories and shelters across the country were tested for the presence of FeLV antigen using two commercial ELISA test kits, PetChek[®] FeLV (IDEXX Laboratories, Inc.) which included both screening and confirmatory protocols and ViraCHEK[®] FeLV (Zoetis, Inc.). There were 82 FeLV-positive samples and 38 FeLV-negative samples identified by the screen. The samples were then tested using three in-clinic tests - IDEXX[®] SNAP[®] Feline Triple[®] Test, Abaxis[®] VetScan[®] Feline FeLV/FIV Rapid Test and Zoetis[®] Witness[®] FeLV-FIV Test following manufacturers' protocols. In-clinic test results were compared to the PetChek[®] and ViraCHEK[®] ELISA results for calculation of sensitivity and specificity. The SNAP[®] Feline Triple[®] Test had higher sensitivity (96.3%; CI-95%:89.2-99.1%) for FeLV antigen detection compared to 75.6% (CI-95%:65.2-83.6%) for the VetScan[®] Feline FeLV/FIV Rapid Test and 82.9% (CI-95%:73.2%-89.6%) for the Witness[®] FeLV-FIV Test. Specificity was 100% for all tests with this sample set. Sensitive screening tests are important in the management and control of this contagious retrovirus infection in cats. Early detection and confirmation of positive screening results, as recommended by American Association of Feline Practitioners, can be used to design programs to lessen the potential for FeLV spread to uninfected cats in the same household or in shelter environment.

Poster 34

Comparative Evaluation of In-clinic Tests for Antibodies to *Anaplasma* and *Ehrlichia* Species in Dogs

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Narrative: *Anaplasma phagocytophilum* is the causative agent of granulocytic anaplasmosis in dogs. A related but distinct bacterium, *A. platys*, also infects dogs and causes infectious cyclic thrombocytopenia. Several *Ehrlichia* species also infect dogs and cause disease including *E. canis* (monocytic ehrlichiosis) and *E. ewingii* (granulocytic ehrlichiosis). Recent large-scale surveys of dogs revealed higher seroprevalence rates for *E. ewingii* (5.1%) and *E. chaffeensis* (1.8%) than for *E. canis* (0.8%) in the eastern half of the United States, especially in the southeastern and south-central regions.^{1,2} Sensitive detection of tick-borne diseases including co- and multiple infections in dogs is diagnostically important for veterinarians and epidemiologically important for public health surveillance. The SNAP[®] 4Dx[®] Plus Test (IDEXX Laboratories, Inc.) identifies exposure to or infection with multiple vector-borne pathogens in a single assay. Recently, two new tests the VetScan[®] Canine *Anaplasma* and *Ehrlichia* Rapid Tests were launched by Abaxis, Inc. In the present study, we evaluated the performance of all three test kits using samples obtained from commercial reference labs and clinics in endemic regions of the US. A total of 234 samples were included in the *Anaplasma* test comparison and 299 samples in the *Ehrlichia* test comparison. Samples were characterized by *A. phagocytophilum* IFA, *E. canis* IFA, or species-specific ELISAs for *A. platys*, *E. chaffeensis* or *E. ewingii*.² Samples were evaluated as per manufacturers' instructions for each test kit. By testing the same sample set, the VetScan *Anaplasma* Test had a very low sensitivity for *A. phagocytophilum*, 29.9% compared to 92.0% for 4Dx Plus. *A. platys* sensitivity was 68.1% for VetScan *Anaplasma* Test compared to 89.4% for SNAP 4Dx Plus. The VetScan *Ehrlichia* Test and SNAP 4Dx Plus had sensitivities of 93% and 100% for *E. canis*, 41% and 69% for *E. chaffeensis*, and 70% and 96% for *E. ewingii*, respectively. The specificity for all three test kits ranged from 83-100%. Detection of antibodies to *E. ewingii* and *E. chaffeensis* is important in the United States, as recent evidence indicates that infections with these *Ehrlichia* spp. is much more common than *E. canis* infections. Based upon the comparative results derived from this study, the VetScan *Anaplasma* and *Ehrlichia* Tests will miss a substantial number of *Anaplasma* and ehrlichial infections detected by SNAP[®] 4Dx Plus. ¹Beall et al. Parasite and Vectors. 2012, 4:29 ²Quorllo BA et al. Infect Ecol Epidemiol. 2014, 4:24699

Poster 35

Evaluation of an improved commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay in cattle in Australia

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Narrative: A commercially available *Anaplasma* species antibody competitive enzyme-linked immunosorbent assay (cELISA) was recently modified by replacing the recombinant major surface protein 5 (rMSP5)-maltose binding protein (MBP) fusion protein with a rMSP5-glutathione S-transferase (GST) fusion protein, improving the diagnostic performance (1). The current study evaluated the rMSP5-GST cELISA for the serological detection of *Anaplasma marginale* and *Anaplasma centrale* antibodies in cattle in Australia. The rMSP5-GST cELISA was performed on 275 *Anaplasma*-negative sera with diagnostic specificity of 98.5%, based on the established cut-off of 30% Inhibition. The commercially available rMSP5-MBP cELISA, when tested with the same set of sera, had a diagnostic specificity of 71.6%. The *Anaplasma*-negative sera used for evaluation were obtained from cattle sourced from an *A. marginale* non-endemic region, where *Rhipicephalus (Boophilus) microplus*, the only tick vector of *A. marginale* in Australia, is absent. In addition, these cattle had no previous *A. centrale* vaccination history. Both cELISAs had a diagnostic sensitivity of 100% when tested on 202 experimentally infected *Anaplasma*-positive sera (96 *A. marginale*-positive and 106 *A. centrale*-positive), defined by *Anaplasma* real-time polymerase chain reaction. This study demonstrates that the rMSP5-GST cELISA has an improved diagnostic specificity and comparable diagnostic sensitivity compared with the commercially available rMSP5-MBP cELISA. The rMSP5-GST cELISA will be a useful serodiagnostic tool for the detection of antibodies to *A. marginale* and *A. centrale* in cattle in Australia in epidemiological studies and for disease or disease-free certification. a *Anaplasma* Antibody Test Kit, cELISA; VMRD Inc., Pullman, WA, USA. 1. Chung C, et al. Improved diagnostic performance of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5-glutathione S-transferase fusion protein as antigen. *Journal of Veterinary Diagnostic Investigation*. 2014;26(1):61-71.

