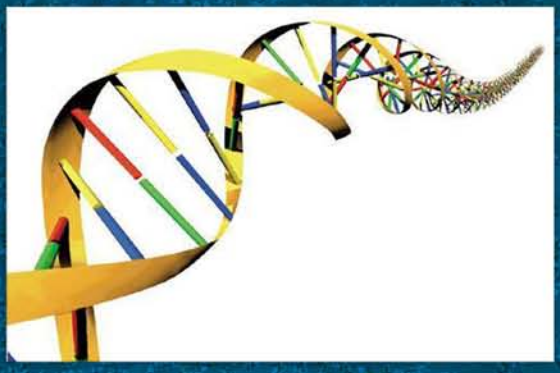
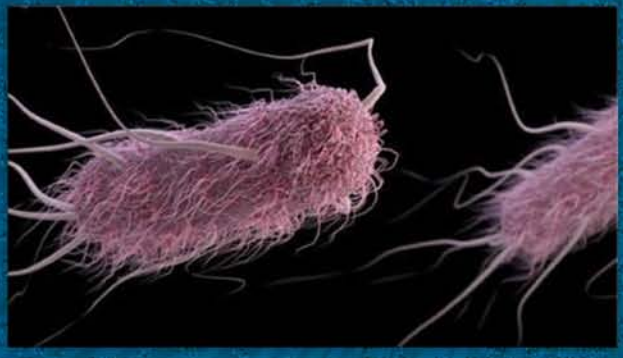
Proceedings of the American Association of Veterinary Laboratory Diagnosticians



Next Gen Sequencing



AMR: Awareness to Solutions

QA

Mycology

60th Annual Conference

Town and Country Resort and Conference Center
San Diego, CA
October 12-18, 2017

Racehorse Pathology

On the cover: Two pictures on the left, AR Global Threat and bacterium, are courtesy of the CDC website.

Middle right: Fungas picture (Mycology) was provided by Dr. Connie Gibas and Dr. Nathan Wiederhold, at the Fungus Testing Laboratory, UT Health San Antonio.

Bottom right: Horse skull and brain (Racehorse Pathology) was provided by Dr. Francisco Uzal at the California Animal Health and Food Safety Lab, University of California, Davis in San Bernardino, CA.

AWARDS

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Henrique Almeida	Iowa State University
Jaber Belkhiria	University of California- Davis
Shubhada Chothe	Pennsylvania State University
Rozalyn Donner	Kansas State University
Elizabeth Houston	Iowa State University
Jennifer Kopanke	Colorado State University
Scott Radke	Iowa State University
Zhenyu Shen	University of Missouri
Arya Sobhakumari	University of California- Davis
Sreenidhi Srinivasan	Pennsylvania State University
Steven Waldrop	Virginia Tech
Yin Wang	Kansas State University
Fangfeng Yuan	Kansas State University
Lorelei Clarke (Pathology)	University of Georgia

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Christina Cappelli	Cornell University
Malgorzata (Margaret) Johnson	Michigan State University
Melanie Koscielny	Ohio Animal Disease Diagnostic Laboratory
Jennifer Rudd	Virginia Tech

2017 ACVP/AAVLD Award

Caroline Andrews

Michigan State University

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.

Acknowledgments

The success of our meeting is a reflection of the hard work, extraordinary dedication and creativity of many people. A special thank you to all who present their studies and findings, to all exhibitors and sponsors, and to everyone who attends this annual event. We would also like to give special recognition to our invited speakers for the AAVLD Plenary Session and the USAHA-AAVLD Plenary Session. Our partnership with USAHA has resulted in outstanding meetings.

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. Jackie Cassarly from Planning Connection, Inc., and Kaylin Taylor from Taylor Made Event Co. 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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Antimicrobial Resistance: Awareness to SolutionsThe Role of Veterinary Diagnostic Laboratories in Addressing the Global Crisis

Saturday, October 14, 2017

Golden West

Moderator: Steve Hooser

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10:15 AM **The role of academic veterinary medicine in combating antimicrobial resistance**
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11:25 AM **Concluding Remarks**

Symbols at the end of titles indicate the following designations:

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+ AAVLD/ACVP Pathology Award Applicant
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

The global antimicrobial resistance predicament: One Health to the rescue

Craig N. Carter

University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY

The acclaimed *Review on Antimicrobial Resistance* sponsored by the Wellcome Trust and the UK Department of Health released its final report in the summer of 2016. It highlights the estimated 700,000 annual human deaths occurring globally related to antimicrobial resistance (AMR). This number could grow to 10 million deaths per year by 2050 with a price tag of $100 trillion of lost global human productivity during the ensuing timeframe. Although there is much uncertainty with the report's projections, there is no question that the problem is vast and growing at an alarming rate. A definition of One Health is the "collaborative effort of multiple disciplines—working locally, nationally and globally—to attain optimal health for people, animals and our environment." The One Health Initiative mission statement reads "One Health seeks to promote, improve, and defend the health and well-being of all species by enhancing the cooperation and collaboration between physicians, veterinarians, other scientific health and environmental professionals by promoting strengths in leadership and management to achieve these goals." It is truly difficult to imagine a health threat that better defines the need for a One Health approach than AMR. To be sure, leaders in veterinary research, industry, academicians, diagnosticians, and yes—politicians--will have key roles in helping finding sustainable solutions to the global AMR predicament. Physician Laura Kahn discusses the many international and political challenges that AMR poses in her recent book *One Health and the Politics of Antimicrobial Resistance*. She emphasizes that food safety, food security and sanitation are keys to the solution and that political leaders will have to decide how to achieve these goals "equitably, judiciously, and sustainably."

Speaker Biography: Craig Carter received his DVM, MS and PhD at Texas A&M University. He ran a large animal ambulatory practice for five years and joined the Texas Veterinary Medical Diagnostic Laboratory as a Clinical Associate. He later assumed the role as Head of Epidemiology and Informatics until 2005. He then joined the University of Kentucky Department of Veterinary Science as a professor of epidemiology. His active duty and reserve military career in the USAF and US Army spanned four decades and three combat deployments, retiring as a full Colonel in 2009. In 2007 he was appointed as Director of the University of Kentucky Veterinary Diagnostic Laboratory where he oversees lab operations, conducts research and works with his graduate students. He served as the Executive Director of the World Association of Veterinary Laboratory Diagnosticians from 2000-2017 and is a past president of the AAVLD. He is currently president of the American Veterinary Epidemiology Society. His research interests are infectious disease epidemiology, real-time health monitoring, clinical decision support and medical information systems. He is active internationally, having lived outside the US for five years and consulted in over thirty countries. He was awarded the AVMA XIIth International Veterinary Congress Prize in 2016.

A One Health approach to antimicrobial resistance surveillance and outbreak response

Megin Nichols, Dawn Sievert

Centers for Disease Control and Prevention, Atlanta, GA

Antimicrobial resistance is a complex and challenging public, animal, and environmental health issue. CDC is committed to transforming how the nation addresses antimicrobial resistance through One Health partnerships with state and federal health and agriculture agencies, academia, laboratories, industry, and consumer groups. With specifically allocated investments, the CDC's Division of Foodborne, Waterborne, and Environmental Diseases has supported the national infrastructure to detect, respond, and prevent resistant infections acquired through food, animal contact, and community interaction.

This plenary session presentation will provide an overview of the current CDC efforts to implement the One Health approach and build the collaborative interactions across stakeholders to address and prevent antimicrobial resistant enteric pathogens. An overview of ongoing activities for antimicrobial resistance will be presented. Examples of outbreaks in which a One Health approach to investigation was utilized will be discussed. Success in this space can only come with continuous collaboration, communication, and planning across the spectrum of human, animal, and environmental sectors to develop surveillance and response strategies for antimicrobial resistant pathogens of human and animal health concern.

Speaker Biography: Megin Nichols, DVM, MPH, DACVPM serves as the Enteric Zoonoses Activity Lead at the Centers for Disease Control and Prevention. In this role, she works on multistate outbreaks of Salmonella and E. coli resulting from exposure to animals, pet products and raw milk. In 2016, Dr. Nichols led the investigation of a multistate outbreak of listeriosis linked to interstate shipment of raw milk. Prior to joining CDC, Dr. Nichols worked as the Principal Investigator of the Active Bacterial Core Surveillance Program at the New Mexico Department of Health for 5 years. She received a Bachelor of Science degree in Animal Science from New Mexico State University, a Doctor of Veterinary Medicine from Colorado State University and a Master of Public Health in Food Safety and Biosecurity from the University of Minnesota. Her areas of interest include: zoonotic disease, food safety, and pediatric health.

Dawn M. Sievert, PhD, MS is the Associate Director of Antimicrobial Resistance in the Division of Foodborne, Waterborne, and Environmental Diseases (DFWED) at the Centers for Disease Control and Prevention (CDC), where she is responsible for leading the Division's efforts and managing the resources to combat antimicrobial resistance related to enteric, fungal, and environmental pathogens. In her prior work at the CDC, she served as Deputy Chief of the Surveillance Branch in the Division of Healthcare Quality Promotion and the Science Lead and Coordinator of the National Healthcare Safety Network. Dawn's work outside of the CDC includes: Chief Scientist with the Lantana Consulting Group, where she led efforts to develop strategies for new models of health information exchange and automation in medicine and public health; Quality Improvement and Infection Control Coordinator with the Detroit Medical Center Healthcare System; and Antimicrobial Resistance Program Lead at the Michigan Department of Community Health. She also has significant experience in laboratory research. Dawn has her PhD in Infectious Disease Epidemiology and her Bachelor of Science degree in Microbiology from the University of Michigan in Ann Arbor, and her Master of Science degree in Epidemiology from the University of Illinois at Chicago.

Integrative approaches/solutions for dealing with AMR

Kerry Keffaber

Elanco Animal Health, Greenfield, IN

AMR emergence is a significance global threat. The relative impact of the use of antibiotics in animals creating these problems for human health is debated. While there is consensus that the use of antibiotics in humans primarily drives the resistance of human concern, there is also agreement that the use of antibiotics in animals creates a risk. Animal agriculture must do our part to protect this resource. Animal, Human and Planet Health are inter connected. One in three people today get the wrong nutrition, including the 800 million malnourished to the 2 billion overweight and obese. Animal protein is critical to balance diets. Growing global population and the expanding middle class, as well as diet trends in the developed world will drive a 60% increase in demand for animal sourced foods in the next 35 years. An untold story of food waste is the 20% of animal productivity lost to morbidity and mortality from disease. 1.6 is the number of Earths we are using each year to produce food. By mid-August, we have used all the resources that should have lasted the full year. We must pursue efforts to optimize remembering that well-meaning actions have both intended and unintended consequences. Even efforts perceived to be helpful such as production of RWA poultry can lead to creating more risk versus a reduction. To insure the long-term effectiveness of antibiotics while protecting the welfare and health of animals and maintaining confidence in how food is raised improving food security we must focus on solutions and being good stewards. With One Health as the foundation, decreasing the need for antibiotic use by discovery and implementation of innovations and the responsible use when they are required are the two cornerstones for collaborative progress.

Speaker Biography: Dr. Keffaber received his DVM and Animal Science degree from Purdue University and completed the Executive Veterinary Program at the University of Illinois. Kerry has been with Elanco Animal Health for 15 years in various roles including Director of Swine Technical Services and Director of Swine Product Development. Currently he is Chief Veterinarian for Scientific Affairs and Policy. Prior to joining Elanco, Kerry was in private practice for over 20 years primarily as a swine veterinary consultant. Dr. Keffaber has authored and co-authored multiple peer reviewed scientific papers, is a past president of the American Association of Swine Veterinarians, AASV Allied Veterinarian of the Year in 2015 and helped coordinate and participate in a veterinary trip with Heifer International to Cameroon, Africa.

The role of academic veterinary medicine in combating antimicrobial resistance

Andrew Maccabe

American Association of Veterinary Medical Colleges, Washington, DC

In 2014, the Association of Public and Land Grant Universities (APLU) and the Association of American Veterinary Medical Colleges (AAVMC) joined forces to create the Joint APLU-AAVMC Task Force on Antibiotic Resistance in Production Agriculture. The task force was comprised of 14 leaders from U.S. agriculture colleges/land grant universities, veterinary colleges and key representatives from the production animal agriculture community and pharmaceutical industry. The task force developed a comprehensive national strategy for diminishing the role antibiotics used in food animal production systems play in the broader antimicrobial resistance (AMR) problem. Their final, which was released in October 2015, detailed a research and educational agenda for addressing the problem.

Crucial to the success of the AMR mitigation effort is the need to educate a wide variety of stakeholders about the proper stewardship and judicious use of antibiotics in production agriculture. To address this substantial task, the Antimicrobial Resistance Core Competencies Working Group, which includes scientists and professors from a group of major universities, was established. The working group identified a broad range of learning outcomes specifically tailored for the discrete stakeholder groups of novice (youth, FFA, 4H), developing (undergraduate and graduate) and professional (veterinary medical).

Antimicrobial resistance looms as one of the greatest public health challenges of our time. Universities are already playing a critical role on this emerging battlefront. Researchers on AAVMC's member institution campuses have the expertise to conduct the scientific investigations that must be completed. University faculty and outreach specialists have the networks and opportunities to play a key role in educating agricultural producers, veterinarians, veterinary students and others about the judicious use of antibiotics in production agriculture. Faculty experts on our campuses are collaborating with scientists and other stakeholders to help executive agencies, Congress and international organizations develop the regulations, policies and resources required to mitigate this threat.

Speaker Biography: Dr. Andrew Maccabe is the Chief Executive Officer of the Association of American Veterinary Medical Colleges (AAVMC). He received his Bachelor of Science and Doctor of Veterinary Medicine degrees from The Ohio State University in 1981 and 1985, respectively.

Dr. Maccabe began his professional career in Jefferson, Ohio where he worked in a mixed animal practice with primary emphasis on dairy herd health. In 1988, he was commissioned as a Public Health Officer in the U.S. Air Force where he managed the preventive medicine activities of several Air Force installations and directed programs in occupational health, communicable disease control, and health promotion.

Dr. Maccabe completed his Master of Public Health degree at Harvard University in 1995. That same year he became Chief of the Health Risk Assessment Branch of the U.S. Air Force where he directed the health risk assessment program for environmental restoration activities throughout the Air Force.

Dr. Maccabe completed his Juris Doctor degree, *Magna Cum Laude,* at the University of Arizona in 2002 and subsequently became the Associate Executive Director at the Association of American Veterinary Medical Colleges where he led programs to advance veterinary medical education. In 2007, he was appointed as CDC's Liaison to the U.S. Food and Drug Administration where he coordinated policies and programs between the two agencies before returning to AAVMC in 2012 as the CEO.

He holds memberships in many professional organizations including the American Veterinary Medical Association, the American Veterinary Medical Law Association, the District of Columbia Veterinary Medical Association, and the Lesbian and Gay Veterinary Medical Association. He is a member of the State Bar of Arizona, the Bar of the District of Columbia, and a Licensed Patent Attorney.

The role of veterinary diagnostic laboratories in addressing AMR

Kenitra Hammac

Comparative Pathobiology, Purdue University, West Lafayette, IN

Veterinary diagnostic laboratories serve as an essential provider of evidence required for evidence based medicine. There is no better example than the antimicrobial susceptibility data provided by diagnostic bacteriology laboratories to inform veterinarians on proper treatment and management of infectious disease cases. While it is critical for laboratories to follow approved methodologies and utilize official interpretation schemes, it is perhaps just as important how the results are communicated to clients, both in writing and during verbal consultations. Veterinary students are not typically trained in the nuances of antimicrobial susceptibility testing and interpretation, so it is imperative that laboratories do not assume veterinarians possess such knowledge when communicating test results. As new treatments are developed to address the growing concern of antimicrobial resistance – new drugs, alternative drugs, phage therapy – veterinary diagnostic bacteriology laboratories must be prepared to provide appropriate evidence regarding the effectiveness of these therapies. Researchers and diagnosticians must come together to develop appropriate test strategies, and the industry must respond quickly to make these tests available. Veterinary students and veterinarians must be educated on the fundamentals of antimicrobial susceptibility testing so they can avoid false assumptions and know when to call their local diagnostic laboratory for assistance interpreting the evidence.

Speaker Biography: Dr. Hammac earned her Bachelor of Science in Microbiology and her Doctorate of Veterinary Medicine from Auburn University. After three years in small animal veterinary practice, she returned to the halls of higher education at Washington State University to pursue a PhD and a residency in clinical microbiology. This year she celebrated her fourth anniversary at Purdue University where she serves as a Clinical Assistant Professor and Head of the Bacteriology section in the Indiana Animal Disease Diagnostic Laboratory. She's an active member of the AAVLD bacteriology committee and the American College of Veterinary Microbiologist's communication and examination committees. Her experience in veterinary practice motivates her daily to provide timely, accurate and useful information to veterinarians and producers, including up-to-date advice on the use of antimicrobials.

Bacteriology 1

Saturday, October 14, 2017

Pacific Salon 2

Moderators: Durda Slavic and Anil J. Thachil

1:00 PM **Vet-LIRN Pilot AMR and WGS Project**
Olgica Ceric, Sarah Nemser, Renate Reimschuessel, Laura B. Goodman, Alma Roy, Yan Zhang, Joy Scaria 8

1:15 PM **Development of a new fluorescent *in situ* hybridization for the detection of *Mycoplasma hyopneumoniae* in lung tissue # * †**
Henrique Meiroz de Souza Almeida, Jennifer M. Groeltz-Thrush, Eric Burrough, Maria M. Merodio, Aric J. McDaniel, Bailey Lauren Arruda, Rachel J. Derscheid, Pablo E. Pineyro 9

1:30 PM **Isolation and molecular characterization of *Campylobacter* species isolated from nonhuman rhesus monkeys, *Macaca mulatta*, in a laboratory animal facility**
Sung Guk Kim, Lillie M. Sims, Christine V. Summage-West, Heather K Bogy, Joanna Deck, Angela Paredes, Seongwon Nho, Steven L. Foley 10

1:45 PM **Evaluation and validation of MALDI-TOF for identification of bacteria and yeasts from animal specimens**
Thomas J. Inzana, Sharon Hurt, Amanda Carbonello 11

2:00 PM **Investigation of the pathogens contributing to naturally occurring outbreaks of infectious bovine keratoconjunctivitis (pinkeye) using Next Generation Sequencing**
Eman Anis, Serina Doolittle, Lee Jones, Roy Berghaus, Rebecca P. Wilkes 12

2:15 PM **Detection and differentiation of Oomycota pathogens infecting dogs (*Pythium insidiosum*, *Lagenidium* spp. and *Paralagenidium karlingii*) by FRET-qPCR**
Terri Hathcock, Joseph Newton, Amelia White, Priscilla Barger, Anwar Kalalah, Chengming Wang 13

2:30 PM **Rapid typing of opportunistic bacterial pathogens using MALDI-TOF mass spectrometry; discriminating *Mannheimia hemolytica* major genotypes 1 and 2 using a biomarker based proteomic approach**
John Dustin Loy, Michael Clawson 14

2:45 PM **Identification of *Edwardsiella piscicida* using MALDI-TOF Biotyper in-house library §**
Christina Cappelli, Rebecca Franklin-Guild, Anil J. Thachil 15

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† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Vet-LIRN Pilot AMR and WGS Project

Olgica Ceric[1], Sarah Nemser[1], Renate Reimschuessel[1], Laura B. Goodman[2], Alma Roy[4], Yan Zhang[3], Joy Scaria[5]

[1]Food and Drug Administration, Center for Veterinary Medicine, Veterinary Laboratory Investigation and Response Network (Vet-LIRN), Laurel, MD; [2]Cornell University, Population Medicine & Diagnostic Sciences, Ithaca, NY; [3]Ohio Department of Agriculture-ADDL, Reynoldsburg, OH; [4]Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA; [5]South Dakota State University, Veterinary and Biomedical Sciences, Brookings, SD

Antimicrobial resistance (AMR) is a major public health threat for humans and animals worldwide. Antimicrobial drugs have been widely used in human and veterinary medicine for more than 50 years; however, when used inappropriately they can increase the risk of creating antibiotic resistant bacteria. In order to successfully monitor the antimicrobial susceptibility of bacterial pathogens it is vital that veterinary diagnostic laboratories be incorporated into the nation's surveillance activities. In 2016, Vet-LIRN developed a collaborative pilot project to assess the antimicrobial susceptibility of selected veterinary pathogens and to collect sequence information on a subset of those isolates. Goals of the pilot included developing the infrastructure to share antimicrobial susceptibility testing (AST) results between veterinary laboratories, to demonstrate laboratory AST proficiency, and to develop whole genome sequencing (WGS) capability in the Vet-LIRN network. In 2017, Vet-LIRN laboratories started collecting three veterinary pathogens: *Salmonella sp*. from any host, and pathogenic *E. coli* and *S. pseudintermedius* isolates from dogs. Twenty Vet-LIRN laboratories (Source labs) will collect and test four isolates of each pathogen per month. The isolates, associated metadata and AST data will be submitted to four Regional WGS laboratories. A subset of the isolates, approximately 240, will be sequenced during 2017. WGS information on resistance genes will be compared with AST data. As of May 1, 2017, 17 Vet-LIRN source laboratories submitted 129 *Salmonella* isolates, 132 pathogenic *E. coli* isolates, and 135 *S. pseudintermedius* isolates. The information from the pilot project will be used to design additional surveillance studies to facilitate data sharing and appropriate selection of antibiotics when treating these pathogens in practice.

Development of a new fluorescent *in situ* hybridization for the detection of *Mycoplasma hyopneumoniae* in lung tissue # * †

Henrique Meiroz de Souza Almeida[1], Jennifer M. Groeltz-Thrush[1], Eric Burrough[1], Maria M. Merodio[1], Aric J. McDaniel[2], Bailey Lauren Arruda[1], Rachel J. Derscheid[1], Pablo E. Pineyro[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA; [2]Department of Veterinary Microbiology and Preventative Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA

Swine enzootic pneumonia is a major respiratory disease of swine and is considered an important health challenge to the global swine industry. The etiologic agent is *Mycoplasma hyopneumoniae* (*Mhp*), a bacterium lacking a cell wall that attaches to the cilia of the respiratory epithelium of bronchi in the lungs of infected animals. A diagnosis of swine enzootic pneumonia is generally achieved by molecular detection (PCR) and confirmation of suggestive histological lesions. However, *in situ* diagnosis relays in antigen detection by immunofluorescence and most commonly immunohistochemistry (IHC). Variation in antigen presentation, amount of antigen in tissues, as well as tissue preservation make IHC results variable and often do not correlate with nucleic acid detection by PCR. Fluorescent *in situ* hybridization (FISH) is a diagnostic tool used to detect nucleic acid in tissues using fluorescent-labeled RNA, DNA, or nucleotide probes targeting specific genomic regions of the agents. The data available regarding the use of FISH for the detection of *Mhp* in tissue samples of naturally-infected animals is limited. The aim of this study was to develop and test a new set of fluorescent-labeled (fluorescein) nucleotide probes in lung tissue of naturally and experimentally-infected pigs. In order to validate the probes and hybridization protocol, IHC-positive lung sections from experimentally-infected animals were selected. A previously described nucleotide probe targeting nucleotide 1046-1063 region of 16S rRNA, and two newly designed nucleotide probes targeting 1284-1303 and 1891-1910 regions of 23S rRNA were 5'labeled with fluorescein. Genetic analyses of the probes sequences showed 100% identity with *Mhp* sequence available and no cross matching with other *Mycoplasma* species was observed. All individual probes or a cocktail containing a combination of the three probes at equal concentration (30ng/μL) were evaluated using the same hybridization conditions. Probe-specific binding control was evaluated using a 5' fluorescein-labeled reverse-complemented probe. Preliminary results showed the presence of fluorescent-labeled bacteria in the apical surface of the columnar epithelium of bronchi and bronchioles. In addition, no fluorescent bacterial was observed in slides hybridized with reversed-complemented probes, reinforcing specific binding to the bacterial rRNA. No difference in detection rate was observed amongst individual 16S and 23S probes or combination of probes. Further studies evaluating sensitivity, specificity, and agreement of this new *Mhp*-FISH protocol, IHC and real-time PCR are necessary.

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† Graduate Student Oral Presentation Award Applicant

Isolation and molecular characterization of *Campylobacter* species isolated from nonhuman rhesus monkeys, *Macaca mulatta*, in a laboratory animal facility

Sung Guk Kim[1], Lillie M. Sims[1], Christine V. Summage-West[1], Heather K Bogy[1], Joanna Deck[2], Angela Paredes[1], Seongwon Nho[2], Steven L. Foley[2]

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Campylobacteriosis is the most common foodborne disease worldwide. Every year over 1.3 million people in the United States are affected with *Campylobacter.* Nonhuman primates are also susceptible to *Campylobacter* infections and it raises occupational and public health concerns among the research personnel and public who are in contact with infected nonhuman primates. A total of 155 rhesus monkeys, *Macaca mulatta*, were screened and 14 bacterial isolates were preliminarily identified as *Campylobacter* species based on morphological characteristics using Gram staining, scanning electron microcopy (SEM) and biochemical assays including the oxoid biochemical identification system (OBIS). Further speciation was performed to differentiate *Campylobacter* species at a species level. The current standard method to differentiate *C. jejuni* subsp. *jejuni* from other *Campylobacter* species is based on the presence and expression of the *N*-benzoylglycine amidohydrolase (hippuricase) gene known to be present only in *C. jejuni* subsp. *jenuni*. However, the inconsistent results of the huppuricase assay and the reports of hippurate-negative *C. jejuni* subsp. *jejuni* strains need further defined methods to differentiate *Campylobacter* species. Real-time PCR, 16S rDNA sequencing, automated biochemical-based identification systems (Vitek2C and BioLog), fatty-acid profile-based identification system (MIDI) and MALDI-TOF-based identification system (Biotyper) were employed to further speciate the 14 *Campylobacter* isolates from rhesus monkeys. Real-time PCR methods were cumbersome and impractical for targeting multiple genes for each *Campylobacter* species. However, our results suggested a real-time PCR method could be useful for the identification of *C. jejuni* subsp. *jejuni* targeting a unique sequence like the hippuricase gene (*hipO*). The two culture-based automated identification systems, BioLog and Vitek 2C, did not provide reliable and reproducible results with the 14 nonhuman primate *Campylobacter* isolates. The analysis of the nucleotide sequences of the 16S ribosomal RNA gene identified the 14 *Campylobacter* isolates as 9 *C. coli*, 4 *C. jejuni* subsp. *jejuni* and 1 *C. fetus* subsp. *venerealis*. The MIDI system identified the 14 isolates as 8 *C. coli*, 5 *C. jejuni* subsp. *jejuni* and 1 *C. fetus* subsp. *venerealis*. The Biotyper system identified the 14 isolates as 7 *C. coli*, 6 *C. jejuni* subsp. *jejuni* and 1 *C. fetus* subsp. *venerealis*. At most 2 of the 14 isolates were called differently either *C. jejuni* subsp. *jejuni* or *C. coli*, while all *Campylobacter* reference strains and *C. fetus* subsp. *venerealis* primate isolate were correctly called by all the methods employed. Phylogenetic analysis was performed using dendrograms prepared by PFGE, 16S rRNA sequences, fatty-acid profiles-based, and protein profile-based methods, respectively. Most *Campylobactor* isolates grouped into species-specific clusters.

Evaluation and validation of MALDI-TOF for identification of bacteria and yeasts from animal specimens

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Conventional assays and kits based on biochemical and physiological properties have been inadequate to correctly identify many bacterial isolates from animals to species and even genus level because of inadequate databases for these isolates. While whole genome or 16S rRNA sequencing (Seq) can be used to identify most, if not all, such isolates, sequencing is too expensive and time consuming for routine identification (ID). The application of matrix-assisted laser desorption/ionization-time of flight (MALD-TOF) mass spectrometry may revolutionize the ID of bacteria and yeasts, including isolates from animals, and is rapid (~ 6 minutes) and inexpensive (a few cents/sample). Two-hundred seventy bacterial and 5 yeast isolates were tested and ID was compared to TREK Sensititre, RapID Ana II, API, or other diagnostic tests. Discordant results were resolved by 16S rRNA-Seq. For Gram-negative enteric rods (GNER) MALDI-TOF agreed with TREK for 61 of 62 isolates, and was validated for all 62 GNER isolates tested. MALDI-TOF agreed with other assays for 21 of 31 isolates of Gram-negative non-fermentative rods (GNNFR). Six isolates of GNNFR identified by MALDI-TOF, but not other assays, were verified by 16S rRNA-Seq, and 2 were not identified to genus level (3 to species level). ID of 31 of 37 fastidious Gram-negative rods (FGNR) were in agreement between MALDI-TOF and other assays. Two FGNR isolates identified only by MALDI-TOF were verified by 16S rRNA-Seq, but 2 isolates were not identified. MALDI-TOF agreed with other assays for 12 of 31 Gram-positive bacilli (GPB), was verified for 14 of these GPB isolates by 16S rRNA-Seq, but was incorrect or there was no ID for 3 isolates. Of 77 Gram-positive cocci (GPC), MALDI-TOF and other assays agreed on 58 isolates to genus level and on 43 isolates to species level. MALDI-TOF was incorrect for 1 GPC isolate correctly identified by other assays, but only MALDI-TOF ID was validated for 20 isolates by 16S rRNA-Seq; there was no MALDI-TOF ID for 3 isolates. Of 32 anaerobe isolates MALDI-TOF and other assays agreed for 24 isolates to genus level (18 to species level). MALDI-TOF ID was incorrect for 3 anaerobe isolates identified by other assays, but MALDI-TOF only was correct to genus level for 14 isolates (7 to species level) by 16S rRNA-Seq. Few yeasts have thus far been tested, but MALDI-TOF correctly identified *Candida* isolates to species level, but not *Malassezia pachydermatis*. In conclusion, MALDI-TOF correctly identified to genus level 95% of animal bacterial isolates (257 of 270 isolates to genus; 255 to species level). Other conventional assays were highly accurate for ID of GNER, but could only identify 39-83% of other bacterial isolates. We conclude that next to DNA sequencing ID of bacterial pathogens from animals by MALDI-TOF is highly accurate, and the only reliable method for ID of most non-enteric bacterial isolates from animals.

Investigation of the pathogens contributing to naturally occurring outbreaks of infectious bovine keratoconjunctivitis (pinkeye) using Next Generation Sequencing

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Infectious bovine keratoconjunctivitis (IBK) is an important ocular disease which affects cattle worldwide. Three infectious agents have been associated with pinkeye, including *Moraxella bovis, Moraxella bovoculi, and Mycoplasma bovoculi*. However, the only one of these organisms that has been shown experimentally to produce IBK is *Mor bovis*. Colonization of the eyes of cattle by *Mor. bovis and Mor. bovoculi* can occur in the absence of clinical disease and both organisms can be cultured from the eyes of healthy cattle, suggesting that there are intrinsic changes that may occur to resident flora or the involvement of additional unrecognized organisms that contribute to IBK. To identify these changes, swabs were collected from the eyes of 100 cattle representing 15 different herds. Of these 15 herds, 11 had current cases of pinkeye, 3 had a past history of pinkeye but no current cases, and 1 farm had no history of pinkeye. Extracted DNA samples from the swabs were subjected to 16SrRNA gene PCR and next generation sequencing (NGS). The types of organisms detected from the eyes of cattle were similar across the herds and there was no difference in the total number of bacterial groups detected among IBK cases and controls. However, the numbers of the different organisms detected varied between the two groups. There was no difference in *Mycoplasma* species or *Mycoplasma bovoculi* between the two groups, though some studies suggested that *Mycoplasma species* are important to the development of pinkeye. In contrast, there were differences in *Mor. bovis,* Mor. *bovoculi* and *Moraxella* "slash" calls (unspeciated *Moraxella*) between cases of pinkeye and normal eyes. Additionally, there were other groups of bacteria that differed between the eyes with IBK and normal eyes. These results suggest that there is a correlation with increased numbers of *Mor. bovis* and *bovoculi* with IBK cases, but there was not a correlation between *Mycoplasma* species and pinkeye. This study also suggest that there were changes to the microbiomes of the animals that had pinkeye. Alteration of the ocular microbiota composition may have a predisposing role, enhancing bacterial infection and the occurrence of clinical IBK. Future studies are required to evaluate if these changes are permanent or if there is a shift in the microbiome following recovery from the infection and how antibiotics might affect the microbiome.

Detection and differentiation of Oomycota pathogens infecting dogs (*Pythium insidiosum*, *Lagenidium* spp. and *Paralagenidium karlingii*) by FRET-qPCR

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For years, *Pythium insidiosum* was considered the only pathogenic Oomycota in dogs. This changed when an undescribed Oomycota in the genus *Lagenidium* spp. was identified also as the cause of subcutaneous and systemic disease in dogs. Another Oomycota pathogen, antigenically and morphologically similar to *Lagenidium* spp., and also associated with dog cutaneous to subcutaneous infections, is *Paralagenidium karlingii*. Because the morphological and clinical features of the pathogenic Oomycota in dogs are similar to true fungi, these pathogens are often misidentified. Laboratory differentiation between these three groups of canine pathogenic Oomycota (*P. insidiosum*, *Lagenidium* spp. and *P. karlingii*) is of clinical relevance because their prognosis and treatment are markedly different.

The infections caused by the mammalian pathogenic Oomycota are devastating and often fatal in dogs. Currently diagnosis is based on serology, culture and/or histopathology but these can be time consuming and can leave questions as to the species of Oomycota involved. We established a PCR which can sensitively and specifically detect mammalian pathogenic *Oomycota* (*P. insidiosum*, *Lagenidium* spp. and *P. karlingii*) allowing for quick and reliable diagnosis of these potentially serious infectious agents in the clinical laboratory.

The rRNA sequences representing pathogenic *Oomycota* in dogs, non-pathogenic Oomycota and true fungi which allowed for the identification of a highly conserved region as primers for specific amplification of pathogenic Oomycota in dogs. In addition, variable regions were identified within the PCR amplicon and probes developed making it possible to differentiate between *P. insidiosum*, *Lagenidium* spp. and *P. karlingii* based on high-resolution melting curve analysis in a single-tube fluorescence-resonance energy-transfer (FRET) PCR. The FRET-qPCR was established to specifically amplify pathogenic Oomycota in dogs with a sensitivity of a single copy of the ITS gene per reaction. High-resolution melting curve analysis enabled us to distinguish between *P. insidiosum* (47.5 °C), *Lagenidium* spp. (53 °C) and *P. karlingii* (57 °C). The total time from DNA extraction of clinical specimens to determination of Oomycota species is approximately 2.5 hours.

The FRET-qPCR established in this study is both highly sensitive and specific for canine pathogenic Oomycota and will have a significant impact on patient success rates due to improving diagnostics and the initiation of more timely treatment, and this enables a quick detection and differentiation of *P. insidiosum*, *Lagenidium* spp. and *P. karlingii* will facilitate more effective management and treatment of canine oomycosis.

Rapid typing of opportunistic bacterial pathogens using MALDI-TOF mass spectrometry; discriminating *Mannheimia hemolytica* major genotypes 1 and 2 using a biomarker based proteomic approach

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Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry is an emerging technology in veterinary diagnostic microbiology. Bovine respiratory disease (BRD) is one of the most costly diseases to cattle production and *Mannheimia haemolytica* is the most commonly detected bacterial agent associated with BRD. Recently, whole genome sequencing was employed on 1,133 *M. haemolytica* isolates from North American cattle and a nucleotide polymorphism typing system was developed. Two major genotypes were discovered (1 and 2). Genotype 1 *M. haemolytica* were mostly isolated from the nasopharynx of cattle without BRD. In contrast, genotype 2 *M. haemolytica* predominantly associated with the lungs of cattle with BRD and integrative conjugative elements that contained antimicrobial resistance determinants. The objective of this study was to develop a rapid MALDI-TOF mass spectrometry biomarker based proteomics classification system that allows for discrimination between *M. haemolytica* genotypes 1 and 2 using mass spectra. Thirty four isolates were used in this study that had been subjected to whole genome sequencing and nucleotide polymorphism based typing, and that represented the major *M. hemolytica* genotypes 1 (n=23) and 2 (n=11) and subtypes 1b (n=6), 1b recombinant (n=1), 1c (n=3), 1e (n=2), 1f (n=4), 1i (n=7), 2b (n=6), 2c (n=2), 2d (n=1) and 2e (n=2). Biological (n=3) and technical (n=12) replication were utilized to collect 36 independent spectra from each isolate. Comparisons were made between each major genotype and subtype using a bioinformatics approach and a "Peak Statistic Table" was generated for each comparison which was used to develop a biomarker based model to objectively resolve genotypes. Following data collection and analysis, a 9,494 Da peak was detected for all genotype 2 isolates and absent for all genotype 1 isolates. Conversely, a 9,523 Da peak was detected for all genotype 1 isolates and absent for all genotype 2 isolates. The biomarker based model was able to correctly classify all isolates into genotype 1 or genotype 2 when the majority of spectra were used for overall classification. Additionally, the direct smear extraction method, which is routinely used in diagnostic workflows for bacterial identification was able to generate spectra that enabled all isolates to be correctly classified. In summary, a MALDI-TOF MS assay was developed that provides an effective, simple, and cost effective way to rapidly type *M. haemolytica* into clinically and biologically relevant genotypes. This assay should greatly enhance the ability of veterinary diagnostic labs to interpret *M. haemolytica* cultures, especially from nasal swabs or the upper respiratory tract where both genotypes are likely to be present. Assay data can be rapidly gathered in routine diagnostic workflows and the results integrated into diagnostic testing reports for enhanced bovine respiratory disease diagnosis.

Identification of *Edwardsiella piscicida* using MALDI-TOF Biotyper in-house library §

Christina Cappelli, Rebecca Franklin-Guild, Anil J. Thachil

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has become widely accepted among veterinary and human laboratories for identifying clinical isolates of bacteria using the Bruker database. Recently, more laboratories are building their own libraries from species they have validated through biochemical tests or polymerase chain reaction (PCR). *Edwardsiella* is group of Gram negative bacteria that can cause disease in fish, reptiles, birds, and mammals. Specifically, *Edwardsiella piscicida* cause pathogenicity in economically important fish including catfish, eels, and tilapia. The Bruker MALDI Biotyper system currently identifies this taxon as *Edwardsiella tarda*. Therefore, DNA analysis using 16s rRNA, *rpoB* gene sequencing, or *gyrB* gene sequencing is required for species-specific identification. Due to time sensitivity, rapid detection of this species is ideal. For this study, two isolates characterized as *E. piscicida* by molecular analysis were used as reference spectra for our in-house Bruker MALDI-TOF biotyper library. Bacterial isolates were prepared by tube extraction method, as described by the manufacturer, to disrupt cells and obtain a mass spectrometry profile (MSP) for each. Custom MSPs were created and added to the MALDI-TOF library database to use as a reference, to test 36 *E. piscicida* isolates previously identified by MALDI-TOF as *E. tarda* with scores above 2.0. Upon addition of the library all 36 isolates were identified as *E. piscicida* with a score above 2.0. This data suggests that use of the Bruker MALDI Biotyper with added in-house spectral library will result in both improved overall reliability and rapid identification of *E. piscicida*.

§ AAVLD Laboratory Staff Travel Awardee

Bacteriology 2 / Parasitology

Sunday, October 15, 2017

Pacific Salon 2

Moderators: Subhashinie Kariyawasam and Olgica Ceric

7:45 AM **Novel IVD tools for diagnosing filariasis in humans and animals**
Andreas Latz 18

8:00 AM **Effects of biological materials and collection media on PCR detection of *Tritrichomonas foetus* ◊**
Kris A. Clothier, Bret R. McNabb, Jeff Ondrak 19

8:15 AM **Forty eight hour incubation of field mimic *Tritrichomonas foetus* positive TF Transit Tubes shows improved real-time PCR threshold cycle values compared to non-incubated and PBS samples ◊**
Brandon Kennedy Font, Stephen Chamberlain, Suzanna Leckman, Tiffany Brigner 20

8:30 AM **Evaluation of an indirect ELISA using recombinant Serpin of *Trichinella spiralis* as antigen**
Vladislav A. Lobanov, Kelly Konecsni, Yunxiu Dai, Alvin A. Gajadhar 21

8:45 AM **Development and performance evaluation of enzyme linked immunosorbent assay and lineblot for serological diagnosis of leishmaniasis in dogs**
Andreas Latz 22

9:00 AM **Monitoring feedlot cattle for seroconversion to *Histophilus somni* # * †**
Kelli Bechtler, Lynette Corbeil, Donal O'Toole, Kerry Sondgeroth 23

9:15 AM **Break**

10:00 AM **Characterization of hypermucoviscous *Klebsiella pneumoniae* isolated from 4 horses with severe hemorrhagic pneumonia**
Barbara A. Byrne, Carsten Struve, Dane M Whitaker, Krista E Estell, Monica R. Aleman, Madelyn E. Arbios, Esteban Soto 24

10:15 AM ***Streptococcus bovis* complex isolates associated with neoplasia in companion animals**
Stephen Cole, Molly Church, Shelley C. Rankin 25

10:30 AM **Antimicrobial activity of bovine NK-lysin-derived peptides on bovine respiratory pathogen *Histophilus somni* ◊**
Rohana P. Dassanayake, Shollie Falkenberg, Robert E. Briggs, Fred M. Tatum, Randy E. Sacco 26

10:45 AM **SODAPOP: A Novel Educational Tool for Veterinary Antimicrobial Selection**
Stephen Cole, Shelley C. Rankin 27

11:00 AM **Urine for a Surprise Pussycat: Evaluation of the use of an expanded quantitative urine culture.**
Stephen Cole, Shelley C. Rankin, Emily Knebel 28

11:15 AM **An outbreak of reproductive salmonellosis in farmed mink in Utah**
Jane Kelly, Arnaud J. VanWettere, Chad Clancy *29*

11:30 AM **Development and performance evaluation of a phase specific enzyme linked immunosorbent assay and lineblot for serological diagnosis of Coxiella / Q-fever infection in humans and animals**
Andreas Latz *30*

Symbols at the end of titles indicate the following designations:

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

Novel IVD tools for diagnosing filariasis in humans and animals

Andreas Latz

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Filariasis is a parasitic disease caused by an infection with roundworms of the Filarioidea type. These are spread by blood-feeding black flies and mosquitoes. This disease belongs to the group of diseases called helminthiases. Eight known filarial nematodes use humans as their definitive hosts. These are divided into three groups according to the niche within the body they occupy. Lymphatic filariasis (*Wuchereria bancrofti, Brugia malayi,* and *Brugia timori*), subcutaneous filariasis (*Loa loa*, *Mansonella streptocerca* and *Onchocerca volvulus*) and serous cavity filariasis (*Mansonella perstans* and *Mansonella ozzardi*). In addition there are many species known to infect animals like *Dirofilaria spp* or *Acanthocheilonema spp*. Most of these parasites are causing zoonotic diseases and can infect both, humans and animals. Therefore tools for monitoring acute and past diseases in animals and humans are needed to efficiently manage the disease. The aim of this work was to develop an ELISA to detect anti-filariasis antibodies (total anti-filaria antibodies and anti-filaria-IgG_4 antibodies) and filariasis antigen in human as well as veterinary specimens. Novel protocols have been established to develop the new ELISA systems. Predefined human samples from acute as well as past infections of lymphatic filariasis, subcutaneous filariasis and serous cavity filariasis as well as veterinary samples from dogs and cows have been used for the development and validation of the test systems. The human and veterinary total antibody detection assay as well as the human IgG_4 detection assay show a sensitivity and specificity of over 95%. The human and veterinary antigen detection assay shows a sensitivity and specificity of over 98%. The human total antibody assay has already been certified according to Directive 98/79/EC of the European Parliament. With the newly developed ELISA system it is possible to discriminate acute and past filariasis infections in both, humans and animals. This will be extremely helpful in diagnosing acute infections and monitoring treatment success in the human as well as animal population (livestock, pets and wildlife). Serological surveys in the animal field will help to improve the risk assessment for the human population in certain endemic regions.

Reference(s):

Tambo, Ernest; Ai, Lin; Zhou, Xia; Chen, Jun-Hu; Hu, Wei; Bergquist, Robert et al. (2014): Surveillance-response systems: the key to elimination of tropical diseases. In Infectious diseases of poverty 3, p. 17. DOI: 10.1186/2049-9957-3-17.

Effects of biological materials and collection media on PCR detection of *Tritrichomonas foetus* ◊

Kris A. Clothier[1], Bret R. McNabb[3], Jeff Ondrak[2]

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In spite of regulatory programs present in many states and countries to address *Tritrichomonas foetus* infection, this pathogen continues to represent a major economic problem to the cattle industry. Due to its insidious nature and lack of clinical signs in infected adult cattle, *T. foetus* can go unrecognized in a herd for many years. The advent of molecular detection has dramatically increased the sensitivity and specificity of *T. foetus* diagnosis over previously used culture methods. Sample condition is also a major contributing factor to accurate detection. The purpose of this study was to evaluate the effects of exposure to a variety of biological materials on the PCR detection of *T. foetus*. A standard inoculum of one of three strains of *T. foetus* were used to inoculate 0.9% saline (n=45), lactated Ringers solution (LRS; n=45), or InPouch® (Biomed Diagnostics) media (IP; n=45.) Samples were then spiked with either freshly collected semen, urine, or blood to mimic conditions which may be present during field sample collection. At the laboratory, saline and LRS were inoculated into modified Diamond's media and incubated at 37 °C for 48 hours; IP samples were incubated at the same temperature for 24 hours. Aliqouts were collected and tested in triplicate by PCR. In IP media, urine had the most detrimental effect on detection of *T. foetus* with higher cycles to threshold (Ct) values identified in urine spiked samples over blood, semen, and control groups across all strains evaluated. Ct values were not significantly different in samples containing blood or semen than in control samples. *T. foetus* detection was less affected in samples inoculated into modified Diamond's media, with no differences in Ct values between treatment groups; however, samples submitted in LRS had lower mean Ct values than samples submitted in saline. Overall, media containing urine had fewer samples identified as "positive" and more samples classified as "inconclusive" using laboratory cut-offs than those containing blood or semen. Sample integrity can impact identification of this pathogen and the present study shows that minimizing urine contamination can improve PCR detection of *T. foetus* in preputial samples.

◊ USAHA Paper

Forty eight hour incubation of field mimic *Tritrichomonas foetus* positive TF Transit Tubes shows improved real-time PCR threshold cycle values compared to non-incubated and PBS samples ◊

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In vitro culture of the protozoan *Tritrichomonas foetus* is the traditional method of diagnosing bovine trichomoniasis in order to meet regulatory requirements and to control for the reproductive and monetary losses absorbed by the cattle industry due to this sexually transmitted disease. In more recent decades, real-time PCR assays have continued to work in tandem with traditional culture/transport systems (e.g., InPouch™ TF system) and also with the TF Transit Tube sample transportation for **'**PCR use only' system to increase the overall sensitivity and specificity of *T. foetus* detection. Current discussions in the veterinary parasitology literature revolve around the optimization and application of best practices regarding *T. foetus* bovine sample collection, transport, storage temperatures, *in vitro* culture dynamics and real-time PCR analysis. Here we performed a simple two lab experiment on a set of 30 field mimic samples spiked with 100 *T. foetus* cells and 250 µl of bull smegma. Field mimic culture/transport systems included InPouch TF, TF Transit Tubes and PBS tube samples. After 24 h ambient transportation times, these systems were exposed to two temperature treatments for 48 h. Ambient vs. 35 ± 2°C samples were analyzed via real-time PCR to observe for any threshold cycle variations due to incubation temperatures or culture/transport systems. Our findings suggest that bovine sample collection in PBS or lactated Ringer's is not optimal for *T. foetus* real-time PCR. While additional investigation is ongoing to examine (1) optimal *T. foetus* recovery as a function of incubation time, (2) effects of gaseous micro-environments for both pure and mixed microbial *T. foetus* cultures, and (3) potential limited *T. foetus* recovery caused by contaminating bacterial blooms, our data show that real-time PCR sensitivity of *T. foetus* was enhanced after 48 h incubation in both the InPouch TF and TF Transit Tube systems.

◊ USAHA Paper

Evaluation of an indirect ELISA using recombinant Serpin of *Trichinella spiralis* as antigen

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Due to their zoonotic potential, infections of food animals with parasitic nematodes of the genus *Trichinella* pose food safety risks with concomitant economic implications for industrial meat production. Food safety testing for *Trichinella* spp. relies upon direct detection of first-stage larvae (L1) by methods based on artificial digestion of muscle tissue. However, the enzyme-linked immunosorbent assay (ELISA) utilizing antigen represented by a complex of excretory-secretory (E-S) proteins from *in vitro*-cultured L1 of *T. spiralis* is commonly used for surveillance and epidemiological investigations in susceptible domestic animals and wildlife. A fraction of these E-S proteins shares an immunodominant carbohydrate antigen epitope containing tyvelose (TSL-1) that is conserved among all known species and genotypes of *Trichinella.* Production of the E-S antigen is a lengthy process requiring laboratory animal use to propagate the parasite. Recombinant antigen production is generally a more resource-efficient, safe, fast and easier-to-standardize process compared to the isolation of somatic or secreted antigens of cultured pathogens. We produced serine protease inhibitor (Serpin) of *T. spiralis* as secreted protein in a yeast expression system and evaluated its performance as an antigen in indirect ELISA. Using this assay, antibodies to *T. spiralis* were consistently detected in pigs experimentally infected with low doses of this parasite. The kinetics of production of Serpin-specific antibodies were similar to those of the antibody responses to E-S antigen in two pigs infected with 300 L1/animal, whereas there were one and three week delays in Serpin-specific seroconversion in pigs infected with 120 and 50 L1, respectively. The diagnostic specificity of both E-S ELISA and Serpin ELISA was 97.9 % for sera collected from 1,056 Canadian sows (*Trichinella*-negative). However, there was a markedly higher level of non-specific reactivity of serum antibodies from these pigs with the Serpin antigen that resulted in a cut off value of more than twice that of the E-S ELISA. This would likely negatively affect the diagnostic sensitivity of Serpin ELISA. Furthermore, we demonstrated that the specificity of recombinant Serpin antigen may be restricted to *T. spiralis*, as pre-immune and immune sera from pigs experimentally infected with *T. britovi* or *T. pseudospiralis* could not be reliably discriminated by Serpin ELISA.

Development and performance evaluation of enzyme linked immunosorbent assay and lineblot for serological diagnosis of leishmaniasis in dogs

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Canine leishmaniasis is a zoonotic disease transmitted by the bite of an infected phlebotomine sandfly. *Leishmania infantum* is the most common and important cause of canine leishmaniasis worldwide. Other *Leishmania spp.* reported from dogs include *L. mexicana*, *L. donovani*, and *L. braziliensis*. Leishmaniasis can be categorized by two types of diseases in dogs: a cutaneous reaction and a visceral reaction also known as black fever, the most severe form of leishmaniasis. Infection does not invariably lead to illness. In fact, most infected dogs remain asymptomatic and may never develop clinical manifestations. In endemic regions, the prevalence of disease is often less than 10% and only about 1 in 5 infected dogs are considered likely to develop clinical disease. Diagnosis of canine leishmaniasis is based on the presence of clinical signs together with positive specific antibody assay. The aim of this work was to develop a serological ELISA assay to detect IgG and IgM antibodies against Leishmania in serum or plasma samples derived from all mammals. Microtiterplates were coated with antigen preparations of *Leishmania infantum*. The presents of antibodies against Leishmania is detected by proprietary technology. A sample collection of about 200 positive samples and 400 negative samples was used for development and evaluation of the assay. Evaluation of the assay took place in different European laboratories. To fulfill the current needs we have developed ELISA assays for Dengue (IgG, IgM,μ-capture), Chikungunya (IgG-capture, μ-capture) and Zika (μ-capture) to detect pathogen specific IgG or IgM antibodies. With the help of the capture technology it is possible to reduce cross reactions to other pathogens, especially other flaviviruses. Here we show the performance characteristic of the newly developed assay. Due to the improved antigen design, purification method and test setup a sensitivity and specificity of 96,9 and >98% respectively could be achieved. This new Leishmania ELISA can be used to diagnose disease in symptomatic as well as in asymptomatic dogs to be able to administer proper treatment. In areas where Leishmania is endemic and transmitted by insect vectors, it is an important agent of human disease and dogs are considered the most important peridomestic reservoir host. The new ELISA can be used to monitor seroprevalence in the animal population to help to control and manage the disease in these regions. In addition this ELISA will be a tool for screening imported or returning animals from endemic countries.

Monitoring feedlot cattle for seroconversion to *Histophilus somni* # * †

Kelli Bechtler[1], Lynette Corbeil[2], Donal O'Toole[1], Kerry Sondgeroth[1]

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Histophilus somni (*H. somni*) is a bacterial agent of the Bovine Respiratory Disease (BRD) complex whose influence on BRD in the United States is not well defined. It is a gram negative bacterium that normally resides in the upper respiratory tract of cattle. Histophilosis occurs when *H. somni* colonizes the lower respiratory tract, contributing to severe respiratory disease and when introduced to the bloodstream can cause septicemia, myocarditis, and thromboembolic meningoencephalitis. Histophilosis is associated with stress and transportation in recently weaned cattle and clinical signs often manifest within 60 days of transport to the feedlot. *H. somni*, especially as part of the BRD complex, can result in great financial and production losses to feedlots.

The primary study objective is to determine *H.somni* antibody levels in a group of feedlot calves over time. Longitudinal blood sampling occurred from intake, day 30 and day 60. Antibody levels from cattle in two feedlots in Colorado and Wyoming were evaluated by ELISA. This ELISA detects the presence of antibodies to the IbpA (subunit A5) antigen, a protein that is not present in a minority of strains of *H. somni* from asymptomatic carriers.

No cutoff values have been established for the IbpA ELISA, thus a 20% increase in OD (optical density) readings between sampling time points is being utilized until validation is complete. Preliminary findings indicate that 20.6% (64/310) of all calves have an OD increase by Day 60. Of the 317 cattle that were sampled, 31.9% (101) were treated for clinical illness between arrival to Day 60. Of these treated cattle, 21.8% (22/101) have an OD increase by day 30, and, 31.6% (32/101) by day 60. This is compared to the healthy or untreated cattle in which 14.5% (46/310) and 11.9% (37/310) have an OD increase at day 30 and day 60, respectively. These results suggest that exposure to *H. somni* does occur post feedlot entry and may be another factor contributing to BRD severity.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Characterization of hypermucoviscous *Klebsiella pneumoniae* isolated from 4 horses with severe hemorrhagic pneumonia

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Klebsiella pneumoniae was isolated from transtracheal wash fluid, pleural fluid, and/or lung tissue samples from 4 horses who were treated at the University of California, Davis William R. Pritchard Veterinary Medical Teaching Hospital for hemorrhagic nasal discharge and severe pneumonia. All 4 horses were euthanized; 3 underwent necropsy examination where the primary pulmonary lesion was severe necrohemorrhagic pneumonia. A total of 12 *K. pneumoniae* isolates with differing colony morphologies were obtained from ante- and post-mortem samples from the 4 horses. Isolates were examined for mucoviscosity using the "string test" method, their identity was confirmed molecularly, and they were characterized using endpoint PCR for the *mag*A, wzy_{K2}, $wzx_{K5,}$ *rmp*A, *iut*A, *iro*D, *fyu*A, *fim*H, *mrk*B, *clb*B, *all*S and *kpn*P genes. Furthermore, 11 isolates from 3 horses were genotyped by pulsed-field gel electrophoresis (PFGE). Five out of 12 isolates exhibited the hypermucoviscous (HMV) phenotype including isolates from three of the four horses. All isolates were capsular type 5 and *rmp*A positive regardless of HMV phenotype. Virulence gene PCR demonstrated identical profiles for all isolates. They were *fyu*A, *iro*D, *fim*H, and *mrk*B positive and negative for the other virulence-associated genes. Examination of PFGE pulsotypes revealed isolates from 2 horses from the same location were indistinguishable and the isolates from the 3rd horse were unrelated to the other horse isolates but indistinguishable from one another. This finding suggests that HMV *K. pneumoniae* may be capable of transmission between horses. Hypermucoviscous *Klebsiella pneumoniae* should be considered as an etiologic agent in severe hemorrhagic pneumonia in horses.

Streptococcus bovis complex isolates associated with neoplasia in companion animals

Stephen Cole, Molly Church, Shelley C. Rankin

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The *Streptococcus bovis* complex (SBC) is a group of closely related, Gram-positive bacteria that has been associated with bacteremia, endocarditis and gastrointestinal neoplasia in people. Several studies have shown the potential for SBC to be directly involved in oncogenesis, but the etiology is still debated. The aim of this study was to investigate if SBC is associated with neoplasia in companion animals. Microbiology records from a Microscan Walkaway were searched for isolates identified as "*Streptococcus bovis* complex" by the PC20 panel. The complete medical record of all dogs and cats from which SBC was isolated were reviewed for signs of neoplasia (including biopsy and diagnostic imaging reports). Between 2003 and 2017, the clinical microbiology laboratory identified 21 SBC isolates from 20 dogs and 1 cat. In human patients the type of neoplasia is associated with a particular SBC biotype, therefore SBC isolates were characterized by biotyping based on mannitol and B-glucuronidase results. Positive specimens included urine (8/21), respiratory samples (3/21), dermatologic samples (3/21), liver/bile (2/21) abdominal swabs (intraoperative) (2/21), lymph node aspirate (1/21), bone (1/21) and synovial fluid (1/21). Of the 21 patients, 10 (47.6%) concurrently or previously had suspected or diagnosed neoplasia. A confirmed or suspected diagnosis of gastrointestinal cancer was made for 60% (6/10). The remaining four cases had lymphosarcoma (2/10) or transitional cell carcinoma (2/10). Biotype II/1 is most commonly associated with gastrointestinal cancer (non-colonic) in people. In this study, significantly more ($p=0.04$) neoplasia-associated isolates (7/10; 70%) were classified as biotype II/1 when compared to non-neoplasia associated isolates (3/11; 27.3%). Colonic neoplasia is more commonly associated with biotype I in people; however, the only case of colonic neoplasia in this study was associated with biotype II/1. Many physicians consider isolation of SBC as indication for further diagnostics (i.e. colonoscopy or ultrasound) to rule out concurrent neoplasia. This study suggests that veterinarians could also consider this approach. SBC are morphologically similar to *Enterococcus* spp. in culture therefore, accurate identification of SBC in veterinary microbiology labs is critical in order for this to be a useful clinical tool. Finally, this may also be a naturally occurring animal model of bacterial oncogenesis that warrants further investigation.