Poster 36

Using veterinary diagnostic laboratories in the Vet-LIRN network to investigate canine diagnostic samples to understand adverse event reports following jerky pet treat ingestion

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Narrative: Since 2007, FDA has received over 5,000 reports of pet illnesses potentially associated with the consumption of jerky pet treats (JPT). FDA reviews medical records for reported cases when available and instituted owner dietary history interviews in order to better understand the role of JPT in the dog's diet. A specific cause of the reported illnesses has not been identified. Approximately 60% of reported illnesses are gastrointestinal and/or liver disease, 30% renal and/or urinary disease, and 10% other types of disease. About 5-6% of all reports suggest Fanconi Syndrome (FS), a broad defect in proximal renal tubular reabsorption. Affected animals show nonspecific clinical signs and are frequently recognized due to the presence of glucosuria despite normoglycemia. FS in dogs can be genetic in origin (primarily the Basenji and Labrador Retriever) or acquired (any breed, many causes including toxicant exposure). Because acquired FS is believed to be rare in pets, in 2012, FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) began collaborating with veterinarians and its nationwide network of diagnostic laboratories to further investigate the potential association between a variety of reported canine clinical illnesses and JPT consumption. In addition to the testing previously conducted, as of January 2015, Vet-LIRN coordinated diagnostic testing for JPT cases, including antemortem and postmortem tests such as fecal culture, toxicology screens (including heavy metals), leptospirosis and Lyme disease serology, immunohistochemistry, and electron microscopy. Vet-LIRN coordinates repeat testing of symptomatic FS dogs testing positive with the urine Fanconi panel performed by the University of Pennsylvania's PennGen Metabolics Genetics Laboratory. Repeat urine testing is performed approximately 2 months after an initial PennGen FS positive result, at which time ~70% of dogs continue to test PennGen FS positive. Vet-LIRN continues to monitor FS positive dogs to see how long markers of FS remain in the urine after JPT are withdrawn. As of February 2015, Vet-LIRN coordinated necropsies for 75 cases of various illness types involving dog deaths, which owners reported as being potentially associated with JPT consumption. The actual cause of death in over half of these cases was determined to be due to other causes such as widespread cancer, Cushing's disease, mushroom toxicity, parvovirus enteritis, bacterial meningitis, abscess, pneumonia, cardiac lesions, infarcts, or internal bleeding secondary to trauma. Collectively, Vet-LIRN collaborated with 20 of its 36 network laboratories for the JPT case diagnostic sample testing. Vet-LIRN's ability to leverage the resources of network veterinary diagnostic laboratories to investigate consumer adverse event reports provides data previously unavailable to the FDA, thereby increasing the government's ability to identify emerging threats.

Poster 37

Reproductive Failure in Cattle Caused by Locoweed Ingestion

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Narrative: Locoweed intoxication (also known as locoism) is one of the most common plant intoxications in the American Southwest. It is the result of livestock chronically ingesting certain species of the plant genera *Astragalus* or *Oxytropis*. The definitive identification of *Astragalus* and *Oxytropis* species is best performed by plant experts with plants that are producing buds, flowers or fruits. The species of *Astragalus* and *Oxytropis* that cause locoism produce the indolizidine alkaloid swainsonine. However, not all species of *Astragalus* and *Oxytropis* produce swainsonine. It has recently been determined that similar to the ergot alkaloids responsible for ergotism and fescue toxicity, swainsonine production in *Astragalus* and *Oxytropis* is associated with infection of the plant by a fungal endophyte. The fungal endophyte infecting *Astragalus* and *Oxytropis* species of plants is *Undifilum oxytropis*. Swainsonine inhibits lysosomal α - mannosidase and Golgi mannosidase II resulting in acquired α - mannosidosis. Locoism in cattle manifests as four conditions. The most recognizable manifestation of locoism is the classical neurological disease. Chronic ingestion of locoweeds by cattle can also manifest as weight loss and deteriorating body condition. Cattle with high altitude disease that ingest locoweeds also exhibit worsening congestive heart failure. Perhaps one of the more devastating manifestations of locoweed intoxication is reproductive failure, which can manifest as abortions, neonatal loss, hydrops and congenital abnormalities including limb deformities. In the spring of 2015, we received cases of reproductive failure in cattle from multiple herds that were ingesting locoweeds. There was up to fifty percent calf loss in the herds due to locoism. Most of the calf loss was due to abortion of late gestation fetuses and birth of weak and mentally abnormal calves that quickly died after birth. There were lesser numbers of abortions earlier in gestation as well as cows that lost weight, were mentally abnormal and rare cows had hydrops. There were no reports of calves with congenital abnormalities. The calves that were necropsied had few gross lesions with equivocal enlargement of the thyroid gland in sporadic calves. Microscopically, the most consistent lesions were cytoplasmic vacuolization of the neurons that was most visible in Purkinje cells of the cerebellum and neurons of the brainstem nuclei. In addition to cytoplasmic vacuolization in neurons, cytoplasmic vacuolization was consistently present in hepatocytes and renal tubular epithelial cells with occasional cytoplasmic vacuolization in thyroid epithelial cells and chorionic epithelium of the placenta. The microscopic lesions and clinical history, including the history of cattle ingesting locoweeds, were diagnostic of reproductive failure due to chronic ingestion of locoweed.

Poster 38

Quantitation of Ergovaline in Bovine Serum

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Narrative: The detection of ergovaline is essential in determining extent of exposure of grazing mammals after ingestion of endophyte-infected (*Neotyphodium spp.*) forage. Ergovaline is the most prevalent of all ergopeptine alkaloids in grasses that are prone to endophyte infection such as perennial ryegrass and tall fescue. Levels of ergovaline analyzed in serum will allow for an enhanced interpretation of exposure on an individual animal basis. Previous methods allowed for the quantitation of alkaloids in endophyte-infected tall fescue with storage and transport considerations currently being a focus in our lab. The purpose of this study is to launch a technique for the detection of ergovaline, at low ppb levels, for definitive interpretation of ergovaline exposure. Bovine serum was spiked with ergovaline and ergotamine (IS) standard solutions, in varying concentrations (1-250 ppb or ng/mL), to simulate naturally incurred serum samples. Proteins are precipitated from serum samples, using optimized organic solvents (acetonitrile and methanol), to reduce matrix effects and increase analytical column longevity. Samples are then centrifuged (16,000g) and the supernatant is then chilled (4°C for 5 minutes) to continue deproteinization followed by additional centrifugation. The resulting supernatant sample is evaporated to dryness using nitrogen in a dry heat block (60°C). The residue is re-dissolved with mobile phase solvent, vortexed for full reconstitution and centrifuged again. The supernatant is transferred for analysis by reverse phase high pressure liquid chromatography (HPLC) with fluorescence detection using excitation and emission wavelengths of 310nm and 410nm, respectively. Preliminary experimental data indicate matrix effects are minimal due to deproteinization of the sample before the concentration procedure. Based on previously established methods, an elution profile for ergovaline, ergotamine and their respective isomers was established with a total run time of approximately seventeen minutes. Ideal resolution between analyte peaks allows for optimal specificity at low ppb levels. A range of linearity for this method has been established for the 1.0-50.0 ppb range with standard spiked serum calibration curves with correlation coefficients better than 0.995. The method sample preparation is simple and does not require specialized equipment or solutions allowing for quick and easy analyses. Continuing experiments include optimizing the protein precipitation separation, assessing sensitivity at sub-ppb levels from standard overspiking, and establishing a range for levels in serum after exposure. Accuracy and precision analyses are underway and will be established as method validation continues.

Poster 39

Expression Analysis and Immunoreactivity of Rift Valley Fever Virus Gc Glycoprotein

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Narrative: Rift Valley Fever virus (RVFV) is a mosquito-borne zoonotic pathogen that belongs to the genus *Phlebovirus* of the family *Bunyaviridae*. In ruminants, RVFV causes a high rate of spontaneous abortion and neonatal mortality, whereas infections in humans are often characterized by febrile illness that in some cases can progress to more severe disease, including hemorrhagic fever, blindness, and neurological disorders. While RVFV has been mainly reported in Africa, introduction into the US carries a potential risk due to the presence of competent vectors. The genome of RVFV is composed of three negative-stranded RNA segments, small (S), medium (M), and large (L). The S segment encodes the nucleocapsid (N) protein, which makes up the basic structure of the virus and the M segment encodes the C-terminal and N-terminal glycoproteins, Gn and Gc, which form sub-unit structures on the surface of the viral envelope. The structural proteins Gne, Gc and N (encoded by S segment) are targets for host antiviral immune response. The primary objective of this study is to determine the performance of a modified truncated version (where the transmembrane and cytosolic domains have been deleted), of the Gc glycoprotein (Gc ectodomain, Gce) compared to full-length Gc as a potential target for immunodiagnostic assay development. The Gc ectodomain was amplified by PCR using target-specific primers and then cloned into pFastBacCTopo plasmid, to create a donor plasmid, pFastGce. This plasmid was used to create a recombinant bacmid for rescue of recombinant baculovirus in *Spodoptera frugiperda*, Sf9, insect cells. Expression of Gce and other RVFV structural proteins, N and Gn, was accomplished using the respective recombinant baculoviruses. Analysis of the immunoreactivity profile of Gce glycoprotein by western blot and enzyme-linked immunosorbent assay (ELISA) using anti-sera (from sheep experimentally infected with wild type RVFV) and a panel of anti-RVFV Gc monoclonal antibodies demonstrated an immunoreactive profile similar to the full-length version of the protein. These results indicated that truncated Gce could serve as a useful diagnostic antigen for monitoring RVFV infection/vaccination, as well as a potential target for vaccine development.

Poster 40

Detection and genomic characterization of a PRCV variant in the United States

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Narrative: First identified in Belgium in 1984, porcine respiratory coronavirus (PRCV) is a spike (S) gene deletion mutant of transmissible gastroenteritis virus (TGEV). PRCV was detected in the United States in 1989. Although PRCV has been known over three decades, the majority of the genomic studies have been focused on the partial sequencing of S, ORF3a, and ORF3b, and so far, there is only one complete genome sequence available. In this study, a PRCV variant OH7269 was reported and genomically characterized. In comparison with the PRCV ISU-1, OH7269 has two deletion regions (a 648-nt deletion and a 3-nt deletion) in the spike gene. Moreover, it has two additional deletion regions in the intergenic region and ORF3a, suggesting OH7269 is a PRCV variant. A newly developed differential RT-PCR confirmed that pigs were infected by PRCV only instead of both PRCV and TGEV. Overall, our study indicates that PRCV continuously evolves in the field and highlights the importance of continued surveillance.