Antimicrobial activity of bovine NK-lysin-derived peptides on bovine respiratory pathogen *Histophilus somni* ◊

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Bovine NK-lysins, which are functionally and structurally similar to human granulysin and porcine NK-lysin, are predominantly found in the granules of cytotoxic T-lymphocytes and NK-cells. Although antimicrobial activity of bovine NK-lysin has been assessed for several bacterial pathogens, not all the important bacterial pathogens that are involved in the bovine respiratory disease complex have been studied. Therefore the objective of the present study was to evaluate the antimicrobial activity of bovine NK-lysin-derived peptides on bovine respiratory pathogen *Histophilus somni*. Four, 30-mer peptides corresponding to the functional region of NK-lysin helices 2 and 3 were synthesized and assessed for antibacterial activity on four bovine pneumonic *H. somni* isolates. Although there were some differences in the efficiency of bactericidal activity among the NK-lysin peptides at lower concentrations (2 - 5 μM), all four peptides effectively killed most *H. somni* isolates at higher concentrations (10 - 30 μM) as determined by a bacterial killing assay. Confocal microscopic and flow cytometric analysis of Live/Dead Baclight stained *H. somni* (which were preincubated with NK-lysin peptides) were consistent with the killing assay findings and suggest NK-lysin peptides are bactericidal for *H. somni*. Among the four peptides, NK2A-derived peptide consistently showed the highest antimicrobial activity against all four *H. somni* isolates. Electron microscopic examination of H. somni following incubation with NK-lysin revealed extensive cell membrane damage, protrusions of outer membranes, and cytoplasmic content leakage. Taken together, the findings from this study clearly demonstrate the antimicrobial activity of all four bovine NK-lysin-derived peptides against bovine *H. somni* isolates.

◊ USAHA Paper

SODAPOP: A Novel Educational Tool for Veterinary Antimicrobial Selection

Stephen Cole, Shelley C. Rankin

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Antimicrobial resistance in bacterial pathogens has dictated a need for veterinarians to to practice "antimicrobial stewardship." Veterinary educators lack effective tools to teach stewardship but recent trends in veterinary education that include case-based teaching methods may be an effective solution. SODAPOP is a mnemonic tool designed to integrate case-based learning of antimicrobial selection into the veterinary curriculum. The teaching tool suggests that students consider the **s**ource and **o**rganism before they **d**ecide to treat with antimicrobials. Then the susceptible **a**ntimicrobials are considered with regard to contraindications in the **p**atient. Ultimately, the **o**ptions are weighed and a **p**lan formulated. The aim of this study was to evaluate the efficacy of SODAPOP to teach fourth-year veterinary students during a clinical rotation. An online survey was administered to 20 students during the first half of a two-week rotation before any clinical microbiology instruction. All surveys were evaluated by the Institutional Review Board and a waiver for approval granted. The pre-survey evaluated (1) students' confidence in antimicrobial selection for three categories: urinary (UR), respiratory (RE) and skin (SK) infections. Participants then viewed an instructional video on the concept of SODAPOP. The post-survey (1) re-evaluated students' confidence in antimicrobial selection and (2) evaluated their views on SODAPOP as a tool. The average weighted-confidence value (5-point scale) increased post-intervention for all categories (UR_{pre}- 2.9, UR_{post}- 3.5, RE_{pre}- 2.7, RE_{post}- 3.3, SK_{pre}- 3.45, SK_{post}- 3.75). The majority of students felt positively (somewhat agree, agree, strongly agree) that SODAPOP is a valuable tool for veterinary students (19/20; 95%), that they would use the tool in the future (19/20; 95%) and that SODAPOP made them consider factors they do not normally consider when choosing an antimicrobial (17/20; 85%). Veterinary clinical microbiologists often play a role in the education of veterinary students with regards to antimicrobial choice. This study shows that SODAPOP is a potentially valuable tool in case-based education for antimicrobial choice.

Urine for a Surprise Pussycat: Evaluation of the use of an expanded quantitative urine culture.

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Feline lower urinary tract disease (FLUTD) encompasses a common, but very complex spectrum of diseases that includes urinary tract infections (UTI), urolithiasis and feline idiopathic cystitis (FIC). FIC is clinically indistinguishable from UTI; however, urine culture is negative for FIC patients. Recent development and implementation of the Expanded Quantitative Urine Culture (EQUC) method suggests that healthy women may have "normal" bacterial inhabitants of the urinary tract that vary from women with chronic urinary tract disease. The EQUC method enhances the detection of microorganisms by using increased volumes of urine with a variety of culture media and atmospheric conditions. The aim of this study was to compare the isolation rates of organisms cultured by EQUC from FLUTD and Non-FLUTD urine samples. To avoid inclusion of contaminants, only urine obtained via cystocentesis from two clinically distinct groups of patients (FLUTD and non-FLUTD cats) was cultured. In total, 11 samples were categorized as FLUTD and 20 were categorized as non-FLUTD based on the final diagnosis in the medical record. EQUC testing was positive for 10/11 (91%) FLUTD and 10/20 (50%) non-FLUTD patient samples. Coagulase-negative *Staphylococcus* (CoNS) species were the most prevalent organisms from both groups. 18/31 [58%] of non-FLUTD isolates, and 8/21 [38%] of FLUTD isolates were CoNS. This proof-of-concept study showed that EQUC can be used to isolate bacteria from feline urine samples that were negative by standard culture. The study suggests that rates of culturable organisms vary between FLUTD and non-FLUTD cats. This difference may reflect an etiology of a disease that is currently considered idiopathic. Alternatively, even if not causative, the differences in microbial populations could potentially be leveraged as diagnostic biomarkers for a disease currently considered a diagnosis of exclusion. Future studies are warranted given the different bacterial populations and will use next generation sequencing techniques to evaluate the bacterial flora of the feline urinary bladder.

An outbreak of reproductive salmonellosis in farmed mink in Utah

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Between April and May 2017, 46 adult mink from nine farms were submitted to the Utah Veterinary Diagnostic Laboratory for necropsy. Mink originated throughout the state of Utah and all were part of a large cooperative. All but one animal were gravid or recently gravid females and had lesions of metritis and placentitis. Other lesions included peritonitis (from ruptured uteri), interstitial pneumonia, splenitis, and fetal bronchopneumonia. No enteric lesions were present. Aerobic, anaerobic, *Salmonella*, and *Campylobacter* cultures of the uteri, liver or spleen were performed. *Salmonella* Reading was isolated from the uteri of only two animals. However, *Salmonella sp*. was detected by polymerase chain reaction and/or immunohistochemistry from the uterus/placenta of mink from every farm. *Campylobacter sp.* was not detected on bacterial culture. Abortion and death in the female mink were attributed to *Salmonella sp*. metritis and placentitis with secondary septicemia. *Salmonella* Reading has been isolated from several domestic livestock species and has been associated with foodborne illness and osteomyelitis in humans and with enteric and systemic disease in poults. All mink farms involved in this outbreak belong to a cooperative and receive food from the same feed mill. The feed is the suspected source of *Salmonella* in this outbreak.

Development and performance evaluation of a phase specific enzyme linked immunosorbent assay and lineblot for serological diagnosis of Coxiella / Q-fever infection in humans and animals

Andreas Latz

Research and Development, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany

The pathogen *Coxiella burnetii* is a gram negative bacterium and belongs to the family Rickettsiaceae. It is the causative agent of Q-Fever, an acute rickettsial disease. It can be considered the most infectious disease in the world, as a human being can be infected by a single bacterium. It can be found worldwide, including tropical countries, with the exception of New Zealand. In Europe it appears as hepatitis rather than pneumonia as in the United States. The most common manifestation is flu-like symptoms. The fever lasts approximately 7 to 14 days. The disease can progress to an atypical pneumonia, which can result in a life threatening acute respiratory distress syndrome (ARDS). Occasionally, Q-fever causes hepatitis, which may be asymptomatic or becomes symptomatic with malaise, fever, liver enlargement and pain in the right upper quadrant of the abdomen. The chronic form of Q-fever is very similar to inflammation of the inner lining of the heart, which can occur months or decades following the infection. It is fatal if left untreated, however with the correct treatment the mortality rate falls under 10%. Acute infection: IgM and IgG antibodies to phase 2 antigen are present. Chronic Infection: IgG and IgA antibodies to phase 1 antigen are present. The aim of this work was to develop a serological assay to detect IgG and IgM antibodies against Coxiella in serum, plasma and milk. Native antigen preparations were used to coat 96 well microtiterplates and to print lineblots. For the detection anti-human IgG and IgM antibodies or a protein A/G conjugate, able to detect IgG and IgM simultaneously, are used. Samples used for the evaluation originated from humans, cows, sheep and goat with a special emphasis on bovine Coxiella samples. For the human as well as for the veterinary assay, values for sensitivity and specificity of > 90% could be achieved for both, Phase 1 and Phase 2. Cut off values have to be adjusted for each species. Veterinary data still needs more validation. This assay will help to understand outbreak situations and to diagnose acute and chronic infections and therefore aid to prevent spreading of the disease and will increase human as well as veterinary welfare.

Epidemiology 1

Saturday, October 14, 2017
Pacific Salon 6-7

Moderators: Pam Hullinger and Karyn Havas

1:00 PM **Modeling suitable areas for avian influenza in California with MaxEnt and Random forest # † ◊**
Jaber Belkhiria, Robert Hijmans, Walter Boyce, Beate Crossley, Beatriz Martínez-López 32

1:15 PM **An evaluation of active surveillance testing protocol options for early marketing during an outbreak of highly pathogenic avian influenza**
Peter Bonney, Sasidhar Malladi, Amos Ssematimba, Todd Weaver, David A. Halvorson, Carol Cardona 33

1:30 PM **Performance of an automated whole-house poultry vaccination system ◊**
Joseph L. Purswell, Scott L. Branton 34

1:45 PM **Assessing the performance of diagnostic tests in detecting low pathogenic avian influenza viruses in pooled swab samples ◊**
Amos Ssematimba, Sasidhar Malladi, Peter Bonney, Cristian Flores, Jeannette Munoz, David A. Halvorson, Carol Cardona 35

2:00 PM **Mycobacterial diversity in birds at San Diego Zoo**
Josephine Braun, Wayne Pfeiffer, Jennifer Burchell, Carmel Witte, Bruce Rideout 36

2:15 PM **Avian influenza in the United States' commercial gamebird industry: Occurrences and selected practices as potential pathways to infection**
Kaitlyn St. Charles, Amos Ssematimba, Carol Cardona. 37

2:30 PM **Monitoring dairy cattle health and husbandry including by use of drones**
David J. Wilson, Lindsey Cheetham, Kerry A. Rood 38

2:45 PM **Employing the RADAR approach to improve AAVLD and quality system compliance**
Susan L. Martin, Thomas James Reilly, Shuping Zhang 39

Symbols at the end of titles indicate the following designations:

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

Modeling suitable areas for avian influenza in California with MaxEnt and Random forest # † ◊

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The unique peculiarities of the state of California such as the coexistence of different types of poultry operations (i.e., organic vs commercial, backyard flocks, live bird markets, etc.), as well as of many areas where wild and domestic birds co-occur, makes it a perfect place for the potential emergence of Highly Pathogenic Avian Influenza outbreaks. The 2014-2015 outbreaks of HPAI in California and other US states highlight the urgent need to develop and implement solutions to protect the poultry industry against this devastating disease. Disease distribution models were used to generate high spatial resolution map of the suitability for Avian influenza and potential emergence of HPAI outbreaks. Two algorithms, Random Forest and MaxEnt, were utilized. Both models were trained with Presence-Background and Presence-Absence data and several environmental predictors specific to disease epidemiology in the state. Overall, both models performed well (AUCc > 0.7 for data testing) particularly the models trained with Presence-Background data (AUCc >0.85). The resulting high resolution maps identified suitable areas across the state, particularly in coast and the valley. Environmental predictors that contributed to the prediction of AI suitability in most models were land cover, distance to coast, and broiler farm density. Suitability maps predicted 6 of the 8 counties where HPAI was detected during the 2014-2015 HPAI outbreak. This study provides further insights into the spatial epidemiology of AI in California, and may be useful to guide risk-based surveillance and outreach efforts and increase producers' awareness to implement more cost-effective interventions.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

An evaluation of active surveillance testing protocol options for early marketing during an outbreak of highly pathogenic avian influenza

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The pre-movement isolation period (PMIP) is a period of heightened biosecurity (as compared to the standard biosecurity protocols outlined in the Secure Poultry Supply Plans) to be implemented during an outbreak of highly pathogenic avian influenza (HPAI) in the days immediately prior to the start of load-out of broilers or turkeys to slaughter. A 5 day PMIP for broilers and 8 day PMIP for turkeys (the suggested length for the PMIP in current HPAI response guidelines), in combination with diagnostic testing, is expected to ensure a 95% chance of detection of virus in a broiler or turkey house prior to load-out, given that introduction of HPAI is prevented during the PMIP. However, in some cases, such as in a newly established or expanded HPAI Control Area, an integrator may want to send flocks to processing as soon as possible (early marketing) to reduce the density of birds in the HPAI Control Area and the risk of HPAI spread. In this case, individual poultry producers may be unable to implement the PMIP for the full length of time, which would result in a decreased likelihood of virus detection before load-out. This decrease may be offset in part by increasing and/or adjusting sampling times for diagnostic testing. We used within-flock HPAI disease transmission models parameterized for the 2015 EA/AM HPAI H5N2 strain and active surveillance simulation models to assess the effect of several diagnostic testing protocols by varying the amount, type, and timing of the testing for broilers and turkeys. The active surveillance protocol options evaluated were based on dead bird testing with RRT-PCR (Influenza A matrix gene real time reverse transcription PCR) and antigen capture tests using lateral flow devices. The probability of detection and mean number of infectious birds in undetected houses at the start of load-out were predicted for each surveillance protocol for multiple days post-exposure. The results will aid in the assessment of the potential use of early marketing as a control option during a HPAI outbreak.

Performance of an automated whole-house poultry vaccination system ◊

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Recent experience with large-scale disease events has highlighted the need for improved response to minimize their impacts to animal agriculture. Response to these events relies on veterinary care to limit mortality and morbidity, containment to limit the spread of the disease, and vaccination to provide resistance to infection. Current vaccine administration methods for loose housed poultry such as drinking water administration or portable spray units produce variable results with limited efficacy. The objective of this study was to characterize the performance of a novel whole-house spray system for loose housed poultry to improve vaccination program efficacy and to reduce adminstration time, labor, and human-to-bird contact. The vaccination system was tested in commercial pullet houses stocked with approximately 10,000 White Leghorn layer pullets. A total of five flocks were vaccinated for Newcastle Disease Virus (NDV) and Infectious Bronchitis Virus (IBV) over a two year period. Three flocks were vaccinated with a prototype system, with a secondary house was vaccinated via man-portable backpack sprayer as a control treatment. The fourth and fifth flocks were vaccinated with a commercial version of the automated spray system. Results show that the prototype automated spray system had a mean seroconversion rate of 69% compared to 41% for the man-portable backpack sprayer for IBV during the first three flocks. Results for NDV seroconversion were 69% and 22% for the automated and backpack sprayers during the first three flocks, respectively. Performance of the commercial system was similar to the prototype system, with seroconversions ranging from 60 to 100%.

◊ USAHA Paper

Assessing the performance of diagnostic tests in detecting low pathogenic avian influenza viruses in pooled swab samples ◊

Amos Ssematimba[1], Sasidhar Malladi[1], Peter Bonney[1], Cristian Flores[2], Jeannette Munoz[2], David A. Halvorson[1], Carol Cardona[1]

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Low pathogenic avian influenza (LPAI) viruses are important precursors to their more devastating highly pathogenic counterparts and such mutations have been reported in some of major outbreaks worldwide. Routine sampling and testing of birds is a vital component of the surveillance protocols implemented to ensure their early detection in poultry flocks. There are several aspects of sampling protocols that influence detection chances. These may include the status of the birds sampled (healthy, sick or dead), sampling methodology, sample storage and transportation and pooled sample composition.

In this study, two experiments were performed involving inoculation of broiler chickens with the low pathogenicity chicken/Pennsylvania/04 H5N2 or H7N2 virus subtype and taking regular swab samples for subsequent testing using and molecular diagnostic real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) and two antigen capture tests. The first experiment, aimed assessing the effect of sample composition on virus detection rate, involved testing pooled samples obtained by mixing the individual swabs to create pooled samples containing either 4, 5 or 10 negatives swabs mixed with one positive swab. The second experiment was aimed at calculating the sensitivities of two antigen capture tests and involved testing the individual single swabs using the two tests with rRT-PCR test as the reference test. In the analysis, rRT-PCR cycle threshold (CT) values are summarized and the proportions detected by antigen capture are obtained and compared between the two antigen capture tests using one-sided fisher's exact test.

Generally, the mean CT value for the H7 samples were slightly lower than that for the H5 samples, the detection rates were found to be significantly higher in the combined pools of 5 and 6 swabs compared to the pools of 11 and FluDetect test was found to detect slightly more positive samples than VetScan for both virus subtypes and at the different CT ranges assessed. For both tests, the highest percentage of positives detected was for H7 samples with CT≤ 30 giving "sensitivities" of 68% and 49% for FluDetect and VetScan respectively.

Much as pooling a bigger number of swab samples increases the chances of having a positive swab included in the sample to be tested, this study's outcomes indicate that this practice may actually reduce the chances of detecting the virus since it result into lowering the virus titer of the pooled sample. Hence there is a need to optimize sample pooling for effective surveillance.

◊ USAHA Paper

Mycobacterial diversity in birds at San Diego Zoo

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Avian mycobacteriosis is a troubling disease facing bird collections and conservation programs around the world and has led to euthanasia of exposed but clinically healthy suspects in the past. *Mycobacterium avium* and *Mycobacterium genavense* are the most common etiologic agents, though several other mycobacterial species and subspecies have been identified in infected birds. Various DNA fingerprinting techniques suggest a diversity of strains within the individual species, but there is concern that lack of resolution may limit differentiation between these strains.

The goal of this study was to determine the number of mycobacterial strains and their genetic relatedness in birds in a single population over a long time period using whole-genome sequencing. Altogether, 123 mycobacterial isolates were cultured from tissues of 105 birds that were diagnosed with avian mycobacteriosis at the San Diego Zoo and its Safari Park between 1992 and 2015. Isolates were submitted for whole-genome sequencing using Illumina sequencers. Computational workflows were developed to identify mycobacterial species and the number of single-nucleotide polymorphisms between individual strains. This genomic information was then used to group closely related strains into genomic clusters and create phylogenetic relationships.

Nine species of mycobacteria were identified, of which *M. avium* and *M. genavense* were most common, in 49 and 48 birds, respectively. Interestingly, *M. avium* strains were more diverse genomically than *M. genavense* strains. Isolates from 31 birds infected by *M. avium* could be grouped into nine genomic clusters, whereas isolates from 40 birds with *M. genavense* were in ten such clusters. Most birds were found to harbor a single mycobacterial species and strain, but 15 birds were infected with more than one strain of the same species, while two birds had infections from two different mycobacterial species.

These data are allowing us to further explore the epidemiology of transmission.

Acknowledgments

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Avian influenza in the United States' commercial gamebird industry: Occurrences and selected practices as potential pathways to infection

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The commercial upland gamebird industry is a substantial sector of the United States' overall poultry industry with an economic worth of an estimated $5 billion as of 2014. As in all types of commercial poultry operations, disease management and prevention is a challenge to upland gamebird operations. A viral disease of particular concern is avian influenza (AI), a virus that continuously demonstrates the capability for worldwide economic devastation in the poultry industry as a whole.

Currently, limited literature exists that captures a comprehensive overview of AI outbreaks involving commercial US gamebird farms. Consequently, there are gaps in knowledge in regards to what types of pathways exist for AI infection to occur on gamebird operations and how frequently AI occurs in gamebird operations in comparison to other types of poultry. In this study, a literature review was conducted as an attempt to fill these gaps in knowledge. Two industry practices that are hypothesized to lead to increased risk of AI infection have specifically been targeted for investigation, namely; the practice of having direct engagement with live bird markets (LBMs) and the practice of raising domestic and/or game ducks together with other gamebird species. The results of this study will inform risk assessments for AI introduction and spread in the gamebird industry.

This study focused on outbreaks involving commercial gamebird operations raising any of the dominant species, namely, ring-necked pheasant, quail, and chukar, as well as the minor species of wild turkey. For the purposes of comparison, AI outbreaks involving commercial turkey and chicken premises were also extracted. Outbreak data were gathered from case reports, annual reports, and outbreak summaries available online and in conference proceedings. In some cases, additional outbreak-specific information was sought via personal communications.

Analyses of the extracted AI outbreak data involving US commercial gamebird operations revealed that the first outbreak was recorded in 1980 and since then 27 outbreaks have been reported. Based on the retrieved information, AI outbreaks were more than ten times as likely to involve commercial turkey operations than commercial gamebird operations, and more than twice as likely to involve commercial chicken operations (broiler and layer combined) than commercial gamebird operations. Finally, out of the 27 gamebird outbreaks extracted, over half were found to involve gamebird premises that had direct connections to LBMs and/or raised ducks alongside the gamebirds on site.

Monitoring dairy cattle health and husbandry including by use of drones

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Unmanned Aerial Vehicles (UAV, drones) have been studied previously for observation of beef cattle. Animal flight resulted when flying below 30m (100 ft). Current objectives were to learn to fly UAVs, evaluate UAV for dairy cattle disease (Dz) monitoring, and observe how low dairy animals would tolerate the UAV being flown. Current Dz detection and recording methods for dairy cows and calves were also assessed. Experts in piloting and operating UAV assisted in hands on flight training and selection of commercial drones. UAVs (2) were purchased; flying, still and video photography, and data handling skills were acquired. A questionnaire about Dz recording was developed for farm personnel, and live color and thermal images and videos were captured by UAV on 4 dairy farms with large housing areas. By first flying at 30 m, then reducing flight altitude in 8 m increments, acclimation of cows and calves was achieved in < 15 min. Final tolerated height was approximately 4.3 m (14 ft). Ear tags showing animal ID were clearly legible. Thermal imaging recorded cows' skin temperature within 0.1° C, but sun vs. shade or black and white hide color on the same cow affected the observed temperature by as much as 17° C. Overcast conditions or cows entirely in shade were ideal. Cows with metritis or in estrus were detected by thermal imaging, but clinical mastitis was not readily detected by UAV. All farms used multiple methods to record Dz. Methods of recording cow Dz: white board 75% of farms, cell phone 50%, notebook to computer 50%, direct to computer 25%, text 25%. Calf records: white board 75%, chart 50%, hutch cards 50%, notebook 25%, calendar 25%, kids tell 25%. Cow Dz: mastitis, abortion, metritis, milk fever, lameness, obturator nerve paralysis, LDA/RDA, and diarrhea were recorded on all farms, acidosis, ketosis and keratitis 75%, twisted cecum 25%. Calf Dz: respiratory Dz was recorded on all farms, diarrhea 75%, diarrhea severity and appearance 50%, respiratory severity 50%. Recording of treatment (Tx): Cows' mastitis, metabolic, reproductive Txs and surgery were recorded on all farms. Calves' diarrhea, respiratory, follow up Txs were recorded on 75% of farms. However, only 50% of farms recorded cow and calf treatments in a permanent form after the meat or milk withhold period ended. Disease records were used by: herdsperson, head calf raiser, calf feeders on 100% of farms, veterinarian 75%, owner 75%, head milker 75%, nutritionist 50%, foot trimmer 25%. Three producers owned UAV, and interest of farm personnel in UAV for possible animal and facility monitoring was high on all farms. Further research in UAV disease monitoring, including via thermal imaging, is needed.

Employing the RADAR approach to improve AAVLD and quality system compliance

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BACKGROUND: Continued compliance with an organization's quality system and the AAVLD standard is an ever-present objective for all accredited veterinary diagnostic laboratories. Between AAVLD audit cycles, continued compliance can easily become a slippery slope and difficult to sustain. Internal audits can be an effective method to measure the degree of compliance and improve it.

OBJECTIVE: The overall aim of this project was to improve the effectiveness of our internal audits, thereby improving AAVLD and VMDL Quality System compliance and accreditation readiness.

METHODS: We adopted the RADAR approach: Rigorous Audits Develop Accreditation Readiness. Conducting rigorous audits means probing deeply into a lab section's activities and examining records in detail to check for compliance on three different levels: AAVLD Standards, VMDL Quality System, and section-specific SOPs.

We employ a three-phase process of pre-audit, audit, and post-audit phases. The pre-audit phase includes examination of documents for adherence to document control requirements and examination of case reports along with their respective submission forms. Pre-audit activities utilize standardized monitoring sheets which are set up with appropriate criteria to check compliance. Open CAPAs and any pending items from the previous audit are also reviewed.

During the audit phase, we inspect the section's activities using an audit checklist modeled on the AAVLD standards, compare documents in use to a list of current approved versions, and conduct a method and/or a vertical audit. We ask open-ended questions, request personnel to demonstrate or explain how they meet various standards, and incorporate checks for compliance with recent revisions to our Quality System.

Post-audit, a comprehensive written report describing findings and nonconformances is prepared and delivered to the Section Leader. Nonconformances are written in the form of a requirement and nonconformance evidence. In addition to the report, the post-audit phase focuses on conducting follow-ups with the lab section audited to assess progress with their responses to the nonconformances. The follow-up activity is a key factor to ensure completion of corrective actions.

RESULTS: Implementation of the RADAR approach contributed to improved compliance to both our Quality System and the AAVLD standards. A June 2016 AAVLD on-site audit identified twenty-seven nonconformances, a decline from sixty-five identified during the previous audit cycle. Although not realized until after the AAVLD on-site visit, the RADAR approach assisted with accreditation readiness by helping prepare personnel for their interactions with the AAVLD site visit team.

Epidemiology 2

Sunday, October 15, 2017
Pacific Salon 6-7

Moderators: Marisa Rotolo and Brant Schumaker

7:45 AM **Prevalence, species distribution, and mechanism of methicillin resistance in *Staphylococcus* from companion animals in the Midwest U.S.**
Kayla Schaefer, Lei Dai, Adam Krull, Eric Burrough, Qijing Zhang, Orhan Sahin *42*

8:00 AM **Recurrence of canine *E.coli* urinary tract infections is unrelated to biofilm formation * †**
Tessa LeCuyer, Margaret A. Davis, Thomas E Besser *43*

8:15 AM **PRRSV IgM/IgA ELISA detects infection in the face of circulating maternal IgG antibody * †**
Marisa Rotolo, Ji Ju, Luis Gabriel Gimenez-Lirola, Ronaldo Magtoto, Chong Wang, Dave Baum, Marlin Hoogland, Rodger Main, Jeff Zimmerman *44*

8:30 AM **Diagnostic data standardization for next generation health information management tools**
Leticia Linhares, Marisa Rotolo, Bret Crim, Kate Mueller, Michael Martin, Randy Berghefer, Rodger Main. *45*

8:45 AM **Using the pig trade networks and the geographical distance among farms to model the spatio-temporal dynamics of porcine reproductive & respiratory syndrome status at farm level ◊**
Sara Amirpour Haredasht, Dale Polson, Rodger Main, Kyuyoung Lee, Derald Holtkamp, Erin Lowe, Beatriz Martínez-López *46*

9:00 AM **Smart-Epidemiology - Big data analytical platforms for the near real-time risk assessment, surveillance and modeling of infectious diseases: practical examples and illustration of benefits for the swine industry ◊**
Beatriz Martínez-López, Dale Polson, Erin Lowe, Sara Amirpour Haredasht, Kyuyoung Lee, Derald Holtkamp, Zachary Whedbee, Bret Crim, Rodger Main *47*

9:15 AM **Synchronization of swine diagnostic results across four swine interest VDLs * †**
Marisa Rotolo, Leticia Linhares, Bret Crim, Kate Mueller, Michael Martin, Ram Raghavan, Gary A Anderson, Jon Greseth, Jane Christopher-Hennings, Mary Thurn, Stephanie Rossow, Jerry Torrison, Rodger Main *48*

9:30 AM **Break**

10:15 AM **Modelling the impact of climate factors on the dynamics of carcass condemnation in cattle slaughter plants in Northern and Southern California from 2005-2015 ◊**
Sara Amirpour Haredasht, Dale A. Moore, Noelia Silva-del-Río, Anita Edmondson, Beatriz Martínez-López *49*

10:30 AM **A multiyear cross-sectional study of antibiotic resistance in companion animal *Escherichia coli* isolates.**
Gabriel Keller Innes, Meghan F. Davis, Shelley C. Rankin *50*

10:45 AM **Quality Assurance in laboratory central receiving: Using the laboratory information system Orchard© Harvest™ to identify, classify, and monitor growing trends in order entry errors to aid in the quality improvement process §**
Jennifer Rudd *51*

11:00 AM **An assessment of veterinary prescription practices and factors influencing usage of antimicrobial drugs**
Daniel Taylor, Elaine Scallan, Jennifer Martin *52*

11:15 AM **Evaluation of the risk of *B. abortus* introduction and spread into California †**
Wen Chi Lee, Anita Edmondson, Alyssa Louie, Beatriz Martínez-López *53*

11:30 AM **Seroprevalence of *Brucella ovis* and associated risk factors in Wyoming domestic sheep # †**
Molly Jeanne Elderbrook, Todd Cornish, Brant Schumaker, Dannele Peck, Kerry Sondgeroth *54*

11:45 AM **Using a novel real-time PCR assay to investigate the epidemiology of brucellosis in the Yellowstone National Park bison herd # † ◊**
Noah Hull, Suelee Robbe-Austerman, Jacob Berg, Sierra Amundson, Ashley Smith, Callie Klinghagen, William Laegreid, Christine Quance, Brant Schumaker *55*

Symbols at the end of titles indicate the following designations:

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

Prevalence, species distribution, and mechanism of methicillin resistance in *Staphylococcus* from companion animals in the Midwest U.S.

Kayla Schaefer[1], Lei Dai[2], Adam Krull[1], Eric Burrough[1], Qijing Zhang[2], Orhan Sahin[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA;
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Methicillin-resistant *Staphylococcus* spp. (MRS) (primarily methicillin-resistant *Staphylococcus pseudintermedius* [MRSP] and to a lesser extent methicillin-resistant *Staphylococcus aureus* [MRSA] and coagulase negative *Staphylococcus* spp. [MRCoNS]) are among the leading causes of both community and hospital-acquired skin, ear and wound infections in dogs and cats with zoonotic potential. Resistance to methicillin in Staphylococci is typically mediated by *mecA* gene, carried on the staphylococcal chromosomal cassette *mec* (SCC*mec*), which encodes PBP2a protein that has a low affinity for majority of other beta-lactam antibiotics. Considering the fact that MRS are also frequently resistant to other important veterinary antibiotics such as aminoglycosides, fluoroquinolones, lincosamides, macrolides, tetracyclines, chloramphenicol and trimethoprim-sulfamethoxazole, there is only a limited option to treat such serious infections caused by these pathogens. The goal of this study is to determine prevalence, species distribution, and mechanisms of methicillin resistance in *Staphylococcus* spp. isolated from the clinical specimens of dogs, cats and other companion animals submitted to Veterinary Diagnostic Laboratory (VDL) at ISU between 2012-2017. Overall phenotypic methicillin resistance rates were 20.3% in *S. pseudintermedius*, 37.2% in CNSG (including *S. epidermidis*), 16.1% in CPSG (including *S. aureus*), and 24.1% in all *Staphylococcus* spp. Presence of PBP2a (using a immunochromatographic assay) varied considerably among MRS by species: 86.5% in MRSP, 51.5 in CNSG, 86.4% in CPSG with an overall carriage of 73.1% in all MRS. These findings indicated a substantial increase in methicillin resistance in clinical *Staphylococcus* spp. isolated from dogs and cats in the U.S. in recent years. These results also suggested the existence of other mechanism(s) than presence/expression of *mecA* in MRS from pets. Detailed studies on the mechanism of resistance as well as patterns of cross-resistance to other antimicrobials and genetic diversity of MRS are currently being performed. The knowledge generated from this study will be valuable for both therapeutic and epidemiological purposes.

Recurrence of canine *E.coli* urinary tract infections is unrelated to biofilm formation * †

Tessa LeCuyer, Margaret A. Davis, Thomas E Besser

Washington State University, Pullman, WA

Published literature on the molecular characteristics of pathogens that cause recurrent urinary tract infection (UTI) in dogs is limited. *Escherichia coli* is the most frequently isolated organism from canine UTI. One study of 12 dogs treated for recurrent *E.coli* UTI in the 1970s and 80s determined that 75% of the dogs were infected by the same 1 or 2 strains, defined by pulsed-field gel electrophoresis, on multiple occasions. Both intracellular colonization and biofilm formation in the urinary bladder are proposed mechanisms of bacterial persistence in the bladder and survival in the face of antimicrobial therapy. We collected *E. coli* isolates from five veterinary diagnostic laboratories from April, 2015 through September, 2016 and identified 87 urinary isolates of *E. coli* from 42 dogs with recurrent UTI, including 9 dogs from Washington, 5 from North Dakota, 8 from Indiana, 5 from Ohio, and 13 from California. The number of *E. coli* isolated from these dogs ranged from two (N=29 dogs) to five (N=1 dog). Multi-locus sequencing typing of the isolates was performed by sequencing seven housekeeping genes and comparison to the EnteroBase database. Biofilm formation *in vitro* was performed by static biofilm assays with crystal violet staining. Twenty-eight dogs had the same *E. coli* sequence type (ST) isolated at all visits and 12 dogs had a new ST at each visit; two had a new ST followed by reversion to the original ST. Interestingly, the rate at which dogs had a change in ST varied with the location from which the isolates originated. Rates of ST change observed were: Ohio 86% (6/7), Indiana 50% (4/8), Washington 22%(2/9), California 21%(3/14), North Dakota 0% (0/5). This variability may be related to patient populations, with laboratories that service larger referral-based caseloads having a higher proportion of dogs with new STs isolated from subsequent UTI episodes. This may indicate that patients with other co-morbidities are more likely to have re-infection of the bladder rather than persistance of a single ST. Biofilm producing strains were infrequent (8%, 7/87) and were isolated on more than one occasion from only two dogs. Extracellular biofilm formation has been suggested as a mechanism for *E. coli* persistence in canine recurrent UTI, but in our study biofilm production was uncommon. The variation in the likelihood that a recurrent UTI will be of the same ST as the previous infection that we observed between patient populations may be of interest for future investigation.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

PRRSV IgM/IgA ELISA detects infection in the face of circulating maternal IgG antibody * †

Marisa Rotolo[1,2], Ji Ju[3], Luis Gabriel Gimenez-Lirola[2], Ronaldo Magtoto[2], Chong Wang[3,1], Dave Baum[2], Marlin Hoogland[4], Rodger Main[2], Jeff Zimmerman[1]

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Oral fluids (OF) are used extensively for PRRSV surveillance: qRT-PCR testing detects active infection and antibody ELISA is useful for establishing prior exposure. However, in weaned pig populations originating from PRRSV infected and/or vaccinated sow herds, maternal antibody (IgG) cannot be differentiated from IgG antibody produced by the pigs in response to infection or vaccination. To address this problem, we developed and evaluated IgM- and/or IgA-specific oral fluid ELISAs as a means to detect PRRSV infection in weaned pig populations with circulating maternal antibody. Oral fluid samples were collected from 3 wean-to-finish (WTF) sites (Sites A, B, C) beginning within 2 weeks of placement. Pigs originated from PRRSV vaccinated and/or exposed sow herds, but the pigs themselves were not vaccinated for PRRSV. Each site had 3 WTF barns and each barn had 36 occupied pens (~25 pigs per pen). By arrangement with the producers, pigs were not moved during the sampling period. Samples were collected weekly from every occupied pen for 9 samplings. A total of ~2,916 OF samples were collected, i.e., 3 sites x 108 pens per site x 9 samplings. All oral fluid samples were randomized (random.org) and tested by PRRSV qRT-PCR. These results were used to establish the PRRSV status of each pen. OF samples were also tested for IgG, IgA, IgM, and the combination of IgM/IgA using ELISAs developed in our laboratory. ELISA cutoffs, diagnostic sensitivity, and diagnostic specificity were determined by ROC analyses. Results: **PRRSV qRT-PCR**: On Site A, 3.7% of pens were positive at the 1st OF sampling, with all pens in all 3 barns positive ≥ 1 times by the 9th sampling. Site B was negative throughout the collection period, except for one positive pen at the 9th sampling. All samples from Site C were negative. **PRRSV Antibody**: Among the 3 sites 90.6% (278/307) of the OF samples collected on the 1st sampling were IgG positive, but 273 of these were from qRT-PCR-negative pens. In contrast, IgA, IgM, and IgM/IgA ELISA responses paralleled with qRT-PCR positivity. An ROC analysis estimated the diagnostic sensitivity and diagnostic specificity of the combined IgM/IgA ELISA at 72% (95% CI 67, 77) and ≥ 99% (95% CI 99, 100), respectively.

Discussion

Swine practitioners need both nucleic acid- and antibody-based tests to track PRRSV in the field. This study suggested that the PRRSV IgM/IgA ELISA could be used to detect active infection in populations of weaned pigs in the presence of circulating maternal antibody.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Diagnostic data standardization for next generation health information management tools

Leticia Linhares[1], Marisa Rotolo[1], Bret Crim[1], Kate Mueller[1], Michael Martin[2], Randy Berghefer[1], Rodger Main[1]

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Successful regional disease control and/or elimination programs require standardized terms for efficient communication, decision making, and benchmarking of disease status. Exchanging diagnostic information across laboratories is challenging when local systems have different ways of identifying, reporting, and/or archiving the same terms. There is an increasing demand on behalf of the veterinary medical communities for the veterinary diagnostic laboratories (VDLs) to standardize diagnostic data. Diagnostic data standardization is the process of unifying the language used for communicating diagnostic results across VDLs, irrespective of lab-specific nomenclature or laboratory information management system (LIMS). The first step in data standardization is to map lab-specific test codes to a universal standard code. Logical Observation Identifiers Names and Codes (LOINC®) is a universal code system that enables the exchange, pooling, and processing of diagnostic data. Thus, there is the need to create LOINCs for each diagnostic test, measurement, or observation that has a clinically different meaning. LOINC codes distinguish diagnostic terms across 6 parts: 1) pathogen or analyte (component): what is being measured or observed/tested (e.g., PRRSV RNA, potassium); 2) property: what about it is being measured or tested (e.g., Ct value, mass concentration); 3) collection time: the interval of time over which an observation was made (e.g., point in time); 4) specimen type (system): type of sample (e.g., urine, tissue); 5) scale: how the test result is quantified or expressed (e.g., quantitative, ordinal, nominal, narrative); and 6) test method: assay used to run the test (e.g., polymerase chain reaction). Currently, existing LOINCs can be found at https://search.loinc.org/searchLOINC. New LOINCs can be requested by submitting a dossier of information to the LOINC committee using a free software package (RELMA®). In a highly collaborative 15-month project involving four swine-oriented VDLs and the USDA NAHLN, we are making great progress in developing a comprehensive library of LOINCs for terms routinely used on swine diagnostic cases. The project will enable HL-7-messaging more than 600 LOINCS, from which 380 are new. All LOINCs will become freely available to the NAHLN labs via a web-portal (e.g., intuitive drop-down menu driven search engine application) that aims to greatly enhance the ease by which end-users can identify and subsequently utilize appropriate LOINCs. While the current project is focused on swine diagnostics, the system being developed has direct application across species. Creating and adopting the use of universally recognized data standards are foundational elements necessary to establish the scalable systems of connectivity and web-based analytical tools necessary to support the needs and demands of the 21st century animal agriculture in North America.

Using the pig trade networks and the geographical distance among farms to model the spatio-temporal dynamics of porcine reproductive & respiratory syndrome status at farm level ◊

Sara Amirpour Haredasht[1], Dale Polson[2], Rodger Main[3], Kyuyoung Lee[1], Derald Holtkamp[3], Erin Lowe[2], Beatriz Martínez-López[1]

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a RNA virus of the family Arteriviridae that causes reproductive failure in breeding stock and respiratory disease in piglets. In the US, it has been estimated that the annual economic impact of PRRSV for the pig industry is US$664 million. A better understanding of PRRSV transmission dynamics is key for the successful PRRSV control and elimination in endemic settings.

In this research we used a two-step parameter-driven (PD) Bayesian approach to model the spatio-temporal dynamics of PRRSV and predict the future PRRSV status at farm level. We used data from >500 production sites from 2012-2015 regarding the PRRSV status, the pig trade network and the geographical location and distance among farms (i.e., distance was used as a proxy of airborne transmission). We evaluated the role of geographical distance and/or pig trade in PRRSV status by using five PD models with different weights matrices: (i) geographical distance weight, defined as the inverse distance between each pair of farms in kilometers, (ii) pig trade weight, defined as the absolute number of pig movements between each pair of farms, (iii) the product between the distance weight and the standardized relative pig trade weight, (iv) the product between the standardized distance weight and the standardized relative pig trade weight, and (v) the product of the distance weight and the pig trade weight. The model that included the pig trade weight matrix provided the best fit with an area under the ROC curve (AUC) of 0.88 and an accuracy of 85% (105/124). Our results emphasize the importance of pig trade in PRRSV transmission in the endemic setting under study. The modeling approach of this study may be easily adapted to other production systems to characterize the PRRSV transmission dynamics under diverse epidemiological settings. This method will be incorporated into Disease BioPortal (http://bioportal.ucdavis.edu) and made available to producers and industry stakeholders so they can use it in a user-friendly way to help prioritize interventions and support timely decision-making.

◊ USAHA Paper

Smart-Epidemiology - Big data analytical platforms for the near real-time risk assessment, surveillance and modeling of infectious diseases: practical examples and illustration of benefits for the swine industry ◊

Beatriz Martínez-López[1], Dale Polson[2], Erin Lowe[2], Sara Amirpour Haredasht[1], Kyuyoung Lee[1], Derald Holtkamp[3], Zachary Whedbee[1], Bret Crim[3], Rodger Main[3]

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Platforms for near real-time risk assessment, spatio-temporal visualization and molecular epidemiology that are operational and easily accessible to producers, veterinarians and diagnostic laboratories are key to support the cost-effective prevention and control of infectious diseases. This is particularly true in the swine industry in the US where the size and complexity of the operations as well as the type and frequency of movements facilitates pathogen transmissions and make challenging the early detection of pathogens and effective intervention. The introduction into and rapid spread of porcine epidemic diarrhea virus (PEDV) across the Western hemisphere, with estimated losses of 3 million pigs in 2013 alone, reflects the urgent need to implement risk-based, more cost-effective surveillance programs in order to better manage the transmission and circulation of these and other diseases in swine industry. We have been working to develop an information management platform using the Disease BioPortal which includes automation of diagnostic data interpretation and transfer for near real-time risk assessment and surveillance for supporting risk-based, more cost-effective programs for infectious diseases globally. Specifically here, we illustrate the capabilities of the Disease BioPortal platform using examples of some of the most economically important swine diseases in the US: PRRS, swine influenza and mycoplasma.

The most recent version of the Disease BioPortal incorporates Big Data analytical capabilities as well as automation of diagnostic data transfer, extended analytical and user-friendly visualization tools adapted to the swine industry. This platform allows users to integrate and analyze swine information at different levels (genomic to phenomic and beyond). As a result, producers and veterinarians can use the Disease BioPortal platform to conduct outbreak investigations; generate site, flow, system, area and network level health reports; as well as test hypotheses about the potential direct (e.g., animal movements) or indirect (e.g., airborne spread) transmission of diseases between swine operations with advanced analytical methods such as social network analysis, trend analysis and multi-dimensional space-time-genomic capabilities.

One of the distinct capabilities of this web-based system is the near real-time integration of different data streams that traditionally have been presented separately and were not available until long after the outbreak, thus were unable to be used to support timely risk assessment and risk-based surveillance strategies. We believe that these types of platforms represent the future of more cost-effective decision support and more effective prevention and control of infectious diseases globally.

◊ USAHA Paper

Synchronization of swine diagnostic results across four swine interest VDLs * †

Marisa Rotolo[1], Leticia Linhares[2], Bret Crim[2], Kate Mueller[2], Michael Martin[3], Ram Raghavan[4], Gary A Anderson[4], Jon Greseth[5], Jane Christopher-Hennings[5], Mary Thurn[6], Stephanie Rossow[6], Jerry Torrison[6], Rodger Main[2]

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Diagnostic test result nomenclature, format, and structure of otherwise equivocal data being stored and reported varies widely across VDLs and laboratory information management systems. Similarly, the submission, premises, and animal level identifiers captured and reported in VDL records vary. Thus, establishing and adopting the use of universally recognized veterinary diagnostic data standards and electronic Health Level-7 (HL7) messaging are necessary to create sustainable and scalable systems of seamless connectivity across VDLs in the NAHLN. Our highly collaborative efforts aim to establish the ability to electronically generate and message the full complement of swine diagnostic test results to third party database applications using standardized Logical Observation Identifier Names and Codes (LOINC®) and HL7 messaging at 4 swine interest VDLs. Accomplishments to date have included: formulating a comprehensive list of submission, premises, and animal level identifiers that need to be capable of being incorporated into the veterinary diagnostic record and USDA NAHLN HL-7 message; determining the information (data) needed to be reported for each of the various types of quantitative and qualitative diagnostic assays conducted on swine samples; and deciding upon the granularity of diagnostic (assay) result identity needed by end users to collate and differentiate amongst diagnostic result information received from VDLs. These accomplishments provided the direction necessary to determine the content of the multitude of standardized diagnostic result codes (LOINCs) to be obtained and to ensure the updated USDA NAHLN HL-7 message readily accommodates the result content to be reported. We then assembled a comprehensive list of the diagnostic assays, analyses, and/or other evaluations routinely conducted on swine diagnostic submissions. The compilation of this work identified more than 720 standardized LOINCs that are necessary to report the results from the diagnostic testing and analyses routinely conducted on swine submissions. We are systematically working through the process of obtaining a greatly expanded formulary of LOINCs for VDL utility. An updated version of the USDA NAHLN HL-7 message capable of capturing this more comprehensive set of submission, premises, and animal level identifiers and diagnostic result information has been developed. These updates are within the overarching framework of the existing USDA NAHLN message structure. A web-based HL-7 message validator has been created for use in testing and providing feedback to VDLs on their messaging capabilities. An intuitive (web-based) LOINC search engine is being developed that will enable users to more readily find the appropriate LOINCs. These precedent-setting efforts aim to deliver a significant advancement in enhancing the connectivity and value of swine diagnostic information across VDLs.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Modelling the impact of climate factors on the dynamics of carcass condemnation in cattle slaughter plants in Northern and Southern California from 2005-2015 ◊

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California ranks fourth in cattle harvested within the US with 1,181,631 head slaughtered in 2015. From all reported carcasses condemned from 2005-2015 in US slaughter plants, 21% occurred in California. Carcass condemnations are associated with farm management practices but also with environmental and climatic factors. A better characterization of temporal and spatial characteristics of condemnation reasons and factors contributing to their incidence in California will help to better prevent them and improve industry profitability.

In this study we aimed to show the ability of our approach to quantify the seasonal components of the carcass condemnation numbers in Southern and Northern of California. We used a multiple-input, single-output (MISO) model to understand the impact of climatic factors on carcass condemnation numbers in Southern and Northern California.

First we used cross-correlation coefficient (CCF) analysis between each of the 35 reasons for carcass condemned case in each region and climate data such as Southern Oscillation Index, Palmer Z-index, modified Palmer drought severity index, cooling degree days, el Niño, standard precipitation index and Pacific decadal oscillation to select input variables with the strongest linear relationship with the number of carcass condemnations. Then, selected inputs were used to model the temporal dynamics of the carcass condemnation using a MISO model.

The selected condemned cases for the MISO model based on the CCF analysis were malignant lymphoma, septicemia, emaciation and pericarditis. Across the 10 year-period, temporal dynamics of these condemnation cases in Southern and Northern California were well-captured by phenomena such as El Niño, Standard Precipitation Index and Pacific Decadal Oscillation with coefficients of determination (R^2) ranging from 0.51 to 0.72. The decreasing precipitation of the last year (i.e. time delay of 11-12 month) will increase the septicemic condemned carcasses a year after and hydrological drought of a last year can increase the emaciated cases in north California a year after. The decrease in the precipitation of a two seasons and a year before will increase the malignant lymphoma condemned carcasses in north and south of California, respectively. Pericarditis condemned carcasses in south of California will increase by hydrological drought of the last season.

We found an association between climatic factors and four of the most incident condemnation cases in California: septicemia, pericarditis, malignant lymphoma and emaciation. This data-based modelling approach can be used in real-time to inform syndromic and risk-based surveillance programs. It may also help us better understand and forecast the impact that climatic change may have on cattle condemnations. This study aims to increase awareness of producers and policy makers of management practices and policies to mitigate the number of condemnations while improving cattle welfare and industry profitability.

◊ USAHA Paper

A multiyear cross-sectional study of antibiotic resistance in companion animal *Escherichia coli* isolates.

Gabriel Keller Innes[2], Meghan F. Davis[2], Shelley C. Rankin[1]

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Over 2 million people contract an antimicrobial resistant infection each year in the United States. This results in an estimated $35 billion in lost productivity and $20 billion in medical bills. Many bacterial pathogens contribute to this problem including antibiotic resistant *Escherichia coli.* Although many *E. coli* Isolates remain susceptible to antibiotics, surveillance trends of isolates from humans and livestock animals suggest a temporal increase in the prevalence of antimicrobial resistance. Organisms that are multiple drug resistant (MDR), pose a significant human and animal health threat. Unfortunately, there is no universally accepted definition for MDR in human or veterinary medicine and this complicates surveillance and control efforts. The objectives of this study were to apply two definitions of MDR to a veterinary data set of companion animal *E. coli* isolates to determine if MDR varied by year, season, and/or infection location. Prevalence of MDR was determined by implementing the guidelines used by the SENTRY Antimicrobial Surveillance Program (STR) and comparing those with the Dutch Working Party on Infection Prevention guidelines to define Highly Resistant Microorganisms (DGR). Data on antibiotic resistance of *E. coli* isolates from companion animals were obtained from a United States academic tertiary referral veterinary hospital from 2003 to 2014. Logistic regression was used to model the relationship between year, month, and infection location against the outcome of MDR. An annual increase in MDR was observed using both definitions: STR Odds Ratio (OR) 1.01 [95% Confidence Interval (CI): 0.99-1.03, $p>0.05$]; DGR OR 1.04 [95% CI: 1.02-1.07, $p<0.001$]. Skin and respiratory isolates had a statistically significant greater odds of being MDR with both STR and DGR criteria compared to the more common urinary isolates. The odds for MDR among skin isolates were: STR OR 1.86 [95% CI: 1.65-2.09] and DGR OR 1.85 [95% CI: 1.56-2.20] respectively. For respiratory isolates, the odds of MDR were: STR OR 2.36 [95% CI: 1.98, 2.83] and DGR 2.36 [95% CI: 1.87-2.97] respectively. Isolates that were collected in the summer months (July, August, and September) had a significantly decreased odds of being designated as an MDR isolate with the STR criteria than the other seasons, OR 0.77 [95% CI: 0.66-0.90, $p<0.001$); however this finding was statistically insignificant using the DGR criteria, with OR 0.82 [0.66-1.02, $p>0.07$). **T**he conclusions with regard to temporal trends in MDR varied between the two criteria studied. This indicates that a universal standard criterion is required to increase translatability of the definition of MDR across One Health studies.

Quality Assurance in laboratory central receiving: Using the laboratory information system Orchard© Harvest™ to identify, classify, and monitor growing trends in order entry errors to aid in the quality improvement process §

Jennifer Rudd

VMRCVM/VTH, Blacksburg, VA

A laboratory's specimen receiving area is the epicenter of action and therefore can be the area that has the highest rates of human-associated errors and the most challenging area to maintain quality. A true commitment to quality means taking a proactive stance and watching for patterns that lend themselves to consistent quality improvement (MacCormack). A laboratory information system (LIS), like Orchard© Harvest™, can be a great tool to a quality manager by increasing the ability to identify, classify, and monitor growing trends in order entry errors. Virginia Tech Animal Laboratory Services used Orchard© Harvest™ to develop and evaluate a large number of rejected or cancelled tests by creating a classification structure for test rejections and implementing an automatic log feature to aid in the monthly monitoring of rejected or cancelled tests. The Quality Manager then employed strategies to identify root cause of some of the most common rejection reasons associated with order entry errors and employed an improvement strategy to reduce the rate of test rejections due to order entry errors. Improvements to the HL-7 interface for data transfers between the electronic medical record software of the veterinary teaching hospital and LIS, standard operating procedures, and laboratory receiving personnel training program has reduced monthly order entry errors 40% to 67% since 2014. Rejection monitoring has become an essential tool in ViTALS' quality assurance program and foundational concepts of classification and monitoring can be applied to other laboratory areas and functions to help a quality manager spot areas for improvement, as well as, areas of focus for internal audits.

§ AAVLD Laboratory Staff Travel Awardee

An assessment of veterinary prescription practices and factors influencing usage of antimicrobial drugs

Daniel Taylor[1], Elaine Scallan[1], Jennifer Martin[2]

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The mitigation of antimicrobial resistant (AMR) microorganisms is arguably one of the most important challenges facing public health and food safety. Although significant strides aimed to reduce the use of clinically important antimicrobial drugs (AMD) have commenced, there is a lack of critical information regarding veterinary prescription practices, veterinary perceptions of antimicrobial use policies, and motivating factors influencing AMD usage. AMR is a complex issue involving a plethora of dynamic social, political, and economical factors. Given the rapidly evolving landscape surrounding use of AMD in animals, we believe that generation of accurate usage data and an assessment of veterinary perceptions and attitudes regarding AMD use is imperative for the direction of development of effective tools and trainings aimed at mitigating AMR.

During 2016-2017, we developed and launched a study to assess veterinary prescription practices, perceptions and factors influencing usage of antimicrobial drugs among veterinarians who prescribe antimicrobial drugs (AMD) to beef cattle, dairy cattle, swine and poultry (i.e., turkey, broiler chickens, and egg–layers).

Veterinarians were surveyed using a short, 15-minute online survey, which included demographic questions, disease scenarios and attitude questions. Disease scenarios were created for feedlot cattle, backgrounding cattle, dairy cattle, swine and poultry, and veterinarians were asked to provide treatment recommendations based on the specie-specific scenario. The study also included willing participants maintaining a prescription diary for six-weeks in order to validate survey responses and gain insight into comprehensive AMD use practices outside of the survey's scope.

As of 5/15/17, we have collected 157 responses from production animal veterinarians. With the data obtained from the online survey and diary component, we will be able to assess current training and educational gaps and needs, which will lead to the development of effective trainings, tools and other resources for veterinarians aimed at preventing antibiotic resistance and promoting antibiotic stewardship. Additionally, our results provide baseline information on prescribing practices and create a validated tool for collecting future data on livestock antimicrobial use.

Evaluation of the risk of *B. abortus* introduction and spread into California †

Wen Chi Lee[1], Anita Edmondson[2], Alyssa Louie[2], Beatriz Martínez-López[1]

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California is a leader in agriculture and for livestock production and trade, particularly cattle. Therefore, it is crucial to maintain the highest standards for animal health and welfare in order to sustain this high productivity and continue having access to competitive national and international markets. The evaluation of cattle movement into the state may be used to conduct risk assessments and identify highly connected areas where risk-based surveillance and outreach activities could be allocated to better prevent the introduction and spread of diseases into California. In this study, we first aim to characterize the nature, extent and temporal-spatial patterns of cattle movement into California as a preliminary but fundamental step to better understand livestock trade dynamics and evaluate their association with the potential risk of disease introduction into the state. Second, we assessed the risk of reintroduction of bovine brucellosis into California (classified as brucellosis free since 1997) from other US states, particularly the Greater Yellowstone Area within Montana, Wyoming and Idaho where the disease is endemic in bison and elk. The risk of brucellosis reintroduction was estimated considering two scenarios: one with the current mandatory calfhood brucellosis vaccination program in place, and the other under the potential scenario of not requiring vaccination. For such purposes, we used a combination of social network analysis and stochastic risk assessment to quantitatively assess the risk of reintroduction of brucellosis into California, and its potential consequences. Results suggest that bovine brucellosis risk of re-introduction both in the scenario with and without vaccination is very low and it is concentrated is few counties located in the Central Valley of California. To the best of our knowledge, this is the first study to assess the risk of brucellosis reintroduction into California under two different scenarios (with and without vaccination). Results are expected to inform policies to better prevent and control potential reintroduction of brucellosis into free areas of the US, as well as to inform the decision to continue or end the brucellosis vaccination program in California.

† Graduate Student Oral Presentation Award Applicant

Seroprevalence of *Brucella ovis* and associated risk factors in Wyoming domestic sheep # †

Molly Jeanne Elderbrook[1], Todd Cornish[1], Brant Schumaker[1], Dannele Peck[2], Kerry Sondgeroth[1]

[1]Veterinary Sciences, University of Wyoming, Laramie, WY; [2]Agricultural Research Service, United States Department of Agriculture, Fort Collins, CO

Brucella ovis (*B. ovis*) is the main causative agent of Ovine brucellosis in domestic sheep in the United States, which causes acute to chronic reproductive problems in those affected. Decreased fertility in rams, lowered conception rates in ewes, and premature lambs with low birth weights are the most common signs of disease in a flock. Infection is introduced into a flock after infected sheep are purchased or after disease-free sheep are exposed to infected sheep on shared grazing allotments or open rangeland. Venereal transmission occurs directly between rams and ewes during the breeding season and between rams outside the breeding season. Vaccines are not licensed for use in the U.S., and few effective treatments leave a test and cull management strategy as the best option once disease manifests in a flock. Research regarding the importance of *B. ovis* in domestic sheep has been done in many major sheep-producing countries, but none have looked at prevalence and associated risk factors of *B. ovis* in North American flocks.

This research study was designed to 1) estimate the animal and flock-level seroprevalence of *B. ovis* in Wyoming domestic sheep and 2) describe epidemiological risk factors associated with disease positive sheep and flocks.

A total of 2,340 sheep from 18 non-random, owner-selected operations were sampled between August 2015 and May 2016 in Wyoming, and animal-level information (e.g. gender, age, and breed) and flock-level information (e.g. location, flock size, breeding and biosecurity practices) was gathered at time of sampling or via epidemiological questionnaire. To bolster sample size for seroprevalence estimation, samples submitted to the Wyoming State Veterinary Laboratory (WSVL) for *B. ovis* testing were included. Results from the National Veterinary Services Laboratory (NVSL) *B. ovis* indirect Enzyme-Linked Immunosorbent Assay (ELISA) were used to calculate *B. ovis* seroprevalence at the animal-level using 2,340 samples collected from individual sheep and at flock-level using 55 flocks that were either sampled or had samples sent in to WSVL for *B. ovis* testing in the state of Wyoming. Possible animal-level risk factors analyzed includ region, gender, age group, and primary breed type. Flock-level risk factors analyzed include region, flock size, and ram to ewe ratio. Preliminary results found statistically significant differences between seroprevalence proportions among age groups ($p=0.0325$) and primary breed types ($p=0.0276$) at the animal-level.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Using a novel real-time PCR assay to investigate the epidemiology of brucellosis in the Yellowstone National Park bison herd # † ◊

Noah Hull[2], Suelee Robbe-Austerman[3], Jacob Berg[2], Sierra Amundson[2], Ashley Smith[2], Callie Klinghagen[2], William Laegreid[2,1], Christine Quance[3], Brant Schumaker[2,1]

[1]Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, WY; [2]Veterinary Sciences, University of Wyoming, Laramie, WY; [3]National Veterinary Services Laboratories, United States Department of Agriculture, Ames, IA

Previous descriptive and analytical epidemiologic analyses of bovine brucellosis (*Brucella abortus*) in the Greater Yellowstone Area defined disease status based on ante-mortem testing (serology) and/or the current "gold-standard" of bacteriologic culture. These findings need to be reassessed in light of novel diagnostics with higher sensitivity than the "gold-standard." Previously, we presented on a novel real-time PCR validation for *B. abortus*, including the differentiation of vaccine strains. Our novel assay had two to three times the sensitivity of culture while maintaining perfect specificity. In the winter of 2016-17, we collected samples from 159 Yellowstone National Park bison (*Bison bison*) at slaughterhouses in Montana. We also collected demographic information: sex (male or female), body condition score (1, 1+, 2, 2+, 3, 3+, 4, 4+ or 5), pregnancy status (bred or not bred), and age (adult, yearling, or calf). Our sample included 126 females (79.2%), of which 66 (52.4%) were pregnant. The sample was predominantly adults (106 of 159; 66.7%). Serology (fluorescence polarization assay; FPA) was run on all animals: 71 animals (44.7%) were classified as seropositive (ΔmP >20); 12 (7.5%) were suspect (ΔmP 10-20); and 76 (47.8%) were negative (ΔmP <10). A stepwise multivariate regression model was run with the outcome of FPA status (positive, suspect, or negative) with all demographic covariates listed above. The best fit model included age and body condition score; however, our R^2 value was low (0.1661). Real-time PCR identified 107/159 (67.3%) as positive. Of these 107, 36 (33.6%) were FPA negative, 61 (57%) were FPA positive, and 10 (9.4%) were FPA suspect. Interestingly, of the 36 that were FPA negative, 25 were in juvenile animals (calves). Cohen's kappa coefficient was calculated on adult animals based on FPA status (positive and negative only) and PCR status, kappa = 0.524 indicating moderate agreement between the two assays. Tooth aging, currently in progress, will allow us to evaluate demographic factors associated with discordant results. We hypothesize that classifying disease status based on PCR test result (positive or negative) will better inform the model. Comparisons will be presented for serology vs. culture, and PCR vs. culture. Additionally, data on ante-mortem samples will be presented.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Molecular Diagnostics and Bioinformatics 1

Saturday, October 14, 2017
Royal Palm Salon 2-3

Moderators: Laura B. Goodman and Sunil kumar Mor

1:00 PM **Validation of a multiplex quantitative PCR for differentiation of *Salmonella* Typhimurium and I 4,[5],12:i:- from other *Salmonella* spp. on porcine samples * †**
Samantha Naberhaus, Adam Krull, Laura Bradner, Karen Harmon, Paulo Arruda, Bailey Lauren Arruda, Orhan Sahin, Amanda Kreuder 57

1:15 PM **QIAxcel Advanced System and multiplex PCR for *Clostridium perfringens v*irulence gene detection §**
Melanie Koscielny, Yan Zhang 58

1:30 PM **Cross-validation of the real-time quaking induced conversion (RT-QuIC) amplification assay and comparison to conventional immunohistochemistry in the antemortem detection of chronic wasting disease infection in elk. # †**
Rozalyn Donner, Joanne Tennant, Davin Henderson, Matteo Manca, Sabine Gilch, Naveen Kondru, Aaron D Lehmkuhl, Bruce Thomsen, Edward Hoover, Anumantha Kanthasamy, Byron Caughey, Nicholas James Haley. 59

1:45 PM **A real-time PCR panel for detection of tickborne pathogens in veterinary specimens # †**
Zhenyu Shen, Roger Stich, Michael Z Zhang, Jefferson W. Mitchell, Shuping Zhang 60

2:00 PM **Use of Intergenic Sequence Ribotyping (ISR) to genotype *Salmonella sp* isolates from clinical cases of commercial birds and poultry environment in Mississippi**
Martha Pulido-Landinez, Jessica Hockaday, Jay Kay Thornthon 61

2:15 PM **Genotyping *Mycoplasma bovis* field isolates using virulence based multi locus sequence typing method**
Srivishnupriya Anbalagan, Jessica L. Peterson, Joshua D. Elston, Angie Cunningham 62

2:30 PM **Development of a novel two-target PCR assay for the detection and differentiation of *Brachyspira hyodysenteriae* and *Brachyspira hampsonii*.**
Karen Harmon, Laura Bradner, Phillip Gauger, Eric Burrough, Hallie Warneke 63

2:45 PM **Sequencing and phylogenetic characterization of *Brucella canis* isolates from Ohio §**
Leyi Wang, Mary Beth Weisner, Jeff Hayes, Jing Cui, Yan Zhang. 64

Symbols at the end of titles indicate the following designations:

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

Validation of a multiplex quantitative PCR for differentiation of *Salmonella* Typhimurium and I 4,[5],12:i:- from other *Salmonella* spp. on porcine samples * †

Samantha Naberhaus, Adam Krull, Laura Bradner, Karen Harmon, Paulo Arruda, Bailey Lauren Arruda, Orhan Sahin, Amanda Kreuder

Iowa State University, Ames, IA

The detection of *Salmonella* from porcine enteric samples is vital to the swine industry to implement necessary treatment and management decisions. Unfortunately, the current turn-around times for samples submitted to a diagnostic lab for *Salmonella* isolation, identification, and serotyping are upwards of six weeks primarily due to awaiting NVSL serotyping results. This delay in determination of serotype greatly limits the ability of producers to efficiently implement treatment protocols that are appropriate for the infecting serotype. While serogrouping can suggest pathogenicity, the Group B serogroup contains both highly pathogenic and less pathogenic serotypes. *Salmonella* Typhimurium and I 4,[5],12:i:- (the monophasic Typhimurium variant) have been shown to be associated with enteric disease with histologic lesions consistent with Salmonellosis in swine cases submitted to the Iowa State University VDL. Conversely, serotypes such as *Salmonella* Agona, Derby, and Heidelberg contribute to over a quarter of the Group B Salmonella isolated, but are rarely associated with clinical disease. With the majority (68%) of all porcine Salmonella belonging to Group B, the aim of this work was to validate a multiplex quantitative PCR to detect and differentiate the pathogenic *Salmonella* Group B serotypes. This qPCR was designed to have the ability to rapidly differentiate samples containing *Salmonella* serotypes Typhimurium or I 4,[5],12:i:- from samples containing other less pathogenic serotypes. The qPCR can detect as low as 500 CFU/mL from a sample tested directly and as low as 5 CFU/mL from a sample following an 18-24 hour enrichment in Buffered Peptone Water. Through the validation, the qPCR has been shown to correctly exclude 28 commonly isolated serotypes of *Salmonella* in addition to 7 other non-*Salmonella* fecal organisms. This qPCR can be used directly on a variety of sample types, including feces, colon mucosal scrapings, and oral fluids, all of which are common samples submitted for culture and molecular testing. Use of this qPCR is expected to have similar sensitivity and specificity to culture with significantly improved time to final diagnosis.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

QIAxcel Advanced System and multiplex PCR for *Clostridium perfringens v*irulence gene detection §

Melanie Koscielny, Yan Zhang

ANIMAL DISEASE DIAGNOSTIC LABORATORY, OHIO DEPARTMENT OF AGRICULTURE, REYNOLDSBURG, OH

Clostridium perfringens is commonly found in the intestines of humans and animals and is shed in feces that can contaminate food and water supplies, causing severe toxin-mediated diseases including food poisoning, gastrointestinal disease, gas gangrene and related necrotic conditions in animals as well as in humans, particularly in children and the elderly. Five strain types of *C. perfringens* exist, classified as Type A (*cpa*+), Type B (*cpa+, cpb+, etx*+), Type C (*cpa+, cpb*+), Type D (*cpa+, etx*+) and Type E (*cpa+, iA*+).

We developed a multiplex PCR using the QIAGEN QIAxcel Advanced capillary electrophoresis system to detect and differentiate six virulence genes (*cpa, cpb, cpb2, cpe, iA,* and *etx*) of *C. perfringens*. The QIAxcel Advanced System separates nucleic acid molecules by application of an electrical current to a gel-filled capillary that are detected as they migrate towards the positively charged terminus. Twelve samples are analyzed in as little as 3 minutes, with up to ninety-six samples set up in a single run. Resolution down to 3-5 bp for fragments <500 bp is possible with as little as 0.1 ng/µl of starting material.

Here, we demonstrate that multiplex PCR, in combination with the QIAxcel Advanced System, is a powerful tool for rapid screening of multiple DNA targets for *C. perfringens* virulence genotype testing. Sensitive, efficient detection of the five strain types of *C. perfringens* is possible using the capillary electrophoresis system. Automation minimizes human error and improves inter- and intra-assay reproducibility, and its fast separation and automated data analysis is very useful in a veterinary diagnostic lab.

§ AAVLD Laboratory Staff Travel Awardee

Cross-validation of the real-time quaking induced conversion (RT-QuIC) amplification assay and comparison to conventional immunohistochemistry in the antemortem detection of chronic wasting disease infection in elk. # †

Rozalyn Donner[1], Joanne Tennant[3], Davin Henderson[3], Matteo Manca[4], Sabine Gilch[5], Naveen Kondru[6], Aaron D Lehmkuhl[7], Bruce Thomsen[7], Edward Hoover[3], Anumantha Kanthasamy[6], Byron Caughey[4], Nicholas James Haley[1,2]

[1]College of Veterinary Medicine, Midwestern University, Glendale, AZ; [2]Department of Basic Sciences, Midwestern University, Glendale, AZ; [3]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO; [4]TSE/Prion Biochemistry Section, Rocky Mountain Laboratories, NIH, Hamilton, MT; [5]Ecosystems and Public Health, University of Calgary, Calgary, AB, Canada; [6]Biomedical Sciences, Iowa State University, Ames, IA; [7]USDA-APHIS, Ames, IA

Chronic wasting disease (CWD) is an efficiently transmitted prion disease of elk and other members of the cervid family. The disease has been found in both wild and farmed cervids, and has been reported in 24 states, 2 Canadian provinces, South Korea, and most recently Norway. While there are no approved antemortem tests for CWD, immunohistochemical analysis of rectal biopsies and recto-anal mucosa- associated lymphoid tissue has been extensively evaluated for the identification of infected deer and elk. Recently, several studies have shown that the diagnostic sensitivity of real-time quaking-induced conversion (RT-QuIC), a developing amplification assay, may equal or exceed that of conventional immunohistochemistry when examining rectal biopsies. In the present study, we compared the sensitivity and specificity of conventional immunohistochemistry to RT-QuIC analyses performed by a number of labs across North America in a cross-validational study. Samples were collected from an ongoing longitudinal study of approximately 450 farmed elk in a CWD endemic area, where animals could be biopsied and tested repeatedly for evidence of infection. All adults were sampled and tested on a yearly basis, and prevalence estimates ranged from 10-22% depending on assay and sampling year. Intra- and inter-assay results were compared using discrepant analysis - one of the few methods available, in the absence of a secondary comparative test, for comparing a developing test to a gold standard. The findings of this study will prove useful for the continued development of both ante- and postmortem testing strategies for not only chronic wasting disease, but other prion diseases as well.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

A real-time PCR panel for detection of tickborne pathogens in veterinary specimens # †

Zhenyu Shen[2], Roger Stich[1], Michael Z Zhang[2], Jefferson W. Mitchell[2], Shuping Zhang[2]

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Tickborne disease is a growing threat to canine and human health. Tickborne pathogens include species in the genera *Anaplasma, Ehrlichia, Rickettsia, and Borrelia* (such as *A. phagocytophilum*, *A. platys, A. bovis, E. canis*, *E. chaffeensis*, *R. rickettsii*, and *B. burgdorferi*). Some of the tickborne pathogens share common tick vectors and coinfection with two or more pathogens is common. The goal of this study was to develop a SYBR Green-based real-time PCR panel for simultaneous detection of *Anaplasma* spp*., Ehrlichia* spp*., Rickettsia* spp*., and Borrelia* spp. in veterinary specimens. Assays (Ana/Ehr-16S, Ehr-16S and Ric-16S) were designed to amplify the 16S ribosomal RNA genes of *Anaplasma/Ehrlichia*, *Ehrlichia* and *Rickettsia*, respectively, while Bor-P66 was designed to target P66, a membrane-associated protein gene of *Borrelia* spp. Genus-specific primers were designed based on the genomic DNA sequences deposited in Genbank. PCR assays were optimized using gBlock gene fragments for each target at 10^7 to 10^1 copies per reaction. The assays were validated using known negative clinical samples spiked with the gene fragments (10^3, 10^2, and 10^1 copies per reaction). The assays were used to determine the prevalence of pathogens in ticks, including *Amblyomma americanum*, *A. maculatum*, *Dermacentor albipictus*, *D. andersoni*, *Ixodes minor*, and *I. scapularis* which were collected from Missouri elk.

Following extensive optimization, PCR amplification efficiencies achieved 99.99% for Ana/Ehr-16S, 99.97% for Ehr-16S, 99.91% for Ric-16S, and 99.96% for Bor-P66. Specificities of the assays were examined among the four genera using the gene blocks and genomic DNA of other bacterial pathogens including *Leptospira* sp., *Salmonella enterica* Typhimurium, *Lawsonia intracellularis*, and *Mycoplasma bovis* and no cross reaction was observed. The analytical sensitivities were 10 copies of gBlock per reaction at the following Ct values: 33.18±0.12 for Ana/Ehr-16S, 33.40±0.43 for Ehr-16S, 34.83±0.82 for Ric-16S, and 33.66±0.83 for Bor-P66. The assays could detect 10 copies of gBlock per reaction for canine, equine and tick samples at Ct values between 34.01 and 36.15. Based on validation data, Ct≤36.5 was chosen as the cutoff value for positive results.

Analysis of tick samples revealed 23.4% (15/64), 4.7% (3/64), 89.1% (57/64) and 4.7 (3/64) of the ticks positive for *Anaplasma, Ehrlichia, Rickettsia, and Borrelia spp,* respectively. Data from the present study indicate that the newly developed PCR panel is a valuable tool for rapid detection of tickborne pathogens in veterinary specimens.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Use of Intergenic Sequence Ribotyping (ISR) to genotype *Salmonella sp* isolates from clinical cases of commercial birds and poultry environment in Mississippi

Martha Pulido-Landinez, Jessica Hockaday, Jay Kay Thornthon

Population and Health - MVRDL, Mississippi State University, Pearl, MS

Bacteria of the genus *Salmonella* play a predominant role in avian medicine and public health, especially those transmitted by poultry products. Sometimes the diagnostic resources available for this condition are limited to the determination of the presence or absence of *Salmonella spp*. The lack of availability of diagnostic tests to characterize Salmonella isolates impedes the effective control of this pathogen because information is not obtained about patterns of distribution, resistance and adaptation characteristics for each serotype, and potential sources. Furthermore, the high cost and the long turnaround time for the complete identification of Salmonella serotypes may interfere or delay control programs. To assess diversity of *Salmonella enterica* serotypes present in poultry and their environment in Mississippi, *Salmonella* isolates are collected and characterized by intergenic sequence ribotyping (ISR). A sequence based method that assesses single nucleotide polymorphisms occurring around a 5S ribosomal gene. Additionally, in all clinical cases antimicrobial resistance patterns (ARP) have been determined using minimum inhibitory concentration values.

ISR have been used to assign serotype to all *Salmonella* enterica isolates from clinical cases and environment in MS during the last two years. The most frequent identified serotypes are: Kentucky, Enteritidis, Typhimurium, Braenderup, Infantis and Mbandaka. ISR provided a very important information about the ecology of *Salmonella enterica* on-farm and clinical cases. The ISR turnaround time usually takes no more than 3 days. The antibiograms demonstrated differences between *Salmonella* serotypes and among isolates of the same serotype. All isolates have been 100% susceptible to enrofloxacin and trimethoprim/sulfamethoxazole. The number of antimicrobials to which the isolates were resistant ranged from two to nine. Variation in antibiograms within clinical samples demonstrates that it is important to survey isolates periodically from a region to follow epidemiologic trends.

Genotyping *Mycoplasma bovis* field isolates using virulence based multi locus sequence typing method

Srivishnupriya Anbalagan, Jessica L. Peterson, Joshua D. Elston, Angie Cunningham

Diagnostics, Newport Laboratories, Inc., Worthington, SD

Mycoplasma bovis is an important bovine pathogen associated with bovine respiratory disease complex and is responsible for substantial economic loss worldwide. Epidemiological studies using different typing methods such as pulse-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) analysis, and multiple-locus variable-number tandem repeat analysis (MLVA) showed that *M. bovis* isolates are heterogeneous. However the results from these routine typing were not reproducible between different laboratories. Therefore it is important to develop a reliable and comparable molecular typing to assess the genetic variation of *M. bovis*. Virulence based multi locus sequence typing (VMLST) is a highly discriminatory and unambiguous method for characterizing bacterial isolates. In this study a VMLST was developed for *M. bovis*, virulence genes encoding hemolysin-related protein (Hem), p40 (adhesion), p48 (major surface lipoprotein), p80/81 (lipoprotein), cysteine protease, glyceraldehyde-3-phosphate dehydrogenase, and variable surface lipoproteins (VspE, VspI, VspK, and VspO) were selected as targets. For each target, multiple sequence alignment of up to 50 sequences from GenBank was conducted with MegAlign (DNAstar, Madison, WI). Primers were designed to amplify either the entire gene or the most variable portion of the gene. Each primer was checked to see if they amplified the desired product, which was also confirmed by sequencing. Finally a multiplex PCR assay was established and the assay was performed on a total of 74 *M. bovis* field isolates. Amplified products were sequenced in Ion Personal Genome Machine (PGM) sequencing platform (Life Technologies, Grand Island, NY) by standard methods. Sequence reads were assembled into contigs using SeqMan NGen program (DNAstar) and compared to reference *M. bovis* sequences from GenBank. Phylogenetic analysis identified 3 individual clusters. Percent identity between isolates varied from 77.7% to 99.8%. Most of the variation was due to *vsp's* which are known to be highly variable due to DNA rearrangement events that occur at high frequency within a single strain. The locus encoding Vsp proteins are also known to undergo spontaneous on-off switching to generate antigenic divergence and altered expression. As expected our results indicated that there were differences in length of *vsp's* tested contributing to sequence divergence in isolates. In particular, we identified *vspI* and *vspO* had 2 different isoforms each varying by 500bp and 100 bp in length respectively. There were several isolates that lacked some or a combination of *vsp's* tested in the assay. Genetic variation observed between the isolates suggests that this assay is ideal for selecting seed isolates for *M. bovis* autogenous vaccine which will help prevent and control *M. bovis* infection. VMLST presented in this study provides a fast, reliable, and cost-effective method for typing *M. bovis.*

Development of a novel two-target PCR assay for the detection and differentiation of *Brachyspira hyodysenteriae* and *Brachyspira hampsonii*.

Karen Harmon, Laura Bradner, Phillip Gauger, Eric Burrough, Hallie Warneke

VDPAM, Iowa State University, Ames, IA

Swine dysentery (SD), a severe enteric disease in pigs, has reemerged in North America during the last several years. In the US, SD is associated with strongly beta-hemolytic *Brachyspira hyodysenteriae* (*B hyo*) or *Brachyspira hampsonii* (*B hamp*). Development of a PCR test to detect and differentiate these two organisms is complicated by the degree of genetic recombination within this genus. Typically, identification by PCR as well as sequencing have targeted the NADH oxidase (*nox*) gene, but those results do not always correlate with results obtained by targeting *16S rRNA* or hemolysin A (*tlyA*). Hence, using only one target, even in what is considered a highly conserved region of the genome, can be problematic. To address this challenge, we developed two separate multiplexed PCR assays, one targeting the *nox* gene and the other the *tlyA* gene, based on sequences from GenBank. The results of both assays are used in tandem to identify and differentiate *B hyo* and *B hamp*. If both targets yield a positive result for either agent, the test is reported as positive for the respective agent. If both targets are negative, the test is reported as negative for the respective agent. If only one target is positive, the result is considered inconclusive with a recommendation for selective bacterial culture, characterization of hemolytic phenotype, and speciation by MALDI-TOF MS or *nox* gene sequencing. Initially, the assays for each target and agent were run separately on isolates from clinical samples (5 *B hamp*, 4 *B hyo*, 5 *B. innocens*, 3 *B. intermedia*, 6 *B. murdochii*, and 3 *B. pilosicoli*) where identity had been confirmed by MALDI-TOF MS. The results for *B hyo* and *B hamp* agreed with culture identification for 100% of the isolates tested. Subsequently the assays were combined into two multiplexed assays, one for *nox* gene and one for *tlyA* gene, each detecting and differentiating the two *Brachyspira* species. The combined assays correctly identified 100% of the *B hamp* and *B hyo* isolates. In addition, one *B. intermedia* isolate exhibited a Ct of 33.3 for *B hamp* with the *tlyA* PCR assay, but was negative for both *B hyo* and *B hamp* by the *nox* gene PCR assay. In a diagnostic scenario, this sample would be classified as inconclusive and flagged for follow-up testing as described above. Evaluation was also performed on clinical specimens. In no case did culture identify a *B hamp* or *B hyo* which the PCR did not detect, but the converse did occur. However, culture frequently confirmed the respective species from other samples in the case, which may suggest that in the discrepant samples the PCR detected DNA from nonviable organisms. For several cases, weakly beta hemolytic *B. pilosicoli*, *B. murdochii* or *B. innocens* were confirmed by culture and MALDI-TOF MS but were negative by the PCR.

This new PCR algorithm will enhance confidence of detection of *B hyo* and *B hamp* in clinical specimens as it is more robust to genetic recombination than traditional single target assays.

Sequencing and phylogenetic characterization of *Brucella canis* isolates from Ohio §

Leyi Wang, Mary Beth Weisner, Jeff Hayes, Jing Cui, Yan Zhang

Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH

Brucella canis is one of four known species (*B. suis*, *B. abortus*, *B. melitensis*, and *B. canis*) causing infection in both human and animals. *B. canis* was first isolated as the causative agent of abortion in 200 beagles in the US in 1966. Since then, *B. canis* has been identified from several American, European and Asian countries. In this study, we performed whole genome sequencing of 38 *B. canis* isolates from Ohio in 2016. These 38 isolates were from 11 cases submitted from five different locations. For sequence analysis, we also downloaded sequences of over 20 isolates available from online databases. A total of 63 *B. canis* isolates were included for the analysis. Core genome MLST analysis indicated that all of the 63 *B. canis* isolates form two genogroups, I and II. The 38 Ohio isolates closely correlate with each other and form a cluster under genogroup I. Within the cluster, the 38 isolates form five different subclusters, reflecting their geographical differences. Unlike the less geographical diversity of genogroup I, genogroup II includes isolates from Asia, America, Africa, and Europe. In addition, two geographical clusters (Asia and South America) forms within genogroup II. Overall, this study is the first time to characterize *B. canis* isolates of the diverse geographical locations across five decades. The findings from this study will improve investigation of canine brucellosis outbreaks in future.

§ AAVLD Laboratory Staff Travel Awardee

Pathology 1

Saturday, October 14, 2017

Pacific Salon 1

Moderators: Francisco R. Carvallo and Ann Britton

1:00 PM **Multiple episodes of sodium monofluoroacetate toxicity in a calf ranch**
John Adaska, Guillermo Rimoldi, John Champagne, Patricia C. Blanchard, Robert H Poppenga, Michelle Mostrom 66

1:15 PM **Hepatotoxicity associated with *Trema tomentosa* in possums**
John Mackie, Claude Lacasse 67

1:30 PM **The pathology of cholecalciferol toxicoses in San Clemente Island foxes**
Leslie Willis Woods, Winston Vickers, Birgit Puschner, Jesse Maestas, Patricia Gaffney, Robert H Poppenga, Melissa Booker, David Garcelon, Nathan Gregory 68

1:45 PM **Mortality due to toxoplasmosis in suburban Eastern Fox Squirrels in Michigan**
Scott D Fitzgerald, Amit Kumar, Julie R. Melotti, Thomas M. Cooley 69

2:00 PM **Zebra alert! Eastern equine encephalitis in puppies in the Midwest and Northeast**
Caroline Andrews, Jodie Gerdin, Jon Patterson, Scott Fitzgerald 70

2:15 PM **Large B-cell lymphosarcoma in the mesenteric lymph node of a Thoroughbred racehorse: early detection associated with chronic inflammation before development of clinical signs + †**
Fraser Ian Hill, Amy Kelly, Alexandra Davis, May Pui Ying Tse 71

2:30 PM **Granulomatous dermatitis in a one year old domestic short hair cat + †**
Krista Eraham 72

2:45 PM **Infectious keratoconjunctivitis in free-ranging mule deer (*Odocoileus hemionus*) from Wyoming and identification of a novel alphaherpesvirus**
Juan Francisco Muñoz-Gutiérrez, Kerry Sondgeroth, Elizabeth Williams, Donald L Montgomery, Terry Creekmore, Myrna M. Miller 73

Symbols at the end of titles indicate the following designations:

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# AAVLD Trainee Travel Awardee
+ AAVLD/ACVP Pathology Award Applicant
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Multiple episodes of sodium monofluoroacetate toxicity in a calf ranch

John Adaska[1,3], Guillermo Rimoldi[2], John Champagne[4,3], Patricia C. Blanchard[1,3], Robert H Poppenga[5,3], Michelle Mostrom[6]

[1]California Animal Health and Food Safety Laboratory System, Tulare Branch, Tulare, CA; [2]Clemson Veterinary Diagnostic Center, Columbia, SC; [3]University of California, Davis, Davis, CA; [4]Veterinary Medical Teaching and Research Center, Tulare, CA; [5]California Animal Health and Food Safety Laboratory, Davis, CA; [6]Veterinary Diagnostic Laboratory, North Dakota State University, Fargo, ND

Over a one year period a large California calf raising operation experienced 4 separate episodes of sudden death losses in 4 to 6 month old steers. In each event the deaths occurred in 1 to 3 contiguous pens on one of three properties owned by the operation but eventually all three properties were involved. In all four episodes, the animals appeared fine at the evening feeding and then at the morning feeding large numbers were found dead and dying. The calves that were still alive had stiff gait and were often dribbling urine but did not show respiratory signs until they would be down and agonal. Over the course of the year, 37 calves were submitted for postmortem examination. Calves consistently showed moderate to large numbers of suffusive hemorrhages on the epicardial surface of the heart as well as moderate to large amounts of fluid in the pericardial sac. Animals often also had pulmonary edema and/or moderate amounts of watery fluid in the thoracic and abdominal cavities. On histologic examination the hearts often had variable degrees of myofiber degeneration characterized by hypereosinophilia and sarcoplasmic vacuolation with mild interstitial infiltrates. Beacause the calves were not found until the morning, they had largely cleaned the feed bunks and representative feed samples were not available. Midway through this series of events the ranch switched from monensin to lasalocid for control of coccidia. Rumen content was negative for oleandrin and grayanotoxins. In animals with heart lesions, sodium monofluoroacetate (compound 1080) was detected at ≥ 10 ppb in kidney (17/18 samples tested) and liver (5/6 samples tested). Based upon the detection of 1080 at concentrations consistent with exposure/intoxication and the failure to detect another cause for the death losses, a diagnosis of 1080 toxicosis was made.

Hepatotoxicity associated with *Trema tomentosa* in possums

John Mackie[1], Claude Lacasse[2]

[1]QML Vetnostics, Murarrie, QLD, Australia; [2]Australia Zoo Wildlife Hospital, Beerwah, QLD, Australia

The common brushtail possum (*Trichosurus vulpecula*) is a semi-arboreal marsupial native to Australia. Its diet consists mainly of leaves of eucalypts and other species and also includes fruits and flowers. Two sub-adult common brushtail possums which were being hand-raised were given a new browse type by their wildlife carer. Within 2-3 days both possums exhibited a sudden onset of depression and bloating and they died a few hours later. At necropsy, the liver was enlarged and mottled and the small intestine and caecum were congested. Histologically, in one possum there was centrilobular hepatocellular necrosis. In the other possum, there was necrosis and loss of hepatocytes throughout the lobules with preferential loss of periportal hepatocytes. In addition, this possum had marked biliary hyperplasia with a cribriform growth pattern and occasional bridging of portal areas. Inspection of the new browse type revealed plants (including berries) of *Trema tomentosa* (syn *Trema aspera;* poison peach). The wildlife carer commented that the possums had eaten a lot of it. *Trema tomentosa* contains an uncharacterised glycoside and has been reported to cause acute hepatoxicity in cattle, goats, deer, horses and camels (as well as experimentally in mice and guinea pigs). The typical histologic lesion is centrilobular (zone 3/periacinar) hepatocellular necrosis. This case extends the range of animals reported to have been naturally poisoned by *Trema tomentosa*. The difference between the two possums regarding histologic changes highlights the variation which may occur in naturally occurring toxicoses. The marked biliary hyperplasia observed in one possum is an atypical reaction to *Trema tomentosa* and is a graphic demonstration of the proliferative capacity of the putative hepatic stem cell niche in the canals of Hering.

The pathology of cholecalciferol toxicoses in San Clemente Island foxes

Leslie Willis Woods[1], Winston Vickers[5], Birgit Puschner[5], Jesse Maestas[3], Patricia Gaffney[1], Robert H Poppenga[1], Melissa Booker[2], David Garcelon[4], Nathan Gregory[4]

[1]California Animal Health and Food Safety Laboratory System, Davis, CA; [2]Naval Base Coronado, San Diego, CA; [3]Fox Monitoring - San Clemente Island, San Diego, CA; [4]Institute for Wildlife Studies, San Diego, CA; [5]University of California, Davis, CA

Rodenticides such as bromethalin, zinc phosphide and cholecalciferol have mostly replaced anticoagulants since the EPA's prohibition of second generation anticoagulants in domestic-use rodenticides which went into effect in 2011. The Institute for Wildlife Studies/U.S. Navy chose to use cholecalciferol rodenticide to avoid the secondary poisonings by anticoagulant rodenticides prior to the EPA regulations were implemented in order to control the rising island rat population, and began to experience San Clemente Island fox mortalities associated with the cholecalciferol rodenticides. Case records of Channel Island foxes submitted to the California Animal Health and Food Safety Laboratory were reviewed for all cases with and without a diagnosis of systemic mineralization from 2010 through May 2017. Of 305 Island fox necropsies, 115 foxes were from San Clemente Island and of 115, 10 foxes were diagnosed with systemic mineralization. Systemic mineralization was not diagnosed as a cause of death in foxes from any of the other islands (Santa Rosa, Santa Catalina, San Miguel, Santa Cruz). Systemic mineralization was found in all size blood vessels in many tissues including the heart, skeletal muscle, lungs, stomach, spleen, kidneys, and tongue. Mineralization of the myocardium and skeletal muscle, gastric mucosa and muscular layers of the stomach, pulmonary interalveolar septa, and renal tubular basement membrane were also present.

Concentrations of vitamin D_3 in kidneys of foxes without systemic mineralization ranged from 3-12 nMol/L. Concentrations in kidneys of foxes with systemic mineralization ranged from 10 nMol/L to 55 nMol/L (10, 15, 15, 26, 28, 38, 55 nMol/L). In addition, blood from 95 live-captured foxes was analyzed for Ca, P, K, BUN, creatinine, albumin, alanine aminotransferase, alkaline phosphatase, amylase, total bilirubin, glucose, Na, globulin, and total protein. Samples were also analyzed for vitamin D_3, PTH, and ionized Ca^{2+}. Fifteen foxes (16% of those tested) had vitamin D_3 concentrations greater than 215 ng/mL; BUN and creatinine were also significantly higher in this group. Overall, vitamin D_3 concentrations correlated positively but weakly with all of the other parameters of interest except P, K, and PTH. Four foxes with vitamin D_3 levels above 450 ng/mL had levels of Ca^{2+} x P above 60 ng/dl. Two of these foxes were in such poor condition that they died or were euthanized after capture, and necropsy results confirmed systemic mineralization consistent with cholecalciferol toxicity.

Secondary poisoning due to cholecalciferol rodenticide has not been documented and not thought to occur. Investigation on whether these cases were due to primary or secondary poisoning revealed that caching of feed by rodents was likely causing primary cholecalciferol toxicosis in the island foxes. Replacing pellet rodenticide with large blocks of rodenticide resolved the problem and no further new toxicoses were seen.

Mortality due to toxoplasmosis in suburban Eastern Fox Squirrels in Michigan

Scott D Fitzgerald[1], Amit Kumar[1], Julie R. Melotti[2], Thomas M. Cooley[2]

[1]Diagnostic Center for Population & ANimal Health, Michigan State University, Lansing, MI;
[2]Wildlife Disease Laboratory, MI Department of Natural Resources, Lansing, MI

During the summer and fall of 2015, multiple die-offs of wild Eastern Fox Squirrels (*Sciurus niger*) were reported in suburban residential areas of southeastern Michigan. Representative animals were submitted to the Wildlife Disease Laboratory, Michigan Department of Natural Resources, for diagnostic evaluation. Tissues were collected in formalin and submitted to the Diagnostic Center for Population and Animal Health, Michigan State University. The squirrels were reported to be in fair to good condition with moderate fat deposits in their bodies. There was no evidence of trauma on the carcasses. The tissues most commonly affected included lungs, liver, and heart. Spleens and brain were also affected occasionally. In lungs, the common findings included moderate to marked interstitial pneumonia with accumulation of fibrin and infiltrates of lymphocytes, plasma cells and neutrophils, necrosis, and alveolar edema. There were multiple hypereosinophilic oval protozoal cysts which measured around 20 um surrounded by a thin cyst wall containing numerous 1-2 um, elongate bradyzoites consistent with *Toxoplasma* cysts. Many of the scattered alveolar macrophages contained 2-3 um, round to crescent-shaped, basophilic, intracytoplasmic tachyzoites. Liver had multifocal areas of necrosis and vacuolar degeneration with adjacent hepatocytes containing the hypereosinophilic *Toxoplasma* cysts. Brain had mild multifocal perivascular infiltrates of lymphocytes and plasma cells. There were a few multifocal areas of necrosis containing protozoal cysts in clusters with no to minimal inflammation. A section of spleen had multifocal areas of marked lymphoid hyperplasia. Cysts in all of the organs were confirmed to be *Toxoplasma gondii* using immunohistochemical staining methods. Toxoplasmosis is recognized as a common infectious cause of disease and mortality in wild squirrels, and is clearly associated with domestic cats as the primary host of this parasite, explaining why these die-offs were predominantly seen in suburban or urban areas with high cat populations.

Zebra alert! Eastern equine encephalitis in puppies in the Midwest and Northeast

Caroline Andrews[1], Jodie Gerdin[2], Jon Patterson[3], Scott Fitzgerald[3]

[1]National Cancer Institute, Frederick, MD; [2]Antech Diagnostics, Hunt Valley, MD;
[3]Michigan State University, Lansing, MI

Eastern equine encephalitis virus (EEEV) is an alphavirus within the family Togaviridae that is classified as a select agent and is capable of causing mortality in humans and a number of veterinary species. The virus is spread by mosquitoes and has recently been postulated to overwinter in snakes. Herein we describe three cases of EEE in puppies in Michigan and New York. Two puppies were euthanized following an acute history of seizures and obtundation. A littermate of one of these puppies died two weeks earlier following a history of anorexia and fever. All three puppies lacked significant gross lesions at autopsy and tested negative for rabies virus. In all three puppies, histologic examination revealed a necrotizing, neutrophilic and lymphoplasmacytic meningoencephalitis with strong positive immunohistochemical labeling for EEEV antigen in neurons and glial cells. The diagnosis of EEE was confirmed by PCR in one puppy and cell culture in the other two dogs. EEE is rare in dogs and pathologic descriptions have only been reported from puppies in the Southeast. The clinical and pathologic features of the cases described here are similar to those previously reported. The initial clinical signs of EEE in puppies are typically nonspecific, including anorexia, fever, and diarrhea, but rapidly progress to severe neurologic disease characterized by seizures and recumbency. Although rare, EEE should be considered as a differential diagnosis for neurologic disease in puppies, especially after more common etiologies, such as canine distemper, rabies, and toxoplasmosis, have been ruled out.

Large B-cell lymphosarcoma in the mesenteric lymph node of a Thoroughbred racehorse: early detection associated with chronic inflammation before development of clinical signs + †

Fraser Ian Hill[1], Amy Kelly[2], Alexandra Davis[2], May Pui Ying Tse[3]

[1]School of Veterinary Medicine, City Univeristy of Hong Kong, Hong Kong, Hong Kong; [2]Vetiernary clinical services office, The Hong Kong Jockey Club, Hong Kong, Hong Kong; [3]Biomedical Department, Cornell Univeristy, Ithaca, NY

Even though lymphosarcoma is relatively rare in horses it is still the most common neoplasm encountered. In this report, we describe an investigation of the cause of chronic inflammation in a clinically normal five-year-old racing Thoroughbred horse, finally confirmed as B-cell lymphosarcoma. Over a 14 week period the horse was regularly evaluated for persistent inflammation without a source of inflammation being found, nor any response achieved after treatment. Eventually a mass was identified by transabdominal ultrasound and rectal palpation. At post mortem a mass in the mesenteric lymph node was sampled. Multiple biopsies were collected. Tissue samples were fixed and stained with H & E. Additional sections were stained for B lymphocytes (CD20, CD79a, PAX), T lymphocytes (CD3), histiocytes (IBA1), and epithelial cells (CK AE1/AE3). Histopathology revealed a dense infiltrate of round cells into a light fibrovascular stroma, completely replacing any normal architecture. Individual cells had minimal, lightly eosinophilic cytoplasm and moderately pleomorphic nuclei containing round, normochromic to hyperchromic nuclei. Mitoses ranged from 5-8 per high-powered field (400x) and were atypical. Immunohistochemical staining: Neoplastic cells showed moderate to strong cytoplasmic immunoreactivity for CD20 and CD79a along with strong nuclear immunoreactivity to Pax confirming their identity as neoplastic B-lymphocytes. Markers for T lymphocytes (CD3) and histiocytic cells (IBA1) found normal non-neoplastic populations of these cells scattered throughout the tumour. CK AE1/AE3 immunostaining did not identify any epithelial cells. From these findings, a diagnosis of large B-cell lymphosarcoma was made. If unexplained chronic inflammation develops in a horse, neoplasia should be considered as a possible aetiology.

+ AAVLD/ACVP Pathology Award Applicant
† Graduate Student Oral Presentation Award Applicant

Granulomatous dermatitis in a one year old domestic short hair cat + †

Krista Eraham

SpecialtyVETPATH, Sammamish, WA

This case report describes the clinical, histopathologic and histochemical features of granulomatous cutaneous disease consistent with mycobacterial infection in a 1 year old domestic short hair cat. Clinically the disease was characterized by multifocal, firm, nodular, well circumscribed and occasionally ulcerated lesions initially affecting the head and progressing to involve the distal limbs. Histologically lesions were comprised by unencapsulated nodules that elevated the epidermis, expanded the dermis and effaced adnexa. Nodules were composed of sheets of histiocytes and Langhans-type giant cells. Fite's staining revealed numerous slender, acid-fast rods within histiocytes, consistent with the lepromatous form of feline leprosy, an unusual finding in feline dermatopathology. This case prompted a review of the literature regarding cutaneous mycobacterial infections in domestic cats, feline leprosy syndrome, and histochemical and molecular techniques helpful in reaching a definitive diagnosis, such as acid-fast staining and PCR for Mycobacteria, as culturing organisms from skin lesions is extremely difficult.

+ AAVLD/ACVP Pathology Award Applicant
† Graduate Student Oral Presentation Award Applicant

Infectious keratoconjunctivitis in free-ranging mule deer (*Odocoileus hemionus*) from Wyoming and identification of a novel alphaherpesvirus

Juan Francisco Muñoz-Gutiérrez[1], Kerry Sondgeroth[1], Elizabeth Williams[1], Donald L Montgomery[1], Terry Creekmore[2], Myrna M. Miller[1]

[1]Veterinary Sciences, University of Wyoming, Laramie, WY; [2]Wyoming Game and Fish Department, Laramie, WY

This retrospective study describes the clinicopathological findings, relative prevalence, and pathogens associated with infectious keratoconjunctivitis in mule deer (*Odocoileus hemionus*) from Wyoming. Seventeen cases with ocular lesions were identified among 1,036 mule deer necropsy submissions (1.6%) in an approximately 16-year period. Sixteen (94%) cases were observed in winter and many corresponded to male (15 cases, 88%) and juvenile (13 cases, 76%) deer. Blindness was the most commonly reported clinical sign (10 cases, 59%). A herpesvirus was detected in 4 (23%) cases of bilateral necrotizing bulbar conjunctivitis. Phylogenetic analysis of glycoprotein amino acid sequences consistently identified this virus as a novel alphaherpesvirus. In 2 of these herpesvirus positive cases, *Actinomyces* sp and *Moraxella ovis* were also identified. *Trueperella pyogenes* was identified in 4 (23%) cases of unilateral ulcerative keratitis, keratoconjunctivitis, and panophthalmitis. *M. ovis* was cultured from 3 (18%) cases of bilateral conjunctivitis and keratoconjunctivitis. In the remaining cases, isolates included *M. bovis* in 1 (6%), *Staphylococcus* sp and *Streptococcus* sp from 2 (13%), *Flavobacterium* sp and *Pseudomonas* sp from 1 (6%), *Escherichia coli* and *Enterobacter* sp from 1 (6%), and pestivirus in 1 (6%). No pathogens were identified in 2 cases. Based on these findings, the relative prevalence of keratoconjunctivitis in mule deer from Wyoming appears to be low and this disease is most commonly associated with infection by a novel alphaherpesvirus, *T. pyogenes,* and *M. ovis*.

Pathology 2

Sunday, October 15, 2017

Pacific Salon 1

Moderators: Jenee Odani and Akinyi Nyaoke

7:45 AM **Winter time hyperthermia in slaughter weight cattle**
Dale Miskimins, Steve M Ensley, Tim Sahli, Russ Daly *76*

8:00 AM **An unusual case of brodifacoum toxicosis in a whelping dog**
Scott D Fitzgerald, Jennifer Martinez, John P. Buchweitz *77*

8:15 AM **Cause of mortality diagnoses in domestic sheep in the Intermountain West**
David J. Wilson, Thomas J Baldwin, Jane Kelly, Arnaud J. VanWettere, Gordon J. Hullinger *78*

8:30 AM **Congenital ocular abnormalities in free-ranging white-tailed deer: a retrospective case series from the southeastern US from 1976-2016 and review of the literature # + * †**
Lorelei Lynne Clarke, Kevin Niedringhaus, M. Kevin Keel, Paige Carmichael, Heather Margaret Anne Fenton *79*

8:45 AM **Diagnostic investigation of conjunctivitis outbreaks in gilt developer units**
Drew Magstadt, Kent Schwartz, Baoqing Guo, Hai Thanh Hoang, Kyoung-Jin Yoon *80*

9:00 AM **When the doctor consults Dr. Google: an information literacy exercise for fourth year veterinary students. ◊**
Danielle Darracq Nelson, Suzanne Fricke *81*

9:15 AM ***Salmonella* Heidelberg: An emerging problem in the dairy industry**
Donald C. Sockett, Nicole A. Aulik, Kathleen M. Deering, Rachel F. Klos, Ann M. Valley *82*

9:30 AM **Break**

10:15 AM **A retrospective pathology study (2007-2015) of the features and causes of myocarditis in young dogs**
Jordan Ford, Laura McEndaffer, Alex Molesan, Melissa Laverack, Randall Renshaw, Kathleen Kelly *83*

10:30 AM **Polyomavirus infection in Gouldian finches**
Francisco A Uzal, Beate Crossley, Hon Ip *84*

10:45 AM **Thyroid activity and survival in Maine moose ◊**
Anne Lichtenwalner, Ann Bryant, Lee E. Kantar, Matthew R. O'Neal. *85*

11:00 AM **Chronic suppurative bronchopneumonia associated to *Candida glabrata*, perforated gastric ulcer and peritonitis in a bottlenose dolphin (*Turisops truncatus*) #**
Albert Canturri, Lola Perez, Maria Lourdes Abarca, Juncal Garcia, Eva Martinez-Nevado, Mariano Domingo *86*

11:15 AM **Fatal ruminal tympany secondary to esophageal and oropharyngeal obstruction caused by an *Actinobacillus lignieresii* pyogranuloma in a steer # + * †**
Melissa Macias Rioseco, Pedro Sitjar, Dario Caffarena, Maria Laura Casaux, Federico Giannitti, Martin Fraga, Franklin Riet-Correa. 87

11:30 AM **Detection of caprine arthritis encephalitis virus using next-generation sequencing technology and potential applications for routine use**
Eric Burrough, Ying Zheng, Qi Chen, Pablo E. Pineyro, Rodger Main, Ganwu Li. 88

11:45 AM **Identification of a divergent porcine astrovirus type 3 in central nervous system tissue from swine with neurologic disease and encephalomyelitis: Diagnostic investigation, virus characterization and retrospective analysis of historic cases. +**
Franco Sebastian Matias Ferreyra, Paulo Arruda, Melissa Hensch, Igor Renan Honorato Gatto, Ben Hause, Ganwu Li, Qi Chen, Ying Zheng, Chenghuai Yang, Karen Harmon, Laura Bradner, Phillip Gauger, Kent Schwartz, Bailey Lauren Arruda 89

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◊ USAHA Paper

Winter time hyperthermia in slaughter weight cattle

Dale Miskimins[1], Steve M Ensley[2], Tim Sahli[3], Russ Daly[1]

[1]Veterinary & Biomedical Sciences Department, South Dakota State University, Elkton, SD;
[2]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA; [3]Tim's Vet Service, Aberdeen, SD

A group of apparently healthy slaughter weight beef cattle were taken to a packing plant in December 2016. The cattle were held overnight. The following morning 24 of 359 were found dead. One additional animal had to be euthanized. The cattle had blood tinged froth coming from the nostrils. Three cattle were necropsied with no definitive lesions. The local veterinarian sent tissue specimens and bedding to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University. A diagnosis needed to be determined quickly so the remaining carcasses could be utilized if possible. The Christmas holiday season was fast approaching and there was a need for answers. A complete diagnostic workup started. Iowa State University was contacted for extensive toxicology testing. The initial results were negative for significant lesions, bacteria, viruses and toxic agents. A site visit was made to the abattoir to observe facilities and visit with company personnel. A final diagnosis of hyperthermia was determined.

An unusual case of brodifacoum toxicosis in a whelping dog

Scott D Fitzgerald[1], Jennifer Martinez[2], John P. Buchweitz[1]

[1]Diagnostic Center for Population & Animal Health, Michigan State University, Lansing, MI;
[2]Small Animal Clinical Sciences, University of Florida, Gainesville, FL

A seven-year-old female Weimaraner was presented to emergency service after 3 hours of active labor with no puppies produced. Hemoabdomen and hemothorax were present at the time of surgery, and prothrombin time (PT) and activated partial thromboplastin time (aPTT) were both found to be within normal ranges. Surgical C-section was performed, four dead puppies and five live puppies were delivered. Since hemostasis was difficult to achieve, a hysterectomy was performed, and the dog died as the surgery was completed. At necropsy, the pleural cavity contained 1.5 L of unclotted blood, while the peritoneal cavity was relatively normal and no obvious hemorrhage was associated with the surgical sites. All 4 dead fetuses were opened, and all four pleural cavities were filled with unclotted blood. An anticoagulant screen was performed on the adult dog's liver utilizing a modified high-perfromance liquid chromatography method, and brodifacoum was identified at a level of 0.024mg/kg. This case is unusual in that the PT and aPTT were within reference ranges, but brodifacoum was present in sufficient amounts to potentially result in this dog bleeding to death, and also is suspected to have crossed the placenta and caused hemothorax and death in four of nine puppies in utero. Other differentials including liver disease, DIC, Hemophilia A, Hemophilia B, and various clotting factor disorders were ruled out based on the normal PT and aPTT. While Von Willebrand disease generally is not commonly associated with abnormal clotting times, it has not previously been reported to be associated with bleeding to death in both an adult dog and unborn puppies. Therefore, we suggest this case represents an unusual manifestation of brodifacoum anticoagulant poisoning.

Cause of mortality diagnoses in domestic sheep in the Intermountain West

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Domestic sheep (*Ovis aries*) (n=258) were necropsied from 2009 through 2016 at the Utah Veterinary Diagnostic Laboratory. Sheep were from farms or range flocks in Utah (93%), Idaho (6%) or Wyoming (0.4%). Sheep were presented in groups of 1-3 with a mean of 1.1 animals per submission. Primary cause(s) of mortality was diagnosed in 240 sheep (93%). Cause of death was established based on reported clinical signs, pathology, and results of ancillary tests. Tests included bacteriology, histology, molecular diagnostics, immunohistochemistry, and vitamin and mineral analyses including inductively coupled plasma–mass spectroscopy. Among the 240 sheep where cause(s) of death were established, 179 (75%) died from a single disease or condition, whereas 61 (25%) had 2 diseases or conditions contributing to death. Six diseases accounted for 138 (58%) of the diagnoses across all ages of sheep: abortion 56 (23%) (22% of bacterial abortions were detected as chlamydial), parasitism 26 (11%) (73% of parasitism deaths were from *Haemonchus contortus*), bacterial pneumonia 18 (8%), enteritis/enterotoxemia 17 (7%), bloat 13 (5%), and selenium deficiency 8 (3%). No cause of death was evident in 18 sheep (7%). No age was provided for 52% of sheep, so except for fetuses, based on literature and expert opinion, their age was estimated from body weight. Of the 48% of sheep with age provided, 91% matched with the body weight estimates. Sex of 24 sheep (9%) was not recorded. Age, weight and sex categories: Fetus (n = 60) mean 3.5 kg, 35 M, 11 F; 4 Wk Lamb (n = 27) 1-28 d, < 8 kg, mean 5.8 kg, 12 M, 9 F; Young Lamb (n = 69) 29-180 d, 8 - 35 kg, mean 22.0 kg, 35 M, 32 F; Older Lamb (n = 28) 181-365 d, 36 - 54 kg, mean 45.4 kg, 15 M, 11 F; Adult (n = 74) >365 d, > 54 kg, mean 80.3 kg, 51 M, 23 F. Main causes of death by age group: Fetus: abortion 50 (83%), including congenital defects 6 (10%) (67% of congenital deaths were clones); 4 Wk Lamb: congenital defects 6 (22%) (all included cleft palate, all clones), parasitism 3 (11%); Young Lamb: parasitism 15 (22%) (67% *H. contortus*), pneumonia 10 (15%) (90% bacterial pneumonia), enteritis/ enterotoxemia 9 (13%), bloat 6 (9%) (83% frothy bloat); Older Lamb: parasitism 7 (25%) (57% *H. contortus*; 43% *Fasciola hepatica*), pneumonia 3 (11%) (all bacterial), clostridial enterotoxemia 3 (11%); Adult: unknown 10 (14%), bloat 5 (7%) (60% gas bloat), parasitism 4 (5%) (all *H. contortus*), peritonitis 4 (5%), pneumonia 4 (5%) (all bacterial), urolithiasis 4 (5%) (50% urethral rupture). Control measures for the most common causes of domestic sheep mortality include vaccination and clean lambing environment to reduce abortions and enterotoxemia, control of *H. contortus* parasite infestation, prevention and treatment of bloat and pneumonia, and adequate mineral supplementation.

Congenital ocular abnormalities in free-ranging white-tailed deer: a retrospective case series from the southeastern US from 1976-2016 and review of the literature # + * †

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Congenital ocular abnormalities in free-ranging cervid species have been previously reported as individual cases from various regions of the United States and include microphthalmia, anophthalmia, congenital cataracts, dermoids, and colobomata. A common underlying cause for these abnormalities, such as nutritional deficiencies, environmental toxin exposures, or genetic mutations, has not been established. This retrospective study summarizes existing case reports and includes cases of suspected congenital ocular abnormalities in free-ranging white-tailed deer cases submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS) in Athens, GA. Of 3645 accessions reviewed, 16 case records qualified for the study. Fifteen cases had been published previously in the literature, including a single SCWDS case of corneoscleral choristomas. Conditions described in the remaining SCWDS cases included microphthalmia (8/15), congenital cataracts (3/15), anopthalmia (2/15), colobomata (1/15), anterior dysgenesis (1/15), ectopic lacrimal gland tissue (1/15), and congenital blindness with corneal opacity (1/15). The majority (11/15; 73%) of the SCWDS cases were male fawns with an average age of 4 months at presentation, which is consistent with previously described cases. Most animals had bilateral abnormalities with few extra-ocular congenital abnormalities reported, also consistent with existing reports. Cases were variably tested for various pathogens at the time of submission; two cases were seropositive for bluetongue virus antibodies. Spatiotemporal clustering of cases was not evident. Further surveillance of white-tailed deer populations and awareness amongst wildlife biologists may help in the identification of other similar cases and potential etiologies.

AAVLD Trainee Travel Awardee
+ AAVLD/ACVP Pathology Award Applicant
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Diagnostic investigation of conjunctivitis outbreaks in gilt developer units

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Conjunctivitis can occur at any age in individual pigs; however, outbreaks of conjunctivitis do occur sporadically, usually between 12 and 26 weeks of age. Etiology is probably multifactorial and risk factors and pathogenic microflora likely vary from case-to-case. Outbreaks of conjunctivitis have been reported as associated with the presence of CSFV, SIV, PRV, PRRSV, PCMV, *Chlamydia, Mycoplasma* and a host of other bacteria that have been demonstrated in conjunctival swabs or extension of rhinitis in affected pigs. Reported here is a summary of diagnostic investigations of ongoing conjunctivitis outbreaks occurred at multiple gilt developer units in a large production system.