Poster 41

Screening of archived paraffin-embedded tissues from equine surgical skin biopsies for the presence of *Bovine Papillomavirus-1&2* by a Taqman real-time PCR ◊

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Narrative: Equine sarcoid is the most common neoplasm in Equidae and accounts for over half of all skin neoplasms in this family. Equine sarcoids have been associated with *bovine papillomavirus* (BPV)- type 1 & 2 (BPV 1&2) and are routinely diagnosed based on histologic features. In some cases there is difficulty differentiating sarcoids from other spindle cell tumors, or granulation tissue. In these cases the absence/presence of BPV 1&2 may aide diagnosis. A qPCR assay targeting a gene fragment of E5L2 from BPV 1&2 was designed and the limit of detection was determined to be two copies (cutoff value Ct 38.0) using 10-fold serial dilutions of a plasmid containing BPV target DNA. Archived paraffin embedded tissue from 98 equine skin biopsy cases were evaluated in this study. Based on histological findings, thirty-one of these cases were diagnosed as sarcoids. Thirty of these cases tested positive and one tested negative for BPV 1&2 by qPCR. The case testing negative was a periocular tumor diagnosed as an occult sarcoid. Specificity of the assay was determined using 62 equine skin biopsy cases diagnosed as various conditions other than sarcoid by histologic findings. Fifty five of 62 were negative and seven were positive for the presence of the BPV 1&2 by qPCR. The qPCR products from the seven positive cases were cloned and results confirmed as BPV by sequencing. Histologic diagnosis of the 7 cases in this group included squamous cell carcinoma (2), granulation tissue (2), peripheral nerve sheath tumor (1), botryomycosis (1), and allergic dermatitis (1). The significance of the presence of BPV 1&2 in these seven cases could not be determined. Five of BPV 1&2 positive cases by qPCR were inconclusive by histologic evaluation; differentials considered were sarcoids, spindle cell tumors, or granulation tissue. Of these five cases, three were positive and two were negative for the presence of BPV 1&2 by qPCR. The findings in this study are consistent with the association of BPV-1&2 with equine sarcoids. Knowledge of the presence or absence of BPV 1&2 in some of these cases would have aided the pathologist in interpretation of histologic findings. The developed qPCR assay using paraffin embedded tissues may prove helpful to both elucidating the role of BPV 1&2 in some pathologic conditions in equine skin and interpreting the histologic findings by the pathologist.

◊ USAHA Paper

Poster 42

Inonotus tropicalis Systemic Mycosis in a Dog *

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Narrative: A 9-year-old female spayed French bulldog was referred to the Louisiana State University Veterinary Teaching Hospital for cardiac evaluation due to ascites. The dog had a chronic history of allergic dermatitis and had received various formulations and doses of glucocorticoids continuously for 8 years. Clinical findings included cachexia, flaky skin, a draining tract over the right hip, peripheral and sternal lymphadenomegaly, mild anemia, mild neutrophilia and monocytosis, moderate basophilia, left ventricular concentric hypertrophy, and severe pericardial effusion with tamponade. Cytology of the sternal lymph node demonstrated pyogranulomatous inflammation with fungal hyphae. The dog was euthanized, and at necropsy all peripheral lymph nodes and the cranial mediastinal lymph nodes were enlarged up to 3 times normal size. Increased serosanguineous fluid was noted in the thorax (400ml) and pericardium (60ml). Approximately 70% of the left ventricular myocardium was transmurally effaced by multifocal to coalescing, firm, white, ill-defined nodules. Approximately 15% of the right ventricular myocardium was similarly effaced. The left ventricular to right ventricular wall ratio was increased at 4 (reference range up to 2.5). The mitral valve leaflets had incidental endocardiosis. Additional gross pathologic findings included ascites, hepatomegaly (5.2% of body weight; reference range up to 4%), focal pale thickening of the gastric serosa and muscularis, diffusely red bone marrow, and a firm subcutaneous swelling over the right flank which oozed a thick, red fluid containing multiple 2mm diameter, green granules. On histopathological evaluation, the majority of the myocardium and epicardial fat was transmurally infiltrated and disrupted by multifocal to coalescing granulomatous inflammation characterized by epithelioid macrophages, multinucleate giant cells, and fewer lymphocytes and plasma cells. Within infiltrated areas there were numerous, 5 to 7µm wide, 200µm long hyaline fungal hyphae that were hyaline via H&E staining, argyrophilic via GMS staining, septate, and had variably parallel walls with occasional areas of bulging and segmental undulation. Similar inflammatory cells and fungal hyphae were seen in the cranial mediastinal lymph node, lung, gastric wall, liver, spleen, bone marrow, and brain. In the dermis and subcutis of the skin of the right flank pyogranulomatous inflammation associated with colonies of filamentous bacteria, suggestive of *Actinomyces*, was seen; however, culture was negative. Culture and rRNA sequencing of the isolate from the antemortem sternal lymph node sample identified the wood decay fungus *Inonotus tropicalis*. To our knowledge this is the first reported case of disseminated *I. tropicalis* infection in a dog. The one other reported case of canine *I. tropicalis* also involved a cranial thoracic lymph node. Immune suppression by glucocorticoids is suspected to have contributed to pathogenesis in the present case.

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EXHIBIT DIRECTORY



American Association of
Veterinary Laboratory Diagnosticians

Exhibit Directory

Providence, RI
October 22-28, 2015

Advanced Technology Corp. VADDS

Booth 32

79 North Franklin Turnpike, Suite 103
Ramsey, NJ 07446
www.vetstar.com
Contact: Joseph Bove
201.934.7127
jbove@vetstar.com

Advanced Technology Corp. invites you to visit our booth to learn about VADDS, our comprehensive, budget-friendly veterinary LIMS system. Stop by to learn about new developments that make VADDS the global leader in veterinary LIMS, and see how VADDS can work for you.

Anaerobe Systems

Booth 10

15906 Concord Circle
Morgan Hill, CA 95037
www.anaerobesystems.com
Contact: Sandy Burg
408.782.7557
sandyburg@anaerobesystems.com

Manufacturer of Pre-Reduced Anaerobically Sterilized (PRAS) culture and transport media. Media is sterilized, poured, and packaged without oxygen. Ready to use out of the package with no need to pre-reduce the media. Manufacturer and distributor of ergonomic and maintenance free anaerobe chambers. New web-based anaerobe educator training software.

BioCheck

Booth 17

3 Southgate Road, Bldg 1, Unit 2
Scarborough, ME 04074
www.biocheck.com
Contact: Tim Goode
207-809-9509
timgoode@biocheck.com

BioCheck is a veterinary diagnostics company specializing in diagnostic test kits (qPCR and ELISA) for the Poultry and Swine industry. These diagnostic tests provide information about the health status of the herds of Swine or flocks of

chickens tested. If test results indicate animal health problems, the farmer can take corrective action.