The age of affected pigs ranged from 6 to 24 weeks, with most occurring from 12-20 weeks-of-age. The clinical severity ranged from mild to severe, with blindness as an outcome in some individuals; however, most cases resolved over time and were asymptomatic by breeding age. Response to antimicrobial interventions was reported as poor. In the index case, conjunctival swabs and conjunctival biopsies were collected from affected and unaffected cohorts for diagnostic investigation. Specific PCR demonstrated the presence of *Mycoplasma hyorhinis* (MHR), and pan-family or genus PCRs demonstrated herpesvirus (porcine lymphotropic herpesvirus) and *Chlamydia*, but not adenoviruses, caliciviruses, paramyxoviruses, pestiviruses, or picornaviruses, in swabs from both clinically affected and unaffected animals. Bacterial culture demonstrated mixed populations of bacteria, with consistent presence of *Streptococcus porcinus* and *Staphylococcus chromogenes* and inconsistent presence of a potpourri of bacteria, including MHR and various species from genera *Aerococcus, Staphylococcus, Pasteurella, Escherichia, Streptococcus, Proteus*, and more.

An addition submission from a related site stocked with gilts from the same flow and genetic lines as the first case included conjunctival swabs as well as fresh and formalin-fixed conjunctival biopsies from 10 affected pigs and 20 unaffected cohorts for a case-control study. *Chlamydia* was unequivocally demonstrated in all swabs by PCR with a low to moderate Ct. MHR was also present in all swabs by PCR as well, but with very high Ct. Interestingly, adenovirus was detected in a portion of conjunctival swabs (n=7) from affected pigs but was not detected in swabs from clinically unaffected pigs by pan-family PCR. Histologic evaluation of conjunctival biopsies consistently demonstrated some level of lymphocytic conjunctivitis in all pigs, with lesions much more severe and chronic in clinically affected pigs. IHC and/or ISH investigation for target agents has yet to be completed.

This case report highlights the complexity of potentially pathogenic and environmental flora found in dense swine populations. Cause(s) of conjunctivitis are likely multifactorial, perhaps with presence of certain agents necessary for clinical expression.

When the doctor consults Dr. Google: an information literacy exercise for fourth year veterinary students. ◊

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Veterinarians play an important role in public health, and face increasing oversight from regulatory agencies. This focuses greater attention on the decentralized nature of information resources for veterinarians. Described herein is an innovative case-based learning exercise co-developed by a diagnostic pathologist and DVM-librarian focused on quality information seeking for animal and human health regulatory and reporting decisions. The small-group session takes place in the final year diagnostic block at Washington State University's College of Veterinary Medicine. Individual students each lead their rotation mates through a pre-selected regulatory scenario discussion. These scenarios are designed to challenge students to efficiently research governmental websites, interpret regulations, and discover valuable information resources in order to make critical decisions. Scenarios address the following essential questions:

Who do you contact when faced with a public health concern and/or a reportable condition, and what agencies handle regulatory testing and reporting at the local, state, federal and international levels?

How do you confirm a reportable diagnosis - what samples are submitted to what laboratory and by whom, and how might those results be achieved in the most timely fashion?

What is the timeline for reporting? For suspected conditions? For definitively diagnosed conditions?

Which government agencies regulate food for animals, food for people, dietary supplements, drugs, biologicals, and pesticides?

What is the field veterinarian's role with respect to treating and diagnosing disease in recently and illegally imported animals?

Where can you find quality consumer information to communicate with animal caregivers from a variety of backgrounds?

What strategies can you use to navigate government, organizational and laboratory websites, legal code and literature databases to find the most current and accurate information?

Students research how to handle these scenarios, and present their recommendations including web references for group discussion. In the process, students indirectly cover the concepts of information currency, bias, authority, gaps, organization, and permanency. Students also develop basic practical game plans and contact lists for common public health concerns requiring rapid diagnosis such as rabies, regulatory testing such as raw milk dairies, and confusing reporting scenarios such as dietary supplements versus feeds. Altogether, participants discover how critical thinking in both the creation and use of online information resources impacts animal and human health.

◊ USAHA Paper

Salmonella Heidelberg: An emerging problem in the dairy industry

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Beginning in late 2015, the WVDL started receiving diagnostic samples from dairy calves (less than 3 weeks of age) that died less than 6-8 hours after they were first noticed to be ill or they were found dead. Some of the calves had diarrhea and a fever (>40 °C) but many calves did not. Upon postmortem examination, the only consistent finding was enlarged mesenteric lymph nodes in 25-50 % of affected calves. A mild interstitial pneumonia and a fibrinous peritonitis was also observed in less than 10% of affected calves. Histological examination found an acute, necrotizing and suppurative gastroenteritis with lymphoid depletion in 25-50% of affected calves with 25-50% of the calves examined having no significant gross or microscopic lesions.

Consistently, *Salmonella* Heidelberg was found in large numbers in multiple organs (lung, kidney, spleen, liver, small and large intestine and mesenteric lymph nodes) indicating that the calves died of bacteremia/septicemia. *Salmonella* Heidelberg isolates were sent to the Wisconsin State Laboratory of Hygiene for molecular finger printing either by pulse field gel electrophoresis (PFGE) or whole genomic sequencing (WGS). Antimicrobial susceptibility testing (Sensititre, Thermo Fisher Scientific, Madison, WI) was done on all the *Salmonella* Heidelberg isolates.

As of 06-01-17, the WVDL has isolated *Salmonella* Heidelberg from 32 different premises located in 5 different states (IN, SD, MN, MO and WI). The majority of the livestock operations (80%) were located in Wisconsin. At least two-thirds of the isolates were obtained from dairy beef operations that experienced high death loss (25-65%) in groups of calves that were received 5-7 days earlier.

All the S*almonella* Heidelberg isolates were multi-drug resistant (only susceptible to gentamicin) and are very closely related when examined by PFGE or WGS. The dominant strain (dendogram: #PF6X01.0523) of *Salmonella* Heidelberg differs by only 8-12 base pairs (bp) when WGS is done. It is important to understand that this strain of *Salmonella* Heidelberg is quite different from previous isolates that have been found in cattle, swine and poultry.

The PF6X01.0523 strain of *Salmonella* Heidelberg can cause severe mortality (> 25%) in well managed dairy operations that incorporate best-practices which includes the industry standards for colostrum management, nutrition, space and minimization of heat and cold stress (calf comfort). There are no recommended antimicrobial drugs that are effective against this bacterium so treatment of clinically affected animals can only be supportive in nature and it is often not effective. Control of *Salmonella* Heidelberg in affected herds should focus on proper cleaning and disinfection of close-up pens, calving pens, warming boxes, calf barns, calf feeding equipment and livestock trailers. High-pressure washers should not be used for cleaning because they do not effectively remove biofilms and they cause cross-contamination of the environment.

A retrospective pathology study (2007-2015) of the features and causes of myocarditis in young dogs

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Myocarditis can cause death or devastating permanent heart damage. As veterinary research is largely limited to postmortem case reports and case series, epidemiologic and etiopathological data describing canine myocarditis are lacking but viral infections or bacterial agents are often suspected. Our retrospective study identified myocarditis in 5.5% (95% Confidence Interval (CI) 4.6%, 6.7%) of young dogs two years of age or less in the Cornell Anatomic Pathology database (106 out of 1911 total canine cases). Using archived autopsy samples, we demonstrated a significant association of CPV-2 with myocarditis in young dogs by polymerase chain reaction (PCR) and sequencing (p=0.003) which was present in 12/40 (30%; 95% CI 18, 45) of cases having a diagnosis of myocardial necrosis, inflammation, or fibrosis and 2/41 (5%; 95% CI 0.1,16) of controls without myocardial diagnoses. CPV-2 VP2 mRNA was amplified by RT-qPCR in the 12 of the 12 cases and 2 controls having amplicon sequences matching CPV-2 sequences in Genbank. The median viral mRNA quantity (minimum, maximum) of PCR-positive cases was $8.77x10^4$ ($2.23x10^1$, $2.10x10^7$) copies suggesting viable myocardial virus while both controls had comparatively low copy numbers. Testing for herpesvirus (PCR and ISH) and adenovirus (PCR) identified herpesvirus in one additional case; however, the etiology of myocarditis was undetermined in 27/40 (69%) of the myocarditis/fibrosis cases. Sixteen of these cases had histologic features of myocardial viral infection. Overall, viral causes were identified or suspected in 28/40 (70%; 95% CI 55, 82) of cases, a similar prevalence to epidemiologic data associating viral infection with myocarditis in humans. In eight cases lacking detectable CPV DNA, myocardial perivasculitis/vasculitis and myocarditis with prominent neutrophils and/or macrophages were histologic features to suggest bacterial agents. Our retrospective study lays the groundwork for understanding the etiopathogenesis of this devastating disease in dogs; however, additional research is needed to identify the causes of myocarditis and to develop testing strategies for the clinical identification of canine myocarditis. Identifying the causes of canine myocarditis is crucial information for general practitioners, emergency clinicians, cardiologists, pathologists, and diagnostic labs.

Polyomavirus infection in Gouldian finches

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Avian polyomavirus causes disease primarily in psittacines. Infection by this virus has been described, albeit rarely, in other avian species including finches. Little is known about the pathogenesis and pathology of polyomavirus infection in finches. Three juvenile Gouldian finches from an aviary of approximately 200 birds presented with lethargy over a week period. Two of these birds were submitted to CAHFS, euthanized, and full necropsies were performed. Gross lesions were similar in both birds and consisted of a moderate to large amount of yellowish fluid feces in the rectum, soiled vent, and severely enlarged spleen. Microscopic lesions included multifocal lympho-plasmacytic and necrotizing hepatitis and splenitis, and interstitial, lympho-plasmacytic nephritis. Large, amphophilic, glassy, intranuclear inclusion bodies were frequently seen in endothelial cells of liver, spleen, kidney, and occasionally in hepatocytes. Virus isolation utilizing a primary chicken embryo fibroblast cell line propagated a virus with morphology compatible with polyomavirus in tissue culture supernatants. Sequence analysis of a 269-bp fragment confirmed presence of finch polyomavirus. Other ancillary tests, including routine aerobic and anaerobic bacterial cultures, culture for *Clostridium difficile*, PCRs for *Salmonella* spp, avian paramyxovirus and avian influenza, heavy metal screen and fecal float, were unrewarding. A diagnosis of polyomavirus infection was established based on the microscopic changes, isolation, and sequence verification of this virus. Information on polyomavirus infection in finches is scant. In psittacines, polyomavirus generally causes disease in fledgling animals, and disease in adult animals is rare unless they are immunosuppressed. In this case, no immunosuppressive factors were found. There are several different but closely related strains of avian polyomaviruses, each with a distinct tropism for various avian species. Full-genome sequencing of the isolated polyomavirus is currently under way which may help to determine if virulence of the polyomavirus affecting these finches was higher than that of other polyomaviruses.

Thyroid activity and survival in Maine moose ◊

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Maine moose numbers are estimated to remain high, but losses of young moose occur yearly, and usually are associated with heavy parasite burdens. Nutritional deficits may contribute to these losses, and multiyear evaluations have shown some mineral deficits, notably selenium, as well as poor body condition in moose mortalities. Between 2014 and 2017, the Maine Department of Inland Fisheries and Wildlife captured and radio-collared adult and calf moose using a helicopter team. A pool of approximately 100 animals has been maintained, with necropsies conducted on any animals that die during the study, and replacement animals collared during the late winter, when deep snow conditions allow humane capture of the animals. At capture, all animals were vigorous and were visually assessed as being generally healthy. Hematologic parameters were recorded, tick counts were conducted, and hair and fecal samples collected. Animals were also sampled after death, monitored via radio tracking. Tissues and blood were collected at field necropsy. 36 moose have died from the initial year of the study, 25 from the second year, 49 from the third year, and 11 from the fourth (current) year. The majority of yearly mortalities are calves. In mortalities analyzed to date, histology showed both thyroid glands in 38 of 46 moose to be inactive (2014-2017); of 11 moose necropsied so far in 2017, 2 had apparently normally active thyroid glands (score 1), 3 had moderately inactive glands (score 2), and 6 had very severely affected the thyroid glands, with numerous follicles empty of colloid (score 3). Only 1 mortality occurred in the fall (score 2), and the remaining 45 died between January and May, with no apparent effect of month on thyroid score. However, the lack of mortality in the summer and fall limits conclusions regarding seasonality of thyroid activity in these moose mortalities. In White Tailed Deer, serum thyroid hormone levels fluctuate with season and nutritional level. For moose at capture, and for those radio-collared moose from which blood could be collected after death (n=33), blood was also submitted for mineral analysis; liver was submitted in moose mortalities. Samples were analyzed at Michigan State University for cobalt, copper, iron, manganese, molybdenum, selenium and zinc. When compared with values established for cattle, very low selenium (mean serum SE 9.1 ± 3.3 ng/ml vs bovine normal mean of 70-100 ng/ml; mean tissue SE 0.05 ± 0.01 PPM versus a bovine mean of 0.2 PPM) along with marginal copper, cobalt and molybdenum, were noted. Selenium acts as a vital antioxidant, and is known to be deficient in Maine soils. Lack of selenium has been implicated in bovine hypothyroidism. Nutritional status in combination with parasite stressors, such as high concentrations of winter ticks and lungworms, may contribute to the relatively high mortality of Maine moose calves.

◊ USAHA Paper

Chronic suppurative bronchopneumonia associated to *Candida glabrata*, perforated gastric ulcer and peritonitis in a bottlenose dolphin (*Turisops truncatus*) #

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Infections caused by opportunistic pathogens have been increasing in the last decades, both in human beings and animals, associated to immunosuppression states and, in part, to antibiotic and antimycotic resistance. In this work we describe the case of a bottlenose dolphin (*Tursiops truncatus*) belonging to a dolphinarium that had a history of anorexia, apathy, progressive emaciation and leucocytosis during the last two months. Previously to this process, the animal was treated with itraconazol because of a positive serology to *Aspergillus spp*. The dolphin was treated with antibiotics, antimicotics, nonsteroidal anti-inflammatory drugs and gastric protectors unsuccessfully. Serology against *Candida spp.* was negative. Four hours after the spontaneous death of the animal, post-mortem examination was performed. There was a diffuse subacute fibrinous peritonitis derived from a focal complete perforation in the initial section of the third stomach. Regarding the respiratory system, there was a multifocal fibrino-necrotizing tracheitis and an extense suppurative bronchopneumonia, more evident in the ventral portions of both lungs. By means of conventional histological study and Grocott staining, abundant fungal hyphae compatible with *Aspergillus spp.* were seen in the tracheal lesions. Likewise, in the lung there were abundant 2-3 um in diameter yeast-like structures compatible with *Candida spp*. both free and inside the cytoplasm of foamy macrophages. The same type of yeasts-like structures were seen admixed in the inflammatory infiltrates of the gastric ulcer and the peritoneum. The corresponding bacteriological and mycological study identified these yeasts as *Candida glabrata* (previously named *Torulopsis glabrata*). Additionally, other bacteria considered normal flora were isolated. *C. glabrata* is a *Candida* species that acts as an opportunistic pathogen in human beings, although its pathogenicity is considered inferior to that of *C. albicans*. The detection of *C. glabrata* is becoming more and more frequent in humans, especially in weakened and immunosuppressed patients, in which it can be lethal. Numerous *C. glabrata* strains are resistant to triazoles (fluconazole, itraconazole). In cetacean, *C. glabrata* can be detected from the upper respiratory tract (blowhole) of free-ranging bottlenose dolphins, but its association with disease remains controversial.

AAVLD Trainee Travel Awardee

Fatal ruminal tympany secondary to esophageal and oropharyngeal obstruction caused by an *Actinobacillus lignieresii* pyogranuloma in a steer # + * †

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A 390 kg, male, Hereford steer on pasture presented acute signs of hypersalivation, dyspnea and severe abdominal distention of the left flank. At physical examination, the animal had a cardiac rate of 89 beats per minute and a normal respiratory rate of 10 breaths per minute (bpm). There was severe abdominal distention in the left paralumbar fossa, along with severe abdominal pain. The animal was unresponsive and based on these clinical signs, a diagnosis of severe ruminal tympany was made. A longitudinal 2.5 cm transmural perforating incision was done using a knife blade in the left paralumbar fossa to relieve the ruminal tympany, and 10 mg/kg IV of methylene blue were administrated by the farmer. Minutes later, the abdominal distention minimized but the respiratory frequency decreased to 7 bpm and the animal died. At autopsy, a multinodular firm mass, measuring 11 x 7 x 3 cm, was observed in the dorsal portion of the oropharynx. The mass was obstructing the lumen of the oropharynx and proximal esophagus, and had an irregular whitish surface with hemorrhages and yellow foci. Two similar ovoid nodules measuring 3 cm, with an irregular yellow cut surface surrounded by a white fibrous capsule, were found in the left and right caudal pulmonary lobes. There was diffuse pulmonary congestion and edema. In the mucosa of the esophagus at the level of the thoracic inlet, there was a well-demarcated transverse line that separated the diffusely congested anterior segment from the diffusely pale anterior portion ("bloat line"). The main histologic lesion was pyogranulomatous oropharyngitis and pneumonia. The pyogranulomas showed necrotic centers with cellular debris occasionally containing gram-negative coccobacilli, surrounded by acellular, club-shaped, deeply eosinophilic palisading material typical of the Splendore-Hoeppli reaction, further surrounded by an inflammatory infiltrate composed of neutrophils, macrophages and occasional multinucleated giant cells. *Actinobacillus lignieresii* was isolated from the oropharyngeal and pulmonary pyogranulomas. This represents an unusual fatal case of ruminal tympany secondary to oropharyngeal and esophageal obstruction caused by an *A. lignieresii* pyogranuloma, that probably resulted in failure of eructation. There are few cases of ruminal tympany due to mechanical obstruction of the esophagus reported in the literature, with neoplasia, lymphadenomegaly, abscesses or foreign bodies being the main associated underlying conditions in cattle.

AAVLD Trainee Travel Awardee
+ AAVLD/ACVP Pathology Award Applicant
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Detection of caprine arthritis encephalitis virus using next-generation sequencing technology and potential applications for routine use

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Pathogen detection in veterinary diagnostic laboratories commonly involves a combination of culture-based (i.e bacteriological isolation and virus isolation) and culture-independent techniques that include antigen and nucleic acid detection (i.e immunohistochemistry, PCR and *in situ* hybridization). Most laboratories have a number of routine assays available for commonly encountered agents and animal species; however, there is significant variability among laboratories regarding the breadth and depth of assays available for a given animal species. For example, at the Iowa State University VDL, small ruminant submissions comprise a minority of total case submissions and accordingly the laboratory maintains a small number of specific PCR assays relevant for these animal species. Additionally, it is often difficult to remain current on available assays at other VDLs and out-sourcing samples increases total turnaround time. In the case of this report, respiratory disease in 4 to 6-week-old goat kids was reported with increasing morbidity and mortality throughout the winter. The submitted lung tissue samples were severely consolidated with a cobblestone appearance on cut section. Microscopically there was severe lymphohistiocytic interstitial pneumonia with marked type II pneumocyte hyperplasia, syncytial cells, and variable purulent bronchopneumonia. A viral etiology was suspected and available PCR assays at the ISU VDL for BRSV and PI3 were negative as was virus isolation. Bacterial culture identified *Mannheimia haemolytica, Bibersteinia trehalosi*, and a *Mycoplasma* sp. Given the increasing morbidity and high index of suspicion of a viral etiology, next-generation sequencing (NGS) was performed and resulted in the detection of a high number of hits with 98.9% homology with the GAG gen of caprine arthritis encephalitis virus (CAEV) as well as detection of multiple bacteria including *M. haemolytica, B. trehalosi, Pasteurella multocida,* and multiple *Mycoplasma* spp. While not a common presentation, juvenile pneumonia is consistent with CAEV infection and aligns with the reported clinical history. NGS technology provides the potential for simultaneous detection of an unlimited number of agents and, as in the case of this report, can be very useful for detecting uncommon pathogens or common pathogens in uncommon species in a given laboratory. Where specific confirmation of disease associated with detected pathogens is desired, serial testing with direct detection techniques such as *in situ* hybridization can be readily implemented. As NGS technology becomes more affordable and more widely available it may ultimately improve diagnostic sensitivity and become a mainstay for pathogen detection in routine veterinary diagnostic pathology.

Identification of a divergent porcine astrovirus type 3 in central nervous system tissue from swine with neurologic disease and encephalomyelitis: Diagnostic investigation, virus characterization and retrospective analysis of historic cases. +

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Porcine astroviruses (PAstV) are a cluster of non-monophyletic viruses composed of five different lineages (PAstV 1-5) belonging to the genus *Mamastrovirus*, family *Astroviridae*. All five lineages have been detected in swine feces submitted to the Iowa State University Veterinary Diagnostic Lab (ISU VDL). Although astroviruses have been identified in a variety of mammals and birds, infection is often considered asymptomatic. In humans, astroviruses have been implicated in cases of gastroenteritis. Recently, a subset of astroviruses have been linked to central nervous system (CNS) disease in humans, cattle, sheep and mink. Over a 9-month period, swine neurologic cases originating from a multisite swine production farm representing a population of 4-12-week-old pigs and adult sows were submitted to the ISU VDL. Affected swine exhibited signs which ranged from hind limb weakness to quadriplegia and occasionally convulsions with a case fatality in pigs and sows of 75% and 100%, respectively. As conventional pathogens, including Porcine reproductive and respiratory syndrome virus type 1 and 2, Porcine circovirus 2, *Suid alphaherpesvirus 1*, *Teschovirus A*, *Sapelovirus A* or "atypical porcine pestivirus", were not detected by PCR in affected nervous tissue and no bacterial pathogens were isolated by culture, unbiased next-generation sequencing was used to identify and genetically characterize a divergent porcine astrovirus type 3 in CNS tissue of one piglet and three sows with neurologic signs and encephalomyelitis. Metagenomic sequencing from CNS samples identified the complete genome (6461 nt) of the virus and full genome phylogenetic comparison placed it under the same cluster as other PAstV-3, yielding the highest homology (92.2% nt identity) with PAstV3/US/MO/123, which was detected in 2013 in swine feces with unknown clinical significance. Notably, the complete genome sequence of this divergent PAstV-3 shared 49.4%-53.5% nt identity to those of previously described neurotropic astroviruses from humans, cattle, sheep and mink while it shared only 39.2% nt identity to PAstV-1, 37.7%-38.4% nt identity with PAstV-2, 36.6%-38.5% nt identity to PAstV-4, and 35.3%-36.9% nt identity to PAstV-5, respectively. Approximately one-third of swine neurologic cases with histologic lesions suggestive of a viral etiology submitted to the ISU VDL over the previous 30 months did not have a definitive etiologic agent confirmed. Due to the high percentage of neurologic cases without a definitive etiology, a retrospective analysis of swine neurologic cases of unknown etiology and lesions compatible with a viral agent was undertaken. Nucleic acid was extracted from formalin fixed paraffin embedded CNS tissue. PastV-3 was detected by RT-qPCR in other cases spanning multiple years. In summary, this is the first report to identify and genetically characterize a novel PAstV3 in CNS tissue of swine with neurologic disease and viral encephalomyelitis.

+ AAVLD/ACVP Pathology Award Applicant

Serology

Saturday, October 14, 2017
Pacific Salon 4-5

Moderators: Neil Pople and Orhan Sahin

1:00 PM **Development of a multiplex assay for the serologic diagnosis of *Brucella canis* infection**
Cassandra M. Guarino, Colleen Eade, Lauren Griggs, Staci Nugent, Elizabeth Altier, Hannah James, Heather Freer, Sanda Asbie, Anil J. Thachil, Craig Altier, Bettina Wagner *91*

1:15 PM **Performance of a synthetic OPS antigen-based DIVA assay for the diagnosis of *Brucella abortus* in cattle** ◊
Andrew Johnson, John McGiven, Sampath Srikanth, Siddra Hines *92*

1:30 PM **Validation of a commercial rLPS-based antibody ELISA for *Brucella ovis* and *Brucella canis*** ◊
Andrew Johnson, John McGiven, Sampath Srikanth, Siddra Hines *93*

1:45 PM **Performance of antibody ELISAs for TGEV/PRCV differential diagnosis** ◊
Ronaldo Magtoto, Dave Baum, Jianqiang Zhang, Qi Chen, Ji Ju, Korakrit Poonsuk, Pablo E. Pineyro, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola. *94*

2:00 PM **Chemical "clean up" of oral fluids does not adversely affect the detection of porcine epidemic diarrhea virus (PEDV) isotype-specific (IgG, IgA) ELISA responses** * †
Korakrit Poonsuk, Luis Gabriel Gimenez-Lirola, Ronaldo Magtoto, Dave Baum, Christopher Rademacher, Justin Brown, Ji Ju, Jianqiang Zhang, Chong Wang, Rodger Main, Jeff Zimmerman *95*

2:15 PM **A highly sensitive and specific multispecies cELISA based on the 3ABC nonstructural polyprotein for the diagnosis of foot-and-mouth disease** ◊
Ethan Adams, Chungwon Joseph Chung, Alfonso Clavijo, Barbara J. Kamicker, David J. Brake, Carey Bandaranayaka-Mudiyanselage, Scott Beeson, Scott Adams, Siddra Hines *96*

2:30 PM **Detection of Rift Valley Fever virus antibodies by a rapid immunochromatographic test**
Bonto Faburay, Kirti Dave, D. Scott McVey, Sonia Dave, William C Wilson, Juergen Richt. *97*

2:45 PM **Number and location of activation induced cytidine deaminase (AID) 'hotspot sequences' in germline variable genes of veterinary and laboratory species**
Randall Lynn Levings *98*

Symbols at the end of titles indicate the following designations:

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

Development of a multiplex assay for the serologic diagnosis of *Brucella canis* infection

Cassandra M. Guarino, Colleen Eade, Lauren Griggs, Staci Nugent, Elizabeth Altier, Hannah James, Heather Freer, Sanda Asbie, Anil J. Thachil, Craig Altier, Bettina Wagner

Population Medicine and Diagnostic Sciences, Cornell Universtiy, Slaterville Springs, NY

Current diagnostic assays to detect *Brucella canis* infection require the regular growth and production of this BSL-3 pathogen. The goals of this project were to (1) identify immunodominant antigens from *Brucella canis* that could be cloned and expressed from a non-pathogenic organism, and (2) investigate the use of these proteins in a multiplex assay for serodiagnosis of *B. canis* infection. *B. canis* lysate was resolved by SDS-PAGE, and serum previously determined to be reactive with *B. canis* antigen in both microagglutination and AGID assays was used to probe immunoblots. Immunoreactive bands were found at ~30KDa and ~9KDa. These bands were excised from an SDS-PAGE gel and analyzed by mass spectrometry. The top identities from each reaction, BP26 and HRL18, respectively, were cloned from *B. canis* genomic DNA and expressed in *Escherichia coli*. Following optimization of expression conditions, the proteins were purified and coupled to beads. Serum dilution and reagent concentrations were optimized using positive and negative sera, as determined by the 'gold standard' microagglutination and AGID assays. 347 samples, including 21 samples confirmed positive by the 'gold standard' assays, were run in parallel with 'gold standard' assays. The HRL18 protein was determined to be inadequate for differentiating positive and negative samples. ROC analysis of the BP26 antibody values revealed 85% sensitivity and 98% specificity in comparison to the 'gold standard', with 12 samples falling into the 'equivocal' range. An additional 530 samples, including 47 samples confirmed positive by the 'gold standard' assays, were screened for BP26 antibody values in parallel with the current 'gold standard' assays. ROC analysis of the BP26 antibody values for the 877 samples reveals 78% sensitivity and 98% specificity, with 73 samples determined to be 'equivocal'. Additionally, 12 out of 25 samples determined to be 'inconclusive' in the 'gold standard' assays had BP26 antibody values in the positive range. Further work is currently underway to test six additional *B. canis* antigens for use in this multiplex assay format in an effort to improve both the sensitivity and specificity of the assay, and to reduce the incidence of 'inconclusive' or 'equivocal' results.

Performance of a synthetic OPS antigen-based DIVA assay for the diagnosis of *Brucella abortus* in cattle ◊

Andrew Johnson[1], John McGiven[2], Sampath Srikanth[1], Siddra Hines[1]

[1]VMRD, Inc., Pullman, WA; [2]Animal and Plant Health Agency, New Haw, United Kingdom

Brucella abortus is one of the causative agents of brucellosis, a zoonotic disease found worldwide in cattle, sheep, goats and swine that results in billions of dollars in economic loss, particularly in endemic areas. Control and eradication of brucellosis can be accomplished through diagnosis and vaccination. The US is considered *Brucella*-free and currently uses the *B. abortus* strain RB51 vaccine as a mainstay of their *B. abortus* control program. For diagnosis of *B. abortus*, the sLPS antigen ELISA is one of the OIE recommended tests. This assay is very sensitive but has a significant number of false positive serum reactors (FPSR), which may result from the shared homology between the sLPS antigen and *Yersinia enterocolitica* O:9 antigens. Synthetic antigens derived from the OPS of Brucella have shown considerable promise in decreasing the number of false positives. A previously published study using a synthetic antigen-based ELISA demonstrated 100% specificity with 125 *Brucella abortus* culture negative samples and 100% sensitivity with 45 culture positive samples. Samples classified as FPSR on the sLPS ELISA (n=125) were also tested. The synthetic antigen ELISA correctly identified 32 of these as negative, resulting in a 25% improvement in specificity with this sample type.

The new VMRD *Brucella abortus* sAg antibody assay designed with this synthetic antigen technology was evaluated using 256 negative samples derived from multiple US cattle herds to determine a specificity of 100%. The assay was then tested on 31 defined samples obtained from the National Veterinary Services Laboratory (NVSL) of the United States Department of Agriculture, including 10 positive check set samples as well as 21 positive control sera representing various states of infection. This included 4 samples from naturally infected animals and 1 from an experimentally infected animal, 8 samples for which the source of infection was unknown, and 8 samples from animals vaccinated with a product other than strain RB51. The VMRD assay correctly identified 26 out of 31 positive samples with a sensitivity of 84%. Based on the same sample sets, the reference cELISA and FPA assays showed a relative sensitivity of 77.4%. The assay appears to not detect antibodies in RB51 vaccinated animals but does detect antibodies in animals vaccinated with *B. abortus* strain 19, performing similar to the cELISA in this respect.

We conclude that the VMRD *Brucella abortus* sAg antibody assay is more sensitive than the FPA or the cELISA assay (84% vs 77%) while showing 100% specificity in a large sample of non-infected animals. Ongoing testing is currently underway for additional species and on samples from endemic areas to better evaluate the performance of this assay in real world situations.

◊ USAHA Paper

Validation of a commercial rLPS-based antibody ELISA for *Brucella ovis* and *Brucella canis* ◊

Andrew Johnson[1], John McGiven[2], Sampath Srikanth[1], Siddra Hines[1]

[1]VMRD, Inc., Pullman, WA; [2]Animal and Plant Health Association, New Haw, United Kingdom

Accurate and consistent serologic diagnosis of *Brucella ovis* and *Brucella canis* have historically challenging for the sheep and dog industries, respectively, affecting animal sales and complicating disease management. Currently for *B. ovis*, diagnosis is performed using ELISA kit components obtained from the USDA-NVSL. These are not assembled into a standardized kit, therefore discrepant results can occur due to variation in individual lab procedures such as plate coating. The assay also has an "indeterminate" range which is problematic for screening purposes, particularly in young ram lambs sold for breeding. Diagnosis of *B. canis* can be even more complicated, and its potentially zoonotic nature generates added concern. False positives are a reported problem with most available testing modalities. As such, current USDA recommendations advocate for a consensus result of up to three different diagnostic tests to confirm a positive result, as no single test is a confirmatory gold standard.

To address these issues and provide a consistent commercial product for diagnosis of both diseases, VMRD Inc. developed an indirect antibody ELISA based on the rough LPS bacterial constituent common to both *B. ovis* and *B. canis*. This project sought to improve specificity and resolution, minimize variation in results between labs, and address the problematic "indeterminate" sample classification. An improved purification method was employed to extract rLPS for plate coating, and species-specific secondary antibodies were utilized for detection. The assay was tested on 482 sheep serum samples, including 30 samples classified as "indeterminate" on the current NVSL ELISA. At a cutoff of 0.3 OD, the assay had a specificity and sensitivity of 100% in comparison to the NVSL ELISA (excluding indeterminate samples). Without a confirmatory gold standard for comparison, it is impossible to reliably classify these indeterminate samples as positive or negative. However, most animals with this status evaluated over time and by multiple methods are found to be truly negative. If these samples are considered negative and the cutoff is increased to 0.5 OD, sensitivity and specificity are both 99.6% with good resolution between sample populations. Canine serum samples (n=136) were also evaluated, with samples classified by immunofluorescence assay. At a cutoff of 0.2 OD, sensitivity was 90.8% and specificity was 100% for *B. canis*. Additional testing will be performed to verify sample classification into positive and negative cohorts.

Overall, an improved, standardized commercial ELISA for *B. ovis* will facilitate appropriate and precise management of sheep flocks to prevent unnecessary economic loss. This is also true for *B. canis*, for which regulations in some states require testing of breeding animals and prohibit sale of puppies born to positive dams. Availability of an accurate and rapid screening method for both diseases would be of great benefit to these respective industries.

◊ USAHA Paper

Performance of antibody ELISAs for TGEV/PRCV differential diagnosis ◊

Ronaldo Magtoto, Dave Baum, Jianqiang Zhang, Qi Chen, Ji Ju, Korakrit Poonsuk, Pablo E. Pineyro, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola

VDPAM, Iowa State University, Ames, IA

Porcine respiratory coronavirus (PRCV) is a natural mutant of transmissible gastroenteritis virus (TGEV) lacking 224 amino acids in the N-terminal portion of the spike (S) glycoprotein. Serum antibodies provide evidence of TGEV or PRCV infection and/or herd immunity. However, antibodies against PRCV can cross-react and cross-neutralize TGEV. Blocking enzyme-linked immunosorbent assays (ELISAs) for differentiation of PRCV and TGEV based on monoclonal antibodies (mAbs) targeting the N-terminal region (300 amino acids) of the S glycoprotein have been described and are currently commercially available. The aim of this study was to evaluate the diagnostic performance of several commercial ELISA kits for the detection and differentiation of TGEV and PRCV antibodies in serum from experimentally inoculated animals.

Forty-eight, 7-week-old conventional pigs from a farm with no history of porcine coronavirus infections were randomized into four inoculation groups: TGEV Miller, TGEV Purdue, PRCV, and a mock infected control group (12 pigs per group; 2 pigs per pen; 6 pens per group). Pig serum samples (n = 528) were collected at DPI –7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42 for antibody (Ab) testing. Three different commercial TGEV/PRCV differential blocking ELISA kits were evaluated: (i) Swinecheck® TGEV/PRCV Recombinant (Biovet, Canada); (ii) Svanovir® TGEV/PRCV-Ab (Svanova, Sweden); (iii) INgezim Corona Diferencial (Ingenasa, Spain).

Antibody response was detected between 7-10 DPI, regardless of the inoculation group or ELISA kit evaluated. Thereafter, the positive rate within each group increased overtime. In the absence of antibodies against other cross-related porcine coronaviruses, the three commercial ELISA kits evaluated had a 99-100% diagnostic specificity. However, a pig-specific two-way serologic cross-reactivity was detected between PRCV and TGEV between 7 and 21 DPI, regardless of the ELISA kit evaluated. The percentage of PRCV false positive results was higher in the TGEV Purdue group compared to the TGEV Miller infected group.

Under the experimental conditions of this study, two-way serologic cross-reactivity between PRCV and TGEV was observed during early infection. This may vary depending on the homology distance of strains present, the commercial test used, and differences at the pig level. This cross-reactivity at early stages post-infection could be resolved in part through the combined use of serologic and PCR-based assays. Nevertheless, our findings support the concept that the accuracy of commercial ELISAs for differentiating PRCV and TGEV at the individual pig level is low, and therefore should be used on a population basis.

◊ USAHA Paper

Chemical "clean up" of oral fluids does not adversely affect the detection of porcine epidemic diarrhea virus (PEDV) isotype-specific (IgG, IgA) ELISA responses * †

Korakrit Poonsuk, Luis Gabriel Gimenez-Lirola, Ronaldo Magtoto, Dave Baum, Christopher Rademacher, Justin Brown, Ji Ju, Jianqiang Zhang, Chong Wang, Rodger Main, Jeff Zimmerman

Iowa State University, Ames, IA

INTRODUCTION

Oral fluids are contaminated with feed, feces, and inorganic particles from the environment which, in the severest cases, may affect test performance. Removal of particulates by centrifugation or filtration is not practical because these procedures require too much time to be performed routinely in high through-put diagnostic laboratories. The two objectives of this study were to evaluate the effect of chemical "clean up" of swine oral fluids on 1) the diagnostic performance of PEDV IgG and IgA ELISAs and 2) the stability of PEDV-specific IgG and IgA in samples held at 4°C over the course of 7 days.

METHODS

The effect of 3 chemical treatments (A, B, C) on PEDV ELISA responses was evaluated using oral fluid samples collected under experimental conditions (Study One) and under field conditions (Study Two). In Study One, oral fluid samples from pigs inoculated with PEDV were collected on day post inoculation (DPI) -3, 0, 5, 10, 15, 20, 25, 30, 35, and 42. In Study Two, oral fluid samples were collected on DPIs -4, 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, and 42.

Each oral fluid sample was split into 4 aliquots. Then each aliquot was treated with one of 3 chemical treatments; the 4th aliquot served as an untreated control. All samples were tested by PEDV IgG and IgA ELISAs at the time of treatment (day post treatment (DPT) 0). Thereafter, treated and control oral fluid samples were kept at 4°C and tested again on DPT 2, 4, and 6. For purposes of comparison, serum samples were tested by PEDV IgG and IgA ELISAs on day 0, then held at 4°C and tested again on 2, 4, and 6.

RESULTS AND CONCLUSIONS

Statistical analysis (nonparametric ANOVA) of oral fluid IgA and IgG S/Ps found that neither treatment, time, or the interaction of treatment*time affected the results ($p > 0.05$). Pairwise comparisons of DPT 2, 4, and 6 results to DPT 0 IgA and IgG S/Ps detected no significant differences ($p > 0.05$), i.e., treated oral fluid samples were stable over time. Thus, chemical treatment removed particulates from oral fluids, but did not affect test performance.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

A highly sensitive and specific multispecies cELISA based on the 3ABC nonstructural polyprotein for the diagnosis of foot-and-mouth disease ◊

Ethan Adams[1], Chungwon Joseph Chung[2], Alfonso Clavijo[4], Barbara J. Kamicker[3], David J. Brake[3], Carey Bandaranayaka-Mudiyanselage[1], Scott Beeson[1], Scott Adams[1], Siddra Hines[1]

[1]VMRD, Inc., Pullman, WA; [2]Plum Island Animal Disease Center, Department of Homeland Security, Greenport, NY; [3]Leidos, Reston, VA; [4]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada

Foot-and-mouth disease (FMD) is extremely contagious, affecting domestic livestock such as cattle, pigs, sheep, and goats. Control of FMD is one of the leading priorities for countries worldwide. An effective diagnostic test must be broadly reactive across all seven viral serotypes and multiple host species, and able to differentiate between infected and vaccinated animals (DIVA capable).

A competitive ELISA (cELISA) has been developed and validated through collaboration between the Institute for Infectious Animal Diseases, Plum Island Animal Disease Center: USDA ARS, USDA APHIS, DHS, and Leidos, and VMRD, Inc. The cELISA detects serum antibodies via inhibition of specific monoclonal antibody binding to an epitope within the FMDV 3ABC non-structural polyprotein (NSP). This enables the assay to be DIVA capable, as 3ABC is only induced in the presence of replicating virus. The cELISA format enhances specificity (Sp) while offering flexibility for multispecies use. Thus far, this assay has been validated for use in cattle, pigs, and sheep, and the USDA has approved pre-licensing serials manufactured by VMRD, Inc. Final licensure is expected in mid-2017.

Serum samples of known infection status were evaluated from 503 FMD negative cattle of U.S. origin (FMD-free), 121 cattle experimentally infected with FMDV isolates representing all 7 serotypes, 117 naturally infected cattle from Cameroon and South Africa, and 52 vaccinated cattle later challenged with live FMDV. A subset of these samples (n=386) was also run in a current commercial FMDV NSP ELISA for comparison. Porcine samples (n=272, 207 negative and 65 positive) were also tested in both assays along with 214 ovine samples, of which 151 were classified negative and 63 positive.

An optimal cutoff of 40% inhibition was determined based on receiver operator characteristic curves. Reactivity to all seven serotypes in experimental infections was demonstrated as well as to five serotypes in naturally infected cattle. DIVA capability was confirmed by negative test results in vaccinated, unchallenged cattle that seroconverted after challenge. In unvaccinated, experimentally infected cattle, the cELISA identified seroconversion in all animals by 7-15 days post infection.

The subset of 386 bovine samples showed a sensitivity (Se) of 99.6% and Sp of 99.3% in comparison to 96.7% and 97.9%, respectively, for the other assay. The complete bovine sample set generated similar values of 99.6% Se and 99.1% Sp. Both the VMRD cELISA and the comparator assay had 100% Sp for porcine samples, however the cELISA had a Se of 96.9% versus 76.9% for the other assay. Finally, the VMRD cELISA had a Se of 76.1% and Sp of 98.6% for ovine samples, with the assay used for comparison demonstrating Se of 68.2% and Sp of 100%. The broad reactivity, DIVA capability, and high performance shown for multiple species demonstrate the value of this cELISA as a critical tool for FMDV control efforts.

◊ USAHA Paper

Detection of Rift Valley Fever virus antibodies by a rapid immunochromatographic test

Bonto Faburay[1], Kirti Dave[3], D. Scott McVey[2], Sonia Dave[3], William C Wilson[5], Juergen Richt[1,4]

[1]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS; [2]USDA-ARS-PA-Center for Grain and Animal Health Research, Manhattan, KS; [3]VecTOR Test Systems Inc., Thousand Oaks, CA; [4]Center of Excellence for Emerging and Zoonotic Animal Diseases, Kansas State University, Manhattan, KS; [5]USDA-ARS, Arthropod-Borne Animal Diseases Research Unit, Manhattan, KS

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic pathogen, belongs to the family Bunyaviridae and genus Phlebovirus. The virus causes disease outbreaks in livestock and humans in sub-Saharan Africa and recently expanded its range into Arabian Peninsula. RVFV contains single-stranded, negative sense, RNA genome that encodes structural proteins, nucleoprotein (N), the glycoproteins Gn and Gc, which are targets for diagnostic and vaccine development. RVFV is classified in the list of Category A agents and poses a threat to agriculture and public health. There is significant risk for introduction of the virus into the United States, which necessitates the development of effective and rapidly deployable countermeasures. The aim of this study was to develop and evaluate the performance of an immunochromatographic lateral flow test (LFT) for rapid detection of RVFV IgG antibodies in target host species. Sheep and cattle were experimentally infected with wildtype RVFV strains or vaccinated with DIVA (differentiate infected from vaccinated animals)-compatible RVFV Gn/Gc subunit vaccine to produce immune sera against RVFV. Using a dual antibody detection format for simultaneous detection of RVFV N and Gn antibodies in host serum, the LFT demonstrated good performance in detecting the kinetics of RVFV antibody response in sheep. The test detected RVFV N antibodies in good correlation with the indirect RVFV N ELISA in experimentally infected sheep and cattle sera revealing sensitivity (using 4-38 dpi sera) and specificity values of 100%, and indirect ELISA agreement value of 90% (using 3-21 dpi sera). The LFT distinguished animals vaccinated with the RVFV Gn/Gc subunit vaccine from non-vaccinated animals. The assay is undergoing further field evaluation of its ability to detect RVFV antibodies in sera obtained from target species exposed to natural infection in endemic settings. The results suggest the LFT device could serve as a useful serodiagnostic tool for rapid detection and enhanced surveillance of RVFV infection, including assessment of seroconversion in hosts vaccinated with the RVFV Gn/Gc subunit vaccine.

Number and location of activation induced cytidine deaminase (AID) 'hotspot sequences' in germline variable genes of veterinary and laboratory species

Randall Lynn Levings

Science, Technology and Analysis Services, USDA-APHIS-Veterinary Services, Ames, IA

Generation of antibody (Ab) diversity is accomplished through five mechanisms: Heavy/Light chain combination, Variable-(Diversity)-Joining gene segment combination, junctional base addition/deletion, gene conversion, and somatic hypermutation (SHM). Different species have been shown to use the mechanisms to varying degrees. Complementarity determining regions (CDRs) are more highly mutated than framework regions (FRs) in the final, affinity-matured Ab. Gene sequence 'hotspots' for the enzyme primarily responsible for SHM (activation induced cytidine deaminase, AID) have been identified - WRCY or its complement RGYW. Species with fewer variable (V) genes (e.g., bovidae relative to muridae or hominidae) might be expected to have V gene segments containing more AID hotspots, so as to 'preposition' the genes for SHM. Germline V sequences identified and annotated in the ImMunoGeneTics (IMGT) database were analyzed for AID hotspot numbers in CDR and FR regions (and diversity genes). Examples of functional germline sequences from six classes of vertebrates were selected. Species included human, laboratory, and farmed animals. Although numbers of sequences studied were not large, general observations could be made. Many hotspots were observed in FRs. The relative frequency of hotspots (hotspots/bases in region) in the CDRs was often zero, but ranged to higher values than for FRs. Strand preference (sense or non-sense) was observed and varied with species and region. No clear pattern of AID hotspot number or location was observed to be associated with phylogeny or diversification strategy (when known). However, 'outlier species/genes' were noted. The results indicate that caution should be exercised in generalizing or extrapolating between species regarding Ab gene sequences. They also reinforce the importance of conducting sequence studies including germline, fetal B lymphocytes, and mature B-cells across many species. Information on the origin and mechanisms of Ab diversity has relevance to vaccination strategies including the choice of antigen and adjuvant.

Toxicology

Sunday, October 15, 2017

Pacific Salon 4-5

Moderators: Lisa A. Murphy and Christina Wilson

7:45 AM **Ionophore toxicosis in swine following concurrent administration of narasin and tiamulin in feed: A case review # * †**
Scott Louis Radke, Drew Magstadt, Steve M Ensley *101*

8:00 AM **Liver dicoumarol concentrations in cattle without evidence of hemorrhagic disorder**
Cynthia Gaskill, Kyle A. Francis, Lori Smith *102*

8:15 AM **Using high resolution accurate mass LC-MS for the identification of an uncommon cardiotoxic glycoside in liver tissue**
Michael Filigenzi, Caitlin Miller, Rakhshanda Javed-Ghaffar, Robert H Poppenga *103*

8:30 AM **Intra-laboratory blinded method test evaluation of an HPLC fluorescent method for quantitation of aflatoxin B_1 and M_1 in animal urine. § ◊**
Xiangwei Du *104*

8:45 AM **A multi-lab evaluation of aflatoxin B1 by ROSA-M Charm Reader in dog food**
Daljit Vudathala, Margie Cummings, Jakub Klobut, Andriy Tkachenko, Renate Reimschuessel, Geraldine Magnin-Bissel, McAlister Troche, Shreelakshmi Gopal, Christina Wilson, Ellen Pace, Karyn Bischoff, Lisa A Murphy *105*

9:00 AM **Break**

9:45 AM **A toxicology screening approach using liquid chromatography - quadrupole orbitrap mass spectrometry**
David J. Borts, Dwayne Edward Schrunk *106*

10:00 AM **Paper spray mass spectrometry for rapid quantitation without sample preparation or chromatography**
David J. Borts *107*

10:15 AM **Incidence of bromethalin poisoning in mammalian species: cases reported at CAHFS between 2013 and 2016.**
Marcos Perez-Lopez, Robert H Poppenga, Arya Sobhakumari, Michael Filigenzi, Leslie Willis Woods *108*

10:30 AM **Diagnostic biomarkers of acute hydrogen sulfide poisoning in cattle**
Wilson Kiiza Rumbeiha, Dwayne Edward Schrunk, Paula Martin Imerman, Steve M Ensley, Scott Louis Radke, Thomas Lapke, Micahel J. Slattery *109*

10:45 AM **Companion animal blood lead testing in Flint, MI**
John Philip Buchweitz, John Kaneene, Vilma Yuzbasiyan-Gurkan, Barbara Daniels, Hilda Mejia-Abreu, Nancy Frank, Daniel Langlois *110*

11:00 AM **Canine bifenthrin intoxication # †**
Jaimie Strickland, Andreas Lehner, Kenneth Harkin, Douglas Mader, John Philip Buchweitz *111*

11:15 AM **Detection of pentobarbital in commercial dog food §**
Margaret Johnson, Andreas Lehner, David Rotstein, Renate Reimschuessel, Sarah Nemser, Jennifer Jones, John Philip Buchweitz *112*

11:30 AM **Chlorinated hydrocarbon intoxication in the environment §**
Dwayne Edward Schrunk, Steve M Ensley, Scott Louis Radke *113*

11:45 AM **Development of a quantitative PCR method for rapid and accurate detection of toxigenic *Microcystis* spp. in fresh water samples**
Jian Yuan, Hyun-Joong Kim, Steve M Ensley, Kyoung-Jin Yoon *114*

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◊ USAHA Paper

Ionophore toxicosis in swine following concurrent administration of narasin and tiamulin in feed: A case review # * †

Scott Louis Radke, Drew Magstadt, Steve M Ensley

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

Following placement within a finishing facility on the 4th of May 2017, approximately two hundred of 1,200 ten-week-old pigs exhibited neurologic/musculoskeletal clinical signs, characterized by dog-sitting, inability to rise, weakness, and tremoring over a period of five days. Clinically affected individuals were first observed four days post-placement within the facility. Following onset of clinical signs, feed was removed from feeders and storage bins and replaced with new feed. Six live pigs and feed samples were received at the Iowa State University Veterinary Diagnostic Laboratory on the 10th of May 2017. Upon arrival, all individuals were reluctant to rise. When made to rise, pigs exhibited increased vocalization and tremoring of the rear limbs prior to collapsing and returning to a sitting position. Necropsy revealed diffusely pale skeletal muscles of the hind limbs and diaphragm in all individuals. Microscopic evaluation of the skeletal muscle and muscular diaphragm revealed a severe subacute necrotizing myositis while cardiac muscle was unremarkable. The toxicology and nutrition section confirmed the presence of narasin, an ionophore labeled for swine, as well as the antibiotics tiamulin and oxytetracycline in the feed. Administration of narasin in the production system was discontinued in November of 2016. Further investigation by the herd veterinarian revealed that the micro ingredient mixing equipment used in the milling process for the first load of feed for this group of pigs had not been used since the discontinuation of narasin within the system. Following discontinuation of narasin administration, the micro ingredient mixer had not been flushed, and narasin was inadvertently mixed into the ration. Tiamulin and narasin are safe when used separately at recommended dosages for labeled indications. However, concurrent administration of both compounds results in the inhibition of liver cytochrome P450 enzymes, by tiamulin, that are required for narasin metabolism resulting in accumulation of the ionophore and skeletal muscle necrosis.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Liver dicoumarol concentrations in cattle without evidence of hemorrhagic disorder

Cynthia Gaskill, Kyle A. Francis, Lori Smith

Dept of Veterinary Science, University of Kentucky, Lexington, KY

Dicoumarol is a fungal metabolite of coumarin, a glycoside found in a variety of plants. Fungi including *Penicillium, Aspergillus* and others can metabolize plant coumarin to 4-hydroxycoumarin and then to dicoumarol, a toxicant that causes coagulopathy and hemorrhage. Dicoumarol intoxication cases are most commonly associated with moldy hay, silage, or pastures containing sweet clover (*Melilotus alba, M. officinalis*) or sweet vernal grass (*Anthoxanthum odoratum*). Dicoumarol causes coagulopathy by inhibiting liver vitamin K epoxide reductase and preventing production of active vitamin K-dependent clotting factors. Presumptive diagnosis of dicoumarol intoxication is based on clinical and post-mortem findings, a history of likely exposure, and elimination of other causes of hemorrhage. Confirmation relies on dicoumarol detection in blood or tissues. Published literature often states that quantitative analysis is unnecessary, as any detection of dicoumarol in blood or tissues is diagnostic. However, modern highly sensitive instrumentation can detect many toxicants at extremely low concentrations. Normal background dicoumarol levels have not been established for livestock tissues, and guidelines for interpreting tissue dicoumarol concentrations are lacking. Study goal: To determine if dicoumarol is detectable in liver tissue from cattle without evidence of hemorrhagic disorder, and to quantitate dicoumarol concentrations when detected. Methods: Dicoumarol analyses were performed on liver tissue from 16 bovines without evidence of hemorrhagic disease on post-mortem examination. Animals included 1 late-term fetus, 2 neonates < 5 days old, and 13 juveniles/adults ranging from 2 months to 8 years (mean: 2 years). Dispersive solid phase extraction (d-SPE) was used to remove matrix interferences from liver extracts prior to analysis by reverse phase ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Negative ion electrospray ionization, combined with multiple reaction monitoring on a triple quadrupole mass spectrometer, allowed quantitation over a range of 50-2500 parts per billion (ppb) using matrix-matched calibrants. Method detection and quantitation limits were 10 and 50 ppb, respectively. Results: Dicoumarol was not detected in fetal or neonatal liver tissue. Dicoumarol was detected in 11/13 juvenile/adult cattle (85%). Concentrations in 7/11 animals were trace (between 10-50 ppb), and > 50 ppb (ranging from 61 to 445 ppb) in 4/11 animals. No animal had evidence of significant hemorrhage on post-mortem examination. Causes of death included pneumonia, enteritis, septicemia, listeriosis, parasitism, blackleg, cardiomyopathy, histophilus, hypomagnesemia, and bloat. Conclusion: Dicoumarol was detected at concentrations as high as 445 ppb in liver from cattle without evidence of hemorrhagic disease. Additional work is underway to better establish interpretive guidelines for tissue dicoumaral concentrations.

Using high resolution accurate mass LC-MS for the identification of an uncommon cardiotoxic glycoside in liver tissue

Michael Filigenzi[1], Caitlin Miller[2], Rakhshanda Javed-Ghaffar[2], Robert H Poppenga[1]

[1]California Animal Health and Food Safety Laboratory, University of California at Davis, Davis, CA; [2]Dept. of Medical Examiner-Coroner, County of Los Angeles, Los Angeles, CA

Cardiotoxic glycosides (CGs) are a class of compounds found in a wide variety of plant species. Plants containing these glycosides are found in most areas of the world and are known to be highly toxic to humans and animals. Hundreds of CGs have been identified but only a few are commonly involved in poisonings in the United States. In our laboratory, we routinely analyze samples for two CGs: oleandrin and perovoside. Oleandrin is the principle toxic component found in *Nerium oleander*, a plant which is found throughout the southern United States. Peruvoside is a toxic component of *Cascabela thevetia* or yellow oleander, a plant found in Mexico and Central America.

Recently, our lab received a request to analyze several human-derived samples for oleandrin. These samples included liver tissue and what was believed to be part of a nut removed from the stomach of the deceased individual. An LC-MS/MS screening analysis of the liver tissue for oleandrin and peruvoside was conducted using a hybrid quadrupole ion trap mass spectrometer. These systems are capable of providing full scan product ion spectra with high sensitivity. Although neither peruvoside nor oleandrin was detected in the sample, a significant peak was present in the acquisition channel for peruvoside. The retention time of this peak was significantly later than that of peruvoside but the MS/MS spectrum showed a cluster of signals in the range of *m/z* 330 – 375 characteristic of a CG. Further analysis of the liver tissue and the nut by high resolution accurate mass LC-MS provided evidence of the presence of several possible CGs in both of these samples. It then came to light that the decedent was suspected of having ingested “pong pong”, a common name for *Cerbera odollam*. *C. odollam* is a plant native to India and southern Asia which contains a number of CGs including cerberin and neriifolin and which is commonly used for the purpose of suicide in those regions. This presentation will discuss the use of high resolution accurate mass LC-MS to detect these putative CGs in both the liver and gastric samples and to positively identify neriifolin.

Intra-laboratory blinded method test evaluation of an HPLC fluorescent method for quantitation of aflatoxin B_1 and M_1 in animal urine. § ◊

Xiangwei Du

VDL, Iowa State University, Ames, IA

Aflatoxin B_1 (AFB_1) is a mycotoxin commonly found in a wide variety of seed and grains used as ingredients in manufacturing animal feeds. A common cause of pet food recalls, AFB_1 is the most potent aflatoxin. It is both hepatotoxic and immunosuppressive. Animals metabolize AFB_1 to AFM_1 and excrete both the parent and the metabolite in milk and urine. Urine is an ideal antemortem diagnostic specimen for aflatoxin exposure because it is noninvasive. Iowa State University VDL has developed a quantitative method for measurement of AFB_1 and AFM_1 in animal urine by high performance liquid chromatography (HPLC) with fluorescence detection. The method has high recovery (> 81%) and high sensitivity, with a method lower limit of quantitation (LLOQ) of 0.3 ng/mL for AFB_1 and 0.5 ng/mL for AFM_1. To evaluate the method, a blinded method test (BMT) organized by the FDA Vet-LIRN (Veterinary Laboratory Investigation and Response Network) was performed. The blinded study consisted of canine urine spiked at low (0.9 ppb for AFB_1 and AFM_1), medium (4.5 ppb for AFB_1 and 5.0 ppb AFM_1), and high (11.0 ppb for AFB_1 and 9.0 AFM_1) levels. Eight replicates were used at the low level, while six replicates were used at the medium and high levels. In addition, two "mystery" samples of canine urine spiked with aflatoxins in the ranges of 0.6 ppb - 13.0 ppb for AFB_1; and 0.7 ppb - 13.0 ppb for AFM_1 were included. Only one replicate was used for "mystery" samples. Due to unscheduled deviations, results of this BMT were unsatisfactory. A major challenge encountered was insufficient volumes of dilution solvent, buffer, and derivatization reagents which ran out during the middle of run. Saved sample extracts were reanalyzed in an unblinded manner. Using results from this second run, we calculated recovery and precision (relative standard deviation). For AFB_1, the recovery ranged from 71.6 to 88.7%, while the RSD ranged from 8.79 to 21.6% at three levels. For AFM_1, the recovery ranged from 65.9 to 87.5%, while the RSD ranged from 11.3 to 14.1% for the three spiked levels. The recovery was excellent for both aflatoxins and was within AOAC and FDA guidelines (50-120%). A repeat intra-laboratory BMT organized by the FDA Vet-LIRN is in progress. This study was funded by FDA grant number 1U18FD005006-04.

§ AAVLD Laboratory Staff Travel Awardee
◊ USAHA Paper

A multi-lab evaluation of aflatoxin B1 by ROSA-M Charm Reader in dog food

Daljit Vudathala[1], Margie Cummings[1], Jakub Klobut[1], Andriy Tkachenko[2], Renate Reimschuessel[2], Geraldine Magnin-Bissel[3], McAlister Troche[3], Shreelakshmi Gopal[4], Christina Wilson[4], Ellen Pace[5], Karyn Bischoff[5], Lisa A Murphy[1]

[1]Pathobiology, University of Pennsylvania, Kennett Square, PA; [2]FDA, VET-LIRN, Laurel, MD; [3]Virginia Tech, Blacksburg, VA; [4]Purdue University, Lafyette, IN; [5]Cornell University, Cornell, NY

Aflatoxins are a group of mycotoxins produced by *Aspergillius flavus* and *A. parasiticum* in warm and humid regions of the world and are found in corn, corn silage, other cereal grains, nuts and oil seeds. FDA has set action levels for aflatoxins in food and animal feed to safeguard human and animal health. Advisory guidelines in animal feed vary from 20 ppb to 300 ppb depending upon the sensitivity of the species and the intended use of an animal product as human food. Pet food falls under the "all other" category with an action level of 20 ppb.

The presence of aflatoxins in commercial pet food has been linked to illnesses and deaths in dogs. When pet food is suspected to be the cause of widespread adverse health effects, rapid identification of a contaminant can be used to initiate a recall to mitigate further harm. In such instances, a reliable method that can be used to screen a large number of samples in a short period of time is critical. The goal of the present study was to evaluate a rapid screening method for the analysis of aflatoxins in pet food at the action level of 20 ppb using lateral flow technology. The performance of the method using the ROSA-M reader was first verified in a single laboratory analysis of 20 blinded samples. This was followed by transfer of the method to three additional laboratories to conduct a blinded multi-lab evaluation. Four laboratories analyzed 36 samples each for a total of blinded 144 samples. The compiled results had a false positive rate of 0% and a false negative rate of 3.12% to meet FDA criteria for an acceptable screening method. A similar second multi-laboratory study is currently in progress using a different source of dog food, and will be followed by a third study using yet another type of dog food to further evaluate the method. Upon successful completion of these multi-laboratory evaluations, the method will be ready to be evaluated by a larger pool of laboratories participating in the FDA's Vet-LIRN program for its usefulness in the event that widespread, rapid emergency aflatoxins testing of pet food is needed.

A toxicology screening approach using liquid chromatography - quadrupole orbitrap mass spectrometry

David J. Borts, Dwayne Edward Schrunk

VDPAM, Iowa State University, Ames, IA

Toxicology screening for complete unknowns is challenging for a number of reasons. Perhaps the greatest challenge is the vast, and constantly growing, array of natural and synthetic toxicants. Another significant challenge is the fact that different toxicant molecules are optimally detected and analyzed using different analytical technologies. Gas chromatography/mass spectrometry (GC/MS) has traditionally represented one of the best options for broad, general, toxicant screening. GC/MS has many strengths for this application including the universal and standardized nature of electron impact (EI) ionization and the existence of many large and well-curated GC/MS spectral libraries. GC/MS is also subject to several limitations, the most important of which is likely that a large fraction of toxicant molecules are not readily amenable to GC analysis. Liquid chromatography/mass spectrometry (LC/MS) has historically been only somewhat useful as a screening tool for complete unknown toxicants. Most LC/MS ionization sources yield intact or 'pseudomolecular' ions that are not highly characteristic of a particular molecular structure. Tandem mass spectrometry (or 'MS/MS') libraries have tended to be small and not well-curated or standardized. A number of different 'targeted' LC/MS screening approaches have been developed, but these are all limited by the finite number of compounds that can be included in a single targeted screening method. We will present a new approach for complete unknown toxicology screening based on ultra-high pressure liquid chromatography (UHPLC) separation with high resolution accurate mass (HRAM) quadrupole orbitrap mass spectrometry. In this approach, a generic sample extract is separated using reversed phase sub-2 micron particle size UHPLC column technology. Detection is initially performed in full mass range mode using an HRAM orbitrap scan, followed by a series of data-dependent tandem mass spectrometry scans ($ddMS^2$) using both the quadrupole and orbitrap mass analyzers of a Thermo Fisher Scientific Q Exactive Focus mass spectrometer. This mass analyzer sequence cycles throughout the LC/MS run, with a full mass range scan typically followed by 3-5 $ddMS^2$ scans. Data collected with this approach is processed using Thermo Compound Discoverer software and tandem mass spectra are searched against the Thermo/HighChem mzCloud high resolution tandem mass spectrometry database. Examples of this approach applied to blinded samples of dog plasma and liver spiked with a series of analytical standard toxicant molecules will be presented. Potential limitations of this approach, including mzCloud library and $ddMS^2$ coverage, will be discussed.

Paper spray mass spectrometry for rapid quantitation without sample preparation or chromatography

David J. Borts

VDPAM, Iowa State University, Ames, IA

Paper Spray Mass Spectrometry (PS/MS) is one of a collection of emerging ambient ionization technologies. Ambient ionization is a form of ionization in which ions are formed in an ion source outside a mass spectrometer without sample preparation or separation. In Paper Spray Mass Spectrometry, an aliquot of a biological fluid (including whole blood) is deposited onto a filter paper substrate and allowed to dry. An appropriate solvent is applied to the paper and allowed to wet the paper by capillary action. As the solvent wicks through the sample, soluble components are extracted into the solvent, leaving behind the majority of proteins and lipids. A high voltage is applied to the moist paper and an electrospray is induced if the paper is cut to a sharp point. As the solvent evaporates from the electrosprayed droplets, gas phase ions of analyte molecules are generated which can be detected by mass spectrometry.

Paper Spray Mass Spectrometry has several advantages over the conventional LC/MS approach to analyte quantitation. These include: elimination of carryover (a new piece of paper substrate is used for each sample), small sample volumes consumed (typically 6 – 12 μl of biofluid), small solvent volumes used (≈ 100 μl), no solvent waste, no (or very limited) sample preparation, no LC system to maintain, and no chromatography to troubleshoot. PS/MS also has a couple of potentially significant disadvantages compared to LC/MS. Limits of detection and quantitation are typically not as low with PS/MS compared to LC/MS and, since there is no chromatographic separation, quantitation of isobaric compounds can be problematic.

Several possible applications for PS/MS in veterinary medicine and toxicology will be discussed and results from selected applications will be presented. In one example, quantitation of nonsteroidal anti-inflammatory drugs directly from equine plasma, without sample preparation or chromatography, will be shown. Other examples of the use of PS/MS for therapeutic drug monitoring (TDM) will also be presented. In the case of TDM, an additional significant advantage of the PS/MS approach is the ability to deliver ‘random access’ quantitative analysis results in near real-time.

Incidence of bromethalin poisoning in mammalian species: cases reported at CAHFS between 2013 and 2016.

Marcos Perez-Lopez[1,2], Robert H Poppenga[3], Arya Sobhakumari[3], Michael Filigenzi[3], Leslie Willis Woods[3]

[1]Toxicology Area, Faculty of Veterinary Medicine, Caceres, Spain; [2]INBIO G+C Research Institute, Caceres, Spain; [3]California Animal Health And Food Safety Laboratory System, School of Veterinary Medicine, UC-Davis, Davis, CA

Bromethalin, a diphenylamine compound with a pale, odorless, and solid crystalline nature, is commonly used as a rodenticide for the control of rats and mice. This compound, not approved by the European Union (according to the EU Pesticides Database of European Commission 2017), is however available in the U.S. since 1985, and is found to be highly toxic to non-targeted species, causing both direct and secondary poisoning. A systematic retrospective study of suspected mammalian cases of bromethalin exposure (based upon detection of the toxic metabolite, desmethylbromethalin, in one or more samples from an individual) recorded at CAHFS between January 2013 and December 2016 was conducted. Recorded variables included the origin, year, month of case submission to CAHFS, as well as species, gender, age of the affected patient, and tissue specimen submitted. When available, corresponding histopathological findings were also recorded. Based on the inclusion criteria, a total of 73 suspected events were confirmed positive for bromethalin exposure during the period between 2013 and 2016. Of these positive cases, 18 (24.7%) were domestic animals (dogs and cats) and 55 (75.3%) included various wild species. Moreover, a clear increase in the number of positive samples was observed during the indicated period (2 in 2013, more than 30 in 2016), with the striped skunk and raccoon representing the highest percentage of reported cases. Neither the gender (male/female: 33/32) nor the age (young/adult: 25/31) had any influence on the number of positive cases. With respect to toxicological analysis, the number of major tissue specimens that tested positive, in descending order, were adipose tissue (n=44), brain (n=19), sera (n=4), liver (n=4), kidney (n=1) and gastric content samples (n=1). The most relevant histopathological findings were noted in the central nervous system with lesions ranging from diffuse, acute white matter spongiosis and vacuolation of the cerebrum and cerebellum to lymphocytic, perivascular and multifocal meningitis. Some cases reported multifocal, variably sized foci of hemorrhage as well as focal satellitosis around neurons in the cerebrum, and occasional shrunken red neurons, thus indicating hypoxia and necrosis. The present study provides useful information on bromethalin exposure and/or intoxications in mammalian species in California. In addition, while detection of desmethylbromethalin is possible in a variety of biological samples, preferred specimens are adipose or brain.

Diagnostic biomarkers of acute hydrogen sulfide poisoning in cattle

Wilson Kiiza Rumbeiha[1], Dwayne Edward Schrunk[1], Paula Martin Imerman[1], Steve M Ensley[1], Scott Louis Radke[1], Thomas Lapke[2], Micahel J. Slattery[3]

[1]VDPAM, Iowa State University, Ames, IA; [2]Rolling Hills Vet Services PC, LaMotte, IA; [3]Risius and Associates, Maquoketa, IA

Hydrogen sulfide (H_2S) is a highly toxic gas and is a common farm hazard. Each year it is responsible for livestock deaths, especially in swine and cattle. It affects the cardiovascular, the respiratory, and the nervous systems. Acute death typically follows sudden exposures to H_2S at concentrations in excess of 10,000 ppm in ambient air. Polioencephalomalacia, commonly observed in ruminants, is an acute manifestation of subchronic or chronic ingestion of high sulfur diets and/or water. Analytical diagnostic confirmation of either condition is still a challenge because of a lack validated diagnostic biomarkers. Because several causes of acute and peracute death in livestock exist, reliable diagnostic biomarkers of acute H_2S poisoning are needed, especially for insurance purposes. In October of 2016 sudden deaths of feeder cattle occurred on two different farms in Iowa. At both farms cattle were housed in mono slope slatted buildings and deaths occurred during manure pit pumping. Ocular fluid and/or urine samples were collected from exposed or dead cattle for determination of thiosulfate and sulfate concentrations, as biomarkers of acute H_2S poisoning. Reports from the literature on human cases and from animal models of acute H_2S poisoning had revealed these two as promising biomarkers of acute H_2S poisoning. Results showed that ocular thiosulfate concentration was increased 9-fold from a mean of 0.34 ppm to a mean of 3.2 ppm on one farm; and 24-fold to a mean of 8.2 ppm on the second farm. This increase in ocular fluid thiosulfate in cattle exposed to H_2S was statistically significant compared to controls ($p< 0.01$). Ocular fluid sulfate was also significantly increased compared to control ($p<0.05$), but only a 2-fold increase at the most. In urine, sulfate concentration was significantly increased from a mean of 279 ppm in controls to 2833 ppm (10-fold increase) in cattle exposed to H_2S by acute inhalation ($p<0.01$). Urine thiosulfate however, was only increased 3-4-fold. Overall, these results suggest that thiosulfate is a better biomarker in ocular fluids, while sulfate is a better biomarker in urine for diagnosis of acute H_2S poisoning by inhalation.

Companion animal blood lead testing in Flint, MI

John Philip Buchweitz[1,2], John Kaneene[3], Vilma Yuzbasiyan-Gurkan[4], Barbara Daniels[4], Hilda Mejia-Abreu[5], Nancy Frank[6], Daniel Langlois[4]

[1]Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI; [2]Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI; [3]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI; [4]Small Animal Clinical Sciences, Michigan State University, East Lansing, MI; [5]Office for Diversity and Inclusion, Michigan State University, East Lansing, MI; [6]Animal Industry Division, Michigan Department of Agriculture and Rural Development, Lansing, MI

Lead is a ubiquitous environmental contaminant. This heavy metal has been molded by anthropogenic processes into a sundry of products for hunting, machinery, and plumbing. This latter use coupled with an aging infrastructure unraveled in a series of events that culminated in the contamination of the Flint, Michigan water supply. In January, 2016 state and federal governments posited declarations of emergency. In response, the Michigan State University College of Veterinary Medicine undertook an initiative to provide free screening for lead exposure to dog owners residing in underprivileged and high-risk areas of Flint between February, 2016 and May, 2016. This presentation will highlight the importance of a "One-Health" approach to researching an environmental contamination incident with an emphasis on multi-disciplinary, multi-institutional partnerships.

Canine bifenthrin intoxication # †

Jaimie Strickland[3,2], Andreas Lehner[2], Kenneth Harkin[4], Douglas Mader[5], John Philip Buchweitz[1,2]

[1]Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI; [2]Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI; [3]Comparative Medicine and Integrative Biology, Michigan State University, East Lansing, MI; [4]College of Veterinary Medicine, Kansas State University, Manhattan, KS; [5]Marathon Veterinary Hospital, Marathon, FL

Bifenthrin is a general use pesticide within the pyrethroid class of insecticides. Sprays, granules and aerosols of bifenthrin are intended for use on various agricultural crops including cereals, cotton, corn, alfalfa, hay, grass seed, some fruits, ornamentals, and vegetables and for use in homes. The pesticide was first registered by the U.S. Environmental Protection Agency (EPA) for use in 1985. Pyrethroids are classified according to their abilities to interfere with sodium channel gating in the central and peripheral nervous systems; Type I pyrethroids (e.g. bifenthrin, permethrin) specifically delay the closure of the sodium channel for shorter times than Type II pyrethroids (e.g. cypermethrin, deltamethrin). When used in accordance with manufacturer instructions, pyrethroid insecticides, such as bifenthrin, are generally considered to have little risk for toxicity in canine populations. However, accidental or inappropriate consumption of sufficient quantities may lead to excessive drooling, vomiting, diarrhea, ataxia, tremors, seizures, and less commonly death. We present the case histories of two canines with progressively worsening tremors from exposure to bifenthrin, the resulting clinical outcomes, and the analytical approach to identifying bifenthrin and its metabolites in feed and tissue matrices by gas chromatography-mass spectrometry (GC-MS) and a unique method involving tandem quadrupole gas chromatography-mass spectrometry, or GC-MS/MS.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Detection of pentobarbital in commercial dog food §

Margaret Johnson[2], Andreas Lehner[2], David Rotstein[3], Renate Reimschuessel[3], Sarah Nemser[3], Jennifer Jones[3], John Philip Buchweitz[1,2]

[1]Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI; [2]Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI; [3]Center for Veterinary Medicine, FDA, Rockville, MD

Sodium pentobarbital is a schedule II barbiturate primarily used alone or in combination with phenytoin for the humane euthanasia of animals. It is not, however, indicated for animals intended for food. In addition to euthanasia, it has also been used for the control of status epilepticus or cluster seizures in dogs and cats when the use of either diazepam or phenobarbital is considered inadequate. Therefore, the finding of pentobarbital in post-mortem veterinary samples generally indicates that either an animal was euthanized, treated for seizures, or was the subject of a secondary poisoning incident. In January of 2017, the stomach contents from an acutely ill dog that presented with neurologic signs and several pieces of meat from a commercial canned dog food were analyzed by the Michigan State University Veterinary Diagnostic Laboratory (MSU VDL) for an unknown toxin or toxicant. Samples were determined to be positive for the presence of pentobarbital in large quantities chromatographically and absent for phenytoin. The Food and Drug Administration has no set tolerance for pentobarbital in pet food. Therefore, its detection at any quantity renders the product adulterated and subject to regulatory action including, but not limited to inspection and recall. A Class I recall which indicates a significant health threat occurred in this case. Commercial feeds incorporating meat products are made in bulk and may not exhibit uniform consistency across an entire lot. The MSU VDL general organic compound screen involves a modified QuEChERS extraction of samples followed by full scan gas chromatography-mass spectrometry (GC-MS) that is sufficient for the detection of pentobarbital in the majority of overt poisoning cases; however, the determination of pentobarbital at lower concentrations requires greater sensitivity. We describe here the validation of a gas chromatography tandem quadrupole mass spectrometer (GC-MS/MS) method for detecting trace quantities of pentobarbital in commercial pet food.

§ AAVLD Laboratory Staff Travel Awardee

Chlorinated hydrocarbon intoxication in the environment §

Dwayne Edward Schrunk, Steve M Ensley, Scott Louis Radke

Toxicology and Nutrition, Iowa State University, Ames, IA

The Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) received and process two cases were chlorinated hydrocarbons were detected in the spring of 2017. Both cases were wildlife samples were analysis indicated chlorinated hydrocarbons as the cause of death. In both cases the chlorinated hydrocarbons that are either no longer available on the market or are not readily available were detected. The first case was a great horned owl that was found convulsing and quickly died. The owl was from a raptor recovery organization located in the Fontanelle Forest reserve near Bellevue, Nebraska. After a necropsy was performed at the Nebraska Veterinary Diagnostic Laboratory the liver was submitted to the ISUVDL for testing. Due to the neurological signs a toxic element screen and a GC/MS screen were performed. While the toxic element produced no significant results, the GC/MS screen was able to detect several chlorinated hydrocarbons. Heptachlor epoxide, ppDDE, dieldrin, and trans-nonachlor were detected by GC/MS. The second case was from a coyote that was suspected of being poisoned. Recently two other coyotes had been found in the general area that this last coyote was found. This area has a recent history of conflict between coyotes and humans, including the miss use of live traps. Several tissues were submitted to the ISUVDL, and the stomach content was analyzed using the GC/MS screen. In this instance, both isomers of endosulfan as well as endosulfan ether were detected in the stomach content.

§ AAVLD Laboratory Staff Travel Awardee

Development of a quantitative PCR method for rapid and accurate detection of toxigenic *Microcystis* spp. in fresh water samples

Jian Yuan[1], Hyun-Joong Kim[2], Steve M Ensley[1], Kyoung-Jin Yoon[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA;
[2]Department of Food Science and Human Nutrition, Iowa State University, Ames, IA

Microcystis is a toxigenic cyanobacteria which is ubiquitously distributed and can form harmful blue-green algal blooms in freshwater in the United States. It is crucial to identify the presence of toxin-producing *Microcystis* spp. because microcystin, the toxin produced by *Microcystis* spp., has considerable adverse impact on animal and human health. Molecular methods are promising and pragmatic for that purpose since: a) the traditional identification method by microscopy cannot differentiate toxigenic *Microcystis* spp. from non-toxigenic ones and b) toxigenic *Microcystis* spp. are known to contain a cluster of genes involving toxin biosynthesis. In this study, we developed two nucleic-acid based rapid quantitative assays to specifically detect and quantify the presence of *Microcystis* spp. and the toxigenic cells based on the 16S rRNA gene and microcystin synthetase C (*mcyC*) gene encoding the enzyme critical in microcystin formation, respectively. The assays, with optimized extraction procedure, appeared to be analytically sensitive (1 to 5 copies/reaction) without cross-reacting with other well-characterized known cyanobacteria. To evaluate its utility in testing field samples, the assays were employed in testing 100 water samples collected from 5 lakes serving nearby swine operations in the U.S. Midwest from August to October in 2016. *Microcystis* was detected in all of the 100 samples, ranging from 17.38 (± 2.35) cells/mL to 7.60 (± 0.18) × 10^4 cells/mL. Eighty two of the tested water samples were found to contain toxigenic *Microcystis* cells, ranging from 1.02 (± 0.04) cells/mL to 1.71 (± 0.05) × 10^3 cells/mL, covering 0.03% - 16.01% of the total *Microcystis* population. It was also observed that the spatial and temporal distribution of toxigenic *Microcystis* was significantly distinct across each lake, and the percentage of toxigenic *Microcystis* spp. varied greatly by time. Results to date suggest that the newly developed PCR method can be used for rapid and specific detection of microcystin-producing *Microcystis* spp. although actual toxin testing has yet to be done. As the assays are in a real-time PCR format, it can provide high-throughput testing capability.

Virology 1

Saturday, October 14, 2017
Pacific Salon 3

Moderators: Tomy Joseph and Phillip Gauger

1:00 PM **Development and optimization PCR assays for rapid identification and authentication of mammalian cell lines commonly used in veterinary virology laboratories ◊**
Amaresh Das *116*

1:15 PM **Demonstration of systemic infection of BVDV vaccine virus after vaccination in presence of persistently infected calves**
Beate Crossley, John Adaska, John Champagne, Shollie Falkenberg, Julia Francis Ridpath *117*

1:30 PM **Validation of a serum – based ELISA for bovine leukemia viral antibodies in milk**
James Evermann, SM Parish, C Merritt, D DeAvila, S Srikanth, A Bronowski *118*

1:45 PM **Improved detection of bovine viral diarrhea virus in bovine lymphoid cell lines using PrimeFlow RNA assay ◊**
Shollie Falkenberg, Rohana P. Dassanayake, Simone Silveira, John D. Neill, Julia Francis Ridpath *119*

2:00 PM **Seroprevalence of a novel ruminant alphaherpesvirus of mule deer.**
Myrna M. Miller, Juan Francisco Muñoz-Gutiérrez, Brittney Rogers *120*

2:15 PM **Identification and genetic characterization of a novel avian deltacoronavirus using next-generation sequencing technology**
Qi Chen, Chenghuai Yang, Ying Zheng, Phillip Gauger, Tavis Anderson, Karen Harmon, Jianqiang Zhang, Kyoung-Jin Yoon, Rodger Main, Ganwu Li *121*

2:30 PM **Whole-genome sequence analysis reveals unique SNP profiles to distinguish vaccine and wild-type strains of Bovine Herpes Virus-1 # * †**
Shubhada Krishna Chothe, Aswathy Sebastian, Asha Thomas, Ruth Nissly, David R Wolfgang, Maurice Byukusenge, Sunil kumar Mor, Sagar M Goyal, István Albert, Bhushan Jayarao, Suresh Kuchipudi *122*

2:45 PM **Determining the effects of climate variables and maternal antibodies on the natural transmission of bluetongue virus in range-pastured beef cattle # * †**
Michelina Meinzer, John Derek Scasta, Brant Schumaker, Myrna M. Miller *123*

Symbols at the end of titles indicate the following designations:

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

Development and optimization PCR assays for rapid identification and authentication of mammalian cell lines commonly used in veterinary virology laboratories ◊

Amaresh Das

Foreign Animal Disease Diagnostic Laboratory, Plum Island Animal Diease Center, Greenport, NY

Continuous mammalian cell lines are routinely used in virus research with applications ranging from virus isolation to production of biologicals. The integrity of the cell lines can be compromised either by spontaneous natural mutations or by cross-contamination with other cell lines. Therefore, it's important to routinely examine the authenticity of cell lines in use. Here we report the development and optimization of new conventional and real time (TaqMan™) cell line specific PCR assays for identification of four most commonly used mammalian kidney cell lines from swine, monkey, hamster and bovine. The primers and probe sequences were designed from the highly conserved mitochondrial genes and analyzed *in silico* by primer BLAST in the NCBI database to ensure their specificity. The assays were optimized using Dream Taq PCR Mastermix (Thermo Fisher Scientific) for conventional PCR and Path-ID qPCR Mastermix for real time PCR (qPCR). Newly developed PCR assays were highly specific for the target cell lines and had no cross-reactivity against other cell lines including sheep (kidney), dog (kidney), cat (kidney), rat (liver), rabbit (kidney), Guinea pig (lung), lamb (kidney-primary) and human (HeLa). The assay specificity was further confirmed by nucleotide sequencing of the PCR products. The sequence analyses revealed 100% identity with the corresponding nucleotide sequences of the respective cell lines in the NCBI database by BLAST. A multiplex qPCR assay was developed for simultaneous detection of up to three cell lines in a single assay (swine/monkey/hamster and swine/monkey/bovine). The multiplex assays exhibited no loss of sensitivity compared to the corresponding singleplex assays. Due to its higher sensitivity (10-1000 fold), qPCR was able to detect traces of contamination of other cell lines that was not detectable by conventional PCR. The newly developed PCR assays can be very useful for routine examination of the identity of the cell lines.

◊ USAHA Paper

Demonstration of systemic infection of BVDV vaccine virus after vaccination in presence of persistently infected calves

Beate Crossley[1], John Adaska[1], John Champagne[2], Shollie Falkenberg[3], Julia Francis Ridpath[3]

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Bovine viral diarrhea virus (BVDV) was detected during routine necropsy of calves, from a well vaccinated, large Jersey/Holstein dairy herd (n=10,000) in California, that succumbed to ill thrift. According to herd management, BVDV has not been considered a problem in the past. The herd had been extensively vaccinated with various brands of modified live vaccines, all of which included the BVDV1a Singer strain. Subsequent testing of ear notches, by antigen capture ELISA, detected 18 positive calves out of 771 tested (2.3%). All dams from positive calves tested negative for BVDV.

Virus isolation was performed and both cytopathic and non cytopathic viruses were detected based on growth patterns in culture. PCR amplification of RNA isolated from isolates followed by sequencing and phylogenetic analysis based on the 5'UTR region of BVDV revealed three different scenarios. Clean sequence matching BVDV1a-Singer, clean sequences, that based on phylogenetic analysis, segregated to the BVDV1b subgenotype and mixed sequence results indicating more than one BVDV present.

Necropsy of the calves revealed a systemic infection of BVDV as demonstrated by real-time PCR in brain, lung, kidney, spleen and liver tissues of all necropsied calves. Calves infected with the Singer strain showed a marginally higher virus concentration in the lung compared to calves infected with the field strain. Two of the calves infected with the Singer strain had never been vaccinated due to their young age, however vaccine strain virus could be propagated from spleen tissue.

The systemic infection of calves with vaccine virus and the presumed transmission of vaccine virus from vaccinated calves to naïve calves were not observed in the efficacy and safety trials required for licensure of the vaccines used in this herd. However, licensure studies are performed using healthy animals in the absence of persistently infected pen mates. The findings suggest that producers cannot rely on vaccination alone to prevent persistent infections and that vaccination of persistently infected animals and/or vaccination herd mates in the face of exposure to persistently infected animals may impact on the transmission and replication of vaccine virus included in modified live vaccines.

Validation of a serum – based ELISA for bovine leukemia viral antibodies in milk

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Bovine leukemia virus (BLV) infection has world-wide distribution in both dairy and beef herds. BLV is naturally spread from dam to calf via blood borne in utero infections and milk-borne peri-natal infections. Although there are several diagnostic assays available for detection of BLV infection, including PCR and antibody detection, the latter is regarded as being very accurate and economical. Serologic assays, such as AGID and ELISA have been successfully utilized to assist in the control of BLV infection, and in some cases, complete eradication of the infection from a given population. This study was initiated in order to encourage compliance of BLV infection control by using milk samples in lieu of serum samples to readily test lactating animals prior to dry off-calving. Two Holstein dairy herds (A and B), with known status of BLV infection, were sampled by collection of a serum and fresh milk samples, and tested using a USDA licensed BLV antibody ELISA kit (BLV Antibody Test Kit, VMRD, Pullman, WA, USA) for serum. Forty lactating cows from each herd were sampled for testing. The serum samples were tested according to the kit insert. The milk samples were initially centrifuged at 2000g for 15 min. A 2ml aliquot was removed from the lactoserum portion of the milk sample and stored at 4C. The milk sample was diluted 1:2 in BLV sample diluent, and tested as for the serum. The ELISA results were analyzed and categorized by herd of origin; positive and negative BLV antibody status; and the correlation between serum and milk results. Herd A was confirmed to be free of BLV infection based upon this sample set, while Herd B was confirmed to be endemic for BLV infection. The milk-based ELISA results demonstrated 100% identification of positive and negative animals with the serum results. The correlation of the ELISA values between the serum and the milk samples was determined to be 97%, which supports the recommended use of the BLV ELISA on milk samples.

Improved detection of bovine viral diarrhea virus in bovine lymphoid cell lines using PrimeFlow RNA assay ◊

Shollie Falkenberg[1], Rohana P. Dassanayake[1], Simone Silveira[2], John D. Neill[1], Julia Francis Ridpath[1]

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Bovine viral diarrhea virus (BVDV) infections, whether as acute, persistent or contributing to co-infections, result in significant losses for dairy and beef producers. While BVDV can be identified by real-time PCR and ELISA, consistent detection and quantification of viral infection at the single cell level is extremely difficult. Detection at the single lymphoid cell level is important due to the nature of the immunomodulation that accompanies BVDV infection. A novel assay based on PrimeFlow RNA technology was adapted for *in-situ* detection of BVDV at the single-cell level. The model used to develop and test this technique included three BL-3 cells lines with three different infection statuses, one was not infected with BVDV, one was infected with BVDV and one was dual infected with BVDV and bovine leukosis virus (BLV). Using RNA probes specific for the BVDV-2a N^{pro}-E^{rns} region, BVDV RNA was detected from both contaminated BL-3 cell lines by flow cytometry and fluorescent microscopy using the novel assay. This is the first report on *in-situ* detection of BVDV at the single-cell level.

◊ USAHA Paper

Seroprevalence of a novel ruminant alphaherpesvirus of mule deer.

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A novel ruminant alphaherpesvirus was identified in adult mule deer (Odocoileus hemionus) that were submitted to the Wyoming State Veterinary Laboratory with a history of an infectious keratoconjunctivitis-like syndrome (IKC) and/or blindness. A retrospective analysis of all IKC cases in mule deer between 2000 and 2016 found that 23% (4/17) were associated with a herpesvirus. These cases consisted of bilateral necrotizing and fibrinosupporative bulbar conjunctivitis. Genetic analysis of four isolates found that they were identical (>99.8% identity). Phylogenic analysis was performed to define relatedness to other herpesviruses based on protein coding sequences for surface glycoproteins gB, gC, and gD. Based on the most conserved of these proteins, gB, this mule deer-associated herpesvirus (MDHV) is most closely related to other ruminant alphaherpesviruses in decreasing order; cervid herpesvirus-1 (CvHV-1, 93% identity), cervid herpesvirus 2 and bovine herpesvirus 1 (CvHV-2, BHV-1, 91% identity), BHV-5 and buffalo herpesvirus (90% identity), and caprine herpesvirus (86% identity). The prototype for ruminant alphaherpesviruses is BHV-1, which causes respiratory and reproductive syndromes in cattle. Clinical signs associated with these syndromes include rhinotracheitis, conjunctivitis, encephalitis and abortion. CvHV-1 causes an ocular syndrome in red deer, characterized by conjunctivitis, hypopyon, corneal opacity without ulceration, nasal discharge and photophobia. CvHV-2 infects reindeer and has been isolated from vaginal swabs, and genital re-excretion can be induced with corticosteroid injection. The distribution and prevalence of MDHV infections, and the potential for cross-infections or serologic cross-reactions with bovine herpesviruses are not currently known. A seroepidemiologic survey was performed on banked serum to establish the prevalence of MDHV infection in three Wyoming mule deer herd units with varying potential for contact with domestic cattle. Microtiter serum neutralization assays were performed using mule deer serum against MDHV or BHV-1. Results for the first herd unit are complete. This herd has limited potential for contact with domestic cattle. The prevalence of anti-MDHV antibodies was 87% (69/80, CI 90%, 83-93). Testing of samples from the other herd units and cross-neutralization assays will be completed in summer 2017.

Identification and genetic characterization of a novel avian deltacoronavirus using next-generation sequencing technology

Qi Chen[1], Chenghuai Yang[1], Ying Zheng[1], Phillip Gauger[1], Tavis Anderson[2], Karen Harmon[1], Jianqiang Zhang[1], Kyoung-Jin Yoon[1], Rodger Main[1], Ganwu Li[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]USDA-ARS, National Animal Disease Center, Ames, IA

Next-generation sequencing (NGS) technologies have played an increasing role in food animal diagnostic medicine and has been offered as a routine diagnostic assay at the Iowa State University Veterinary Diagnostic Laboratory. In this case report, we identified a novel avian deltacoronavirus (DCoV) using NGS. During routine health surveillance testing at a grower-finish swine farm in Illinois, porcine DCoV (PDCoV) was detected as weak positive (Ct 34.2-39) using real-time RT-PCR (rRT-PCR) targeting the nucleoprotein gene in oral fluid samples from clinically healthy pigs. Additional testing was conducted on feces collected from pigs and terrestrial birds located inside a hoop facility and from feed delivered to the farm. Interestingly, several avian fecal samples were tested positive using the ISU VDL PDCoV rRT-PCR (Ct 26.3-37.3). Using NGS technology, full-length sequencing was attempted on the positive avian fecal samples for further characterization of the virus. A viral genome of 25842 nucleotides was *de novo* assembled, and the assembled genome was queried against the GenBank database using the basic local alignment search tool blastn: this revealed the genome to be most closely related to viruses in the genus deltacoronavirus. The virus, designated DCoV USA/IL/ISU690/2017 (hereafter ISU690), shared 87% and 88% identity with PDCoV HKU15/155 (JQ065043) and a sparrow DCoV HKU17-6124 strain (JQ065045), respectively. The genome shared limited identity with other DCoVs: magpie-robin coronavirus (79%), bulbul coronavirus (80%), white-eye coronavirus (80%), and thrush coronavirus (80%). Phylogenetic analysis based on the whole genome sequences of alpha-, beta-, gamma-, and deltacoronaviruses yielded a topology consistent with established relationships in the *Coronavirinae* subfamily. This tree revealed that the ISU690 strain shared common ancestry with both PDCoV and avian DCoVs, but the ISU690 strain was an independent branch suggesting that it is a novel DCoV different from currently described porcine and avian DCoV strains. Several genes (S, E, M, and NS6) of ISU690 showed the highest similarity to PDCoV (from 72%-93%) while other genes (ORF1ab, N, and NS7) showed the highest similarity to sparrow DCoV (from 87%-99%): this pattern of discordant similarity suggests recombination that may have facilitated the emergence of this novel DCoV. Since DCoV in pig samples had high Ct values and sequencing was unsuccessful, it is unknown whether DCoV detected in pig samples was derived from the avian DCoV ISU690 strain or not. More surveillance and sequence data are needed in order to understand the potential interspecies transmission of DCoVs.

Whole-genome sequence analysis reveals unique SNP profiles to distinguish vaccine and wild-type strains of Bovine Herpes Virus-1 # * †

Shubhada Krishna Chothe[3,1], Aswathy Sebastian[2], Asha Thomas[1], Ruth Nissly[3], David R Wolfgang[3], Maurice Byukusenge[1], Sunil kumar Mor[4], Sagar M Goyal[4], István Albert[2], Bhushan Jayarao[3,1], Suresh Kuchipudi[3,1]

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Bovine Herpesvirus-1 (BHV-1) is a major viral pathogen affecting cattle worldwide. BHV-1 causes primarily respiratory illness referred to as infectious bovine rhinotracheitis (IBR), along with reproductive disorder including abortion and infertility in cattle. Modified live vaccines (MLV) are widely used for the prevention of IBR. However, BHV-1 MLVs have been implicated in disease outbreaks including abortions in vaccinated pregnant cattle. A major challenge for the clinical diagnosis of BHV-1 is the inability of the existing diagnostic methods to distinguish between vaccine and wild type BHV-1 strains from a clinical case. In addition, the limited genomic diversity among strains for BHV-1 makes it even harder to differentiate vaccine strains from wild type BHV-1 strains. Hence, there is an urgent need to develop molecular diagnostic methods that would allow not only accurate BHV-1 diagnosis but also to identify if the given clinical case is caused by a wild-type or vaccine strain of BHV-1. We have performed whole genome sequencing of 18 BHV-1 field isolates from Pennsylvania and Minnesota along with 5 commercial BHV-1 vaccines and 2 BHV-1 reference strains. The reference, vaccine and field strains of BHV-1 were mapped to the complete genome sequence of the National Veterinary Services Laboratory (NVSL) reference/challenge Cooper strain of BHV-1.1. Illumina Miseq sequencing produced 35 to 150 base pair long paired reads and the single nucleotide polymorphism (SNP) were called using freebayes. Low quality SNPs with quality score of <10 and read depth of <10 were excluded from the analysis. Based on the unique SNP profiles, all the isolates were clustered into three distinct groups. Vaccine strains 1-4 were clustered in Group 1, vaccine 5 in group 2 and the wild-type strains in group 3. Based on the SNP profiles, we identified field isolates that clustered in group 1 and 2 indicating that they were vaccine strains. We also identified that of the 18 field isolates, only 6 were wild-type strains of BHV-1. Using the BHV-1 SNP profiles, we have developed a SNP based PCR assay which allows differentiation between vaccine and clinical strains of BHV-1.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Determining the effects of climate variables and maternal antibodies on the natural transmission of bluetongue virus in range-pastured beef cattle # * †

Michelina Meinzer[1], John Derek Scasta[2], Brant Schumaker[1], Myrna M. Miller[1]

[1]Veterinary Sciences, University of Wyoming, Laramie, WY; [2]Ecosystem Science and Management, University of Wyoming, Laramie, WY

Bluetongue virus (BTV) is an arbovirus spread by members of the genus *Culicoides* that infects both domestic and wild ruminants. It may lead to a highly fatal hemorrhagic disease in certain species, including mule deer, white-tailed deer, pronghorn, domestic sheep, and bighorn sheep. Cattle are susceptible to infection but generally do not exhibit symptoms. This study examines the effects of climate variables and maternal antibodies on the natural transmission of BTV in range-pastured beef cattle. Each summer for three seasons, 20 maternal antibody positive and 20 maternal antibody negative spring-born calves were tested twice monthly for BTV antibodies using competitive enzyme-linked immunosorbent assays and serum neutralization assays. To identify the duration of passive protection afforded by maternal antibodies, the rate of antibody decay was determined for maternal antibody positive calves. Onset and rate of infections was determined by seroconversion in maternal antibody negative calves and detection of BTV RNA in the blood by RT-PCR, followed by virus isolation attempt from positive samples. In addition, *Culicoides* were sampled from a location near the calves' water source to determine weekly relative vector abundance and compared to climate variables on the day of collection as well as 2-3 weeks prior. Climate variables evaluated for correlation with onset of infections and vector abundance will include high, low, and mean temperature, wind, humidity, and rainfall. The sampled *Culicoides* were also tested for presence of bluetongue virus via RT-PCR. For the first two years of the study, BTV-17 was identified in our study, which has historically been the predominant serotype in Wyoming. Rates of infection for 2015 and 2016 were 38% and 55%, respectively. Maternal antibodies in calves were found to persist to a mean of 18.3 (range: 11.5 – 25.5) weeks of age. It is likely that a similar duration occurs for sheep and wild ruminants, species that are more susceptible to BTV-caused disease. Therefore it can be inferred that maternal antibodies help protect spring-born animals from disease in years with early transmission but will play a lesser role in protecting offspring when transmission occurs in late summer or fall.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Virology 2 / Molecular Diagnostics and Bioinformatics 2

Sunday, October 15, 2017
Pacific Salon 3

Moderators: Rebecca P. Wilkes and Megan Schroeder

7:45 AM **Molecular characterization of pseudorabies virus isolated from a Florida panther**
James Evermann, Annabel G. Wise, Allison J. McKeirnan, Robert G. McLean, Jo Anne C. Crum, Mark Cunningham, Suzanne Mason, Roger K. Maes *126*

8:00 AM **Molecular detection of novel adenoviruses in Chimney Swifts**
Annabel G. Wise, David B. Needle, Dalen W. Agnew, Inga Sidor, Roger K. Maes *127*

8:15 AM **Seroprevalence of Senecavirus A in swine herds on the United States**
Elizabeth Houston, Luis Gabriel Gimenez-Lirola, Ronaldo Magtoto, Dave Baum, Pablo E. Pineyro. *128*

8:30 AM **Practical application of MinION next generation sequencing of Porcine Reproductive and Respiratory Syndrome virus for routine identification and strain typing**
Kevin Lahmers, James Stanton, Tanya LeRoith, Michelle Todd. *129*

8:45 AM **Development and validation of a multiplex real-time PCR assay for the detection and differentiation of PCV2 and PCV3 strains # †**
Yin Wang, Fangfeng Yuan, Elizabeth Porlsen, Russel Ransburgh, Wanglong Zheng, Xuming Liu, Nanyan Lu, Lalitha Peddireddi, Gary Anderson, Jianfa Bai *130*

9:00 AM **Break**

9:45 AM **Development of a quantitative real time RT-PCR assay for sensitive detection of emerging Atypical Porcine Pestivirus associated with congenital tremor in pigs # †**
Fangfeng Yuan, Xuming Liu, Jianfa Bai, Gary A Anderson, Ying Fang, Bailey Lauren Arruda, Paulo Arruda, Lalitha Peddireddi *131*

10:00 AM **Human to swine transmission of novel H3 Influenza A viruses from 2010 to 2017 * †**
Michael Zeller, Amy Vincent, Tavis Anderson, Eugenio Abente, Jianqiang Zhang, Karen Harmon, Phillip Gauger *132*

10:15 AM **Development of a luminex xTAG assay for the detection and differentiation of type 2 PRRSV field strains from the four vaccine strains used in the US # †**
Yin Wang, Russel Ransburgh, Elizabeth G. Poulsen, Wanglong Zheng, Hao Shi, Xuming Liu, Nanyan Lu, Gary Anderson, Jianfa Bai *133*

10:30 AM **A specific, sensitive, and rapid reverse transcription-insulated isothermal polymerase chain reaction on a field-deployable PCR system for Senecavirus A detection in swine samples**
Jianqiang Zhang, Charles Nfon, Chien-Hsien Lee, Chuan-Fu Tsai, Lindsay Fredericks, Qi Chen, Avanti Sinha, Sarah Bade, Karen Harmon, Pablo E. Pineyro, Phillip Gauger, Yun-Long Tsai, Hwa-Tang Thomas Wang, Hsiao Fen Grace Chang, Pei-Yu Alison Lee *134*

10:45 AM **Identification of porcine epidemic diarrhea virus variant with a large spike gene deletion from clinical swine samples in the US**
Jianqiang Zhang, Wannarat Yim-im, Qi Chen, Ying Zheng, Haiyan Huang, Phillip Gauger, Karen Harmon, Ganwu Li. *135*

11:00 AM **Detection of porcine cytomegalovirus using oral fluid lysate**
Susan Schommer, Jessica Owen, Melissa Samuel, Randall Prather *136*

11:15 AM **Application of next-generation sequencing (NGS) technology in food animal diagnostic medicine**
Ganwu Li, Qi Chen, Chenghuai Yang, Ying Zheng, Baoqing Guo, Phillip Gauger, Karen Harmon, Jianqiang Zhang, Kyoung-Jin Yoon, Gregory Stevenson, Kent Schwartz, Eric Burrough, Darin Madson, Paulo Arruda, Bailey Lauren Arruda, Drew Magstadt, Rachel J. Derscheid, Pablo E. Pineyro, Rodger Main *137*

11:30 AM **RT-QuIC: A next generation diagnostic assay for prion detection**
Davin Henderson, Joanne Tennant, Nicholas James Haley, Edward Hoover *138*

11:45 AM **Animal disease diagnosis using Next Generation Sequencing**
Sunil kumar Mor, Todd P. Knutson, Anibal G Armien, Alexander Primus, Sagar M Goyal, Robert E. Porter, Nicholas B.D. Phelps *139*

Symbols at the end of titles indicate the following designations:

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

Molecular characterization of pseudorabies virus isolated from a Florida panther

James Evermann[2], Annabel G. Wise[1], Allison J. McKeirnan[2], Robert G. McLean[3], Jo Anne C. Crum[4], Mark Cunningham[5], Suzanne Mason[1], Roger K. Maes[1,6]

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The epizootiology of pseudorabies virus (PRV) in the United States has been an active area of research for over 25 years. Drivers were the national PRV eradication from domestic swine populations, the continued presence of PRV in feral pigs and the associated potential for cross-species infections. Limited circumstantial evidence indicates that highly fatal PRV infections in the endangered Florida panther (*Felis concolor coryi*) are of feral pig origin, but experimental evidence is lacking. In collaboration with personnel from the Florida Fish and Game Wildlife and the Colorado Center for Disease Control and Prevention, samples from a deceased panther were obtained for virus isolation. Cytopathic changes in CRFK cells were confirmed to be the result of PRV infection, based upon direct FA staining and virus neutralization with PRV-specific reagents provided by the National Veterinary Services Laboratory, Ames, IA. Aliquots of the PRV isolate, identified as WSU 92-10542, were used for further analyses. DNA was extracted using a commercial kit and tested for the presence of PRV DNA. For initial testing we used a real-time PCR protocol which targets a portion of the PRV gD gene and was obtained from Dr. Scherba, University of Illinois. Subsequently, a 712 bp portion of the PRV gC gene was amplified. Amplicons of the expected size were purified and submitted. to the Research Technology Support Facility of Michigan State University for automated bidirectional DNA sequencing. BLAST analysis of sequence data was performed to search for significant similarities to sequences in the GenBank database. The BLAST results showed that the panther-derived sequence was 99% identical to the corresponding sequences of U.S. suid herpesvirus 1 strains. Phylogenetic analysis confirmed that the Florida panther strain forms a well- supported clade with porcine PRV strains from the U.S.

Molecular detection of novel adenoviruses in Chimney Swifts

Annabel G. Wise[1], David B. Needle[2], Dalen W. Agnew[1,3], Inga Sidor[2], Roger K. Maes[1,3]

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Five chimney swift fledglings from a wildlife rehabilitator were euthanized and submitted for histopathology after a history of non-specific illness, progressing to anorexia. Histopathology showed necrotizing ventriculitis with intranuclear inclusion bodies in epithelial cells. Transmission electron microscopy revealed paracrystalline arrays of icosahedral virions with electron-dense nucleoids consistent with adenovirus morphology. A generic adenovirus nested-PCR, targeting an approximately 320-bp region of the DNA polymerase gene, was performed on DNA extracted from pooled FFPE ventriculus tissue. Bidirectional sequencing of the amplified PCR product yielded a unique 272 bp nucleotide sequence that was analyzed by BLAST against the GenBank database. Database sequences producing the most significant alignments to the unknown sequence were those of unclassified adenoviruses from gentoo and chinstrap penguins at 74% sequence identities with a query coverage of only 65%. These were followed by sequences from a bat mastadenovirus and snake adenoviruses at 72% and 69% identities, and query coverages of 63% and 64%, respectively. Based upon the BLAST percentages, it appears that a novel adenovirus had been identified from these chimney swift index cases. To further investigate the association of this new virus with clinical disease in chimney swifts, eight additional cases were collected and analyzed. Histopathology performed on these cases demonstrated milder degenerative ventriculitis, minimal necrosis, and without viral inclusions. The same consensus adenovirus PCR was used to test the 8 cases. Only one sample was found positive for adenovirus DNA, the sequence of which was only 59.9% similar to that of the index case. BLAST analysis of the additional case sequence produced the most significant alignments with amphisbaenian and duck adenovirus sequences at 68% and 69% nucleotide identities, and at query coverages of 91% and 89%, respectively. The predicted partial adenoviral DNA polymerase amino acid sequences (90 a.a. sequence) derived from the index and follow-up cases were aligned with corresponding sequences of representative members of four genera within the Adenoviridae family. Phylogenetic analysis performed on the amino acid sequence alignment data showed that the two novel adenoviruses detected from the chimney swifts are most closely related to members of the Atadenovirus genus. The high A+T content (>60%) of the virus sequences obtained from the swift cases is a characteristic feature of the atadenovirus lineage. In this study, two clearly distinct adenovirus species have been identified in chimney swifts. The marked genetic difference could be the basis of observed differences in pathogenicity.

Seroprevalence of Senecavirus A in swine herds on the United States

Elizabeth Houston, Luis Gabriel Gimenez-Lirola, Ronaldo Magtoto, Dave Baum, Pablo E. Pineyro

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

Senecavirus A (SVA) is a Picornavirus discovered in 2002 and documented retrospectively in United States' swine herds since 1988. Recently, SVA has been associated with idiopathic vesicular disease and increased neonate mortality. Outbreaks of vesicular disease associated with SVA have been reported in multiple countries with increasing incidence since 2014. Previous studies demonstrated that the proportion of seropositive animals, using recombinant SVA-VP1protein (rVP1) ELISA, in farms affected by SVA did not differ between animals clinically and no clinically affected. However, no information is available regarding the seroprevalence or virus circulation on farms that have not reported outbreaks of vesicular disease associated or not with an increment in neonatal mortality. The objective of this study was to determine the seroprevalence of SVA in commercial swine herds in the United States. A total of 5,957 (1,986 sows and 3,971 grower-finisher pigs) serum samples were collected through June to December 2016 at Iowa State College of Veterinary Medicine Veterinary Diagnostic Laboratory, from farms that have had not previously reported breaks of vesicular disease, sudden increment in neonatal mortality, or never being diagnosed with SVA. Thirty animals were selected from each site (n=75 sows; n=144 grower-finishers), identified by unique premise identification number. Sera were diluted 1:50 and tested using a SVA rVP1 indirect ELISA. To confirm seropositive herd status, an indirect immunofluorescence (IFA) was performed on all samples from sites with at least one rVP1 ELISA positive animal. As screening method for detection of viremia real time PCR was completed in pools of five individual animals for all sow sites. Animals evaluated represent 219 unique commercial swine production sites in 19 states. The overall seroprevalence was 28.95 % (CI ±1.99; 26.96% to 30.94%) and 14.23% (CI ±1.09; 13.14% to 15.32%) for sows and grower-finisher respectively. The between farm prevalence demonstrate that 80% (CI ±9.05; 70.95% to 89.05%) of the sows, and 61.81% (CI ±7.94; 53.87% to 69.75%) of the grower-fisher herds had at least one seropositive animal. Within herd prevalence distribution varied from 0-10% in 20% of sows and grower-finisher farms to 90-100% in 3% of the sows farms. The serological status of all herds was confirmed by IFA. Out of 61 sow positive sites, twelve showed one or more positive pool by PCR. Clinically healthy herds of sows and grower-finisher pigs possessed antibody against SVA with variable within herd prevalence. In addition virus circulation was demonstrated in sow herds without presence of clinical signs. These results strongly suggest that SVA is circulating sub clinically or may go undetected in sow farms. The presence of antibodies in grower finisher also indicated that either colostral antibodies may still be persistent or that animals suffered a subclinical infection during the grower-finisher stage.

Practical application of MinION next generation sequencing of Porcine Reproductive and Respiratory Syndrome virus for routine identification and strain typing

Kevin Lahmers[1,2], James Stanton[3], Tanya LeRoith[1], Michelle Todd[1]

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Detection and characterization of pathogens by their nucleic acid is a universally accepted and commonly used approach. This is usually by standard or real-time polymerase chain reactions with established controls and defined validation criteria. However, this requires knowledge of suspected pathogens and is limited in pathogen genotyping. Next-generation sequencing allows for an unbiased identification of organisms in a sample and supplies additional information that can be used to further characterize detected pathogens. We have used the nanopore-based MinION from Oxford Nanopore Technologies to detect and correctly genotype multiple Porcine Reproductive and Respiratory Syndrome virus infections in serum and oral fluids. The MinION uses variations in current through a pore as the nucleic acid flows through the pore and produces long reads, similar to Pacific Biosciences sequencing. These long reads allow for spanning of repetitive regions and coverage of full viral genomes in a single read. This commercially available sequencer is powered by the laptop USB, has a low capital investment and provides real-time sequence information, which can speed bioinformatics and allow for optimization of individual sequencing runs. In addition to identifying PRRS virus, we have also identified co-infections with porcine epidemic diarrhea virus, porcine parvovirus 6, low pathogenicity swine influenza virus, *Haemophilus parasuis*, and *Salmonella enterica.* We have been able to correctly identify and genotype PRRS virus in samples using a custom library for python-based, open-source software, Centrifuge, which includes all bacterial, viral and protozoal refseq genomes as well as a database of 731 PRRS genomes. Analysis using Centrifuge can be completed on a laptop within 90 seconds of full sequencing runs. In summary, nanopore-based sequencing is a financially and technically achievable diagnostic tool, which allows for rapid and effective identification and genotyping of PRRS virus with the added benefit of identifying co-infections.

Development and validation of a multiplex real-time PCR assay for the detection and differentiation of PCV2 and PCV3 strains # †

Yin Wang[1], Fangfeng Yuan[1], Elizabeth Porlsen[1], Russel Ransburgh[1], Wanglong Zheng[1,2], Xuming Liu[1], Nanyan Lu[1], Lalitha Peddireddi[1], Gary Anderson[1], Jianfa Bai[1]

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Porcine circoviruses (PCV) are small non-enveloped icosahedral viruses containing a circular single-stranded DNA genome. PCV1 was first identified in pig kidney cell culture as a nonpathogenic agent. PCV2 found later was the causal agent of porcine circovirus diseases (PCVD), including postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory diseases complex (PRDC), enteric disease and reproductive failure. In the past years PCV2 has under gone significant genomic changes, and has evolved into different genotypes including PCV2a, 2b and 2d. In 2015, PCV3 was first isolated from a sow farm in North Carolina, US. Accumulated evidences indicated that PCV3 is a causal agent of PDNS, reproductive failure and multisystemic inflammation. In this study, a multiplex real-time PCR assay was developed for PCV3 and PCV2 detection and differentiation. Eighteen complete genome sequences of PCV3 were collected from NCBI and analyzed for the assay design. Several atypical circovirus identified in beef and pork that showed high identity to PCV3 especially in the replicase region were also analyzed. The unique PCV3 capsid gene was used for primers and probe design, which has 100% coverage to the 18 PCV3 strains. PCV2 has a very divergent genome with a large number of full genome sequences available. A collection of 1,907 PCV2 full or near-full genome sequences from NCBI were analyzed and 2 sets of primers and probes, targeted in the ORF3 and ORF1 regions, were designed for PCV2 detection. The coverage for each set is 90.5% and 94.5% respectively with an overall coverage of 98.9%. The 3 sets of primers and probes were multiplexed, and analytically analyzed using a 10-fold serial dilutions of a cloned complete genome of PCV3 and a half genome of PCV2. The detection limit for both PCV2 and PCV3 were 10 copies per reaction, which corresponds to Ct 37.0 for PCV3, and Ct 35.2 for PCV2. Correlation coefficients were 0.997 and 0.995, and PCR amplification efficiencies were 103.1% and 102.7%, respectively, for PCV3 and PCV2. Of 592 pig diagnostic samples, 119 (20.1%) were tested positive (Ct<37) for PCV3. Twenty PCV3 full-genomes were sequenced, from which 17 distinct PCV3 genomes were identified. When analyzed with the 18 published sequences, they share above 97% nucleotide identities at the full-genome level, and above 97.3% and 96% for the replicase gene and the capsid gene respectively. The assay detected PCV2 on all 12 diagnostic samples that were previously tested PCV2-positive by KSVDL.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Development of a quantitative real time RT-PCR assay for sensitive detection of emerging Atypical Porcine Pestivirus associated with congenital tremor in pigs # †

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Atypical Porcine Pestivirus (APPV) is reported as the etiologic agent for type AII congenital tremors in newborn piglets. APPV was first identified through metagenomic screening of PRRSV positive samples and subsequently reported in the US swine herds in 2014; since then it has been reported in multiple countries. Initial PCR-based diagnostic tests to detect APPV were designed based on the limited sequence information available at that time and are not capable of detecting all APPV strains. A sensitive and reliable PCR-based diagnostic test is critical for accurate detection of APPV. In this study, a quantitative real-time RT-PCR (qRT-PCR) assay was developed using all available and newly generated sequencing information, for reliable detection of all currently known APPV strains. The assay design also included an 18S rRNA internal control of swine to monitor RNA extraction efficiency. A positive control plasmid harboring the amplified APPV-target region was constructed and a serial 10-fold dilutions of RNA obtained from *in vitro* transcription of the plasmid was used to determine the analytical sensitivity/limit of detection (LOD). Individual 18S rRNA and APPV qRT-PCR assays were optimized separately and then combined into a duplex assay. Both individual and duplex assays had correlation coefficients of 0.997 and PCR amplification efficiencies of 91-92%. Comparison of detection limit and analytical sensitivity between individual and duplex assays indicated no inhibition of PCR sensitivity, when both assays are combined. The detection limit for APPV target, based on analytical sensitivity, is~12 copies per reaction, which corresponds to a Ct of ~38 for both individual and duplex reactions. Assay specificity was verified using nucleic acids of other closely related pestiviruses and also the nucleic acids from clinical samples that are positive for other common swine pathogens and no cross reactivity was observed. Testing 30 previously known APPV-positive and -negative clinical samples, submitted to Kansas State Veterinary Diagnostic Laboratory (KSVDL), demonstrated a good sensitivity and specificity. This data further demonstrates the sensitivity and specificity of the APPV duplex qRT-PCR assay and forms the basis for further evaluation of clinical sensitivity and specificity for rapid detection of APPV in swine clinical samples. Screening of 758 porcine clinical samples from KSVDL identified 110 APPV-positive (Ct ≤38) samples suggesting 14.52 % prevalence of APPV in the US swine herds. Among the various sample types tested, oral fluid had lower average Ct compared to serum, feces, and other tissues. Detection of APPV positives cases from post weaning and grower pig populations suggests APPV persistent infections. Further studies are needed to support this speculation. This duplex qRT-PCR assay offers a rapid and reliable detection of APPV in swine and serves as a valuable tool in APPV surveillance and epidemiological investigations.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Human to swine transmission of novel H3 Influenza A viruses from 2010 to 2017 * †

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Influenza A Virus (IAV) is a common cause of severe respiratory disease in swine (IAV-S). The combined effects of antigenic drift and antigenic shift makes influenza a moving target, requiring vaccines to be updated in order to be effective. Further complicating the IAV-S epidemiology is the broad host range making swine populations susceptible to IAVs from other species including human-origin IAV.