BioCheck offers a complete range of products to our customers. The BEAR (BioCheck ELISA Assay Robot) is a complete solution for running ELISA Assays. For easy data management BioCheck has developed a comprehensive, user friendly software package and app. This powerful software package allows users to access data 24/7 and create both individual and trend reports.

BioCheck has a strong reputation in service, our technical support ensures the ease of use for veterinary laboratories and accurate results for end users. BioCheck operates from a USDA licensed center in Scarborough, Maine.

BioMed Diagnostics, Inc.

Booth 01

1388 Antelope Rd
White City, OR 97503
www.biomeddiagnostics.com
Contact: Ravi Vinayak
650-996-4951
rvinayak@biomeddiagnostics.com

BioMed Diagnostics is an innovative manufacturer of microbiology diagnostic devices that save money, time, improve workflow and reduce sample exposure and contamination. Veterinarians, medical professionals, and researchers worldwide accurately identify bacteria, parasites, fungi, and more using Biomed point-of-care ready tests.

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For additional information on these and other products please visit: www.biomeddiagnostics.com.

bioMérieux

Booth 12

595 Anglum Road
Hazelwood, MO 63042
www.biomerieux.com

Contact: Karen Mullen
314.731.8884
karen.mullen@biomerieux.com

A world leader in the field of *in vitro* diagnostics for over half a century, bioMérieux is present in more than 150 countries through 40 subsidiaries and a large network of distributors.

bioMérieux provides diagnostic solutions (reagents, instruments, and software), tools and services to improve productivity and quality that contribute to optimizing laboratory performance every day.

Its products are used for diagnosing infectious diseases and providing high medical value results for cancer screening and monitoring and cardiovascular emergencies. They are also used for detecting microorganisms in agri-food, pharmaceutical, cosmetic products and the veterinary diagnostic market.

Biovet

Booth 03

9025 Penn Avenue South
Minneapolis, MN 55431
www.biovet-inc.com

Contact: Sheila Braun
877.824.6838
biovetusa@biovet-inc.com

Biovet Inc. develops, manufactures and markets animal diagnostic kits. Additionally, Biovet provides animal health and agro-industry specialists with a unique expertise in the diagnostic field. Biovet operates certified laboratories offering a complete array of innovative diagnostic services for veterinarians. The US head office is located in Minneapolis, MN. Biovet employs more than 50 people including 15 scientists. The company is active internationally particularly in North America and Europe.

Bruker Daltonics

Booth 24

40 Manning Road
Billerica, MA 01821
www.bruker.com

Contact: Nancy Salt
978.663.3660 x1492
nancy.salt@bruker.com

Bruker Corporation is a leading provider of analytical systems for diagnostic applications. Led by innovative,

easy-to-use and cost effective systems for Microbial Identification, the industry leading MALDI Biotyper CA System produces identifications in minutes with minimal reagents from primary culture.

Centaur, Inc.

Booth 31

1351 Old 56 Hwy West, Bldg F
Olathe, KS 66061
www.centauranimalhealth.com

Contact: Howard Jones, DVM
800-236-6180
sales@centauranimalhealth.com

Centaur Animal Health is focused on developing innovative diagnostics, pharmaceuticals and nutraceuticals for practitioners and diagnostic labs. Key products include: FP-ELISA II, Centaur's third generation test for Equine Infectious Anemia Virus, Foalchek to measure IgG levels, as well as tests for Feline Leukemia Virus and Canine Heartworm, with others to be available by the end of the year.

Computer Aid, Inc.

Booth 23

470 Friendship Road, Suite 300
Harrisburg, PA 17111
www.compaid.com

Contact: John Kucek
717.651.3000
john_kucek@compaid.com

Computer Aid, Inc. is a global information technology application management and outsourcing firm focused on helping clients gain a competitive advantage through the effective use of IT. CAI was selected by the Commonwealth of Pennsylvania and the **National Agribusiness Technology Center** to develop and maintain the original herds software and after, all of NATC's AgraGuard products. As a \$550 million IT company with more than 30 branch offices worldwide, we are entrepreneurial in focus, specializing in technical and management disciplines associated with business and government IT services and consulting. CAI is the original technical architect for all products in the AgraGuard suite. We work in various agencies including departments of agriculture, veterinary diagnostic laboratories, departments of health and bureaus of plant industry. This work has generated several awards, including the American Council for Technology 2006 Award and multiple honors from NASCIO.

ECL2

Booth 25

PO Box 1731
Grapevine, TX 76099
www.ECL2.com
Contact: Keith Ernst
972.983.3956
kernst@ECL2.com

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www.elsevierclinicalolutions.com
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215-239-3900
b.karafin@elsevier.com

ELSEVIER is a leading publisher of health science publications, providing superior reference information, decision support tools to doctors, nurses, health practitioners and students. Our media spectrum — print, online and handheld, provides information in the most convenient format.

GeneReach Biotechnology Corporation

Booth 06

No 19, Keyuan 2nd Rd
Central Taiwan Science Park
Taichung, 407 Taiwan ROC
www.genereach.com
Contact: Simon Chung
886.4.2463.9869
sales@genereach.com

GeneReach Biotechnology is a worldwide biotechnology company dedicated to bringing the innovation to global health management. By developing, manufacturing and

marketing products for applied nucleic acid detection technology, we offer disease detection platforms, including equipment and reagents, to multiple industries such as aquaculture, agriculture, livestock, companion animals, and in vitro diagnostic industries. We developed and manufacture the world's one and only OIE-certified PCR diagnostic system, IQ2000. Our quality system is in compliance with ISO9001 and ISO13485.

GeneReach has developed POCKIT, a portable PCR platform based on the principle of insulated isothermal PCR. It comes in as a carry-on hard-shell suitcase package for room temperature shipping. The system can take up to eight samples per run. The total run time is less than 1.5 hours. With its high sensitivity and specificity, ease-of-use and short-turn-around-time, POCKIT can provide farmers and field consultants a powerful tool for veterinary disease management.