Control of IAV in the human population may be a potential method to prevent spillover events into swine. When spillover events do occur, swine can be a dead-end host, or a combination of genetic and environmental factors may allow a particular IAV to adapt and persist in swine. To search for possible H3 spillover events from humans to swine, 1,032 H3 hemagglutinin (HA) sequences generated from diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) from 2010 to April 2017 were analyzed. In addition, 41 human reference vaccine sequences and 1,000 randomly selected human-origin IAV in the United States from 2010-2017 were included for analyses. A total of 2,073 H3 sequences were aligned with MAFFT, and ISU VDL sequences that contained insertions or deletions representing less than 95% of the HA sequence were removed. A maximum-likelihood tree following a general time-reversible substitution model with gamma distributed rate variation was constructed from the alignment using FastTree.

We identified six unique transmission events from humans to swine that occurred from 2010 to 2017. The earliest of these spillover events was first detected in 2012, and has become established as a new human-like H3 clade in swine, with a total of 180 sequences to date detected in samples submitted from Illinois, Indiana, Iowa, Minnesota, Missouri, Montana, North Carolina, Ohio, and Oklahoma. Prior phylogenetic analysis by Rajão et al. 2015, based on the HA gene, indicates that this IAV is most closely related to the 2012-2013 WHO designated vaccine strain A/Victoria/361/2011.

Events 2 and 3 were detected in 2013, each a single separate event, but did not persist in the swine population. Spillover events 4 and 5 were detected in 2016, and it remains to be seen if these transmission events are sustained in the pig population. The 6th event resulted in four genetically similar H3N2 IAV-S observed from early May to late April of 2017 from a farm in Oklahoma. An additional closely related case was reported from a farm in Indiana. Phylogenetic analysis of the HA gene indicates this IAV is most closely related to the human-seasonal strains circulating in 2016-2017, and similar to the WHO designated vaccine strain A/Victoria/673/2014 recommended for the 2017-2018 influenza season. These novel HAs may reassort with endemic lineages further affecting transmission, although it remains to be seen if spillover events 2-6 become an established clade in the swine population similar to the first 2012 human-like H3.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Development of a luminex xTAG assay for the detection and differentiation of type 2 PRRSV field strains from the four vaccine strains used in the US # †

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Since the type 2 PRRSV emerged in North America in the late 1980s, it becomes one of the most problematic pathogen in swine production systems, which causes at least $664 million economic losses annually in the US. The viral genome has undergone constant changes and new variants have evolved upon time. This highly divergent nature of PRRSV genomes makes molecular diagnosis very challenging. There are four North American type PRRS vaccines that have been used in the US, namely Ingelvac MLV, Ingelvac ATP, Fostera and PrimePac. Differentiating vaccine strains from the field strains is important to guide and improve vaccine applications. Due to that the vaccine strains are very similar to some of the field strains, it is often difficult to differentiate vaccine and field strains. Currently the most used method of differentiation is by ORF5 sequencing, which is expensive and time consuming. The Luminex xTAG assay is a bead-based nucleic acid detection assay that hypothetically can analyze more than 100 different nucleotide sequences in a single reaction. In this study, a Luminex-based multiplex assay was developed to detect the vast majority of type 2 PRRSV field strains, at the same time to differentiate the four vaccine strains that have been used in the US. A collection of 694 full or near-full genome North American type 2 PRRSV sequences were analyzed. Three pairs of primers targeting in the M, N and NSP2 genes were designed for the virus detection. The coverage for each set is 85.4%, 91.2% and 50.7%, respectively, with an overall coverage of 99.3% by an *in silico* analysis. Four pairs of primers targeting in NSP2 and GP5 genes of vaccine strains were designed for differentiation. In the preliminary experiment using regular primers, the 7-multiplexed assay detected all 12 field strains tested, and differentially detected each of the 4 vaccine strains. Luminex primers and biotinylated primers have been synthesized and tested with the BioRad Bio-Plex 200 system, and resulted good data with relatively high background. Optimized procedure and data on more PRRS field strains will be reported.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

A specific, sensitive, and rapid reverse transcription-insulated isothermal polymerase chain reaction on a field-deployable PCR system for Senecavirus A detection in swine samples

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Senecavirus A (SVA; formerly Seneca Valley virus) has been associated with idiopathic vesicular disease in swine. SVA infection causes vesicular lesions clinically indistinguishable from those caused by other vesicular disease viruses, such as the highly regulated foot-and-mouth disease virus (FMDV), swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV), and vesicular exanthema of swine virus (VESV). When a vesicular disease is suspected, in addition to ruling out FMDV, SVDV, VSV and VESV, a timely detection of SVA can be useful. The TaqMan probe-based insulated isothermal polymerase chain reaction (iiPCR) working on a user-friendly field-deployable PCR system (POCKIT™ combo, GeneReach USA; including an automatic nucleic acid extraction device [taco™ mini] and an iiPCR device [POCKIT™ Nucleic Acid Analyzer]) has potential to serve as an on-site tool for rapid, specific and sensitive pathogen detection. In this study, a SVA RT-iiPCR targeting the SVA *3D* gene was developed, validated and compared to a SVA real-time RT-PCR (rRT-PCR) targeting the 5'UTR, which is routinely used at the Iowa State University Veterinary Diagnostic Laboratory, for SVA detection. Neither the SVA RT-iiPCR nor the rRT-PCR cross reacted with any of the 19 porcine viral pathogens including 4 vesicular disease viruses (FMDV, 20 strains; VSV, 6 strains; SVDV, 2 strains; VESV, 2 strains) and 15 other swine viruses. Limit of detection 95% based on *in vitro* transcribed RNA was 7 genomic copies per reaction for the RT-iiPCR and 3.5 genomic copies for the rRT-PCR. According to testing the serially diluted SVA cell culture isolates, the SVA RT-iiPCR and rRT-PCR had comparable analytical sensitivities. For diagnostic performance evaluation, 125 swine samples (12 vesicular swab, 30 tonsil swab, 25 oral fluid, 28 serum, and 30 fecal swab samples) collected from various states within the USA in 2015-2016 were tested. First, nucleic acids prepared by the MagMAX™ Pathogen RNA/DNA Kit were tested by both PCR assays. Second, the same sample set was tested by the POCKIT™ combo system and compared to the MagMAX™/rRT-PCR system. Among the 125 MagMAX™ extracts, one was negative by the rRT-PCR but positive by the RT-iiPCR, resulting in an agreement of 99.20% (124/125; 95% CI: 96.59-100%, κ=0.98). Among 125 samples, two were positive by the MagMax™/rRT-PCR system but negative by the POCKIT™ combo system, resulting in a 98.40% agreement (123/125; 95% CI: 95.39-100%, κ=0.97). In conclusion, the SVA RT-iiPCR/POCKIT™ combo system showed analytical and diagnostic performances comparable to those of the laboratory MagMAX™/rRT-PCR system for SVA RNA detection. This simple field-deployable system could serve as a tool to help swine vesicular disease diagnosis at points of need.

Identification of porcine epidemic diarrhea virus variant with a large spike gene deletion from clinical swine samples in the US

Jianqiang Zhang, Wannarat Yim-im, Qi Chen, Ying Zheng, Haiyan Huang, Phillip Gauger, Karen Harmon, Ganwu Li

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In April 2013, severe porcine epidemic diarrhea (PED) outbreaks occurred in the US and a highly virulent PED virus (PEDV) strain was identified which was referred to as 'non-S INDEL' strain. In January 2014, a PEDV variant different from the original highly virulent PEDV strains, as reflected by insertions and deletions in the S gene, was identified in the US and designated as 'S INDEL' PEDV. A nucleocapsid (N) or membrane (M) gene-based real-time RT-PCR (rRT-PCR) is generally used for the detection of PEDV from clinical specimens. A spike gene-based multiplex rRT-PCR can be further used to differentiate the non-S INDEL from the S INDEL PEDV strains.

In February 2017, a fecal swab collected from a sow farm in Oklahoma, USA was positive for PEDV by a N gene-based rRT-PCR with a threshold cycle (Ct) value of 15.5; however, S gene-based differential rRT-PCR revealed this sample was negative for S INDEL PEDV (Ct>40) and was weak positive for non-S INDEL PEDV (Ct 36.8). To determine the possible reasons for this observation, the sample was sequenced using next-generation sequencing technology. A PEDV sequence was detected with 27,405 nucleotides nearly spanning the whole genome excluding 33 nucleotides at the 5' end. This PEDV strain was designated as USA/OK10240-8/2017 which was genetically more closely related to non-S INDEL than S INDEL PEDV strains. However, compared to non-S INDEL PEDV strains, the OK10240 PEDV had a large continuous deletion of 600 nt (200 aa) in the S1 region (aa positions 31-230 in the S protein, with positions numbered according to the non-S INDEL PEDV strain USA/IN19338/2013 [GenBank # KF650370]). The remaining genome of the OK10240 PEDV, other than the S deletion region, had 99.7% nt identity to the non-S INDEL PEDV strain IN19338. A gel-based RT-PCR was developed to differentiate the OK10240 PEDV from non-S INDEL PEDV. Twenty more samples were collected from the same farm; all of them contained non-S INDEL PEDV and none of them contained OK10240-like PEDV, indicating the prevalence of OK10240-like PEDV in swine populations may be very low.

It was previously reported that a cell culture-adapted US PEDV strain TC-PC177-P2 contained 591-nt (197-aa) deletion in the S protein (aa positions 34-230) but such deletions were not present in the original clinical samples. A Japanese PEDV strain Tottori2/2014 identified in the clinical sample contained 582-nt (194-aa) deletion in the S protein (aa 33-225). A Korean PEDV strain MF3809/2008 identified in a clinical sample contained 612-nt (204-aa) deletion in the S protein but in a different location (aa positions 713-916). The OK10240/2017 strain described in this study was the first report of a PEDV strain with a large deletion in the S gene identified in clinical swine samples in the US. It remains to be determined whether PEDV variants with large deletions in the S protein would change the pathogenicity and tissue tropism of PEDV.

Detection of porcine cytomegalovirus using oral fluid lysate

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Porcine cytomegalovirus (PCMV) is a betaherpesvirus found endemically in pigs throughout the world. PCMV is normally latent in adult pigs but can cause rhinitis, pneumonia and mortality in pigs less than 3 weeks old. Because of its high prevalence, herd immunity to PCMV generally prevents clinical signs from being observed. Virus shedding begins around 3-6 weeks of age and becomes undetectable at 11-12 weeks; even though the host remains latently infected.

PCMV is a major concern in genetically modified pigs that may be immunocompromised and in animals that will be used for xenotransplant. PCMV has been shown to reactivate when tissues from positive pigs are transplanted to baboons in xenotransplant models, significantly decreasing the survival time of donor organs. Clients of the National Swine Resource and Research Center have requested antemortem selection of PCMV-free pigs to be used as xenotransplant organ donors prior to shipment. A real-time PCR assay (Mueller et al. 2002) was adapted to include an internal control for inhibition and results from antemortem samples (blood, oral fluid) were compared to postmortem spleen. In previous studies virus levels in blood were shown to be below the limit of detection during latent infection; however using our protocol, 13/29 blood samples (44.8%) and 16/29 oral fluid samples (55.1%) tested positive from animals with positive spleens.

Since the levels of virus are expected to be very low, various modifications to the Zymo QuickDNA™ Miniprep Plus extraction were tested to determine if sensitivity could be increased; i.e. increasing sample input volume, concentrating the DNA post-extraction (Zymo Genomic DNA Clean & Concentrator™-10 μL; Genomic DNA Clean & Concentrator™-25 μL, ethanol precipitation), and post-extraction removal of inhibitors (Zymo OneStep™ PCR Inhibitor Removal). While some methods improved the Ct value, none detected additional positives and all added significant processing time and cost. To decrease cost and hands-on processing time while potentially increasing sensitivity, Embryo Lysate Buffer (ELB) with proteinase K was used to directly lyse oral fluids at 55°C. After lysed samples were shown to work directly in our real-time PCR, samples concentrated using CentriVap were lysed with the same conditions and used directly in PCR. Twenty-three oral fluids from individual animals or small groups of animals were tested using both concentrated oral fluid lysed with ELB for 45 minutes and the Zymo Universal DNA extraction kit. Concentrated samples were positive for PCMV in 11/23 samples; 10/23 were positive with the kit despite samples using direct lysate having more instances of inhibition. We demonstrate an inexpensive, low cost, and hands-off lysis method that requires little specialized equipment can be used to detect a DNA pathogen in oral fluid and may have broader applications in the field.

Application of next-generation sequencing (NGS) technology in food animal diagnostic medicine

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Conventional diagnostic tests for pathogens are generally narrow in scope and may lack the ability to detect multiple pathogens if present in clinical cases. Failure to detect significant agents and intervene infection in a timely fashion contributes to continued transmission and increased economic losses in production animal settings. In addition, the appearance of re-emerging and newly emerging pathogens also underscores the need for rapid and comprehensive diagnostic assays that require no prior knowledge of the presence or identity of potential infectious agents. Unbiased next-generation sequencing (NGS) technologies hold the promise of identifying most potential pathogens in a single assay without prior knowledge of a specific target. Given sufficiently long read lengths, multiple hits to the microbial genome, and a well-annotated reference database, nearly all microorganisms can be uniquely identified on the basis of their specific nucleic acid sequence. Thus, NGS is an attractive tool and has wide microbiological applications, including infectious disease diagnosis at veterinary diagnostic laboratories. Since April of 2016, NGS has been offered as a routine diagnostic assay in the Veterinary Diagnostic Laboratory at Iowa State University. Here, we will present the applications of this unbiased tool in detection of mixed infections in clinical samples; identification of new viral variants and novel viruses; and virulence typing and resistome (a set of antibiotic resistance genes) identification of bacterial pathogens. Several recent cases will be highlighted, in which we have identified two novel recombinant PRRSV variants, a novel PEDV variant with a large deletion in S gene, a novel deltacoronavirus, an astrovirus 3 in pigs with encephalomyelitis, a novel adenovirus of birds, and uncommon pathogens such as caprine arthritis encephalitis virus and porcine hemagglutinating encephalomyelitis virus. NGS has provided an unprecedented opportunity to ‘cast a wide net’ and survey the full breadth of known and novel pathogens in clinical specimens. It may ultimately become a mainstay for pathogen detection in routine veterinary diagnostic investigation. However, it should be noted that NGS technology is a method of detection that does not confirm pathogenicity and aligning results with the clinical context of the case with other diagnostic assays is of the utmost importance.

RT-QuIC: A next generation diagnostic assay for prion detection

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Prion diseases including scrapie, bovine spongiform encephalopathy (BSE) and chronic wasting disease (CWD) are lethal neurodegenerative diseases cased by a pathogenic prion conformation. Detection of prion diseases has be confounded by difficulty in distinguishing the pathogenic conformer from the cellular form of the protein found abundantly in the brain as well as other tissues. Current assays approved for the detection of prion diseases rely on the relative protease resistance of the prion protein agent to discriminate it from the cellular form. Recently, techniques utilizing the replicative potential of the prion protein conformer have lead to an increase in sensitivity greater than 10,000 fold in a rapid assay format. Prions, as protein-only pathogens, have the ability to convert the cellular form of the prion protein to the pathogenic conformation. The RT-QuIC assay uses a cellular form of the prion protein produced in bacteria as a substrate for the sensitive amplification of prions found in tissues and excreta. We have successfully used the RT-QuIC assay to detect prions at early time points of disease, for antemortem testing using rectal biopsies, and for detection of prions in shed excreta including saliva, urine and feces. In this respect, the sensitive detection of prions in the RT-QuIC assay parallels the advancements in nucleic acid containing pathogens detection following the advent of PCR. Moreover, we have developed methods for quantification of prion burdens in tissues and excreta with techniques similar to real-time PCR. Here, we present advancements in techniques for the detection of prions at extremely low levels in excreta as well as sensitivity advancements for antemortem detection of CWD.

Results: In the analysis CWD seeding activity by RT-QuIC we found that most (81.8%) (9 of 11 tested from 3 animals) fecal samples from deer infected with CWD for one year or longer contained prion seeding activity. Applying this approach to elk living in a natural environment, we demonstrated that up to 40% (12 of 27) of asymptomatic, CWD(+) animals could be identified by RT-QuIC analysis of fecal samples.

Conclusions: Based on analysis of elk on ranch land, we propose that RT-QuIC analysis of feces may be used to determine whether a population may contain CWD-positive animals. In the future it may be possible to estimate the prevalence of CWD in a given landscape based on the relationship between positive fecal samples and IHC-positive rectal biopsies. However, understanding how environmental factors influence seeding activity of feces on the landscape will be necessary to better understanding the potential of CWD surveillance with feces.

Animal disease diagnosis using Next Generation Sequencing

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Methods for nucleic acid sequencing have improved greatly since the first version of the Sanger technique and has facilitated the development of very powerful tools for detecting and identifying viral and bacterial pathogens. The ongoing development of high-throughput sequencing (HTS; also known as next-generation sequencing or NGS) has resulted in a dramatic reduction in DNA sequencing costs, making the technology more accessible to the average laboratory. The hypothesis-free metagenomics strategy enables NGS to simultaneously detect mixed infections of different microorganisms and identify novel and/or uncharacterized pathogens directly from clinical samples without any prior knowledge of the pathogen(s). The University of Minnesota Veterinary Diagnostic Laboratory has made major investments in this technology, which is available for use by others. During 2014-16, we used NGS Illumina MiSeq to test clinical samples from several disease conditions of fish, poultry, honey bees, pigs, and wild animals. The samples from poultry were from different disease conditions, e.g., poult enteritis syndrome, mid-growth turkey enteritis syndrome, lameness problem in chicken and turkeys, and drop in egg production in turkey breeder flocks. In almost all samples, multiple infections with emerging and re-emerging viruses were detected. We found novel picornaviruses in several disease conditions of chickens and turkeys; three different types of paramyxoviruses in penguins from Chile; and complete genome of avian influenza and infectious laryngotracheitis virus from chickens in Egypt. By testing 'fish kill' event samples and surveillance samples of baitfish and invasive carp, novel viruses belonging to *Astroviridae*, *Circoviridae*, *Hepadnaviridae*, *Nodaviridae*, *Picornaviridae* and *Totiviridae* were detected. Most of the viruses have never been described/reported and are the focus of ongoing research efforts to better understand their disease-causing potential. From honey bees, viruses such as black queen cell virus, Sacbrood virus, deformed wing virus, Israeli acute paralysis virus, Lake Sinai virus, Aphid lethal paralysis virus, and chronic bee paralysis virus were detected either alone or as mixed infections. This technology should be helpful in detection of variants, novel pathogens, detection of coinfections and in solving cases that are undiagnosed by conventional techniques. The advantages and challenges related to NGS will be discussed in detail.

Saturday Poster Session

Saturday, October 14

Town and Country

Sat-1 **Evaluation of MALDI Biotyper for identification of *Taylorella equigenitalis* and *Taylorella asinigenitalis***
Kristina Lantz *144*

Sat-2 **MALDI-TOF isolate addition: Walking the fine line between improving scores and skewing identifications for the established Bruker library §**
Kyriakos Deriziotis, Rebecca Franklin-Guild, Anil J. Thachil *145*

Sat-3 **Detection of low concentrations of *Salmonella* in food products with an impedance-based biosensor # ***
Zhenyu Shen, Ibrahem Jasim, Mahmoud Almasri, Shuping Zhang. *146*

Sat-4 ***fimH* has a strain and host-cell type dependent role in adherence of *E. coli* O157:H7 super-shedder strains to host cells # ***
Sreenidhi Srinivasan, Robab Katani, Indira Kudva, Lingling Li, Terrance Arthur, Vivek Kapur *147*

Sat-5 **Rough *Brucella neotomae* and *Brucella suis* overexpressing GnRH and FSH: A novel brucellosis immunocontraceptive food safety vaccine # ***
Steven Grant Waldrop, Garrett Paul Smith, Neeta Jain-Gupta, Stephen Boyle, Nammalwar Sriranganathan *148*

Sat-6 **Monitoring of yeast species in the environment of dolphins in human care**
Miranda O'Dell, Carol Wolfgang Maddox, Piper Hodson, Karen Terio *149*

Sat-7 **The VItek family for fast, accuarete and cheap microbioogical testing**
Suan Sanchez, Paula Bartlett *150*

Sat-8 **Comparison of *Salmonella enterica* detection methods * ◊**
Lydia Margaret Hall, Kenitra Hammac *151*

Sat-9 **Comparison of pathogenic *Salmonella* spp. detection from porcine oral fluids, over-the-shoe booties, and fecal samples ***
Samantha Naberhaus, Adam Krull, Bailey Lauren Arruda, Paulo Arruda, Drew Magstadt, Franco Sebastian Matias Ferreyra, Igor Renan Honorato Gatto, Henrique Meiroz de Souza Almeida, Orhan Sahin, Amanda Kreuder *152*

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Symbols at the end of titles indicate the following designations:

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

Poster Sat-1

Evaluation of MALDI Biotyper for identification of *Taylorella equigenitalis* and *Taylorella asinigenitalis*

Kristina Lantz

National Veterinary Services Laboratories, Ames, IA

Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) has become an increasingly popular method for bacterial identification in recent years. Contagious Equine Metritis is a venereal disease of equids of international significance caused by the gram negative organism, *Taylorella equigenitalis*. The prescribed test is by bacteriological culture, however, due to the slow-growing and fastidious nature of the bacteria, culture requires specialized media and extended culture time. In addition, differentiation of *T. equigenitalis* and the closely related *Taylorella asinigenitalis* from each other and from common contaminants of the equine genital tract is difficult and has required advanced diagnostics with specialized reagents. This study evaluated the use of MALDI-TOF identification for detecting and differentiating individual colonies of *T. equigenitalis* and *T. asinigenitalis* directly from culture plates, significantly decreasing the time and cost of diagnosis. The study compared the effects of varying extraction methods, types of culture media and day of culture on the ability to identify these species. This method represents an inexpensive, rapid, and accurate new diagnostic tool for Contagious Equine Metritis diagnosis.

Poster Sat-2

MALDI-TOF isolate addition: Walking the fine line between improving scores and skewing identifications for the established Bruker library §

Kyriakos Deriziotis, Rebecca Franklin-Guild, Anil J. Thachil

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Mannheimia haemolytica (MH) and *Mannheimia varigena* (MV) are two closely related members of the Pasteurellaceae Family of Gram-negative bacteria. Both organisms can be responsible for bovine respiratory disease and bronchopneumonia in calves. With the introduction of MALDI-TOF in diagnostic labs, bacterial identification is often streamlined, and may not require more labor-intensive techniques such as 16S DNA sequencing. While many isolates align with Bruker's isolate library, we encounter occasional isolates that do not provide high enough scores. These isolates need further work-up, and are often put through a biochemical screen or sent to DNA sequencing, which increases costs and delays the final result. Bruker allows for custom isolates to be added to the library, through their MBT Explorer Software Module. Through bioinformatic spectra comparisons and statistical data interpretation, bacterial identification can be optimized as reference spectra, which must meet very specific requirements, are added, created and organized. An ideal isolate can help us differentiate between two similar organisms. A *Mannheimia varigena* isolate met these requirements and was added to our library in the 1st quarter of 2017. An analysis of the past 6 months of *Mannheimia* isolate MALDI-TOF results was completed, with and without the MV isolate addition. Its addition increased the scores of previous MV calls of the 2.0-2.1 range to the 2.1-2.4 range. Of the mixed MH/MV 1.7-1.9 range calls, an increase was noticed to scores above 2.0 for a few isolates. The addition of in-house MV spectra enable us to improve our scores, as well as helping to more efficiently identify some difficult isolates.

§ AAVLD Laboratory Staff Travel Awardee

Poster Sat-3

Detection of low concentrations of *Salmonella* in food products with an impedance-based biosensor # *

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[1]Electrical and Computer Engineering, University of Missouri, Columbia, MO; [2]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO

Nontyphoid *Salmonella* spp. are leading foodborne pathogens worldwide. The bacteria can cause enteritis and septicemia in humans and animals or silent infection in certain animal species. Ingestion of contaminated food/feed and water is a major route of transmission. Diagnosis of salmonellosis is based primarily on bacterial culture and polymerase chain reaction (PCR). These traditional detection methods are not only laborious and time-consuming, but also require specific laboratory settings. The goal of the present study was to develop and validate an impedance-based microelectromechanical system (MEMS) biosensor for accurate and rapid simultaneous detection of multiple serogroups of *Salmonella enterica* in food products and clinical specimens.

The biosensor was fabricated on a glass slide, consisting of 1) a sample inlet; 2) three microchannels split from the inlet where bacteria interact with detecting antibodies; 3) three separate antibody inlets connected to the microchannels; 4) an outlet for waste flow. Each microchannel contained a focusing region and sensing interdigitated electrode arrays (IDEA). The design of the biosensor was based on the following principles. During test, antibodies were added through the antibody inlets and immobilized to the sensing IDEA. A suspension of the testing sample was added through the sample inlet. Bacteria in the sample were concentrated in the focusing region and directed toward the sensing IDEA. The presence or absence of *Salmonella* bacteria in the sample was determined by measuring impedance increase which was triggered by specific antigen-antibody binding. To detect *Salmonella* bacteria, rabbit anti-*Salmonella* antisera against serogroups B, D, and E were pretreated with a cross-linker (sulfo-LC-SPD) and loaded to the antibody inlets. A sample of serially diluted *Salmonella enterica* Typhimurium (serogroup B) culture or ready-to-eat (RTE) turkey and raw chicken spiked with known numbers of *S. enterica* Typhimurium were added to the sample inlets. The impedance of each microchannel before and after loading the samples was measured using an impedance analyzer. The biosensor's capability to differentiate serogroups B, D and E was first proved with pure cultures of *S. enterica* Typhimurium. When applied in spiked food samples, the biosensor was able to detect as low as 7 and 15 CFU/ml *S. enterica* Typhimurium in RTE turkey and raw chicken. In both pure culture test and food product tests no across-reactivity was observed between serogroups. The assay including sample preparation and detection could be could be completed within 2 hours. Data from the present study suggests that the biosensor is suitable for rapid and field or laboratory detection and differentiation of different *Salmonella* serogroups.

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* Graduate Student Poster Presentation Award Applicant

Poster Sat-4

fimH has a strain and host-cell type dependent role in adherence of *E. coli* O157:H7 super-shedder strains to host cells # *

Sreenidhi Srinivasan[1,3], Robab Katani[1,3], Indira Kudva[2], Lingling Li[1,3], Terrance Arthur[4], Vivek Kapur[1,3]

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Escherichia coli O157:H7 (O157) are Shiga toxin-producing food-borne pathogens that are a significant threat to human health, causing severe illnesses including hemorrhagic uremic syndrome and kidney failure. Cattle are the major reservoirs of O157, with asymptomatic animals harboring the organism in their terminal recto-anal junction (RAJ). Some cattle shed ≥ 10^4 CFU of O157/g of feces and are referred to as super-shedders (SS). They are known to play an important role in O157 transmission and prevalence of the disease. Previous work from our lab showed that the SS17 strain of O157 displayed enhanced adherence to bovine rectal cells in a LEE (Locus of Enterocyte Effacement)-independent manner (Cote et al., 2015). We also showed that deletion mutants in SS17 and EDL933 (reference O157 strain) displayed alterations in adherence phenotypes on bovine RAJ squamous epithelial (RSE) cells, indicative of strain and host-cell type dependent role of *fimH* in adherence in both SS17 and EDL933. However, a definitive role for *fimH* participation in the adherence of O157 strains remained unclear as molecular Koch's postulates were unfulfilled. Hence, to better understand the role of *fimH* in O157 adherence phenotype, we constructed a series of *fimH* complementation strains of SS17 and EDL933 using plasmid complementation (pBBR1MCS) and Lambda red recombination techniques. The results of our studies indicate that for strain SS17, both complementation techniques caused a restoration of the wild type phenotype (strong aggregative) on RSE cells, suggesting that, contrary to prevailing dogma, *fimH* is likely to play a role in the adherence phenotype of O157 strains to specific mammalian cells. In contrast, while complementation of Δ*fimH* EDL933 using plasmid-encoded *fimH* restored the adherence phenotype (moderate aggregative), complementation of EDL933 using the recombination method did not. Similarly, the adherence phenotype of both SS17 and EDL933 was restored (to moderate diffuse) on human HEp-2 cells by the plasmid but not by the recombination method. Together, our findings suggest that despite the *fim* switch being permanently turned "off" in O157, additional genetic elements located in the region encoding *fimH* likely permit regulatory override of this switch, suggesting the existence of a complexly coordinated regulatory network underlying the expression of *fimH* in *E. coli* O157:H7.

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* Graduate Student Poster Presentation Award Applicant

Poster Sat-5

Rough *Brucella neotomae* and *Brucella suis* overexpressing GnRH and FSH: A novel brucellosis immunocontraceptive food safety vaccine # *

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While brucellosis has been eradicated from domestic livestock in the United States, the causative agent is still present in elk, bison, and feral swine. The interaction between infected wildlife and domestic livestock along with humans poses a great health risk. In particular, the feral swine population has quadrupled in the past ten years and continues to expand nationwide creating a biosecurity risk to the public and livestock. Furthermore, feral swine are known carriers of zoonotic diseases like leptospirosis and swine influenza, along with brucellosis. The current population control practices have neither minimized their spread throughout the United States nor the conservative $1.5 billion dollars of damage a year to agriculture. Thus, there is a need to efficiently control the feral swine population and prevent the spread of zoonotic diseases, like brucellosis, to domestic food animals. Two rough strains of *Brucella, B. suis* and *B. neotomae,* expressing gonadotropin releasing hormone (GnRH) and/or follicle stimulating hormone (FSH) were created. We are in the process of testing their ability to induce an antibody response in mice. These vaccine strains have the potential to be an effective immunocontraceptive for feral swine management, while reducing the spread of brucellosis to both humans and domestic livestock. As the vaccine strains do not cross react with veterinary diagnostic tests for brucellosis, they could pave the way for novel effective immunocontraceptive tools to be used in wildlife and domestic animal health management.

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* Graduate Student Poster Presentation Award Applicant

Poster Sat-6

Monitoring of yeast species in the environment of dolphins in human care

Miranda O'Dell[3], Carol Wolfgang Maddox[1], Piper Hodson[2], Karen Terio[1]

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Halotolerant and halophilic yeasts have the ability to survive in waters in which dolphins in human care live. Considerable amounts of time and effort are put into the care and training of these dolphins and disease prevention is of the utmost importance. Respiratory lesions and abscesses with *Candida* spp., especially *Candida glabrata*, and other yeasts have been reported and recovered by the Veterinary Diagnostic Laboratory at the University of Illinois at Urbana-Champaign. This study was designed to identify yeasts that may be found within the marine environment of dolphin habitats from several aquaria around the United States. The objective was to determine the potential for yeasts to form biofilms within the environment and serve as a source for reinfection. Personnel at five facilities swabbed 10 environmental areas within the dolphin habitat and submitted them to the VDL for analysis. If yeasts grew from the isolates, identification with MALDI-TOF was first attempted. From closed water systems (n=4), *Rhodotorula sphaerocarpa*, *Rhodotorula mucilaginosa*, *Dothideales/Hortaea werneckii, Cryptococcus* sp. (which sequenced closest to *C. fonsecae*), *Williopsis* sp*., Candida albicans, Meyerozyma (Candida) guilliermondii, Trichosporon* sp., *Cryptococcus curvatus*, *Phaeotheca salicorniae*, and *Candida spencermartinsinae* were recovered. From the only open water system in this study, *Tremallales* sp*., Knufia petricola, Cryptococcus liquefaciens*, and *Cryptococcus flavescens, Dothideales/Hortaea werneckii, Cryptococcus* sp. (which sequenced closest to *C. fonsecae*) were recovered. These results suggested that *C. glabrata* is likely not making biofilms within the dolphin habitat, and colonization of dolphins is likely occurring via contact with other colonized individuals, such as those sharing the environment, or handlers. Most isolates were identified via 18s-23s rRNA sequencing, as MALDI-TOF identifications were only successful with *Rhodotorula mucilaginosa, Candida guilliermondii, Candida albicans, Cryptococcus curvatus, Cryptococcus flavescens,* and *Cryptococcus liquefaciens.* MALDI-TOF was successful in separating *C. glabrata* isolates into subtypes with high correlation to site.

Poster Sat-7

The VItek family for fast, accuarete and cheap microbioogical testing

Suan Sanchez, Paula Bartlett

Infectious Diseases, Athens Veterinary Diagnostic Laboratory/ College of Veterinary Medicine, Athens, GA

In the world we leave today results on diagnostic testing are expected within ours. Bacterial culture has remained entrenched in the 19th century. Bacteria are plated, isolated and then subject to biochemical tests for identification, then susceptibility testing can be performed. Luckily, the Matrix Assisted Desorption Ionization Time Of Flight (MALDITOF) revolution of the 21st century has brought a new way of doing microbiology that does not require additional growth to identify bacteria in a matter of minutes.

The Vitek family of equipment with the assistance of Myla has brought things even further allowing unhindered communication between MALDITOF and fast susceptibility testing to finally push data to your LIM system. The integration of Vitek MS and Vitek 2 in our laboratory has allowed in critical cases to provide identification and susceptibility results within 24hrs; improving animal care and cutting cost to both client and laboratory.

In this poster we will describe the workflow of the VItek system and how it compares with traditional identification and susceptibility testing methods. We will also review the new susceptibility veterinary panels.

Poster Sat-8

Comparison of *Salmonella enterica* detection methods * ◊

Lydia Margaret Hall[3,1], Kenitra Hammac[2,1]

[1]Indiana Animal Disease Diagnostic Laboratory, West Lafayette, IN; [2]College of Veterinary Medicine, Purdue University, West Lafayette, IN; [3]College of Agriculture, Purdue University, West Lafayette, IN

Salmonella enterica is a well-known pathogen which can contaminate Large Animal Veterinary Teaching Hospitals. A previously published study concluded that the use of electrostatic wipes instead of sponges for sampling the hospitals environment may be a more effective method for routine surveillance testing. Although more effective, the study used a unique and longer bacterial culture method for detection. This study aimed to compare bacterial culture methods against previously published procedures to establish rapid and sensitive detection of *Salmonella enterica* contamination. The study design used laboratory based triplicate comparison of 5 different culture methods for the detection of low levels (10^3; 10^2; 10^1; & 10^0 colony forming units per mL) of *Salmonella enterica* utilizing Blood Agar, Buffered Peptone Water (BPW), Tetrathionate Broth with Iodine (Tet), Rappaport-Vassiliadis R10 Broth (RVR-10), Variable Day Incubation (24hr or 48hr per culture media), and XLT4-agar plates (XLT-4) in a 6 day trial. Preliminary results found that the use of RVR-10 is not necessary for effective detection, although the full 6 day culture incubation is still needed for low level *Salmonella* detection. Next steps may include coupling Veterinary Hospital based samples to confirm that RVR-10 is unnecessary when electrostatic wipes are used for sampling.

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster Sat-9

Comparison of pathogenic *Salmonella* spp. detection from porcine oral fluids, over-the-shoe booties, and fecal samples *

Samantha Naberhaus, Adam Krull, Bailey Lauren Arruda, Paulo Arruda, Drew Magstadt, Franco Sebastian Matias Ferreyra, Igor Renan Honorato Gatto, Henrique Meiroz de Souza Almeida, Orhan Sahin, Amanda Kreuder

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Pathogenic serotypes of *Salmonella*, including the commonly known serotype Typhimurium and now the recently emerging variant of Typhimurium known as I 4,[5],12:i:-, represent not only a production concern but also a potential pork contamination problem. Accurate and timely detection of these pathogens is vital to the initiation of appropriate treatment and prevention strategies. The proper sample type to use for herd level surveillance testing is a common question from veterinarians. The goal of this project was to determine the applicability of oral fluids, over-the-shoe booties, and fecal samples for diagnostic testing for pathogenic serotypes of *Salmonella*. Oral fluids, currently widely used for the detection of PRRSV and SIV are quick and inexpensive to collect. Booties, while easy to collect, are not used as frequently for surveillance of pathogens in the swine industry. Fecal samples are a commonly used sample for detection of many viruses and bacteria but are more time consuming to collect than the two previous sample types. In pigs experimentally infected with *Salmonella* serotypes I 4,[5],12:i:- and Typhimurium, fecal samples more closely reflected the level of clinical disease as indicated by temperatures and fecal scores than oral fluids or booties. In terms of quantities of *Salmonella* in the feces, oral fluids were only lowly positive for *Salmonella* during the highest level of disease and were negative for *Salmonella* just 4 days post inoculation (DPI). Over-the-shoe booties were not reflective of the level or stage of disease, with samples positive for *Salmonella* being sporadic throughout the period from inoculation with *Salmonella* until 28 DPI. Based on these results, fecal samples should remain the gold standard for *Salmonella* detection, but oral fluids may also be used at the peak of a *Salmonella* infection.

* Graduate Student Poster Presentation Award Applicant

Poster Sat-10

Serovar distribution and antimicrobial susceptibility of *Salmonella enterica* isolates from avian samples between 2010 and 2016 # *

Ming Yang, Chunye Zhang, William H Fales, Michael Z Zhang, Daniel P Shaw, Shuping Zhang

Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO

Introduction

Contaminated poultry and eggs are important vehicles of *Salmonella* infections. The determination of *Salmonella* serotype distribution and antimicrobial resistance (AMR) of clinical isolates is important to effective prevention, control and treatment of *Salmonella* infections in poultry. The aim of this study was to determine the serovar distribution and antimicrobial susceptibility pattern of *Salmonella enterica* isolated from avian samples submitted to University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL).

Methods

Salmonella culture and antimicrobial susceptibility testing were conducted in house using standard methods and serotyping was performed by the National Veterinary Services Laboratories (NVSL). Minimum inhibitory concentration (MIC) was determined using commercial MIC plates and antimicrobial results were selectively reported. Data were collected from the laboratory information system – Universal Veterinary Information System (UVIS).

Results and Discussion

Between 2010 and 2016, a total of 133 *Salmonella* isolates were recovered from avian species, including 77 turkeys, 19 chicken, 10 quail, 11 pheasants, and 16 unidentified avian species. The top ten most common *Salmonella* serovars were Typhimurium (15.79%), Senfternberg (12.03%), Bredeney (10.53%), Albany (8.27%), Hadar (5.26%), Enteritidis (4.51%), Heidelberg (3.76%), Saint-Paul (3.00%), Reading (3.00%), and Muenchen (3.00%).

From 2010 to 2016, MIC_{90} of amoxicillin, clindamycin, erythromycin, gentamicin, novobiocin, spectinomycin, sulfadimethoxine, sulfathizole, tetracycline, or tylosin was consistently greater than the highest concentrations included in the MIC plate. The MIC_{90} of trimethoprim/sulfa increased from ≤0.5 μg/ml to >2 μg/ml in 2012, 2 μg/ml in 2014, 1 μg/ml in 2016, respectively, whereas the MIC_{50} of tetracycline increased from 1 μg/ml to 2 μg/ml from 2014.

The percentages of total isolates over seven years with MICs greater than the highest testing concentrations were: 100%, erythromycin (MIC>4 μg/ml); 100%, novobiocin (MIC>4 μg/ml); 100%, tylosin (MIC>20 μg/ml); 69.17%, sulfadimethoxine (MIC>256 μg/ml); 53.38%, spectinomysin (MIC>64 μg/ml); 46.62%, gentamycin (MIC>8 μg/ml); 44.36%, amoxicillin (MIC>16 μg/ml); 39.85%, sulfathiazole (MIC>256 μg/ml); 39.10%, tetracycline (MIC>8 μg/ml); 39.10%, oxytetracycline (MIC>8 μg/ml); and 27.07%, ceftiofur (MIC>4 μg/ml).

The percentages of total isolates over seven years with MICs greater than the highest testing concentrations for more than one drugs were: 12.03%, 14.29%, 11.28%, 19.55%, 8.27%, 5.26%, 16.54%, and 12.78% for 10, 9, 8, 7, 6, 5, 4, and 3 antimicrobials, respectively.

The high MIC values from antimicrobial susceptibility testing reveal a high prevalence of multidrug resistant *Salmonella* serotypes in the tested avian samples. Results from the present study warrant further investigation aimed at understanding the role of therapy on AMR among enteric flora of avian species.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant

Poster Sat-11

Serovar distribution and antimicrobial susceptibility of *Salmonella enterica* isolates from ruminant samples between 2010 and 2016 # *

Chunye Zhang, Ming Yang, Michael Z Zhang, William H Fales, John R Middleton, Shuping Zhang

Veterinary Medical Diagnostic Laboratory, Columbia, MO

Introduction

Nontyphoidal salmonellosis is the most common bacterial foodborne illness in the United States. The disease is caused by various serovars of Salmonella enterica. These bacteria also infect animals and the clinical manifestations vary depending on the serovar and host immune status. Surveillance of multi-drug resistance (MDR) is critical component of understanding disease epidemiology and developing prevention and control strategies. The aim of the present study was to determine the serovar distribution and antimicrobial susceptibility pattern of *Salmonella enterica* isolated from ruminant samples submitted to University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL).

Methods

Salmonella culture and antimicrobial susceptibility testing were conducted in house using standard methods and serotyping was performed by the National Veterinary Services Laboratories (NVSL). Minimum inhibitory concentration (MIC) was determined using commercial MIC plates and antimicrobial results were selectively reported. Data were collected from the laboratory information system.

Results and Discussion

Between 2010 and 2016, a total of 212 *Salmonella* isolates were recovered, including 198 from cattle, 7 from sheep, 3 from elk, 2 from alpaca (camelid), and 1 each from a deer and a reindeer. The top ten most common *Salmonella* serovars were Typhimurium (16.03%), Dublin (11.32%), Agona (10.37%), Anatum (6.13%), Meleagridis (5.19%), Montevideo (4.25%), Cero (3.77%), Heidelberg (3.77%), Arizoniae (3.30%), and Muenchen (3.30%).

From 2010 to 2016, MIC_{90} of ampicillin, ceftiofur, spectinomycin, chlortetracycline, neomycin, oxytetracycline, or tylosin was consistently greater than the highest concentrations included in the MIC plate. The MIC_{90} of sulfadimethoxine increased from ≤256 μg/ml to >256 μg/ml in 2011 whereas the MIC_{90} of trimethoprim/sulfa increased from ≤2/38 μg/ml to >2/38 in 2014.

The percentages of total isolates with MICs greater than the highest testing concentrations were: 99.00%, tylosin (MIC>32 μg/ml); 69.34%, sulfadimethoxine (MIC>256 μg/ml); 43.81%, oxytetracycline (MIC>8 μg/ml); 40.38%, chlortetracycline (MIC>8 μg/ml); 32.55%, ampicillin (MIC>16 μg/ml); 29.25%, ceftiofur (MIC>8 μg/ml); 20%, neomycin (MIC>32 μg/ml); and 14.9%, spectinomycin (MIC>64 μg/ml).

The percentages of total isolates with MICs greater than the highest testing concentrations for more than one drugs were: 4.25%, 2.36%, 11.79%, 10.38%, 5.19%, 10.85%, 1.42%, and 33.49% for 9, 8, 7, 6, 5, 4, 3, and 2 antimicrobials, respectively.

The high MIC values obtained in a seven-year span indicate a high prevalence of MDR *Salmonella* in the tested ruminant samples. Results from the present study warrant further investigation aimed at understanding the role of therapy on AMR among enteric flora of ruminants.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant

Poster Sat-12

Application of the matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry method to identify *Moraxella* spp. isolated from cattle

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Infectious bovine keratoconjunctivitis (IBK) is an economically significant disease caused by *Moraxella bovis*. *Moraxella bovoculi* also secretes virulence factors and is the most frequently isolated species from bovine eyes during IBK outbreaks. Distinguishing between *Moraxella* species in clinical laboratory specimens is time consuming and relies on biochemical testing. Nucleic acid assays (PCR RFLP) can distinguish species, but do not work on all strains. Identification of the species during an IBK outbreak is critical to assist veterinarians implement the proper prevention and treatment strategies. The overall goal of this study was to determine the accuracy of the MALDI-TOF MS method as applied to *Moraxella* spp. isolated from bovine eyes and compare it to existing identification methods. *Moraxella bovoculi* (n=250) and *Moraxella bovis* (n=22) were used in this study that had been subjected to whole genome sequencing using MiSeq (Illumina) and subsequent 16S rDNA analysis (*M. bovis*) or whole genome sequence identification (*M. bovoculi*). Isolates represented included isolates from both outbreaks of clinical IBK and those from normal eyes in adults and young cattle. Isolates were subjected to the PCR RFLP test (Angelos 2007) and band sizes were evaluated using capillary gel electrophoresis. For MALDI-TOF MS, spectra were collected using the direct smear method and automated detection (Bruker Biotyper). Identifications were determined using commercial software (Bruker Biotyper) and the manufacturer's database (BDAL) or a modified database developed by the authors (UNLVDC) with additional spectra from reference isolates added, including additional *M. bovis*, *Moraxella ovis*, and *M. bovoculi* isolates. Comparisons were conducted using sequence information as the gold standard for identification. MALDI-TOF MS with the UNL VDC database was able to correctly classify 100% (250/250) of *M. bovoculi* and 91% (20/22) of *M. bovis*. The BDAL database correctly classified 99% (249/250) of the *M. bovoculi* and 45% (10/22) of the *M. bovis* isolates. In comparison, the PCR RFLP test was able to correctly classify 87.5% (210/250) of the *M. bovoculi* and 68% (15/22) of the *M. bovis*. Most of the disagreement was isolates identified as *M. bovoculi* by sequencing and MALDI-TOF MS that did not demonstrate a restriction site with RFLP and had an amplicon size consistent with *M. bovis*. Overall agreement between the MALDI-TOF-MS method and PCR RFLP for *M. bovoculi* was moderate with a kappa statistic of 0.4, and agreement between MALDI-TOF MS and sequencing was nearly perfect with a kappa statistic of 1.0 In conclusion, MALDI-TOF MS provides an effective, simple, and cost effective tool to rapidly identify *Moraxella* spp. isolated from cattle eyes. The enhanced MALDI-TOF MS database with inclusion of additional reference spectra enhances the ability to identify bovine *Moraxella* and database sharing may enable users of the platform to further enhance identification capabilities.

Poster Sat-13

A small device for a big challenge: surveillance of drug resistance in *Mannheimia haemolytica* using nanopore single molecule sequencing technology

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Disruptive innovations in high-speed, long-range, cost-effective direct template nucleic acid sequencing are transforming clinical and diagnostic medicine. With Oxford Nanopore's portable technology, whole genomes of a multidrug resistant and a pansusceptible strain of *Mannheimia haemolytica* were assembled *de novo*. The bacterial strains were isolated from pneumonic bovine lung samples submitted for diagnostic testing to the Oklahoma Animal Disease Diagnostic Laboratory. Sequence assembly produced a complete genome for the non-resistant strain and a nearly-complete assembly for the drug resistant strain at 29X coverage. Functional annotation using RAST (Rapid Annotations using Subsystems Technology), CARD (Comprehensive Antibiotic Resistance Database) and ResFinder databases identified genes conferring resistance to different classes of antibiotics including beta lactams, tetracyclines, lincosamides, phenicols, aminoglycosides, sulfonamides and macrolides. The antibiotic resistance of the *M. haemolytica* strains were also confirmed with the phenotypes determined by minimum inhibitory concentration (MIC) assays, highlighting the potential of whole genome sequencing as a valuable tool in diagnostic medicine.

Poster Sat-14

Simultaneous detection of antibodies to PRRSV 1, PRRSV 2, *APP* 2, *APP* 6, and *APP* 12 using a MFIA

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and *Actinobacillus pleuropneumoniae* (APP) are two major swine pathogens. Two major genotypes of PRRSV (type 1 and type 2) and 16 capsular serotypes of APP (1 to 16) are presently recognized. Serological testing for PRRSV and APP antibodies is usually performed using ELISAs. The most popular ELISAs are using the PRRSV nucleoprotein (NP) and the APP long chain lipopolysaccharides (LC-LPS) as antigens. In order to detect antibodies to PRRSV types 1 and 2 and various APP serotypes as many specific ELISAs as there are antigens have to be performed. A multiplex assay allowing detecting antibodies to all the various antigens at one time in a single well would allow saving a lot of time and reagents.

We have developed a 5-plex Multiplexed Fluorometric Immuno Assay (MFIA) for detecting and differentiating antibodies to PRRSV type 1, PRRSV type 2, APP2, APP6, and APP12 in swine serum samples. For that purpose five sets of microbeads with unique fluorescent dyes were coupled to PRRSV type 1 and type 2 NP, APP2, APP6, and APP12 LC-LPS. The mix of the 5 bead sets and serum samples were added to a microtiter plates (one well per sample) and incubated on a plate shaker at room temperature for 60 minutes at 800 rpm. Antigen-antibody complexes were then detected through successive incubations at room temperature for 2 x 30 minutes at 800 rpm with biotinylated species-specific anti-swine IgG and streptavidin coupled to phycoerythrin (SA-PE). Incubations were followed by wash steps to remove unbound serum constituents and reagents. Finally MFIA plates were read and analyzed using a fluorescence analyzer measuring the intensity of SA-PE fluorescence of each bead set. S/P ratios of the fluorescence index of the SA-PE on each set of antigen-coated beads were calculated and a ROC analysis was performed to determine the assay threshold for each antigen. The assay detects and differentiates antibodies to the five selected antigens with diagnostic performances (sensitivity, specificity, reproducibility) similar to those of regular individual PRRSV and LC-LPS ELISAs. Detailed results will be presented at the meeting.

Poster Sat-15

Assessment of vulnerability in commercial poultry farms in Egypt for control of avian influenza + † ◊

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Avian Influenza (AI) is a transboundary animal disease with a huge socio-economic effect on poultry sector. Egypt witnessed the first outbreak of AI due to HPAIV H5N1 clade 2.2 in February 2006 with estimated loss 1 billion US$. The source of the outbreak is migratory birds (late 2005). Highly fissured poultry-human interface leads to spill-over infections into humans (pandemic risks). Serious and frequent outbreaks in poultry on-going despite massive intervention by cull/control and vaccination campaigns. Vaccine escape mutants emerged and started to circulate. The research results showed the possibilities of mutation of the Low Pathogenic Avian Influenza virus (LPAIV) into Highly Pathogenic Avian Influenza Virus (HPAIV) under unfavorable production practices in Egypt. The aim of this study was to conduct a qualitative risk assessment for the possibilities of the re-occurrence of AI outbreak in Egypt. A cross-sectional analytical study was conducted through interviews using structured questionnaire during July 2015 to March 2016. Eighty six questionnaires were distributed to poultry producers with respondent's rate of 35.6%. All the data collected were analyzed using the software programme SPSS version 22. Results revealed that 50% of producers had higher level of education and this can ease their understanding and adoption of the study recommendations. Knowledge, Attitude and Practice (KAP) of producers towards prevention and control of AI disease scored 86.4%, 80.8% and 25.0 % for good level, respectively. These levels are not enough to protect poultry sector from the disease occurrence and spreading. Risk factors associated with poultry farms, 11.5% of farms did not use all in all out system of production, and 30.6% of farms did not experience any biosecurity measures and 73.8% did not hygienically dispose dead birds particularly during disease outbreak. Moreover, no program for pest control in 87.0% of targeted farms. 100% of the producers were not satisfied with the rate and time of compensation. The study highlighted the necessity to restructure the farm distribution, needs to establish a system to enforce the veterinary rules and legislation, urgent needs for effective extension services, availability for diagnostic facilities and training institutes.

+ AAVLD/ACVP Pathology Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Poster Sat-16

Development of a real-time biosecurity score based on self-assessment of management accountable for increased risk of LPAI and HPAI introduction and spread in poultry farms in USA * † ◊

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The numerous (>220) outbreaks in more than 20 US states during 2014-2015 epidemic of highly pathogenic avian influenza (HPAI), primary H5N8, that affected commercial turkey, poultry operations and backyard flocks highlights the urgent need to develop and implement solutions to protect US poultry industry against this devastating disease. These recent outbreaks have highlighted the need to integrate the environmental, climatic and anthropogenic factors (e.g. biosecurity and management practices) that are associated with an increased risk for HPAI outbreaks in poultry operations (POs). All those aspects combined with extension tools that increase the awareness, provide recommendations and education of producers would lead to the implementation of appropriate biosecurity and management practices on farms located in high-risk areas, which is key to prevent and mitigate the devastating consequences of HPAI outbreaks. The overall goal of this study was to gather information about the biosecurity practices in diverse poultry production systems as well as to develop an online biosecurity scoring system that provides real-time biosecurity scores and recommendations to improve biosecurity on poultry farms. A systematic literature review was conducted to inform the development of a semi-structured questionnaire. Options/answers under each question were divided into 4 categories: high risk, medium risk, low risk and preventive factors and scored according to literature review and expert opinions. An overall biosecurity score was then calculated by summing up individual scores for the options/answers for different questions. On completion of the online survey, a farmer gets this real-time biosecurity score and a customized (based on their responses) list of recommendations to improve the overall score when necessary. Completion of this study provides the farmers a better understanding about their farm biosecurity practices and their potential risk for HPAI exposure. Moreover, the overall, combined, analysis of the biosecurity scores and management practices for each of the poultry production systems will provide valuable information on the strengths and vulnerabilities of poultry industry regarding HPAI exposure to better inform risk mitigation strategies. Results of this survey and the list of customized recommendations would foster the awareness among the farmers and facilitate the improvement of biosecurity practices to more cost-effectively prevent and control future HPAI outbreaks in the US poultry industry.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Poster Sat-17

Drug Residue Avoidance * ◊

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Antibiotics are used in food-producing animals to treat, prevent and control diseases caused by harmful bacteria. Any animal that receives antibiotic therapy cannot, by law, be sent to slaughter until the drug has been reduced to a specified level and deemed safe for human consumption. Drug concentrations above this level are illegal and known as violative residues. It is the responsibility of the producer to ensure the health, safety and well-being of their animals while remaining in compliance with state and federal laws. Following labels and abiding by withdrawal times are crucial parts in protecting our food supply chain. Diseases can have a devastating impact on animal productivity and production. Animal health affects food safety and food safety affects public health. Consumers have expressed concern regarding the health impact of drug residues in their food. These concerns include the potential for a transfer of antibiotic resistance and allergic or hypersensitivity reactions. All drugs should be used according to label directions and in a judicious manner. Drug residue avoidance begins by working with your veterinarian to put into place best management practices or "BMPs" and standard operating procedures or "SOPs" for your farm or operation. Following the formation of these BMPs and SOPs, all employees and stakeholders must be regularly trained and adherence verified. Reading and following product label directions, maintaining good records and adopting a quality assurance program that encompasses a wide array of topics from drug storage, administration techniques and humane animal handling practices, all contribute to maintaining the safest food supply chain in the world. The Kansas Department of Agriculture received a grant through the Food and Drug Administration (FDA) with the goal to educate and promote the prevention of illegal drug residue in animal derived foods produced in Kansas through educational training and outreach. The purpose of this project was to create and disseminate educational materials to limit the occurrence of drug residues. This was accomplished through three strategies; brochures, PowerPoint slide sets and online training modules. These materials were created in a species-specific packaging to effectively communicate to the various different industries. The five species covered in this project were beef, dairy, swine, poultry and small ruminants. Upon completion, these materials will be made available on the Kansas Department of Agriculture's website.

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster Sat-18

Use of interactive, near real-time GIS in veterinary diagnostic laboratories for increased situational awareness of confirmed animal diagnoses—is it time for a national diagnosis map?

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Simple GIS mapping tools have been utilized for decades to display the distribution and frequency of animal health events. However, legacy mapping tools are feature poor and labor intensive. Today's GIS tools enable near-real-time, interactive maps that are dynamically updated and published as large data sets change. This enables any laboratory to build and maintain broad-based mapping products with minimal technical labor. In addition, maps can be built such that the user controls how the data is displayed such as ranked summaries of diagnoses and etiologies and the identification of statistical disease clusters. These methods improve situational awareness of animal disease for practicing veterinarians and producers. Unfortunately, the data streams that are used in many health mapping systems are derived from unreliable and non-validated sources. On the other hand, fully accredited veterinary diagnostic laboratories render highly accurate confirmed diagnoses based on validated methods and strong quality assurance/control programs.

The thematic GIS products produced by the University of Kentucky Veterinary Diagnostic Laboratory are fully interactive. The user can easily mine data for any county and species through simple mouse clicks. The data can be updated hourly, daily, weekly, and monthly based on the laboratory preference. Any given laboratory might wish to publish 200 disease/etiology distribution maps for the various domestic and other species. The chore of manually updating maps alone could require 1-2 full-time GIS technicians. Utilizing the Kentucky model, maps change dynamically as new case data is captured by a Laboratory Information Management System (LIMS) or other source which includes date case submitted, tests performed, the test results, breeds, species and county. Finally, multiple maps can quickly be produced for each disease or etiology for the current year (or many years) or season.

Of course, the limitations of diagnostic laboratory case data must always be kept in mind. A laboratory collects and archives disease and etiological data only from cases submitted to the laboratory (the iceberg phenomenon). Therefore, using diagnostic laboratory data does not yield a true incidence of disease. The power in diagnostic laboratory confirmed diagnoses lies in the fact that are accurate and timely and suggests that other animals in the same contiguous geographic areas could be affected. This, coupled with the distribution of diseases by near-real-time distribution maps, can help elicit suspicion by practicing veterinarians to look more closely for specific disease problems in an area and also can highlight the need for field investigations by county, state, federal, and university veterinary epidemiologists and public health officials in the case of zoonotic diseases.

Poster Sat-19

Infectious agents identified in aborted swine foetus in Korea (2007~2016)

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Reproductive failures including abortion are one of the major issues in swine industry. Although main etiologic agents in swine abortion are virus such as Porcine Parvovirus (PPV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Porcine Circovirus Type 2 (PCV-2), Japanese Encephalitis Virus (JEV) Encephalmyocarditis Virus (EMCV) and Aujeszkey's Disease Virus (ADV), there are several bacterial agents such as *Brucella,* and *Leptospira*. The goal of our study was to determine the presence of pathogens that are known to cause abortion in swine. One hundred and seventy aborted cases from a nationwide area in Korea for 10 years (2007~2016) were used for this study. The pigs were diagnosed based on the agent detection (bacterial culture, virus PCR) and serology. Brain, lung, heart, spleen, lymph node, liver, kidney, and placenta were collected for pathology and PCR for virus. Bacterial isolation was checked to the lung, stomach contents, body fluid, and placenta. To detect antibodies, body fluid was collected from thorax, abdomen, heart, and et al.

An infectious etiologic agent was found in 121 of 170 cases of abortion (71.2%). These included viral agents (PPV, 73; EMC, 61; PRRS, 31; PCV-2, 27), bacteria (*E.coli*, 2; *Leptospira spp*, 2), and parasite (*Toxoplasma gondii*, 1). Single infection case was 51.2% (62/121), double infection was 38.8% (47/121), and multiple infection was 9.9% (12/121). This study describes the prevalence of infectious agents involved in swine abortion in Korea. This data can be used to prevent swine abortion and in supervising the breeding process in Korea.

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Poster Sat-20

Ceftiofur-resistant *Salmonella* Kentucky isolates from broiler chicken clinical specimens display resistance to β-lactamase inhibitors and reduced susceptibility to third generation cephalosporins

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Salmonella Kentucky is one of the most common serotypes from poultry that exhibits resistance to the third generation cephalosphorin, ceftiofur. Ceftiofur has been used to prevent early embryonic death at hatcheries. Ceftiofur use in livestock and poultry correlates with ceftriaxone resistance to *Salmonella* in human. Although *S*. Kentucky infection in humans is not as common as some other serovars, *S*. Kentucky is ranked ~15 among laboratory confirmed *Salmonella* isolates from clinical/non-human sources and number one among serovars isolated from non-clinical/non-human sources submitted to the National Veterinary Services Laboratories in recent years. Ceftriaxone which is an extended-spectrum cephalosphorin is a critically important antibiotic for the treatment of severe *Salmonella* infections, especially in children, and therefore ceftriaxone resistance in *Salmonella* is a serious public health threat. In this study, *S*. Kentucky isolates (n=71) originated from poultry clinical diagnostic cases submitted to the Animal Diagnostic Laboratory of the Pennsylvania State University during 2008 to 2016 were examined. Seven *S*. Kentucky isolates resistant to ceftiofur with a minimal inhibitory concentration (MIC) of ≥4 μg/ml were further characterized for the presence of extended spectrum β-lactamases (ESBLs) and genes conferring ESBL resistance and were subjected to plasmid profiling, and pulsed-field gel electrophoresis (PFGE). Of the seven isolates, one isolate was resistance to ceftazidime whereas the remaining isolates demonstrated reduced susceptibility to third generation cephalosporins including ceftriaxone. PCR and sequence analysis detected the $bla_{\text{CMY-2}}$ gene in all seven isolates. Three different *Xba*I PFGE patterns were identified among seven isolates and only one isolate harbored a plasmid. Interestingly, no ceftiofur-resistant *S*. Kentucky were isolated from poultry clinical specimens submitted after 2012 which coincides with the order prohibiting certain extra-label uses of cephalosporins in food-producing animals in the United States.

Poster Sat-21

Detection of multiple bovine viral and bacterial respiratory pathogens by real-time TaqMan qPCR

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Bovine respiratory disease complex (BRDC) is a significant economic problem for the cattle industry. This syndrome is caused by the interaction of multiple non-infectious and infectious factors, including multiple viruses (bovine respiratory syncitial virus, bovine herpes virus 1, parainfluenza virus type 3, bovine coronavirus, bovine viral diarrhea virus, influenza virus D) and bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*). The aim of the study was to determine the occurrence of these agents in Quebec cattle using real-time TaqMan PCR assays. Thirty nasal or nasopharyngeal swabs from 20 cases received during a three month period (mid-March to mid-May) were examined using 10 specific real-time PCR (qPCR) for the above mentioned bacterial and viral respiratory pathogens. Samples originated from cattle of various ages (6 months to 3 years) but all with severe respiratory clinical signs. No agent was detected in 4 cases. Mixed infections with up to four agents were common (61%). The agents most frequently identified were *Pasteurella multocida* (46%), *Mannheimia haemolytica* (30%), *Mycoplasma bovis* (30%), and bovine respiratory syncitial virus (23%). Other agents were detected in less than 15% of the cases. Bovine viral diarrhea virus was not detected in any sample. Influenza virus D was detected in two unrelated animals (6%). As far as we know it is the first time that this virus is identified in Canada. Compared to traditional techniques such as bacterial culture and virus isolation qPCR appears as a convenient and performant tool to detect bovine respiratory pathogens.

Poster Sat-22

Spatio-temporal patterns and phylogenetic analysis of Equine Influenza Virus (EIV) from 2011 to 2016 in the U.S. * ◊

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Equine influenza virus (EIV) is considered one of the leading causes of infectious respiratory diseases of equids in the US. Since 1956, two distinct subtypes of EIV (H7N7, H3N8) have diverged to the American lineage and further diverging into the Kentucky, South American and Florida sub-lineages. Nowadays, the Florida sub-lineage is predominant and has evolved into two antigenically different clades: clade 1 viruses endemic in horses from North America and clades 1 and 2 circulating in Europe. The hemagglutinin (HA), one of the surface glycoproteins of EIV, is the primary target of the protective immune response and associated with evasion of antibody neutralization. Therefore, the HA gene is the focus of EIV surveillance and ensure that the vaccines contain epidemiologically relevant strains. In this study, we describe the spatio-temporal distribution and phylogeny of H3N8 EIV isolates collected from outbreaks across the USA from 2011 to 2016. Specifically, we evaluated a high degree of evolutionary changes in HA gene and gauged suboptimal cross-protection level against antiquated vaccine strains. Seventy EIV qPCR positive cases out of the 190 cases were selected for sequencing of the HA1 gene and determining their genetic relationship. The conventional PCR was carried out to sequence HA1 gene in three segments using the three primers: 5' piece, middle piece and the 3' piece. The basic phylogenetic tree isolated EIV was combined with 193 reference EIV HA1 complete sequences isolated from January 1960 to January 2017 all around the world by the maximum likelihood method using PhyML v3.0. A Bayesian molecular clock and a coalescence-based method implemented in BEAST were used to measure the molecular clock and estimate the time of the most recent common ancestor (tMRCA) using a Bayesian Evolutionary Analysis Sampling Trees (BEAST) v1.8.4 software. The ancestor location and viral migration were evaluated to the spatio-temporal diffusion by grouping EIV isolates using the discrete phylogeographic asymmetric model in BEAST. Phylogenetic analysis showed different H3N8 monophyletic clades, linked to strains of different non-US origins and suggesting that EIV outbreaks were caused by different independent introductions, likely linked to international movement of horses, and further spread at regional level. Results of this study supports the importance to continuously update the vaccine strains to achieve adequate population immunity and to improve the epidemiological surveillance and tracing of horse movements to prevent its spread globally and regionally.

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster Sat-23

Real time RT-PCR based retrospective assessment of Mammalian Orthoreovirus type-3 in cases of epidemic diarrhea in US swine

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The outbreaks of porcine epidemic diarrhea virus (PEDV) in May 2013 and porcine delta coronavirus (PDCoV) in February 2014 that caused huge economic losses, raised concerns about other unknown or emerging enteric viruses present in the pig population in the United States. In 2014 an etiological investigation of an epidemic diarrhea outbreak in neonatal pigs revealed the presence of a new pathogenic mammalian orthoreovirus type 3 (MRV3) in feces, and was further detected in the ring-dried swine blood meal from affected farms in Iowa, Minnesota and North Carolina (Narayanappa et al.). Although PDCoV was first detected in 2014, a retrospective study conducted at the Iowa State University (ISU) in 2014 (Sinha et al.) determined that the virus was present in the pig population since August of 2013. Thus, the objective of this study was to evaluate retrospectively if pathogenic MRV3 was present in cases of neonatal diarrhea prior to 2014 in U.S. In addition the experimental design used in this study helped to discern if the MRV3 was present in U.S. around the time of PEDV and PDCoV entry to the U.S. by retrospectively testing samples submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) circa 2013. A total of 611 formalin fixed paraffin embedded (FFPE) neonatal pig intestinal tissues submitted from January 2013 to February 2014 were used in this study. The primers and probe for a real-time PCR assay were designed using Primer Express software to target the conserved regions of the MRV3 Sigma-1 protein gene based on the nucleotide sequences of T3/Swine/FS03/USA/2014 and T3/Swine/BM100/USA/2014 deposited in GenBank (KM820760.1 and KM820750.1 respectively). A plasmid containing the target of the MRV3 PCR assay was used as a positive control, and specificity of the assay was confirmed by performing the PCR assessment on DNA and RNA extracted from *Brachyspira*, *Clostridium*, *E.coli*, *Salmonella*, PEDV, PDCoV, TGEV, PCV-2, PRRSV (types 1 and 2), and porcine rotaviruses (Types A, B, and C). The assay was robust in distinguishing and detecting the MRV3 positive control but no other agents were detected during validation. The assay sensitivity was established by testing serial dilutions of the MRV3 plasmid in triplicate. The standard curve indicated the assay was able to detect the MRV3 RNA in a range of $1x10^3$ to $1x10^{10}$ copies/μL. RNA extracted from the 611 FFPE clinical cases were subjected to the MRV3 real time RT-PCR. As positive control RNA extracted from positive FFPE intestinal section obtained from experimentally infected animals and a plasmid containing the Sigma-1 protein region were used. All of the samples evaluated were negative to MRV3 by real-time PCR assay. Thus, based on the results of this retrospective study on available FFPE samples submitted to the ISU VDL, it appears that the MRV3 introduction first occurred in 2014 and was independent of PEDV and PDCoV introduction to the U.S.

Poster Sat-24

Improved understanding of DNA and RNA fragmentation in formalin fixed, paraffin embedded tissues.

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Formalin fixed, paraffin embedded (FFPE) tissues have become an integral sample type for molecular diagnostic testing. Per the Iowa State University Veterinary Diagnostic Laboratory USDA import permit, tissues from many countries must be inactivated in a minimum of 10% formalin. Therefore, FFPE tissues may be the only sample type available from some international clients. Additionally, FFPE tissues are critical for retrospective testing of emerging diseases or disease outbreaks. It is well known that formalin fixation fragments DNA and RNA present in the tissues. This usually does not pose issues for real-time PCR due to the small target size (100-200 base pairs). However, when a larger product is needed for applications such as sequencing, fragmentation of nucleic acid becomes problematic. The purpose of this investigation was to evaluate sequencing success from FFPE tissue, specifically by examining the impact of the size of the products being amplified and sequenced. Porcine Epidemic Diarrhea virus (PEDV) was selected as a representative RNA virus and porcine circovirus 2d (PCV2d) as a representative DNA virus. Primers were designed to generate various product sizes of PCV2d ORF2 (from about 300 to 800 bp) and PEDV S1 gene (from about 250 to 1000 bp). The primers were designed so an entire sequence could be obtained by aligning multiple smaller sequences. For both agents, five FFPE tissues and five fresh samples were chosen for sequencing. All samples were tested with real-time PCR prior to sequencing. For PEDV, the complete 1000 bp sequence was obtained on 3/5 of the FFPE samples for the 250, 350, and 450 bp fragments. No complete sequences from the FFPE samples were obtained for the 700 and 1000 bp fragments. In contrast, full sequences were acquired from 5/5 fresh samples at all fragment sizes. For PCV2d, a complete 800 bp sequence was obtained from 3/5 FFPE samples for the 300 bp fragment, 1/5 for the 450 bp fragment, 3/5 for the 600 bp fragment, and 1/5 for the 800 bp fragment. Full sequences were obtained from 5/5 fresh samples of each fragment size except 4/5 for the 450 bp fragment. The higher success rate of sequencing of up to 800 bp with PCV2d compared to PEDV may be due to greater stability of DNA vs. RNA in the FFPE tissue. Additionally, a negative correlation was found between the Ct value and the length of DNA/RNA fragments which could be detected for both PEDV and PCV2d. This indicates that ability to obtain a sequence is also dependent on PCR Ct value. The sequences of each virus were compared for consistency between the fragment lengths and between fresh and FFPE samples. The sequences were all highly similar. In summary, sequencing from FFPE tissues can be successful if multiple smaller PCR products are generated for sequencing. A lower Ct also improves success of sequencing from FFPE tissues.

Poster Sat-25

Comparison of three different conventional polymerase chain reaction (PCR) methods to detect and genotype *Gallibacterium anatis* isolates # + † ◊

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Gallibacterium anatis is a naturally occurring commensal bacterial of the upper respiratory system in poultry that may induce upper respiratory signs, decreased egg production, salpingitis, and airsacculitis, this bacterium can produce important health and productive problems in broiler breeder and commercial layer flocks. During the isolation procedures, other bacteria such as *Pasteurella* sp. may show similar phenotypic features, therefore the evaluation of molecular methods for the efficient detection and genotyping of *G. anatis* is necessary. *G. anatis* isolates from 2016 and 2017 were included in this study. During the isolation all of them exhibited β hemolysis, positive oxidase and negative indole reaction among other biochemical features determined by the Sensititre system. DNA from these isolates was extracted, one conventional PCR method was used to amplify the 16s rRNA gene, and two other PCR methods were directed to the conserved parts of the 16S-23S rRNA gene. The three different methods efficiently amplified the targeted regions of all *G. anatis* isolates, and the amplicon exhibited the expected sizes. However, the nucleotide sequence and phylogenetic analyses of these isolates using these three methods showed differences in the clustering of the isolates. The usefulness of these three methods to genotype and classify the different *G. anatis* isolates will be discussed.

AAVLD Trainee Travel Awardee
+ AAVLD/ACVP Pathology Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Poster Sat-26

A novel approach for detection and identification of swine respiratory pathogens

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Swine respiratory tract infections are caused by many pathogens including *Haemophilus parasuis* (HPS), *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus (PRRS) and swine influenza virus (SIV). To efficiently identify all of these pathogens requires an accurate and reliable method that is affordable, rapid and sensitive. Here, we have developed a prototype testing system that uses a multiplex PCR/RT-PCR assay followed by analysis with the QIAxcel Advanced capillary electrophoresis system, which allows simultaneous detection of HPS, *M. hyopneumoniae*, PRRS and/or SIV in oral fluid samples in a single tube. This testing system is sensitive, specific, and cheap with fast turnaround time. In addition, this system is flexible to allow more gene targets to be included for detection of more pathogens.

Poster Sat-27

Developing next-generation sequencing informatics pipelines for unknown pathogen detection, genome assembly, and comparative genomics

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Viral disease outbreaks in production animals (swine, chickens, turkeys, etc.) cause substantial economic losses and pose a constant threat to the agricultural industry. Rapid detection of common viral pathogens by qPCR is effective, yet these methods often fail to identify highly mutable viral subtypes and cannot identify unknown viruses involved in clinical disease. Next-generation sequencing (NGS) of animal samples followed by bioinformatics analysis has allowed us to survey the entire virome of a clinical sample and provide rapid diagnostic information, including full genome assembly and comparative analysis with other viral strains. At the University of Minnesota Veterinary Diagnostic Laboratory, we are currently working on using NGS as a diagnostic test. Our method of pathogen detection begins with RNA/DNA isolation from clinical samples of various animal sources, followed by Illumina MiSeq sequencing. After quality control and read trimming, each read is classified using an ultrafast k-mer search strategy employed by Kraken software and a database of known species, which is 900 times faster than Megablast. We created a custom database containing all GenBank viral sequences and full-length RefSeq genomes from all bacteria, archaea, fungi, protozoa, and plasmids. With individual read classification, extraction of species-specific reads greatly facilitates *de novo* genome assembly. Viral assemblies are annotated and evaluated for SNVs, indels, ORFs, recombination, and phylogenetic analysis. We used our pipeline to examine fecal samples from 25-day old piglets with unexplained diarrhea. We identified and assembled a novel porcine *enterovirus (enterovirus G* EV-G) complete genome that contained substantial nucleotide differences (>30%) compared to known sequences. This virus also contained a novel non-structural protein, similar in sequence to the *Torovirus* papain-like cysteine protease (PLpro), which was likely acquired via a recombination event. EV-G is commonly found in swine from multiple Asian and European countries at very high prevalence (up to 90%), but only one other case of EV-G has been reported in the United States (which did not contain the novel PLpro sequence). We have developed a metagenomics sequencing approach for rapid pathogen detection and genome assembly that provides a new option for veterinary diagnostic investigation.

Poster Sat-28

A novel multiplex field deployable molecular assay for vesicular diseases of pigs

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There are several vesicular diseases of pigs which are difficult or impossible to differentiate clinically: Foot-and-Mouth Disease (FMD), swine vesicular disease (SVD), vesicular stomatitis (VS) and Senecavirus A (SVA). Vesicular diseases have become a major concern in the last several months since several SVA cases have been confirmed in the US.

For FMD testing two independent real-time RT-PCR assays targeting different regions (5'UTR and 3D) of the FMDV genome are routinely used.

For the development of the SVA assay Tetracore collaborated with South Dakota State University (SDSU) and the Pirbright Institute. SDSU assessed whether the genetic differences between current isolates and the isolates obtained prior to 2015 would affect the detection efficacy of the SVA diagnostic test in use at the South Dakota Animal Disease Research and Diagnostic laboratory (ADRDL). For this, the real-time PCR assay currently in use at the ADRDL was tested against several US SVA isolates obtained in 2015 and 2016 and against nine SVA isolates obtained between 1988 and 2002. The SVD assay is currently in validation.

In a field study in Africa the lyophilized FMD reagents and the T-COR8™, a portable rRT-PCR platform, were compared against the gold-standard laboratory-based rRT-PCR and alternative molecular technologies RT-LAMP and RT-RPA. In this ongoing study, a robust sample preparation method for serum, esophageal-pharyngeal fluid and epithelial suspensions were developed to negate the need for RNA extraction prior to rRT-PCR. The final rRT-PCR protocol and associated lyophilized reagents were field evaluated in three endemic settings (Kenya, Tanzania and Ethiopia), consistently detecting both clinical and subclinical FMD infections. The ability of the T-COR8 FMD rRT-PCR test to utilize simple sample preparation, amplification and detection methods offers promise for rapid in situ FMD diagnosis and demonstrates an important transition for FMDV-specific molecular assays into formats suitable for field diagnostic use.

The SVA assay which targets a conserved region of the SVA genome efficiently detected all SVA isolates. Results from the SDSU study provided an improved understanding of genetic diversity of contemporary SVA isolates recently associated with vesicular diseases in the US and in Brazil.

The availability of diagnostic assays allowing for rapid and specific (differential) detection and identification of FMD, SVA and SVD in swine naive populations is mandatory. Testing of suspect cases in field rapidly utilizing T- COR 8 field portable system will help considerably to contain the disease in the event of a future outbreak and to understand the rapid disease progression. Through the cooperation with Pirbright and IZLER we were able to determine and optimize the reagents to detect additional strains of SVD which would have not been detected otherwise. This emphasizes on the importance of worldwide validation during the development of an assay.

Poster Sat-29

A new generation of sample preparation for the purification of nucleic acid from a variety of sample matrices

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Magnetic bead-based isolation of nucleic acid from pathogens has been quickly adapted for veterinary diagnostic testing due to the easy adaptability of this technology to be used with high throughput testing. The MagMAX™ product line from Thermo Fisher has been used in many labs in conjunction with the KingFisher™Magnetic Particle Processor as a way to automate pathogen nucleic acid isolation with magnetic beads from a variety of sample matrices. Labs testing numerous sample types need a simplified workflow that will allow a single kit to be used for multiple sample types. In addition, many labs are also increasingly looking for a single sample preparation product that allows for the flexibility of testing applications beyond real-time PCR such as sequencing. The new MagMAX CORE Nucleic Acid Purification Kit was designed in order to meet these growing customer needs.

The MagMAX Core Kit was used to isolate pathogen nucleic acid from multiple types of animal samples infected with both RNA and DNA viruses. The results show improved recovery of pathogen nucleic acid from clinical animal samples compared to competitor kits based on real-time PCR detection. In addition, we used the MagMAX Core Kit to isolate genomic DNA from animal sources. The isolated genomic DNA was used for fragment analysis by capillary electrophoresis and sequence analysis by next generation sequencing. The data show that the new MagMAX CORE Nucleic Acid Purification Kit performs equally or better than currently available magnetic bead-based isolation kits for both real-time PCR and sequencing applications, and demonstrates that the quality of the isolated RNA/DNA was sufficient enough to be applied to a wide variety of molecular analysis techniques.

In conclusion, the new MagMAX Core kit efficiently isolates microbial RNA and/or DNA as well as genomic DNA from animal sources. Thus the MagMAX kit expands user applications beyond what was designed for many other nucleic acid isolation kits. All MagMAX CORE reagents were designed to be stored at room temperature, the wash solutions are pre-mixed eliminating the need to purchase additional isopropanol and ethanol, and the number of washes were reduced eliminating the need and expense of using two processing plates required for the current family of MagMAX kits.

Poster Sat-30

An IDEXX RealPCR modular panel for the identification of nucleic acid from common swine pathogens

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Real-time polymerase chain reaction (PCR) permits the rapid, sensitive and specific identification of nucleic acid of pathogenic organisms from diagnostic samples. Since many results can be obtained from a single sample, the ability to run a panel of reactions can make diagnostics fast and highly efficient. Counterproductive to this, many real-time PCR cycling protocols differ, thus requiring that reactions be run separately, or utilize test specific reagents (i.e., master mix or positive control). To this end, IDEXX has developed the RealPCR modular platform that allows the identification of nucleic acid of pathogenic organisms, using a single real-time PCR cycling protocol and a combination of target-specific primer/probe mixes paired with common master mixes and a single positive control. The following study was performed to demonstrate the ability of this identification to concurrently identify nucleic acid extracted from, but not limited to, the porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae* (Mhyo), porcine circovirus type 2 (PCV2), influenza A virus (SIV), and/or porcine epidemic diarrhea virus (PEDV) and porcine deltacorona virus (PDCoV). All reactions were performed from the same sample purification and run on the same real-time PCR 96-well plate. RNA targets utilized the RealPCR RNA master mix and likewise, DNA targets utilized the RealPCR DNA master mix. A single positive control containing all listed targets was used to ensure proper reagent functioning, and all reactions contained an internal control targeting either a sample-endogenous RNA or DNA target (internal sample control, ISC) or a spiked unique RNA or DNA target (IPC). Using commonly submitted swine diagnostic sample types of known disease status, a total nucleic acid extraction and purification was performed with the RealPCR DNA/RNA Magnetic Bead Kit. All purified material was used directly in real-time PCR or stored at -20°C until needed. Each reaction contained 10µL of target mix (specific for each nucleic acid target), 10µL of RealPCR DNA or RNA master mix, and 5µL of total purified sample nucleic acid, positive control, or water (as a negative control). A PCR cycling protocol was used that allows for both cDNA production as well as DNA amplification, regardless of the starting material. Results were auto-analyzed allowing the instrument software to determine threshold levels and Ct (crossing threshold) values. All results compared favorably to the known status of the sample.

Poster Sat-31

Serotyping of *Salmonella:* Comparison of Luminex xMAP assay, Check&Trace *Salmonella* kit, and serological method

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Salmonella are major foodborne pathogens, and only a subset of serotypes cause illness in human and animals. *Salmonella* serotyping is an essential and integral part of *Salmonella* surveillance and outbreak investigations. In this study, a total of 188 *Salmonella* isolates (strains) were blind-serotyped using the xMAP *Salmonella* serotyping assay (Luminex, Austin, TX) and Check&Trace *Salmonella* Kit (Check-Points, Wageningen, The Netherlands), and compared with the results obtained from National Veterinary Services Laboratories (NVSL, Ames, IA) and/or a commercial laboratory based on the traditional serotyping by agglutination test. The Luminex assay is a bead (microsphere)-based molecular serotyping method that simultaneously detects genes coding for serotype-specific antigens, and is designed based on 100 common *Salmonella* serotypes by testing 7-O Groups, 35-H antigens and 3 additional targets (*fljB, sdf, Vi*). The Check-Points assay is a microarray-based serotyping method that can identify over 300 serotypes through multiple single-nucleotide polymorphism loci across the genomes with a single test that can be done in one day. The pattern generated from the presence or absence of each locus (corresponding to spots in the Array Tube microarray) straightly interprets a specific serotype. A total of 25 serotypes were identified from the 188 *Salmonella* strains (ranging from 1~30 isolates per serotype). Check-Points detected 184 isolates that matched with traditional and/or Luminex results, and the additional 4 strains resulted in a unique microarray pattern that is translated by the software into "Genova 14959". This unique pattern is potentially a new Anatum variant for Check-Points database. Assuming the serotyping result is correct if it is detected and confirmed by at least 2 of the 3 methods, then the Check-Points assay's correct rate is 97.8% or 100% after the database is updated. Luminex detected 180 samples (95.7%) that matched to the traditional and/or Check-Points results, and the other 8 strains resulted in inconclusive dual serotypes. The traditional serological method detected 169 samples (89.9%) matched with the Luminex and/or Check-Points results, and the serotypes of 15 strains (mismatch rate 8.0%) did not match either Luminex or Check-Points results. The other 4 strains (2.1%) were not viable for agglutination testing. In general, the traditional *Salmonella* serotyping is time-consuming, labor-intensive, and its un-typable rate is relatively high. Both Luminex and Check-Points assays are nucleic acid-based, multiplexed, and high throughput detection methods. Our results demonstrated that molecular serotyping with Check-Points or Luminex assays are accurate and rapid alternatives to the traditional antigen-based method for *Salmonella* serotyping.

Poster Sat-32

Development of pen-side screening and surveillance tools ◊

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MRIGlobal, in collaboration with Kansas State University and with support from the Animal Research Services (ARS), and the Plum Island Animal Disease Center (PIADC), are developing a rapid, sensitive, portable, multiplex veterinary syndromic disease identification system. The workflow system will allow rapid multiplexed pen-side detection, providing advancing situational awareness during outbreaks and provide rapid tools to distinguish between diseases of every day importance in the swine and cattle industries. The developed system will provide end users the opportunity to demonstrate freedom from disease after an outbreak. The system will contain separate sample preparation and analysis/detection components. Sample preparation methods are in development for swine oral fluid, whole blood, vesicular fluid, serum and feces. Multiplex recombinase polymerase amplification (RPA) assays provide pen-side results in less than 15 minutes. Including sample preparation, the total turnaround time will be less than 1 hour for target detection. Four syndromic multiplexed assay panels are in development. The first panel includes USDA-regulated swine vesicular diseases (Seneca Valley A virus (SVV), Swine Vesicular Disease virus (SVDV), and Foot and Mouth Disease virus (FMDV)). Singleplex SVV detection was demonstrated by spiking live SVV into swine serum and oral fluid and extracted using the GeneReach taco™mini. Sample extraction of 8 samples was completed in less than 30 minutes. RPA detection of 50 PFU of SVV in serum and oral fluid was achieved in less than 8 minutes. Multiplex assay development is underway to combine the SVV assay with FMDV and SVDV assays.

Three other assay panels target diseases of concern to commercial industry (swine respiratory, swine diarrheal, and a cattle diarrheal) and have been developed with input from the USDA, The National Pork Board, The National Cattleman's Association and the National Milk Producers Federation. A multiplexed respiratory RPA panel has been developed which includes Porcine Reproductive and Respiratory Syndrome virus (PRRS), Swine Influenza A and *Mycoplasma hyopneumoniae.* In a multiplexed format detection of 31 target copies of Swine Influenza and 63 target copies each of PRRS and *Mycoplasma hyopneumoniae* was achieved in less than 10 minutes. The goal of this proof of concept project is to develop a field deployable multiplexed workflow system to support animal health management and monitoring programs to improve industry preparedness, disease response, and heighten U.S. biosecurity and biocontainment capabilities. The work is funded by the U.S. Department of Homeland Security Science and Technology Directorate (Contract No. DOI D15PC00281).

◊ USAHA Paper

Poster Sat-33

Evaluation of four molecular methods for identification and genetic typing of *Campylobacter* isolates from rhesus monkeys

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The genus *Campylobacter* contains pathogens causing a wide range of diseases, targeting both human and animals. Previously, fourteen bacterial isolates were preliminarily identified as *Campylobacter* species from screening of rhesus monkeys, *Macaca mulatta*. Currently, we conducted several analytical methods to determine identification and characterization of the *Campylobacter* isolates, based on genotype or phenotype such as 1) nucleic acid sequence analysis of 16S rRNA gene, 2) genetic fingerprinting using pulse field gel electrophoresis (PFGE), 3) fatty acid composition using MIDI Sherlock®, and 4) protein profiling using MALDI-TOF MS (Biotyper).

Most of them (13 out of 14) were identified to be *C. coli* and *C. jejuni* and the one remaining isolate was identified as *C. fetus*. Clustering analysis based on 16S rRNA genes and Biotyper peak pattern classified the isolates into three clusters, *C. coli*, *C. jejuni* and *C. fetus*, respectively. Among the 4 methods, the 16S rRNA gene sequence-based method and the protein profile-based method by Biotyper demonstrated best in agreement for the identification and genetic grouping of the primate *Campylobacter* isolates. The sensitivity and specificity of the Biotyper assay were 100% and 93%, respectively, compared to the 16S rRNA sequence method. However, other methods including MIDI showed a limited resolution because of a high genetic and biochemical similarity between *C. coli* and *C. jejuni*.

Overall, genetic method based on 16S rRNA gene sequence was still more reliable because nucleic acid sequences are highly conserved by microbial species, and application of MALDI-TOF MS into the diagnostic laboratory has significantly improved the possibilities of a highly accurate identification of *Campylobacter* species, saving time and cost compared to conventional methods.

Poster Sat-34

Survey of MG/MS infection in backyard avian species using real time PCR ◊

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Mycoplasma gallisepticum (MG) and *M. synoviae* (MS) alone or by co-infection can cause respiratory disease in chickens and some other avian species. 1,337 swabs were randomly collected from 1337 backyard birds and pooled into 294 samples. Each pool consisted of 1-5 swabs with the same species; the avian species including chicken, quail, duck, goose, guinea, peafowl, pheasant, and turkey. The original purpose of these samples were designed for the USDA program, AI/NDV surveillances. The DNA was extracted from 294 pooled samples and tested for MG and MS using real time PCR. Based on the PCR results, 176 samples were positive for either single infections alone or for co-infections, 84 (28.6%) MG positive, 158 (53.7) MS positive, and 66 (22.4%) samples with MG/MS co-infection. The positive species consisted of chicken, duck, guinea, peafowl, pheasant, and turkey.

In this survey, all birds did have obvious clinical signs. This data indicated that MG/MS are very common in backyard birds; and is more prevalent in backyard birds than in commercial poultry. This data also indicate that backyard birds may play a significant role in the prevalence and disease control of MG/MS.

◊ USAHA Paper

Poster Sat-35

A cost effective method for surveillance of PRRSV and influenza viruses A, B, C and D using newly developed multiplex rRT-PCR assays.

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The objective of this study was to develop cost effective methods for respiratory disease surveillance in swine oral fluid and respiratory swab samples, specifically for porcine reproductive and respiratory syndrome virus (PRRSV) and various types of influenza viruses (IV). There are four types of influenza viruses: A, B, C and D. In addition to influenza type A, swine are also infected by three other types of influenza viruses (B, C, and D). Individual real-time reverse transcription polymerase chain reaction (rtRT-PCR) tests for both PRRSV and influenza A virus in swine (IAV-S) are well established and are already commercially available. Here, we reported on the development of a panel of multiplex rtRT-PCR assays that detect the conserved regions of PRRSV and all four types of influenza viruses. This panel of novel assays was designed to provide cost efficient testing to the producer and promote the continued surveillance for both PRRSV and influenza viruses. The characterization of circulating influenza virus types will provide critical information about the potential health risk these zoonotic influenza viruses pose to humans, in addition to developing a guide for strain selection for vaccine production. Screening for various types of influenza viruses by rtRT-PCR is a first but vital step in surveillance. The mechanism by which PRRSV interacts with other pathogens, such as influenza viruses, is still being explored but it is believed that these interactions can increase the severity of pathogenicity. The producer will see cost savings when using the multiplex testing versus the singleplex. In conclusion, having well validated and rapid diagnostic tools such as these new multiplex rtRT-PCR assays will be vital for continued swine health and production while enhancing the One Health Initiative.

Poster Sat-36

Optimal swab location for detection of *Mycoplasma synoviae*, *Mycoplasma gallisepticum*, and infectious laryngotracheitis virus via real-time PCR * †

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Mycoplasma synoviae (MS), *Mycoplasma gallisepticum* (MG), avian influenza (AI), and infectious laryngotracheitis virus (ILTv) are economically significant respiratory pathogens affecting chickens. There is increasing need for rapid detection of all relevant respiratory pathogens in order to properly diagnose flocks suspected of disease. Recently, our laboratory has tested the effect of swab collection sites for detection of MS and MG via real-time PCR. Three respiratory sites, trachea, choanal cleft, and oropharyngeal, were tested and choanal cleft swabs were determined to be best overall for detection of the two species in terms of both average Ct value and percentage of positive results; oropharyngeal swabs were significantly less sensitive for detection. Typically, tracheal swabs are taken for MS, MG, and ILTv detection, while oropharyngeal swabs are taken for AI. In an attempt to standardize collection for these poultry respiratory pathogens, we expanded this study by determining the ideal swab collection site for ILTv detection; additionally, we established the amount of collagen from each sample site to verify successful swabbing. The birds from the previously mentioned study had been co-infected with the Zoetis LaryngoVac ILT vaccine five and six days post MS or MG inoculation, respectively. Approximately one week post vaccination, the birds were swabbed in the three sites in random, rotating order. Swabs were prepped in PBS and DNA extracted via the ABI MagMAX Express-96 Partical Processor. Finally, the extracted DNA was run on the ABI 7500 Fast Real-time PCR System using a previously developed duplex qPCR for detection of ILTv and collagen. Most of the results for ILTv were negative. However, for the positive results, choanal cleft appeared to be the best site with 14/41 (34%) positive; compared to tracheal and oropharyngeal swabs with 5/41 (12%) and 4/41 (10%) positive, respectively. Both MS and MG had similar results with tracheal swabbing giving the lowest mean Ct value (strongest positive) and choanal cleft showing the highest percent positive across sites. As a whole, choanal cleft is the optimal site for MS, MG, and ILTv detection. Determining if a swab of a single location will be adequate to successfully detect each of these pathogens will improve diagnostic testing in terms of time and money and will enhance animal welfare and ease of sampling for poultry growers.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Poster Sat-37

Development of a triplex real-time RT-PCR assay for simultaneous detection and differentiation of Influenza B, C, and D viruses in swine and cattle

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Influenza is a highly contagious viral respiratory disease caused by influenza A, B, C, and/or D viruses (IAV, IBV, ICV, and IDV). IAV is the most common pathogen, and previously was assumed to be the only influenza virus that could infect swine and cattle. Recent evidences demonstrate that ICV and the newly discovered IDV can also infect swine and cattle, and IBV can infect swine. Serological evidence indicated IBV may also affect cattle. Timely diagnosis of influenza viruses is important for prevention and intervention. IAV in human and animals has been well studied and various mature diagnostic assays are available. In this study, a high-coverage, high-throughput, low-cost, multiplex, single-step, TaqMan real-time RT-PCR assay was developed and validated for simultaneous detection of the under-studied and under-diagnosed IBV, ICV, IDV, and efficient differentiation of the four influenza viruses in swine and cattle. Specific real-time PCR primers and probes were designed to target the most conserved gene regions upon the analyses of all available and unique full- or near-full segment sequences of IBV, ICV, and IDV. Primers and probe for the house-keeping gene 18S rRNA were designed to serve as an internal control to monitor RNA extraction efficiency and RT-PCR efficiency. Cloning primers flanking the real-time PCR target regions were designed, and positive control plasmids harboring the real-time PCR target regions were constructed. Both the plasmid DNA samples and the *in vitro* transcribed RNA samples from the respective plasmids were used to determine the analytical sensitivity/limit of detection (LOD). The assay coverage rate (perfect matches of all real-time PCR primers and probe) of IBV qPCR is 95.8% over 5,261 Matrix sequences, ICV qPCR is 99.4% over 157 Matrix sequences, and IDV qPCR is 100% over 23 PB1 sequences. The uniplex and triplex RT-qPCR protocols were optimized and validated for the identification of IBV, ICV, and IDV, with PCR efficiencies (E) 90%~110%, correlation coefficients (R^2) >0.99, and LOD around 10 copies. The triplex assay was also highly specific in detecting one or more of the influenza viruses in various combinations without cross-reactivity, including 11 known virus isolates of IBV, ICV, and IDV, 18 known positive IDV clinical samples, and more than 50 IAV positive samples. A new ICV strain was identified with the new assay and confirmed by sequencing. More validation data will be generated and presented. We are also in the process of adding current USDA IAV real-time RT-PCR assay into the newly established triplex real-time RT-PCR assay, to form a multiplex real-time PCR panel assay for rapid detection and differentiation of all four influenza viruses. (*Supported by SHIC grant #16-255).

Poster Sat-38

Serotyping and selection of *H. parasuis* field isolates for vaccine formulation using whole genome sequencing

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Haemophilus parasuis is the causative agent of Glässer's disease in swine that causes pneumonia, meningitis, polyserositis, and polyarthritis in young pigs. Pigs are usually most susceptible to *H. parasuis* infection from shortly after weaning to about four months of age. Fifteen serovars of *H. parasuis* have been described and the capsular loci of strains from each serotype have been elucidated and described previously. Serovars 4 and 5 appear to be most commonly associated with clinically significant disease. However, other serotypes are frequently isolated from sick animals and several studies have demonstrated that pathogenicity is not strictly linked with serotype. Serotyping has traditionally been accomplished using a panel of serovar specific antisera. However, many isolates remain non-typeable using this method. In addition to serotyping, methods that utilize multi-locus sequence typing and characterization of virulence associated trimeric autotransporter genes have been used to characterize *H. parasuis* strains. The use of these latter two methods to clearly differentiate virulent and avirulent strains has been a subject of debate. However, the presence of virulence genes has not been shown to be associated with immunogenicity or protection and the use of these methods to select isolates for vaccine design may prove to be misleading. Commercial vaccines are available and are effective against disease caused by serovars 4 and 5. However, there are gaps in the immunity leading to susceptibility. Autogenous vaccines are used to protect against *H. parasuis* serotypes that evade immunity induced by commercial vaccines. In order to make efficacious autogenous vaccines it is imperative to determine the serotype unequivocally. Unfortunately, the current methods are ambiguous and it has been suggested that an effective vaccine should include all serotypes isolated from a farm. In the present study, we used whole genome sequencing (WGS) to characterize the capsular loci of several *H. parasuis* field strains. We also submitted many of these isolates for serotyping using antisera specific for serotypes 4, 5, 12, 13, and 14. With the exception of serovars 5 and 12 which bear very similar capsular loci, we found that the structure of the capsular locus could reliably identify the serotype of our isolates. For those isolates that were not serotyped using reference antisera we were able to assign a serotype by map-based comparative genomic analysis. We found that many of these strains had loci identical in structure to less commonly isolated serovars such as 1, 2, and 7. An additional advantage to WGS based analysis is the ability to query the genome of each strain for the presence of bonafide or putative virulence factors and antibiotic resistance genes. This may allow further refinement in the selection of strains from within each serotype for use in vaccine formulation.

Poster Sat-39

Metagenomics for infectious disease research: Pre-sequencing, sequencing, and post sequencing considerations for clinical application

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Next generation sequencing (NGS) based microbial genomics and metagenomics appears to be paramount to 21st century approaches to rapid microbial detection and precise sub-typing of pathogens, commensal microbiota, polymicrobial community, and/or co-infections. It offers unique opportunity to leverage whole genome sequence information to investigate the complete genetic make-up of microbial pathogens to understand evolution, pathogenesis, clonal transmission, and provides opportunity to comprehensively profile hundreds of species in a sample and even discover entirely new infectious agents. Recent advancement in sequencing throughput and turnaround time, automation in nucleic acid extraction and library preparation, and significant advancement in developing ultrafast bioinformatics package (MetaGen) and curated genome databases (GenBook) make this technology highly attractive for infectious disease detection, biosurveillance, emerging and remerging pathogen discovery, polymicrobial infection dynamics, hospital acquired infections and outbreak investigation. However, there is a need for optimization of pre-sequencing, sequencing, and post-sequencing steps in metagenomics workflow as each directly affects the accuracy and precision of the microbial community representation, as well as the ability to make an actionable and timely diagnosis. In pre-sequencing, the various wet laboratory methods currently employed for extraction/library preparation and the need to avoid laboratory contaminants must be considered if accurate and reproducible results are to be obtained for clinical diagnosis. In sequencing, differences among the various NGS platforms must be considered; such as distinct error profiles and sequencing read length, depth and turnaround time. Robust bioinformatics tools that can be seamlessly integrated into multi step workflows are required to address effectively the various challenges and concerns in utilizing metagenomics in complex clinical microbiology and infectious disease diagnostics. Bioinformatics tools must be sensitive, specific, and rapid if they are to be used to obtain actionable results for diagnostic and therapeutic purposes. We have developed MetaGen, a powerful system with informatics tools that address the concerns outlined above, that is, able to tolerate diverse sequencing error rates and employ highly curated databases with greater breadth and depth than comparable databases that are publically available. We have efficiently integrated MetaGen into a clinical metagenomics workflow that includes both pre-sequencing and sequencing methods. The presentation will demonstrate some valuable features that make this technology very attractive and powerful for simultaneous detection and characterization of infectious agents and showcase some examples of how MetaGen is employed today and its future potential in clinical metagenomics will be provided.

Poster Sat-40

Development of quantitative real-time PCR assays to detect *Mycobacterium* spp. in Zebrafish (*Danio rerio*)

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Mycobacterium spp. infections are common in zebrafish kept in research facilities. These comorbidities can substantially modulate the responses of these fish to external and internal stimuli. Therefore, diagnostic tests to detect *Mycobacterium* spp. infections in zebrafish colonies prove essential. Here, we outline the development of quantitative simplex real-time PCR assays to detect the 3 *Mycobacterium* species most commonly identified in laboratory zebrafish. The assays targeted the heat-shock protein 65 gene of *M. marinum*, *M. chelonae*, and *M. haemophilum*. The assays are both highly specific and sensitive for fresh-frozen samples and highly specific and moderately sensitive for formalin-fixed paraffin-embedded (FFPE) samples. Two sampling techniques for FFPE samples of sagittally sectioned zebrafish were evaluated. Both paraffin cores targeting granulomas containing bacteria and scrolls from the entire fish yielded DNA of equivalent quantity and purity. The diagnostic sensitivity of cores was superior to that of scrolls for *M. chelonae* and *M. haemophilum* but not *M. marinum*. The assays are cost-effective and ideally suited to diagnosing common *Mycobacterium* spp. infections in zebrafish.

Poster Sat-41

Untargeted metabolomic analysis of porcine serum and oral fluid following intramuscular administration of a porcine reproductive and respiratory syndrome virus (PRRSV) modified-live virus (MLV) vaccine

Aislinn Anne Pomfret[1], David J. Borts[2], Jeff Zimmerman[2], Yuly A. Henao Diaz[2], Luis Gabriel Gimenez-Lirola[2], Ting-Yu Cheng[2]

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First identified in 1991, porcine reproductive and respiratory syndrome virus (PRRSV) remains an economically important cause of reproductive failure in breeding stock and respiratory illness and death in young pigs. Understanding the pathogenesis of PRRSV and devising methods for controlling its effects remain active fields of study. In this experiment, an untargeted metabolomics approach was used to detect small molecule biomarkers associated with the replication of PRRSV MLV vaccine. Serum and oral fluid samples from 12 pigs collected prior to and following vaccination were analyzed. Samples were extracted by protein precipitation and analyzed using ultra-high-pressure liquid chromatography (UHPLC) coupled with high resolution orbitrap mass spectrometry. Multivariate statistical analyses revealed well-separated clusters between the pre-vaccination and post-vaccination groups in both serum and oral fluid specimens. Variable importance in projection (VIP) plots were used to rank metabolites for their ability to discriminate between these two groups. Receiver-operator characteristic (ROC) curves were calculated to determine the quality of biomarker sets. The resulting ROC curves suggested that the putative biomarkers identified in this study might form the basis of an effective diagnostic test for detecting PRRSV replication. Additional work will focus on metabolite identification, identification of small molecule biomarkers of PRRSV infection, and on potential diagnostic applications, e.g., discrimination between PRRSV vaccinated vs wild-type infected animals and detection of persistently infected animals.

Poster Sat-42

VetMAX pathogen detection kits demonstrate equivalent performance when run on the QuantStudio 5 and 7500Fast Real-Time PCR Systems

Robert Sterling Tebbs, Michelle Swimley, Denisse Meza, Angela Burrell, Quoc Hoang, Adam Allred, Derek Grillo, Rick Conrad

Animal Health and AgriGenomics, Thermo Fisher Scientific, Austin, TX

The Applied Biosystems™ 7500 Fast Real-Time PCR System is commonly used by veterinary diagnostic laboratories for pathogen detection. The Applied Biosystems™ VetMAX™ Gold pathogen detection kits were certified by the USDA for animal disease diagnosis on the 7500 Fast. We sought to verify that the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System performance would be equivalent to the 7500 Fast Real-Time PCR System in terms of providing equivalent diagnostic calls for a diverse set of common veterinary samples. The QuantStudio uses white LED as an excitation source whereas the 7500 Fast uses a halogen lamp, and both instruments use Peltier devices for temperature cycling. The optical performance between the two platforms is equivalent and the data analysis algorithms are designed to show equivalent results.

Eight VetMAX Real-Time PCR Pathogen Detection Kits were evaluated in this study. The kits included the Applied Biosystems™ VetMAX™-Gold SIV Detection Kit, the Applied Biosystems™ VetMAX™-Gold SIV Subtyping Kit, the Applied Biosystems™ VetMAX™-Gold AIV Detection Kit, the Applied Biosystems™ VetMAX™-Gold Trich Detection Kit, the Applied Biosystems™ VetMAX™-Gold BVDV PI Detection Kit, the Applied Biosystems™ VetMAX™-Gold MAP Detection Kit, and the Applied Biosystems™ VetMAX™ NA and EU PRRSV Reagents. Porcine nasal swabs, porcine oral fluids, bovine ear punches, bovine smegma, bovine feces, and avian oropharyngeal swabs were prepared using the Applied Biosystems™ MagMAX™ Pathogen RNA/DNA Kit to extract nucleic acids. A portion of the sample extracts were spiked with control nucleic acid to concentrations ranging between 40 and 2,000,000 copies/reaction. Ten spiked samples and 10 non-spiked samples were tested in triplicate. Real-time PCR solutions were prepared according to the manufacturer's instructions and split between two 96-well plates. One plate was loaded onto the 7500 Fast instrument and one plate was loaded onto the QuantStudio 5 instrument. The results showed 100% positive/negative concordance for detection of pathogen nucleic acid. Results from both instruments demonstrated 100% sensitivity and 100% specificity. The two instruments showed equivalent Cts for each sample set (<0.5 ΔCt).

In addition we tested a new offering, the Applied Biosystems™ VetMAX™ PEDV/TGEV/SDCoV Kit, on both Real-Time PCR systems for LOD, efficiency, and Ct calls. The PEDV/TGEV/SDCoV assay is a complex assay that combines four individual assays into a single tube to detect 3 porcine coronavirus targets and an internal positive control. LOD, efficiency, and average Cts were equivalent between the two instruments.

This study demonstrates the 7500 Fast and QuantStudio 5 platforms give equivalent results for a variety of assays and sample matrices used for animal pathogen detection. This equivalency in performance coupled with the additional features offered with the QuantStudio 5 make it the new instrument of choice for veterinary diagnostic testing.

Sunday Poster Session

Sunday, October 15

Town and Country

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Symbols at the end of titles indicate the following designations:

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

Poster Sun-1

The eastern coyote: Missing link? ◊

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Sylvatic (genotype 8; G8) *Echinococcus granulosus* (EG) was identified during 2012 in the form of lung cysts in Maine moose, which can act as an intermediate host for this cestode parasite. The definitive host, in which the adult cestode reproduces, has been reported to be the wolf (*Canis lupus*). Since wolves are not thought to be present in Maine, a more likely definitive host is the Eastern Coyote (*Canis latrans x Canis lycaon*), due to its relatively large size compared to western coyotes (*Canis latrans*), diverse diet, and the fact that it has been reported to predate upon large game (i.e. deer, moose). Alternatively, domestic dogs might be implicated in the spread of EG in Maine moose. The goal of this study, conducted in collaboration with investigators at the University of Saskatchewan, was to assess whether the Eastern Coyote in Maine is a definitive host of the sylvatic form of EG. With the assistance of Maine IFW, enteric tracts of coyotes (n= 28) trapped or hunted in Maine's Northern Wildlife Management Districts (WMD) were collected by legally permitted hunters and trappers. Tracts were frozen for 14 days at -80 degrees C to inactivate tapeworm ova. Thawed tracts were sectioned, immersed in water, and the mucosa was scraped. The water was then filtered with 850 μm, followed by 212 μm pore size sieves. Parasitic worms in the filtrate were morphologically identified, imaged, and fixed in ethanol. A variety of cestodes and trematodes were found in most intestinal tracts. Based on morphologic characteristics, EG were detected in coyotes collected in three of the six Maine WMD studied. Based on morphology of the adult cestodes collected, it appears that the coyote is a definitive host for the sylvatic strain of *E. granulosus* G8 in Maine moose. DNA from adult cestodes collected in this study will be sequenced by PCR for the mitochondrial CO1 gene to confirm that EG is found in Maine coyotes.

◊ USAHA Paper

Poster Sun-2

A novel application of enzyme-linked fluorescent assay for detection of veterinary *Toxoplasma gondii* infections in multiple host species

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[1]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN; [2]Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN

Toxoplasma gondii is a global zoonotic protozoan parasite of endothermic animals. In addition to causing clinical disease in domestic and companion animals, this parasite threatens captive exotic and wild animals. Due to the consequences of congenital infection by this parasite, several methods for diagnosing *T. gondii* infection in humans are widely available. In contrast, veterinary detection relies primarily on serologic methods, most requiring species-specific conjugates. Our lab has adapted the modified agglutination test (MAT) for simultaneous IgG and IgM detection in warm-blooded animals, testing more than 18,000 samples over 30 years. The commercially produced MAT is ideal in veterinary testing due to the nonspecific reagents suitable for testing multiple host species. However, the federally regulated reagents require special import permits and the necessary tachyzoite propagation remains challenging. To expand the diagnostic capabilities of our lab, we have validated the novel application of an automated platform and assay developed for human diagnostics. Using 312 previously MAT IgG antibody tested frozen serum samples (Negative=154; Positive=158), we evaluated the miniVIDAS® and TOXO Competition (TXC) assay (bioMerieux) for comparison. Representative animal groups included our most common clinical and research submissions: canine (n=62), feline (n=63), macropod (n=62), primate (n=61), and aquatic mammal (n=64). Non-parametric analyses evaluated interrater agreement, homogeneity, and performance between the two assays for combined samples and by stratified animal groups. Overall, TXC was an excellent test compared to the MAT for combined animal groups. Stratified analyses indicated that each canine, feline, macropod, and primate animal group had near perfect or perfect agreement with excellent performance. Among the aquatic mammals, the TXC had a poor to fair performance depending on the species. In this study, we determined that the novel use of the TXC assay has excellent overall performance for IgG detection, particularly in canines, felines, macropods and primates. However, additional work is required to validate this technique for aquatic mammals. This assay represents a valuable tool for detection of *T. gondii* in companion animals, captive exotics, and wildlife.

Poster Sun-3

East meets west: Establishing a veterinary diagnostic laboratory in Hong Kong

Fraser Ian Hill

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In 2008, City University of Hong Kong (CityU) began the process of establishing a School of Veterinary Medicine (SVM) in collaboration with Cornell University. The vision of SVM is to create a centre of excellence in veterinary education and research under the One Health paradigm to enhance public health, food safety, animal care and the prevention and control of infectious diseases in Hong Kong and the region. As part of this process, a veterinary diagnostic laboratory was established to provide diagnostic services to local veterinary practices and animal health facilities in Hong Kong. After incorporation as a company, the laboratory was set-up as a for-profit business owned by City U. In addition, laboratory diagnostics will be taught to veterinary students in their clinical years of training and research project collaboration undertaken.

This presentation will describe the process of developing a full-service veterinary diagnostic laboratory in a new veterinary school in Hong Kong at the interface of western and eastern cultures.

Poster Sun-4

Utilization of the "squash-prep" technique paired with molecular testing to confirm *Angiostrongylus cantonensis* migration as a cause of eosinophilic meningoencephalitis in southeastern wildlife # + * †

Martha Frances Dalton[1,2], Heather Margaret Anne Fenton[1], Michael Yabsley[1,3], Christopher Cleveland[1], Elizabeth Jane Elsmo[1,2]

[1]Southeastern Cooperative Wildlife Disease Study, Athens, GA; [2]Department of Pathology, University of Georgia, Athens, GA; [3]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

Angiostrongylus cantonensis (the rat lungworm) is a nematode that is capable of causing severe neurological disease in both animals and humans. *Angiostrongylus cantonensis*, the rat lungworm, was diagnosed in two nine-banded armadillos (*Dasypus novemcinctus*) and one Virginia opossum (*Didelphis virginiana*) from the southeastern United States. A combination of manual dissection, histological examination, and molecular techniques were utilized to arrive at the final diagnosis. Histological findings in all three cases included eosinophilic meningoencephalitis with variable numbers of nematode larvae in the meninges or the neuroparenchyma. Other histological findings included eosinophilic granulomas in the large intestinal submucosa or in the adventitia of the pulmonary artery. Nematode adults and larvae were manually dissected from the brain tissue using the "squash prep" technique in 2/3 cases. The "squash prep" method includes viewing sections of fresh tissue pressed between two glass plates with a trans-illumination light source to detect nematodes present in the tissue. Nematodes are typically less translucent than the pressed tissue, thus they are often discernible. Nematodes can then be extracted and viewed under dissecting and compound microscopes for morphological identification. This method is a convenient and cost-effective tool that can be utilized in suspected cases of nematode migration. The diagnosis was confirmed by amplification and sequence analysis of the partial cytochrome c oxidase subunit I gene from brain tissue of all three cases. Sequences (704bp) from the two cases from Louisiana were identical and 99.7% similar to worms from an armadillo from Florida. To the author's knowledge, this is the first report of *A. cantonensis* as the cause of neurological disease in an armadillo and provides additional documentation that this nematode can cause disease in wildlife species in the southeastern United States. *Angiostrongylus* spp. are an important cause of eosinophilic meningitis in humans worldwide, thus this parasite is gaining recognition as an emerging zoonosis. This parasite is now endemic in the southeastern US, thus *A. cantonensis* is an important differential for any wild or domestic animal with neurological signs. Increasing awareness of the parasite among biologists, veterinarians, state wildlife agencies, and pathologists will hopefully lead to more accurate documentation of cases and increase public awareness.