IDEXX Laboratories

Booths 29-30

One Idexx Drive
Westbrook, ME 04092
www.idexx.com/production
Contact: Erin Ware
207.556.8313 or 207.210.8288
erin-ware@idexx.com

About IDEXX Livestock, Poultry and Dairy - Livestock and poultry producers, laboratories, veterinarians and dairy processors depend on IDEXX diagnostic technologies to make confident decisions about animal health, disease management and reproductive efficiency, and to ensure consumers have access to safe, healthy food and milk. Reproducibility, reliability and accuracy are three of the reasons why more than 1 billion IDEXX tests—including dairy residue tests and milk-based diagnostics—have been run worldwide since 1985.

Life Diagnostics, Inc.

Booth 15

906 Old Fern Hill Road, Suite 1
West Chester, PA 19380
www.biomeddiagnostics.com
Contact: Dr. Christopher Chadwick or
Laura Chadwick
610-431-7707
info@lifediagnosics.com

Established in 1997, Life Diagnostics, Inc. is a leading manufacturer of ELISA kits, purified proteins and antibodies for preclinical research and veterinary diagnostic applications. Our areas of expertise include cardiovascular, skeletal muscle, acute phase, immunotoxicity and immunology biomarkers in companion animals, poultry and livestock. We have a strong focus on biomarkers for inflammation, muscle injury, and mastitis. The recent opening of our own monoclonal facility allows us to also offer monoclonal antibodies for many of our acute phase biomarkers. All products listed on our website are manufactured by Life Diagnostics, Inc., allowing unparalleled technical advice and customer support.

Liferiver Bio-Tech (United States) Corp.

Booth 21

9855 Towne Center Drive

San Diego, CA 92121

www.liferiverbiotech.com

Contact: Xuping Zhu

415-535-7599

trade@liferiver.com.cn

Liferiver is a leading molecular diagnostics solution provider. The company develops and manufactures over 300 real time PCR based diagnostic kits/reagents and instruments for various infectious diseases, genetic diseases, tumor, organ transplantation as well as others.

One of the important aspects of Liferiver's products is for detecting various animal infections including rare pathogens. We provide over 70 sets of reagents for catching infectious pathogens in various animals including birds, swine, cattle, goats, etc. The products are user-friendly designed with convenient one-step assay, high specificity, sensitivity, and accuracy.

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MACHERY-NAGEL Inc.

Booth 16

2850 Emrick Blvd

Bethlehem, PA 18020

www.mn-net.com

Contact: Dawn Russup

888-321-6224

sales-us@mn-net.com

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PRI Bio

Booth 26

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Dupo, IL 62239

www.pri-bio.com

Contact: Jim Laarman

618.286.5000

jlaarman@progressive-recovery.com

PRI Bio is a global leader in Alkaline Hydrolysis Technologies (Tissue Digesters) and Effluent Decontamination Systems servicing Veterinary Research and Diagnostic Labs; Zootonic Research Facilities; Centers for Disease Control; and Pharmaceutical R&D and Manufacturing operations. For more than 30 years, PRI has advanced the science in hazardous liquid and solid waste treatment, specializing in the design and manufacture of sterilization and digester equipment. PRI's thermal products are currently used in 24 countries worldwide, with over 3,000 units installed.

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Qualtrax

Booth 07

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Blacksburg, VA 24060

www.qualtrax.com

Contact: Terry Driver

540-260-9961 x106

tdriver@qualtrax.com

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QIAGEN, Inc.

Booth 33-34

19300 Germantown Road
Germantown, MD 20874

www.qiagen.com

Contact: Jennine Cannizzo
207.572-8024
jennine.cannizzo@qiagen.com

QIAGEN is the leading global provider of Sample to Insight solutions to transform biological materials into valuable molecular insights. QIAGEN sample technologies isolate and process DNA, RNA and proteins from blood, tissue and other materials. Assay technologies make these biomolecules visible and ready for analysis. Bioinformatics software and knowledge bases interpret data to report relevant, actionable insights. Automation solutions tie these together in seamless and cost-effective molecular testing workflows.

QIAGEN provides these workflows to more than 500,000 customers around the world in Molecular Diagnostics (human healthcare), Applied Testing (forensics, veterinary testing and food safety), Pharma (pharmaceutical and biotechnology companies) and Academia (life sciences research). Further information can be found at www.qiagen.com.

Partner with us to secure animal health

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Quanta Bio

Booth 02

202 Perry Parkway
Gaithersburg, MD 20877

www.quantabio.com

Contact: 800-364-2149
sales@quantabio.com

Best-In-Class

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Robbins Instruments

Booth 04

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www.robbinsinstruments.com

Contact: 800-206-8649
George Mulvaney
Skype: George.Mulvaney1

Robbins Instruments will feature our complete line of veterinary surgical instruments, disposable biopsy punches, curettes, dermatome blades and supplies. **For more information visit www.robbinsinstruments.com**

RWD Life Science

Booth 08

6540 Lusk Blvd, Suite 161
San Diego, CA 92121
www.rwdstco.com

Contact: Danica Wang, Ph.D.
858-900-6602
danica.w@rwdstco.com

Established in 2002, RWD Life Science has been dedicated to being a leading manufacturer in veterinary and research equipment worldwide. We specialize in anesthesia, physiological and surgical equipment, used by veterinarians and pre-clinical researchers. Our mission is to provide high quality products and integrated solutions for our customers with prompt and professional support.

For more information about our supplies, such as Anesthesia Machine, Ventilator, Stereotaxic Instrument, Syringe Pump, Cannula System and Surgical tools, please check our website: www.rwdstco.com.

SAGE/JVDI

Booth 22

2455 Teller Road
Thousand Oaks, CA 91320
www.sagepub.com

Contact: Lisa LaMont
805.410.7239
lisa.lamont@sagepub.com

Founded 50 years ago by Sara Miller McCune to support the dissemination of usable knowledge and educate a global community, SAGE publishes journals, books, and library products spanning a range of subject areas. SAGE remains majority-owned by our founder, who has ensured that the company will remain permanently independent. www.sagepub.com

Tetracore, Inc.

Booth 05

9901 Belward Campus Drive, #300
Rockville, MD 20850
www.tetracore.com

Contact: Alexandra Yanus
240.268.5400
ayanus@tetracore.com

Company Representatives: Dr. William Nelson, Rolf Rauh, John Kelly, and Alexandra Yanus

Tetracore is a leading biotechnology company providing innovative diagnostic assays and reagents for infectious diseases. The Tetracore VetAlert™ product line features real-time Polymerase Chain Reaction (PCR) test kits and reagents for rapid and sensitive detection of animal pathogens. USDA licensed test kits are available for Johne's disease and CSFV, in addition to specific detection reagents for PRRSV, Influenza, PED/TGE/PDCoV, FMDV, ASFV and others.

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Thermo Fisher Scientific (formerly Life Technologies)

Booth 18-20

2130 Woodward Street
Austin, TX 78744
www.thermofisher.com/animalhealth

Contact: Elizabeth Lohse
512-721-3610
Elizabeth.lohse@thermofisher.com

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer.

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Thermo Scientific

See Thermo Fisher Scientific

VMRD, Inc.

Booth 27

425 NW Albion Road
PO Box 502
Pullman, WA 99163
www.vmr.com

Contact: Amanda Ellis
800.222.8673
vmrd@vmrd.com

VMRD was founded in 1981 by D. Scott Adams, DVM, PhD, and currently employs approximately 50 researchers, lab technicians, and support personnel. From its site in Pullman, WA VMRD develops and manufactures diagnostic test kits and related reagents for distribution in more than 55 countries. As a rapidly growing company VMRD strives to preserve its family focused culture and core values of integrity and quality. Its mission to provide high quality products, services and support for customers and a harmonious and rewarding work environment for employees reflects and enforces the company's market reputation for delivering best in class products with a uniquely personal touch. As a result of this clear focus VMRD has a global impact on improvements in animal welfare through the diagnostic laboratories, animal producers, government agencies and veterinarians who use its products. Visit www.vmr.com for more information.

Zoetis

Booth 28

100 Campus Drive
Florham Park, NJ 07932
www.zoetis.com

Contact: Chris Demiris
973.443.3139
chris.demiris@zoetis.com

Zoetis (zô-EH-tis) is the leading animal health company, dedicated to supporting its customers and their businesses. Building on more than 60 years of experience in animal health, Zoetis discovers, develops, manufactures and markets veterinary vaccines and medicines, complemented by diagnostic products and genetic tests and supported by a range of services.

In 2014, the company generated annual revenue of \$4.8 billion. With approximately 10,000 employees worldwide at the beginning of 2015, Zoetis serves veterinarians, livestock producers and people who raise and care for farm and companion animals with sales of its products in 120 countries. For more information, visit www.zoetis.com.

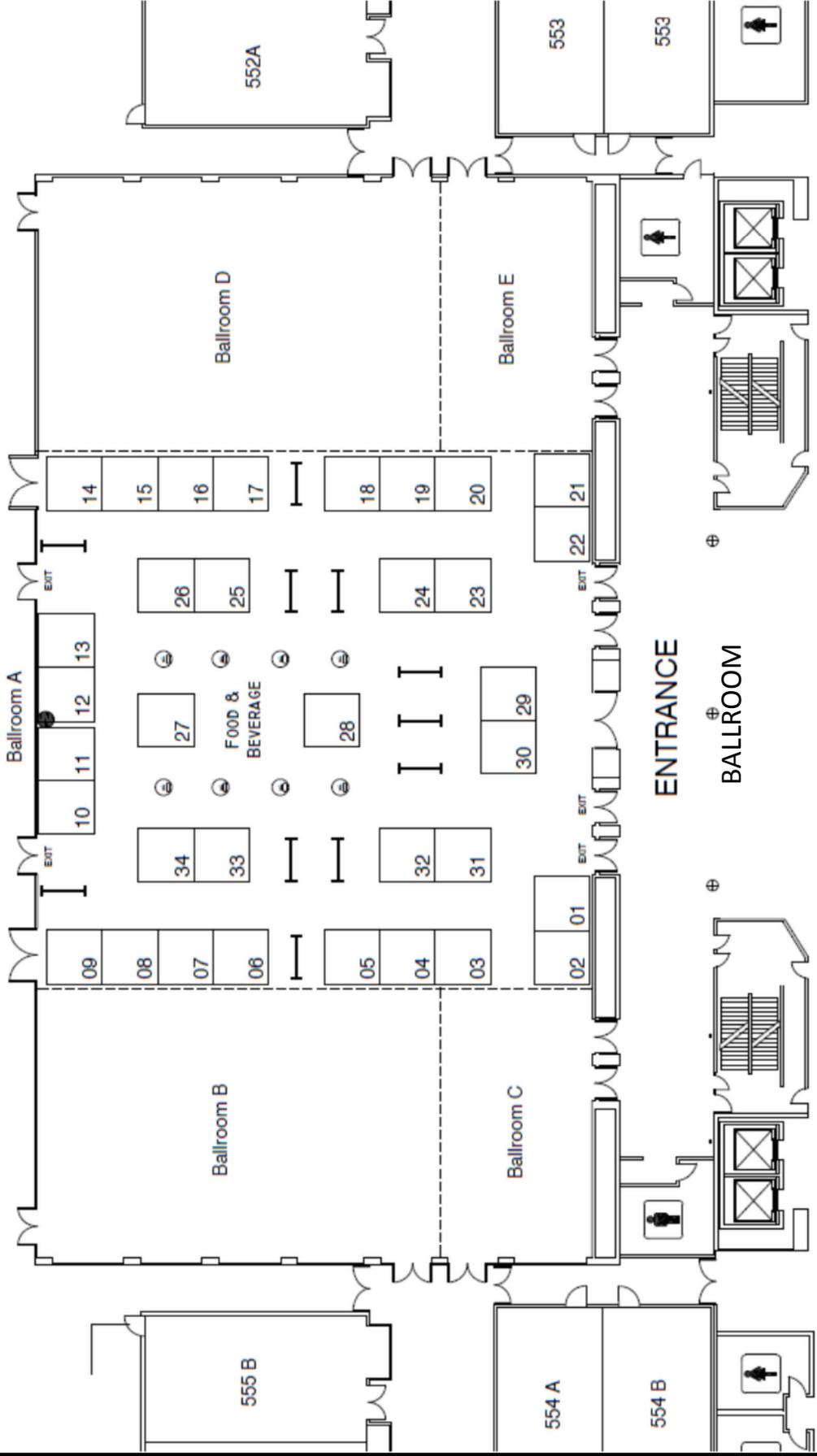
AAVLD/USAHA Upcoming Meetings

October 20-26, 2016
Greensboro, North Carolina

October 10-18, 2017
San Diego, California

EXHIBIT HALL

Rhode Island Convention Center



EXHIBITS AND POSTER SESSION

Ballroom A

2015 EXHIBITORS

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Exhibit Hall Schedule

Saturday, October 24

9:00 am—6:00 pm

Sunday, October 25

9:00 am—1:00 pm

Poster Session

Posters are available in Ballroom A

9:00 am- 6:00 pm Saturday, Oct 24

9:00 am-12:00 pm Sunday, Oct 25

Authors present 3:00-4:00 pm
on Saturday, Oct 24, 2015

SPONSOR PRESENTATIONS

Saturday, October 24, 2015			
IDEXX	6:00-6:30 pm	Room 556	Quality Control and Standardized Workflows in PCR Testing
Biovet	6:00-6:30 pm	Ballroom E	Enter a new era for animal diagnostics with the Biovet Multiplex test!
Thermo Fisher Scientific	6:30-7:00 pm	Room 556	Trends of Bacterial Antimicrobial Resistance Associated with Cattle Affected with Bovine Respiratory Disease Complex (BRDC) in the State of Missouri, USA William H. Fales, MS, PhD
QIAGEN	6:30-7:00 pm	Ballroom E	Real-Time PCR to NGS and Bioinformatics – A QIAGEN Tool Belt for Animal Health

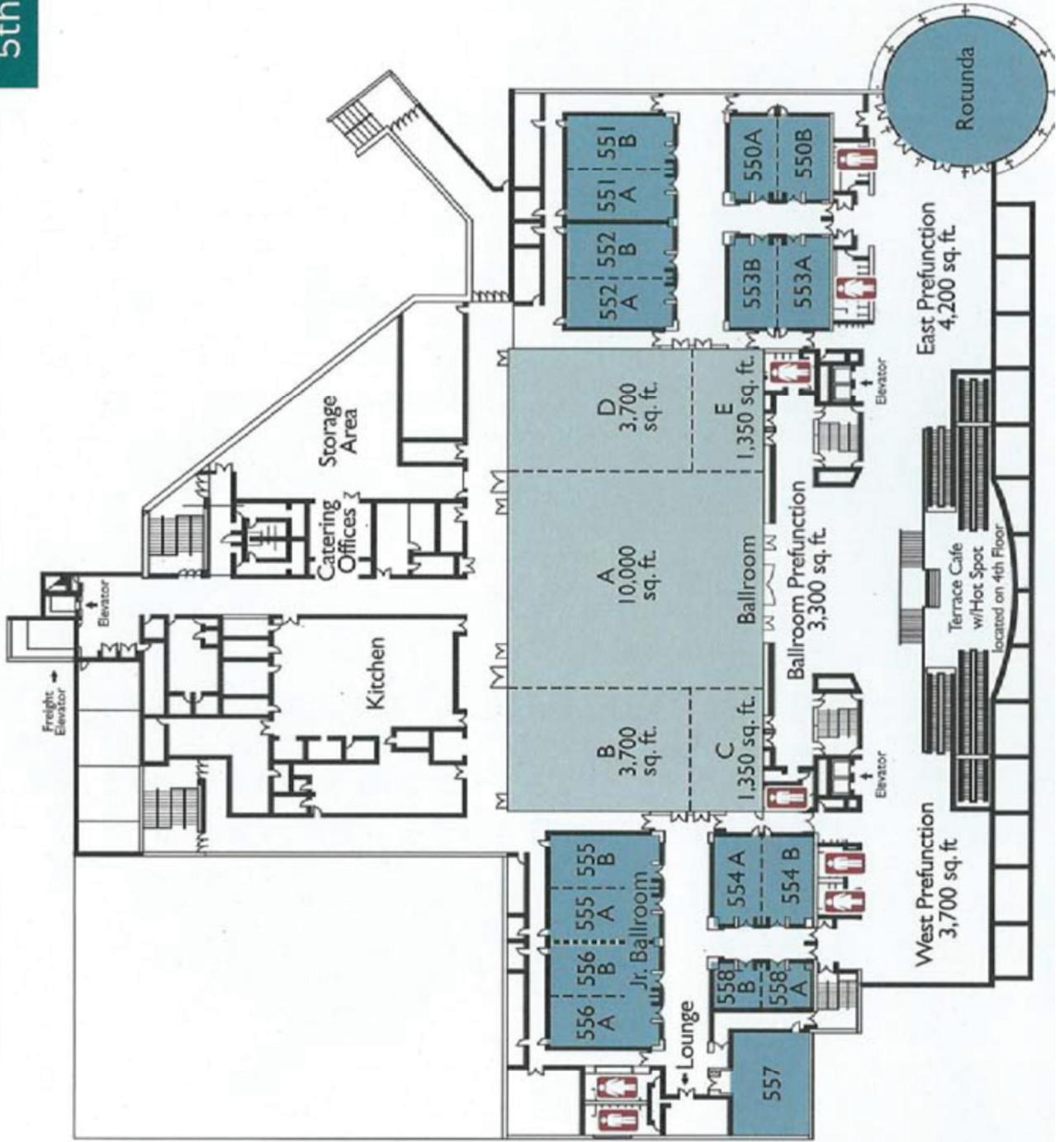
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