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Poster Sun-5

Venoocclusive disease in captive cheetahs (*Acinonyx jubatus jubatus*) # + * †

Rahul Babulal Dange, Leslie Willis Woods, Asli Mete

California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, University of California, Davis, Davis, CA

Veno-occlusive disease of the liver (VOD), an uncommon disease in mammals, is a common life threatening disease of captive cheetahs. Histopathologically VOD is characterized by partial or complete occlusion of the centrilobular or sublobular hepatic veins due to spindle cell proliferation, centrilobular to bridging fibrosis and fibrosis of space of Disse. Previous studies speculated that early lesions are due to subintimal smooth muscle-like proliferation progressing to the chronic stage of fibrosis in the intima and wall of central veins and positive correlation of hypervitaminosis A and VOD in captive cheetahs. The aim of this study was to better characterize the histologic lesions associated with VOD while using smooth muscle actin (SMA) immunohistochemistry (IHC) and Masson's trichrome to evaluate the progression of disease, as well as to investigate the correlation between hypervitaminosis A and VOD. A male and a female cheetah aged 14. 5 and 15 years, respectively, from a private wildlife preserve were euthanized due to anorexia and weight loss with poor prognosis and submitted for autopsy to the California Animal Health and Food Safety lab, Davis. On gross exam, both cheetahs were in very thin body condition and the mucous membranes as well as the generalized subcutis and fatty tissue were icteric. The peritoneal cavity contained 1.5 - 3 liters of milky to pale tan-yellow opaque fluid consistent with ascites, chylous effusion. The livers were diffusely firm, with irregular, bosselated capsular surfaces and few overlying fibrin strands. On cut surface, the hepatic parenchyma was composed of multifocal to coalescing pale, miliary nodular areas. On histopathology, liver of both cheetahs had severe generalized centrilobular and bridging fibrosis, fibrosis of space of Disse, central vein occlusion with spindle cell proliferation and rare recanalization, with mild hepatocellular necrosis, consistent with VOD. Immunopositivity for SMA was observed in small numbers of proliferated cells in the thickened subintima of central veins and space of Disse. Majority of areas of thickening in and around the obliterated central veins and space of Disse did not stain with SMA but did stain with Masson's trichrome, consistent with fibrosis that indicate the advanced stage of the venoocclusive disease in both cheetahs. Both cheetahs had low vitamin A levels in the livers which was not in agreement with previous reports of excessive liver and dietary vitamin A in captive cheetahs with VOD. This study concludes that histopathology, SMA IHC and Masson's trichrome may be effectively used to assess the stage of hepatic disease in VOD and it is proposed to use this method effectively to evaluate cheetahs with nonspecific clinical signs of hepatic disease.

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† Graduate Student Oral Presentation Award Applicant

Poster Sun-6

Pituitary abscesses in four free-ranging white-tailed deer (*Odocoileus virginianus*)

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Intracranial abscess syndrome is a well-recognized cause of morbidity and mortality in white-tailed deer (*Odocoileus virginianus*). While numerous cases of intracranial abscesses have been described in white-tailed deer, reports of intracranial abscesses limited to the pituitary gland, sella turcica, and basosphenoid region are lacking. Unlike intracranial abscess syndrome of white-tailed deer, which is generally associated with extension from antler pedicle lesions and is almost exclusively a disease of bucks, half of the white-tailed deer with pituitary abscesses in this case series were does. Aerobic bacterial culture of pituitary lesions isolated a number of opportunistic pathogens, including *Trueperella pyogenes*, *Streptococcus* sp. and *Staphylococcus* sp.. Two of the affected animals had concurrent bilateral retrobulbar abscesses with suppurative keratitis, and three deer had concurrent suppurative skin wounds. As is suspected to be the cause of pituitary abscesses in cattle, bacteremia should be considered a possible predisposing factor in white-tailed deer. Clinical features of pituitary abscesses in deer are similar to those observed in cattle and include ataxia, lack of fear of humans, hypersalivation, foaming at the mouth, dysphagia, and blindness. Pituitary abscesses are a potentially overlooked cause of neurological disease in white-tailed deer, with clinical symptoms that may mimic other diseases of significant concern, such as rabies infection.

Poster Sun-7

Agarose gel electrophoresis determination of bovine lipoprotein fractions compared to a wet chemistry method

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Quantification of serum lipoproteins provides information relative to the overall metabolic health, degree of lipid mobilization, and hepatic function of a cow. Lipoproteins are macromolecular complexes composed of a triacylglycerol and cholesterol ester core surrounded and stabilized by phospholipids, unesterified cholesterol, and proteins. The major classes of lipoproteins in the cow are separated by density and include (from least to most dense): chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). The gold standard method of quantification of lipoproteins is ultracentrifugation, which is time-consuming, laborious, and impractical for routine diagnostic testing. As such, many investigators and clinicians have relied on the use of automated commercially-available assays developed for the quantification of human lipoproteins. While the major classes of lipoproteins are similar across species, the protein and lipid composition of the lipoproteins is not. Consequently, there is a high probability for generation of inaccurate results using these assays in non-human species and a need to validate them for use in each species of interest. In this study, we prospectively analyzed bovine serum lipoproteins from 56 Holstein cows using horizontal slab agarose gel electrophoresis to quantify both the HDL and LDL fractions by densitometry. These values were compared to values obtained by direct measure of HDL cholesterol, total cholesterol and triglyceride (TG) concentrations on a Roche Hitachi Module P benchtop chemistry analyzer, and calculation of LDL cholesterol. Ultracentrifugation was used to confirm the electrophoretic separation pattern of the lipoproteins. Correlation between these methods was poor for HDL (Passing-Bablok regression line: $y = 30.31 + 0.853x$) and could not be calculated for LDL due to automated HDL values that were equal to, or higher than, the total cholesterol concentration in 25 of the 56 samples. In all samples, LDL was detected by electrophoresis (average of 18% of all lipoproteins, SD = 0.8%, n=56). Additionally, 18 samples had TG concentrations above the reference interval and these samples had an average of 96% of the cholesterol measured as HDL by the automated method, and 78% of their lipoprotein content measured as HDL by electrophoresis. Triglyceride concentrations were moderately correlated with LDL percentage as determined by electrophoresis (Spearman correlation coefficient = 0.63). Given that it is physiologically impossible to have more cholesterol within the HDL fraction than in the total serum fraction, and the increased proportion of triglyceride that is found in LDL and VLDL, our results draw into question the accuracy of automated assays in quantifying bovine lipoprotein fractions.

Poster Sun-8

Sarcocystis calchasi outbreak in free-ranging rock pigeons (*Columba livia*) in California

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Increased intake of rock pigeons (*Columba livia*) by wildlife rehabilitation centers in the San Francisco Bay Area were reported to the Wildlife Investigations Laboratory, California Department of Fish and Wildlife (Rancho Cordova, CA) between March and May 2017. Clinical signs of sick pigeons included head tilt, ataxia, and difficulty flying and standing. Four adult rock pigeons with neurologic disease were submitted to the California Animal Health and Food Safety Laboratory (Davis, CA) for post-mortem examination. At autopsy, the birds were in fair to poor body condition, two were male and two were female, all with inactive gonads, and no bursas. Gross examination revealed non-specific findings such as generalized dark tissues consistent with hyperemia/congestion (3), mycotic pneumonia (1), proventricular glandular hemorrhage (1), and splenomegaly (2). On histopathological examination, all birds had severe granulomatous to lymphohistiocytic meningoencephalitis, rare heterophils, with malacia and adjacent neuronal necrosis. Gram and silver stains were negative on the brain sections. In two birds, there were numerous protozoal cysts in the pectoral and thigh muscles and myocardial sarcoplasm with associated severe granulomatous myositis and myonecrosis. In one bird, there was focally extensive granulomatous myositis in one skeletal muscle section with no protozoal cysts visible. Pigeon paramyxovirus-1 and West Nile virus were ruled out by PCR and the brain lesions were negative for *Sarcocystis falcatula* and *S. neurona* by immunohistochemistry. Polymerase chain reaction was conducted on brain tissue using 16S-23S ITS region and the amplicon sequence was most similar to *S. calchasi.* Pigeon protozoal encephalitis, caused by *S. calchasi,* is reported here for the first time to cause an outbreak of morbidity and mortality in free-ranging rock pigeons in California and should be monitored closely as an emerging pathogen, especially for native columbids.

Poster Sun-9

Cutaneous and renal vasculopathy in a Greyhound dog (*Canis familiaris*)

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A 12-year-10-month-old, male, Greyhound dog presented acute subcutaneous hemorrhage, renal failure and died later. Several renal medullary blood vessels, glomerular capillaries and dermal and subcutaneous blood vessels contained thrombi. The dermis was expanded by edema, hemorrhage, scattered macrophages, lymphocytes, plasma cells and reactive fibroblasts. The inflammatory cells were often observed in perivascular areas (vasculitis). The clinical disease and histopathology is consistent with idiopathic cutaneous and renal glomerular vasculopathy, a form of thrombotic microangiopathy also referred to as “Alabama rot” or “Greenetrack disease”. It is a potentially fatal disease of unknown etiology most commonly observed in young adult racing and training greyhounds and rarely also in other dog breeds. Although cutaneous lesions characterize the disease, four different forms of clinical signs were recorded. A complex pathogenesis was suspected in which genetic predisposition may play a prominent role. This is an interesting case in that diagnosis of the disease is challenging clinically, the underlying cause is unknown and histologically the lesions may vary from case to case as different manifestations of the disease may be observed.

Poster Sun-10

Histopathologic features and smooth muscle actin immunoreactivity in a case of feline restrictive orbital myofibroblastic sarcoma (FROMS)

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Feline restrictive orbital myofibroblastic sarcoma (FROMS) is a rare malignancy of the orbit and adnexa which can be easily mistaken for fibroplasia if the clinician and pathologist are not aware of the entity. Here we present a case of FROMS which illustrates diagnostic features in the adnexa relative to the anterior globe. A 13 year old male neutered domestic medium haired cat presented for a chronic non-healing corneal ulcer and had clinical findings of unilateral ocular discomfort and a thickened superior eyelid which was retracted superiorly with tarsal margin contraction and fixation with lagophthalmos. Chronic keratitis was attributed to lagophthalmos, and the globe and pupil were fixed. The eye was visual, and trigeminal and facial nerve function were intact. Enucleation was performed and the globe, adnexa, and retrobulbar tissue were submitted for biopsy with a suspicion of FROMS indicated in the submission. Histopathologically there was no suggestion of a mass. A poorly demarcated, insidious spindle cell proliferation was arranged in vague streams and bundles, and disrupted adnexal structures including Muller's muscle, adipose and native collagen. A marked chronic keratoconjunctivitis was also diagnosed, whereas retrobulbar tissues were well-differentiated skeletal muscle. FROMS was suspected based on morphology and the diagnosis was confirmed with immunochemistry demonstrating smooth muscle actin expression by the proliferating spindle cells. This case demonstrates diagnostic features in a subtle case of FROMS and emphasizes the importance of including this entity in the differential diagnosis of spindle cell lesions in the feline orbit, periocular tissues, adnexa, and lips.

Poster Sun-11

Small intestinal adenomatous polyps resulting in chronic obstruction in a 3-year old Quarter horse gelding

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A 3-year-old, Quarterhorse gelding was admitted to Kansas State University Veterinary Health Center with a primary complaint of colic. The horse had a 3-month history of recurrent colic and progressive weight loss. On physical exam the horse was quiet alert and responsive. The horse's mucous membranes were pink and capillary refill time was 2 seconds his rectal temperature was 99.6 F (reference range (rr 99-101.5 F)), heart rate 56 beats/min (rr 28-44 bpm) and respiration rate 16 brpm (rr 2-40 breaths/min). Rectal palpation revealed an approximately 5 cm dilated, firm, tubular, mass traversing from left caudal abdomen to mid abdomen. Nasogastric intubation obtained 6L net reflux. A 5 cm diameter small intestinal intraluminal mass was detected on abdominal ultrasound. Complete blood count, serum chemistry, and peritoneal fluid analysis were performed and all results were unremarkable. The horse was humanely euthanized due to pain and financial constraints. Postmortem examination and histopathology revealed a 15 x 5 x 5 cm polyp with multiple smaller satellite polyps that obstructed the distal duodenum and proximal jejunum. Colic was considered secondary to small intestinal obstruction and luminal distension by the adenomatous polyps. **To the authors' knowledge, this is the first report to include a sonographic appearance of a small intestine adenomatous polyp that caused small intestinal obstruction and colic in a 3 year-old horse.**

Poster Sun-12

Cutaneous T cell angioinvasive lymphoma with lung and ocular metastasis in a cat

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History: A Seven year old female spayed domestic long hair cat was presented with a focal subcutaneous nodule that remained unresponsive to antibiotics. On re-examination the cat was presented with generalized lymphadenopathy, corneal edema and multiple irregularly thickened subcutaneous nodules on the entire body. The cat was then treated with steroids, the subcutaneous nodules have regressed but the cat developed severe respiratory distress and became febrile. The condition of the cat continued to worsen and the hence she was euthanized and submitted for autopsy. There were multifocal subcutaneous flat nodules through the body, the lungs had multifocal to coalescing pale poorly demarcated thickened areas which on cut section extend into deep parenchyma. The left eye had corneal opacity and thickening. Microscopic lesions were noticed in the skin, lungs and left eye only. The dermis and subcutis contain scattered to aggregates of round cells that often surround and infiltrate the blood vessels and rarely occlude the vascular lumen (thrombosis) and also infiltrate the adjacent skeletal muscle. The lungs contained multifocal to coalescing large areas of consolidation with scattered perivascular aggregates of round cells with similar morphology as described in the skin. The reminder of the lung parenchyma contained multifocal to coalescing areas of alveolar infiltrates of large numbers of foamy macrophages with occasional type II pneumocyte hyperplasia and mild to moderate interstitial fibrosis. The left eye showed marked thickening of the cornea, iris and ciliary body with multifocal perivascular aggregates of round cells similar to those described earlier in skin. **I**mmunohistochemical staining was positive for CD3 and negative for CD20 and CD18 suggestive of a T cell lineage. This is the third case report of agioinvasive lymphoma in a cat and the first report demonstrating ocular metastasis besides lungs. All reported cases were in female spayed cats with age group ranging from 4 to 17 years.

Poster Sun-13

A tale of two ELISAs: Which serological assay more accurately detects *B. ovis* antibodies in Wyoming domestic sheep? # * †

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Brucella ovis (*B. ovis*) is the primary causative agent of Ovine brucellosis, an infectious, sexually-transmitted bacterial disease of sheep that causes decreased fertility in rams, increased lamb mortality, and infrequent abortions in ewes. A lack of vaccines and efficacious treatments paired with few trade restrictions, sale requirements, and clinical signs leaves few good management strategies once disease manifests in a flock. While epididymitis may be indicative of infection, this clinical sign is present in <50% of infected rams, and laboratory testing is necessary for disease confirmation. Direct diagnosis can be made via PCR or bacterial isolation of *B. ovis* from semen samples or tissues from rams and vaginal discharge or milk from ewes. However, these specimens are not practical when sampling large numbers of animals, thus indirect diagnosis via serological testing is preferred. The NVSL *B. ovis* indirect ELISA is currently the only ELISA utilized in the U.S. for detection of *B. ovis* antibodies in sheep and goat serum; however, IDEXX has created a standardized and commercially manufactured kit that is marketed for serological diagnosis of *B. ovis* in Europe. The antigen used for both assays is a hot-saline water soluble extract of the REO 198 strain prepared according to OIE recommendations, and sensitivities and specificities for both assays are comparable.

The main objective of this project was to compare serological results from the National Veterinary Services Laboratory (NVSL) *B. ovis* indirect ELISA and the IDEXX *B. ovis* indirect ELISA used for diagnosis of *B. ovis* exposure in 2,278 serum samples from Wyoming sheep.

When 2,278 serum samples from Wyoming domestic sheep were tested using the NVSL and IDEXX ELISAs, 0.88% (95% CI: 0.57 – 1.35%; 20/2,278) and 1.14% (95% CI: 0.78 – 1.67%; 26/2,278) were serologically positive for anti-*B. ovis* antibodies, respectively. Although there was no statistically significant difference in the seroprevalence proportions (p= 0.3802), approximately 10% (95% CI: 8.64 – 11.08%; 223/2,278) of the serum samples had discordant results (i.e. one ELISA yielded a positive result and the other yielded a negative or indeterminate result). Results from a commercial AGID kit (TECPAR, Brazil) will be used as a confirmatory test for those discordant results to verify false positive or negative results from the ELISAs. The AGID kit is widely used for serological detection of *B. ovis* antibodies in many South American countries and uses antigen prepared from the *B. ovis* REO 198 strain prepared according to OIE international standards.

AAVLD Trainee Travel Awardee
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Poster Sun-14

Comparison of conventional tube and gel-based agglutination tests for feline AB system blood typing ◊

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Gel technology is widely used in human medicine for blood typing. It carries many advantages over routine tube testing, such as: standardization, stability, smaller sample volume, easy to perform and read, and rapidity. The aim of this study is to evaluate the gel column technique in feline blood typing. The blood type of one hundred and thirty-six blood samples anticoagulated with EDTA or CPDA from feline blood donors, feline blood recipients, health patients and stored units of whole blood was determined using tube agglutination (TUBE) with plasma from type B cats as anti-A reagent, *Triticum vulgaris* lectin as anti-B reagent and PBS for control. Samples positive for type B and AB were back typed with type A RBCs to confirm whether the samples were B (strong agglutination) or AB (absence of agglutination). Samples were blood typed in duplicate using the same anti-A and anti-B reagents in a neutral gel (GEL) column technique (ID-Card NaCl enzyme test and cold agglutinins, DiaMed). Briefly, 25 μL of type B plasma and 25 μL of *Triticum vulgaris* lectin were mixed with 50 μL of a 0.8% RBC suspension (made by suspending 10 μL of the RBC pellet in 1 mL of low ionic strength solution) in the reaction chamber of a gel column identified as A and B respectively. For all samples, a negative control column containing the RBC suspension of interest and PBS was included. The gel columns were incubated for 15 min at room temperature and then centrifuged in a special gel column card centrifuge (ID-Centrifuge 24 S, DiaMed) at 80 *g* for 10 min. Finally, the gel column cards were visually checked to identify positive samples via agglutination reactions. Results were considered valid if the control column was negative. Sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) and Cohen's kappa coefficient (K) for GEL were calculated, considering TUBE as the gold standard technique. Of 136 samples typed with TUBE, 95 (69.8%) were type A, 22 (16.2%,) type B and 19 (14.0%) type AB. All B and AB samples were confirmed by back typing. With GEL 112 samples (82.3%) gave concordant results with TUBE, and 24 samples showed a mixed-field agglutination pattern (presence of a layer of RBCs simultaneously either at the top and at the bottom of the gel in A or in B gel column). If a mixed-field pattern was interpreted as a negative result 135/136 (99.3%) samples showed concordant results and Se, Sp, PPV and NPV (95%CI) were respectively 100% (96.1-100), 100% (91.4-100), 100%, 100% for type A, 95.4% (77.1-99.8), 100% (96.8-100), 100% and 99.1% (94.3-99.8) for type B, 100% (82.3-100), 99.1% (95.3-99.9), 95.0% (72.9-99.2) and 100% for type AB. Strength of agreement was very good (K= 0.98, 95%CI 0.95-1.00). The GEL column technique, using the same anti-A and anti-B reagents as in TUBE test is a sensitive and specific method for blood typing feline samples. Mixed-field pattern should be considered as negative results.

◊ USAHA Paper

Poster Sun-15

Seroprevalence of *Leptospira interrogans* serovars in Oklahoma mares

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The seroprevalence of *Leptospira interrogans* and its related serovars in horses of the southern United States is approximately 77%[1]. This number reflects healthy horses that have been exposed to at least one serovar without previous vaccination. Leptospirosis in horses can cause uveitis, abortion, and kidney failure and is transmitted by contact with contaminated urine, water, or bedding containing the bacteria[2]. Most recent studies suggest *L. pomona* and *L. grippotyphosa* are the principle pathogenic serovars in horses in the United States[3]. Little research has been done on the seroprevalance of equine leptospirosis in Oklahoma, especially on multiple serovars. Blood samples from 107 mares on 3 farms across Oklahoma were collected and a microscopic agglutination test (MAT) was performed and reviewed by two trained technicians examining 6 leptospirosis serovars: *pomona, hardjo, canicola, autumnalis, grippotyphosa* and *icterohemorrhagica*. Animals selected for testing had not been previously vaccinated and were managed under pasture or range conditions. Only 6% (6/107) mares tested were negative (< 1:100) for all serovars tested. Serovar *Leptospirosis autumnalis* was the most frequently identified, with 88% (96/107) horses tested having detectable antibody. There was some variation between farms, with *L. autumnalis* 80% (32/40) at Farm 1, 96% (26/27) at Farm 2 and 95% (38/40) at Farm 3. The other serovars detected at lower frequency were: *L. icterohemorrhagica* 65% (71/107), *L. hardjo* 40% (44/107), *L. pomona* 29% (32/107), *L. canicola* 16% (18/107) and *L. grippotyphosa* 16% (17/107). Positive cut-off level using the Leptospirosis MAT is considered a titer of 1:1600. There were 16 positive samples tested in this study, with a high titer identified of 1:3200. No clinical illness, reproductive failures or ocular lesions were reported in any horses sampled. Five animals tested were positive to two different serovars at the time they were sampled. Positive titers were noted to *L. pomona* 6% (7/107), *L. autumnalis* 6% (7/107) and *L. grippotyphosa* 2% (2/107). This data supports previous studies suggesting horses are exposed to leptospirosis, and that unvaccinated mares under pasture conditions in Oklahoma have slightly higher exposure rates than those reported in other regions. The number of positive titers identified is similar to other surveys and identification of antibodies to serovar *L. autumnalis* requires further investigation.

Poster Sun-16

Swine serosurveillance in Hawaii ◊

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Historical Background

Swine play an important cultural and economic role in Hawaii, and despite Hawaii's relative isolation from the mainland USA and other countries, many swine pathogens have been introduced into the domestic herd. Porcine Respiratory and Reproductive Syndrome virus (PRRSV) has been present in Hawaii since 1992, and both the European and the North American strains have been detected. Porcine Circovirus type 2 (PCV2) was first detected in Hawaii in 2008, and subsequent surveillance in 2009 showed that it had already spread widely throughout the state. A variant strain of Porcine Epidemic Diarrhea virus (PEDV) caused disease in a single Oahu farm in 2014, and investigations revealed other infected farms that did not exhibit clinical signs. Senecavirus A (SVA) was first detected in imported hogs on Oahu in 2013, and sporadically thereafter (2015, 2016, and 2017) in recently imported animals.

Current Study

The State of Hawaii comprises a chain of eight major islands separated by sea, enabling interisland variability in disease introduction and maintenance. Therefore, swine herds on the four main swine producing islands (Kauai, Oahu, Maui, and the Big Island) were included in this study, and serum samples were tested for PEDV, SVA, PRRSV, and PCV2 by the University of Minnesota's Veterinary Diagnostic Laboratory. Results from this ongoing project suggest that there are geographic differences in pathogen occurrence and provide meaningful information that local swine producers, veterinarians, consultants, and regulatory agencies can use in their decision-making process. Current data and maps will be presented.

◊ USAHA Paper

Poster Sun-17

Comparison of three cross-matching methods to detect canine DEA 7 blood incompatibility ◊

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The prevalence of naturally occurring antibodies to dog erythrocyte 7 antigen (DEA 7) in DEA 7–negative dogs has been reported to be up to 50%. The potential risk of delayed transfusion reaction due to these antibodies makes it prudent to consider cross-matching before transfusion, especially when DEA 7 status of the donor dog is unknown. This prospective study compares diagnostic performances of neutral gel column (GEL), standard tube (TUBE) and a point-of-care immunochromatographic strip kit cross matches to identify DEA 7 blood incompatibilities due to the presence of naturally occurring anti-DEA 7 antibodies in canine blood.

Firstly, 42 canine sodium citrate whole blood samples were typed for DEA 7 (by agglutination on gel technique). Of these 2/42 samples were DEA 7-positive (agglutination strength 2+), and 40/42 samples were DEA 7-negative (agglutination strength ≤1+).

Secondly, the 40 DEA 7-negative samples were centrifuged and plasma samples were cross-matched against two samples of DEA 7-positive and three DEA 7-negative RBCs using the GEL technique. Samples that showed >1+ agglutination strength with DEA 7–positive RBCs samples but not with DEA 7–negative RBCs samples were classified as containing naturally occurring anti–DEA 7 antibodies. Samples that showed no agglutination with DEA 7-positive RBCs were classified as containing no anti-DEA 7 antibodies.

Thirdly, the 40 DEA 7-negative plasma samples were cross-matched in double blind fashion with the two DEA-7 positive RBCs samples using TUBE and immunochromatographic kit and results were compared with those of the agglutination on GEL, considered the gold standard technique. A positive/incompatible cross match was identified when agglutination, hemolysis, or both reactions were present with TUBE technique or when a red band, other than the control one, was identified on the immunochromatographic strip. To determine relationship between results obtained with various methods, 2 x 2 tables were used. Cohen's kappa coefficient (K) was calculated with 95% confidence interval (95%CI) between results of GEL and other methods.

With GEL agglutination 21/40 plasma samples showed positive cross-matching and 19/40 showed negative cross-matching. The same results were obtained by TUBE cross match, whilst only 1/40 sample showed positive cross matching with immunochromatography. There was a statistically significant relationship between results of GEL and TUBE methods (P<0.000), but not between GEL and immunochromatography results (P=1,000). Agreement quantified by kappa showed perfect (K=1,000, 95% CI 1,000 to 1,000) agreement for comparison of TUBE to GEL, but agreement equivalent to chance (K=0,0453; 95% CI -0.0427 to 0.133) was seen between GEL and immunochromatography.

GEL column and TUBE crossmatch tests are useful methods to evaluate DEA 7 blood compatibility, whereas the immunochromatography was not able to identify DEA 7 incompatibilities due to anti-DEA 7 naturally occurring antibodies.

◊ USAHA Paper

Poster Sun-18

Modified salmonella ELISA using swine oral fluids

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The use of oral fluids in the US swine industry has become a popular disease monitoring tool. The objective of this study was to adapt a commercial *Salmonella*-Ab ELISA serum test for antibody (IgG) detection in swine oral fluids. Group 1 were known negative serum (n=230) and oral fluid (n=213) samples matched by barn from Sweden. Group 2 were serum (n=3/pen) and oral fluid (n=1/pen) samples matched by pen from 11 week old US commercial pigs vaccinated with a modified-live commercial *Salmonella* vaccine. Natural exposure measured by *Salmonella* culture of oral fluids. Samples were collected per pen, bi-weekly from 14 to 24 weeks of age (total serum n=288, total oral fluid n=96). Receiver Operating Characteristic analysis was performed to establish an experimental cutoff. All Group 1 oral fluids were negative. Group 2 oral fluids were considered positive if 3 out of 3 matched serum tested positive within a given pen. Using the experimental cut-off, oral fluids tested positive 0.95% and 94.74% in Group 1 and Group 2, respectively. The combined data set of both groups gave a predicted specificity of 89.12% and sensitivity of 98.51%. The correlation of serum to oral fluid titer had an R^2 of 0.575 with a P-value of <0.0001. This modified assay proved effective at detecting Salmonella antibody in oral fluids. Further evaluation using a larger set of known exposure status oral fluids can help optimize sensitivity and specificity. This modified oral fluid *Salmonella* ELISA could become a more efficient surveillance and control tool for *Salmonella*.

Poster Sun-19

Blinded Method Test (BMT) - Lessons learned ◊

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U.S. FDA, Laurel, MD

The Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM), Veterinary Laboratory Investigation and Response Network (Vet-LIRN), comprises 38 veterinary diagnostic laboratories across North America. Vet-LIRN has funded several projects to test for food related toxicants or pathogens in animal diagnostic samples. To evaluate performance of chemistry methods, Vet-LIRN Program Office (VPO) conducts Blinded Method Tests (BMTs) with the method originating laboratory followed by a multi-laboratory BMT. In both Single- and Multi-laboratory BMTs, test samples are prepared by VPO and analyzed by participants in a blinded manner. Single-laboratory BMTs have been identified as an essential prerequisite exercise prior to multi-laboratory exercises as they ensure the method works well and may bring to light important aspects to include when transferring the method to other laboratories. Using FDA guidelines for methods validation, VPO can, within a relatively short time period, determine major characteristics of method performance with a high degree of confidence. BMTs were successfully applied for both quantitative and qualitative methods based on different instrument platforms confirming the great flexibility and adaptability of the BMT approach. Key BMT features include: (i) preparation of the test samples, (ii) capturing and processing data and (iii) application of project management tools.

◊ USAHA Paper

Poster Sun-20

Lead poisoning in cattle following feed contamination by tractor battery: A case review * †

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Following the delivery of a total mixed ration of feed to seventy-six 1,000-pound feedlot cattle and fifteen cull cow-calf pairs at two separate sites on the 9th of October 2016, plastic fragments were observed in the feed bunk. Upon further inspection, it was established that a tractor battery, discovered in the grinder-mixer wagon, had been ground into the ration and distributed to cattle. An attempt was made to remove the contaminated feed from the feed bunks. However, much of the feed had already been consumed. Exposed cattle at both sites were reluctant to eat and exhibited increased vocalization, lateral recumbency and paddling, blindness, and head pressing at two days post exposure. Signs progressed to include seizures and tremors the following day. At five and ten days post exposure, approximately forty and eighty cattle had died respectively with deceased animals being disposed of through burial. Whole blood from two affected individuals was received at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) on the 13th of October 2016. The toxicology and nutrition section confirmed lead poisoning as blood lead levels in both samples exceeded 1.2 ppm. Samples taken at necropsy ten days post exposure from a cow calf pair for further lead analysis included liver, kidney, ribs, and milk with all samples possessing high levels of lead. Due to contamination of feed equipment, periodic testing was performed over a period of five months on additional cattle on site that were not directly exposed in order to ensure food safety.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Poster Sun-21

Vet-LIRN proficiency test to detect vitamin E in animal serum

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In September 2016, a proficiency test (PT), to evaluate the performance of individual analysts to detect vitamin E in animal (bovine and swine) serum, was co-organized by Food and Drug Administration's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) in collaboration with the Moffett Campus Proficiency Testing Laboratory. Vitamin E is an essential nutrient for animals and plays a crucial role in reducing the risk associated with oxidative stress like cancer, cardiovascular disease, and neurodegenerative diseases (1). Animals obtain vitamin E through their diet and the adequate range of vitamin E concentration in serum for a healthy bovine is 3-10μg/mL (2, 3). Twelve blind-coded frozen serum samples were sent to 24 analysts at 13 network laboratories. Analysts reported "Detected" or "Not Detected" and the vitamin E (α-tocopherol) concentration as μg/ml. The vitamin E in the samples was naturally occurring, not spiked. Results were statistically evaluated according to ISO 13528:2015 (4) standards. The assigned values and standard deviations were determined by consensus and calculated using algorithm robust statistical method by combining reported replicate results. The assigned values and standard deviations determined for the serum batches S02, S03, and S04 were 0.88 ± 0.31, 3.13 ± 0.82, and 4.59 ± 1.18 μg/mL, respectively. The assigned value and standard deviation were not determined for serum batch S01 due to insufficient quantitative results reported by the participants. S01 had an extremely low vitamin E concentration, which was much lower than most laboratories' Limits of Detection (LOD) and Limits of Quantitation (LOQ). The PT revealed that 79% (19 out of 24) network laboratory analysts had satisfactory performance (expressed as $|z| \leq 2$) for detecting vitamin E in all PT samples. Additionally, the majority of the analysts had coefficient of variations (CV) less than 20% for S03 and S04. As expected, the majority of the analysts (20 out of 24) were not able to quantify, and in some cases detect, the vitamin E concentration in S01 which had extremely low levels of vitamin E.

Poster Sun-22

Carbon monoxide (CO) poisoning in two cats # *

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Carbon monoxide is a colorless, odorless gas, the inhalation of which can be fatal. There is only one report on CO poisoning in cats in the literature. Two adult Singapura brown ticked cats were submitted to the San Bernardino branch of CAHFS for necropsy. These animals had been found dead in an apartment along with their two deceased owners. At necropsy, gross lesions were similar in both cats and consisted of multifocally large and irregular, bright red spots on the skin of the abdomen and the inner surface of ear pinna, bright red muscles and blood. The carcasses, and tissues fixed in formalin retained the bright red discoloration for up to two weeks. Microscopic lesions were also similar in both cats and included diffuse pulmonary congestion and edema, and multifocal intense basophilia of cardiomyocytes. The latter was seen mostly affecting whole fibers but it was occasionally affecting only a portion of the fiber, with a clear transverse line of demarcation from the rest of the fiber. Van Kossa staining of these fibers was unrewarding. Transmission Electron Microscopy (TEM) is currently under way to determine the ultrastructural nature of these changes. Rarely, discrete areas of hypercontraction bands were seen in individual cardiomyocytes. Based on the clinical history, gross and microscopic changes, cyanide or carbon monoxide poisoning was suspected, and frozen muscle and blood from the two animals were submitted for toxicological analysis. The muscle samples were negative for cyanide by distillation method. The blood samples were analyzed for carbon monoxide by a modification of the Comopac® electrochemical gas meter. The blood carboxyhemoglobin (COHb) was measured as % saturation, and values of 57 % and 41 % were found for both cats, respectively. Although there are no established reference values for COHb concentration in cats, these values are considered lethal in humans and it is likely that the same applies to cats. Based on gross and microscopic findings, and the high level of COHb saturation, a diagnosis of CO intoxication was established in these cats. The source of CO is currently being investigated.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant

Poster Sun-23

DDT intoxication of big brown bats in Hamilton, Montana

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Dichlorodiphenyltrichloroethane (DDT) is an insecticidal organochlorine pesticide that that was extensively used in agriculture during the 1950s and '60s and is still used in several countries. In 1972, the U.S. Environmental Protection Agency (EPA) cancelled the registration of DDT in response to increasing evidence of its persistence in the environment, adverse effects on wildlife health and potential for impact on human health. DDT is highly lipophilic and therefore accumulates in fatty tissues in exposed mammals, birds and aquatic organisms. In addition, it is metabolized by animals and breaks down environmentally to the fat-soluble metabolites Dichlorodiphenyldichloroethylene (DDE) and Dichlorodiphenyldichloroethane (DDD). Accordingly, DDT may accumulate in the brains of wild animals and has been shown to cause neurological effects (tremors) when present at lethal doses. We present the case history of big brown bats found dead or convulsing following an acute exposure to DDT, the resulting investigation, and the analytical approach to identifying and quantifying DDT, DDD and DDE in brain, liver and superficially on bat wings by gas chromatography-mass spectrometry (GC-MS) and gas chromatography- tandem quadrupole mass spectrometry (GC-MS/MS).

Poster Sun-24

The effects of formalin fixing on trace mineral status

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The Iowa State University Veterinary Diagnostic Laboratory Toxicology and Nutrition section is routinely asked to perform trace mineral analysis on fixed tissues. However, interpretation of those results is often difficult and may provide more questions than answers. After receiving a request to perform sodium analysis on a fixed brain it was decided that, more information was needed on the effect of formalin fixation. Two small research studies were performed to better understand the effect of formalin fixation on tissues. The first study assessed sodium levels in cerebrum of samples fixed in 10% neutral buffered formalin, 10% non-buffered formalin, and fresh samples for healthy market weight pigs. Sodium levels from samples in 10% neutral buffered formalin are 1.9 to 2.4 times higher than the levels in the fresh samples, conversely the samples fixed in the 10% non-buffered formalin are only 26 to 34% of the levels in the fresh tissue; Sodium levels appear to correlate with the level of sodium in the fixative used. The second study looked at the levels of Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, Se, and Zn in liver. Sodium showed the same effect in liver as was observed in brain. The elements Mg, Mo, and K were lower in both fixing solutions, with K having considerable loss. The potassium level in the buffered fixing solution was 7.6% of the non-fixed liver, while it was 13.6% of the non-fixed liver in the non-buffered fixing solution. Calcium showed minor difference in concentration in the buffered fixing solution as compared to the fresh samples. However, the non-buffered samples were almost three times higher in Ca concentration. For Cd, Co, Cr, Cu, Fe, Se, and Zn the levels in each test group were virtually the same. While it is always preferable to have fresh tissue in order to perform trace mineral analysis it is possible to provide meaningful data when all that is available is a fixed liver tissue sample. Sodium content in brain tissue is dramatically affected by formalin fixation.

Poster Sun-25

Experimental infection of white-tailed deer (*Odocoileus virginanus*) with Heartland Virus # * †

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Heartland virus (HRTV) is a phlebovirus reported to cause illness, characterized most frequently by thrombocytopenia and leukopenia, in humans and has been found in wild-caught *Amblyomma americanum* ticks. Previous serological surveys for HRTV antibodies in wildlife identified seropositive white-tailed deer across a wide geographic area. To better understand the role of deer, if any, in the epidemiology of HRTV, we experimentally inoculated white-tailed deer fawns with HRTV and monitored for clinical disease, viremia, virus shedding, and seroconversion. None of the animals showed signs of clinical disease, viremia, or virus shedding post-inoculation. This was confirmed by RT-PCR. Two wild-caught fawn had pre-existing antibody titers against HRTV. All animals showed minimal humoral immune responses against HRTV following exposure.

AAVLD Trainee Travel Awardee
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Poster Sun-26

Predicting genetic adaptations of a segmented arbovirus (bluetongue virus) using an *in vitro* system # * †

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Bluetongue virus (BTV) (family Reoviridae, genus *Orbivirus*) is a segmented, double-stranded RNA (dsRNA) virus that can cause severe disease in wild and domestic ruminants. BTV is transmitted between ruminant hosts by *Culicoides* midges. Clinical BTV infection manifests as vasculitis, edema, and respiratory distress in susceptible animals, although subclinical infections are also common. BTV's ability to mutate and spread to new regions is poorly understood. As an RNA virus, BTV may be prone to high rates of mutation due to the lack of proofreading abilities of RNA polymerases (genetic drift). BTV also possesses a segmented genome, allowing for frequent reassortment events (genetic shift). However, the specific contributions of genetic shift and genetic drift to BTV evolution are unclear. To investigate the role of mutation in BTV's viral diversification, a field isolate of BTV-17 was used to infect cell monolayers at an MOI of 1 and was passaged under three different conditions in duplicate: (i) 9 serial passages in *Culicoides sonorensis* cell culture (CuVaW3 cells), (ii) 9 serial passages in bovine pulmonary artery endothelial cells (BPAEC cells), or (iii) 9 alternating passages in CuVaW3 cells and BPAEC cells. Aliquots of virus were harvested from each passage and sequenced using next-generation sequencing technologies (Illumina®). LoFreq* variant calling software was used to detect single nucleotide variants (SNVs) and insertions-deletions (indels). Preliminary results indicate that BTV passaged in alternating cell lines has lower rates of single nucleotide variants (SNVs) compared to virus passaged in solely insect or mammalian cells. Segment-specific trends in SNV and indel incidence were also noted, indicating that certain segments may be more conserved to preserve the functionality of viral proteins. Our proposed study system is uniquely valuable for investigating genetic adaptation of a segmented arbovirus and offers a platform to intensively study genotypic and phenotypic changes.

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Poster Sun-27

Comparison of isolation of swine influenza A virus in human colorectal adenocarcinoma (CACO-2) cell line and Madin-Darby canine kidney (MDCK) cell line

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Swine influenza A virus (IAV) is one of the major swine respiratory pathogens and causes substantial economic burden to swine producers. Isolation of IAV is critical for antigenic characterization, pathogenesis investigation, and vaccine production. Currently MDCK (ATCC CCL-34) is the most commonly used cell line for swine IAV virus isolation (VI). In addition to MDCK cells, embryonated chicken eggs (ECE) and/or another cell line is recommended in order to achieve the best VI outcome. However, there are some limitations of using ECE for isolating IAV. No consensus has been reached regarding alternative cell line for isolating IAV. The CACO-2 cell line (ATCC HTB-37) has been reported to support IAV replication with no need of exogenous trypsin during VI process. In contrast, exogenous TPCK-trypsin is generally required for IAV VI in MDCK cells. The objectives of this study were to compare the efficiency of isolating swine IAVs from clinical specimens using MDCK and CACO-2 cells with and without trypsin and also to investigate whether IAVs isolated in CACO-2 cells can grow in MDCK cells and vice versa.

IAV matrix PCR-positive clinical specimens with various threshold cycle (C_T) values were selected for this study. These included 71 lung (Ct 12.7-35.8), 66 nasal swab (NS, Ct 18.3-34.1), and 59 oral fluid (OF, Ct 18.9-36.3) samples. IAV isolation was attempted on each sample under 4 conditions: MDCK with trypsin, MDCK without trypsin, CACO-2 with trypsin, and CACO-2 without trypsin. Cytopathic effect was recorded and the hemagglutination (HA) titer was determined on the cell lysates harvested 5 days post inoculation. The HA-positive isolates were tested by IAV matrix and subtyping PCRs for VI confirmation. Eight randomly selected isolates respectively obtained in MDCK and CACO-2 cells were tested for their growth in both MDCK and CACO-2 cells.

Among the specimens tested, 31 (43.7%), 0 (0%), 36 (50.7%), and 35 (49.3%) lung samples, 22 (33.3%), 0 (0%), 28 (42.4%), and 30 (45.5%) NS samples, and 7 (11.9%), 0 (0%), 6 (10.2%), and 6 (10.2%) OF samples, were VI positive in MDCK with trypsin, MDCK without trypsin, CACO-2 with trypsin, and CACO-2 without trypsin, respectively. There were 7 lung, 8 NS, and 3 OF samples with VI discrepancies between MDCK with trypsin and CACO-2 with trypsin; 6 lung, 8 NS, and 3 OF samples with VI discrepancies between MDCK with trypsin and CACO-2 without trypsin; 5 lung, 4 NS, and 0 OF with VI discrepancies between CACO-2 with and without trypsin. Eight isolates obtained in MDCK cells and 8 isolates obtained in CACO-2 cells were found to grow in both MDCK and CACO-2 cells.

In summary, under the conditions of this study, CACO-2 cell line was overall more sensitive than MDCK and could be a useful alternative cell line for primary isolation of swine IAVs from clinical specimens. Further studies are needed to determine which CACO-2 conditions (with or without trypsin) is superior for IAV VI.

Poster Sun-28

Evaluation of Senecavirus A transmission in mice under experimental conditions # * †

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Senecavirus A (SVA) is an RNA virus of the genus *Senecavirus* in the family *Picornaviridae.* SVA has recently been identified as causative agent of outbreaks of vesicular lesions and increased neonate mortality in U.S. swine production systems. Previously the presence of SVA neutralizing antibodies and nucleic acid have been reported in mouse samples collected from afflicted swine facilities. The objective of this study was to evaluate the transmission of SVA and potential clinical outcome in mice experimentally infected with SVA. In order to evaluate mice strain susceptibility, five (3 male and 2 female), 8-weeks-old BALB/C, SWISS, SJL/J, and C57BL/6 mice, and 4-weeks-old SCID mice, were inoculated subcutaneously in the rear left foot pad with 100 μL of SVA at 1 x 10^9 $TCID_{50}$/mL. Direct-contact transmission was evaluated by adding 1 mock infected mouse, inoculated with 100 μL of RMPI media, to the same cage and kept through the duration of the experiment. Mice were monitored every 4 hs for the first 72 hs post infection, and daily thereafter for 14 days. Body weight and clinical signs of systemic infection were recorded. Feces were collected daily from individual animals, and tested for viral shedding by PCR. Animals were euthanized at 14 dpi. Blood and a full set of tissues (brain, heart, lung, liver, spleen, kidney, and intestine) were collected for viral detection by PCR and histopathological evaluation. Clinically, only BALB/C mice developed mild clinical signs characterized by a rough coat, poor general body condition, and slight decrease on body weight for a period of 3 dpi; however, these changes were not significantly different from other mice strains or sentinel mice. All infected animals, except SCID mice, developed SVA IgG antibody response detected by SVA-rVP1 and whole virus indirect ELISA. Negative control, housed with infected animals, showed no seroconversion. Nucleic acid was detected in all tissue and all mice strains experimentally infected at 14 dpi. SVA nucleic acid was not detected in sentinel mice expect for 2 mice with positive PCR in kidney. Different levels of viral shedding on feces were observed on infected mice throughout the study. Sentinel animals had inconsistent shedding patterns but nucleic acid was detected spanning from 5 to 10 dpi. In conclusion, in experimentally infected mice, SVA is shed through feces, can be detected in multiple tissues, and can induce seroconversion. However, no clinical or histological changes were observed after subcutaneous infection. The viral shedding observed in sentinel mice supports previous field observations suggesting that mice could be a reservoir or vector of SVA in swine herds. The lack of detection of SVA in tissues from sentinel mice could be due to the low infectious dose and viral load occurred during natural infection. Thus, these results suggested that a mouse model could be used to study SVA transmission in swine positive farms.

AAVLD Trainee Travel Awardee
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Poster Sun-29

Differential gene modulation of pattern-recognition receptor TLR and RIG-I-like and downstream mediators on intestinal mucosa of pigs infected with PEDV non S-INDEL and S-INDEL strains

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Porcine epidemic diarrhea virus (PEDV) causes enteric diseases resulting in significant morbidity and mortality in neonatal pigs. PEDV can regulate different immunological pathways *in vitro* but the main effect of this virus on intestinal mucosa is still unknown. The objective of this study was to evaluate the differential gene modulation of distinctive pattern-recognition receptor (Toll-like receptors (TLRs) and retinoic acid-inducible gene (RIG)-I) and its pathway mediators, and how they affect the final cytokine gene expression in intestinal mucosa of neonatal piglets experimentally infected with PEDV. Three groups of ten, five-day-old piglets were inoculated orally with 10 mL of 10^4 $TCID_{50}$/mL of PEDV non S-INDEL USA/IN19338/2013 strain, PEDV S-INDEL USA/IL20697/2014 strain or 10 mL of culture media, respectively. Five pigs from each group were euthanized at day post-inoculation (dpi) 3 and 7, respectively. Sections of intestine were snap frozen and saved at -80 C until further use. Gene expressions were measured on sections of 40 mg of mucosal scrape by ΔΔCT method using a SYBR green qPCR assay. Our results indicate that, PEDV non S-INDEL infection induced a significant downregulation ($p<0.05$) in the expression of TLRs 4, 7, 8 and 9 genes at 3 dpi, while no differences in regulation of TLRs 2, 3 and RIG-I receptors were observed. Conversely, S-INDEL infection significantly upregulated ($p<0.05$) expressions of TLRs 3, 4, 7 and RIG-I receptors at 3 dpi with no change in gene expression of TLRs 8 and 9 receptors. PEDV non S-INDEL infection produced significant downregulation ($p<0.05$) ofTRIF, MYD88, NF-κβ gene expression and one of its downstream adapter proteins (RelA-p65), with no variation in TRAF6 and IRF7 expressions at 3dpi. In contrast, S-INDEL infection significantly downregulated ($p<0.05$) TRAF6 and IRF7 gene expression. Interestingly, S-INDEL infection caused significant upregulation ($p<0.05$) of NF-κβ gene expression compared to both control and non S-INDEL infected groups. Thus, NF-κβ regulation resulted in a significant upregulation ($p<0.05$) of the cytokine molecules TNFα and IFNα mRNA expression induced by S-INDEL infected piglets. In summary, the results from this study demonstrated that the PEDV S-INDEL infection can induce gene expression of pro-inflammatory cytokines TNFα and IFNα in intestinal mucosa through activation of TLR3 or RIG-I receptors, leading to the activation of the NF-κB signaling pathway and nuclear factor translocation. Our results provide novel evidence that supports the evasion strategy of the US PEDV non S-INDEL e strain in its host in first few days of infection and might be an important insight to the pathogenesis of this strain. A study of the TRAF-3/IRF3 signaling pathway is warranted to confirm that, indeed, this secondary pathway plays a role in the immune modulators during the S-INDEL infection in pigs.

Poster Sun-30

Detection and characterization of canine distemper virus from an outbreak in Ohio

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Canine distemper virus (CDV) is an enveloped, negative sense, single-stranded RNA morbillivirus within the *Paramyxoviridae* family. CDV is the etiological agent of a highly infectious disease of domestic dogs, causing high morbidity and mortality rates, particularly in high-density housing situations such as dog shelters. CDV is also prevalent in carnivorous wildlife, including bears, coyotes, foxes, mustelids, raccoons and skunks. In September 2016, a central Ohio dog shelter experienced an outbreak of disease characterized by canine distemper-like signs, including altered behavior, conjunctivitis and severe upper respiratory disease. Necropsy lesions of the two dogs included bilateral mucoid ocular discharge, conjunctivitis and multifocal consolidation of lungs. Histopathology lesions included nonsuppurative conjunctivitis, nonsuppurative meningoencephalitis and suppurative bronchopneumonia. Intracytoplasmic or intranuclear inclusions were observe in multiple tissue types, including epithelial cells of conjunctiva, pancreatic ducts, cholangial cells of biliary ductules, jejunal crypt epithelial cells and transitional epithelial cells of urinary bladder. Canine distemper virus was tested positive using a fluorescent antibody test and confirmed by RT-PCR. The outbreak resulted in the quarantine of nearly 300 dogs; 84 dogs had to be euthanized based on severe clinical signs of distemper or unsuitability for quarantine due to age, behavioral and/or medical problems. Sequencing analysis was performed of the CDV outbreak strain and phylogenetic trees were constructed. The outbreak strain of the CDV virus from two affected dogs examined at the Ohio ADDL shared 90% nt identity of the M gene/M-F intergenic region, 97% nt identity of the N gene, and 93% nt identity of the H gene with CDV viruses from three raccoons in Ohio that were also examined at the ADDL in 2016. Importantly, all three genes of the virus clustered together with two CDV isolates from grey foxes (>99% nt) in southeastern Ohio that were submitted for necropsy and testing in 2016 as well, indicating that the dog shelter disease outbreak was most likely associated with a strain of CDV closely related to a CDV strain actively circulating in gray fox in Ohio in 2016.

Poster Sun-31

Development of a dry room temperature-stable real-time RT-PCR assay for the specific detection of porcine hemagglutinating encephalomyelitis virus (PHEV) ◊

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Porcine hemagglutinating encephalomyelitis virus (PHEV) is a member of the family Coronaviridae. This positive sense ssRNA virus has a crown-like appearance and a large, non-segmented genome. Structural proteins include hemagglutinin-esterase (HE), envelope protein (E), spike glycoprotein (S), membrane protein (M), and nucleocapsid (N). Clinical presentation in suckling pigs is short and include displaying neurological disorders (muscle tremors, paddling, and paralysis), vomiting, and wasting, with mortality rates of 100% in naïve farms of piglets less than three weeks of age. However, PHEV infection in adult animals is subclinical, being a potential threat to a high health gilt herds. There are not previous information describing kinetic of PHEV shedding (i.e., duration and pattern of viral shedding, specimen more suitable for testing) in the field nor under experimental conditions. Moreover, there has been a growing research interest in PHEV as infection and rates and disease severity have increased in some countries. Therefore, the implementation of PCR-based methods will help to identify and subsequently isolate animals who are actively shedding the virus. The objective of this study was to develop a dry room temperature-stable real-time RT-PCR assay for the specific detection of PHEV, to describe and compare the patterns of PHEV shedding in pen-based feces and oral fluid specimens collected from PHEV experimentally inoculated 7-week-old pigs (12 pigs, 6 pens, 2 pigs per pen) over the curse of the infection. PHEV was consistently detected in oral fluid specimens within the first 28 days post-inoculation (DPI) compared fecal specimens where the shedding was just detected within the first 10 DPI. Preliminary results indicated that oral fluids are a suitable specimen for routine PHEV diagnosis and surveillance. This is consistent with the fact that oral fluids have become the preferred sample type used in monitoring for pig diseases. Further studies are being carried out to optimize an easiest and fastest extraction protocol that can be used for blood, feces and oral fluids specimens at the same time. The ultimate goal of this collaborative project between Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) and Tetracore Inc. is to develop and optimize a standardized PHEV rRT-PCR kit of easy implementation in all U.S. VDLs.

◊ USAHA Paper

Poster Sun-32

Adaptation of the porcine epidemic diarrhea virus (PEDV) fluorescent focus neutralization (FFN) assay to a high-throughput format using imaging cytometry ◊

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Since its first report in the US (April 2013), PEDV has spread aggressively throughout farms affecting 36 states. Last year alone (2016), 1092 confirmed cases were reported (www.aphis.usda.gov/animal-health/secd) to the USDA. Because of its highly contagious nature and its consequential economic losses, efforts have been made to develop diagnostic tests able to accurately diagnose and/or monitor PEDV in commercial swine farms, e.g., ELISA, RT-PCR, and immunofluorescence (IFA) assays. Currently, the tests described to detect PEDV neutralizing antibodies in pigs are the serum-virus neutralization (SVN) and fluorescent focus neutralization (FFN) assays. Although both provide high specificity, they are time-consuming and labor intensive. Most importantly, these assays are inherently variable due to the subjective nature in which antibody titers are determined. In the present study, we describe the adaptation of FFN to a high-throughput virus reduction neutralization test using imaging cytometry (SpectraMax i3x and SoftMax Pro 6.5). Results based on testing of samples of precisely-known PEDV status showed three clear advantages over traditional FFN assays: 1) fluorescence reading of a 96-well plate is fast (3-4 minutes); 2) the use of imaging cytometry eliminates the eye strain associated with reading plates under a microscope; 3) reliance on data generated through imaging cytometry eliminates human operator-dependent variation in plate readings and makes determination of virus neutralization titers more consistent and repeatable. We believe this approach can be broadly applicable to a variety of antibody detection assays.

◊ USAHA Paper

Poster Sun-33

Determination of the immunodominant regions of Senecavirus A-VP1 by ELISA epitope mapping # * †

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Senecavirus A (SVA) is an RNA virus of the genus *Senecavirus* in the family *Picornaviridae.* The virus was recently detected in swine production in the U.S., causing sporadic outbreaks of vesicular disease and neonate mortality. The structural VP1 protein has proven highly immunogenic in SVA and other *Picornaviridae* members. However, no information on immunodominant regions of SVA-VP1 is available. The objective of this study is to identify immunodominant regions of SVA-VP1 using ELISA epitope mapping. Experimental anti-SVA polyclonal antibodies (SVA-pAb) and SVA-VP1 monoclonal antibodies (VP1-mAb) were characterized by whole virus (WV) and recombinant VP1-ELISA (rVP1-ELISA), WV and rVP1 western blot, virus neutralization and immunofluorescence assays. The complete SVA-VP1 sequence (264 aa) was truncated into 18 overlapping peptides shifted by 5 amino acids. All peptides were evaluated against SVA-pAbs and VP1-mAbs by indirect ELISA. All experiments were controlled using pre-injection SVA negative mouse sera. A peptide blocking ELISA was used to assess the blocking effect of each peptide on SVA-mAb binding activity to rVP1 immobilized to the surface of polystyrene microplates. The half maximal inhibitory concentration (IC_{50}) was calculated for each peptide comparing 100% binding activity of SVA-mAb to SVA-rVP1. The inhibitory effect of each peptide was also evaluated by a blocking ELISA on known positive serum samples collected during an outbreak of vesicular disease associated with SVA. No significant differences in reactivity was observed between SVA-pAbs and VP1-mAbs using WV and rVP1-ELISAs, Western blot, and IFA. However, SVA-pAbs but not mAbs showed virus neutralizing activity. All VP1-derived peptides were reactive against SVA-pAb and VP1-mAb, with no significant differences in reactivity amongst peptides when tested by ELISA except for peptide-1 (aa 1-20). However, peptides 1, 5,8, and 9 (aa 1-20, aa 60-80, aa 105-125, aa 120-140) showed a potent inhibition in binding activity between rVP1 and VP1-mAb. In addition, several peptides showed variable inhibition of binding activity in sera from naturally infected animals against rVP1. These results suggest that the humoral immune response generated against SVA-VP1 can be defined by a set of linear epitopes. Further investigations warranted to determine whether these set of linear epitopes are indeed responsible of eliciting neutralizing antibodies against SVA.

AAVLD Trainee Travel Awardee
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Poster Sun-34

Serologic cross-reactivity between PEDV and other porcine enteric coronaviruses ◊

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The development of porcine epidemic diarrhea virus (PEDV) antibody-based assays is important for detecting infected animals, confirming previous virus exposure, and monitoring sow herd immunity. However, the potential cross-reactivity among porcine coronaviruses, including PEDV, transmissible gastroenteritis virus (TGEV), porcine respiratory coronavirus (PRCV), and porcine deltacoronavirus (PDCoV), is a major concern for the development of pathogen-specific assays. In this study, 72, 7-week-old pigs were randomized to six groups; each group consisted of 12 pigs in one room, with 6 pens per room and 2 pigs per pen. Each group of pigs was experimentally inoculated with a different porcine coronavirus (PEDV non-S INDEL, TGEV Miller, TGEV Purdue, PRCV, PDCoV, and uninoculated control group). Serum samples (n = 792) were collected from all groups on day post-infection (DPI) –7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42. Virus shedding within groups and absence of cross-contamination between groups was confirmed by rRT-PCR through the observation period (DPI –7 to 42). The antibody response to recombinant polypeptides derived from PEDV structural proteins, i.e., spike (S) nucleocapsid (N), membrane (M), and envelope (E), and to the intact PEDV virion was evaluated using a multiplex fluorescent microbead-based immunoassay (FMIA) and a whole-virus (WV) ELISA. The final aim of this study was to identify highly sensitive and specific PEDV antigen targets for the antibody-based differential diagnosis of coronavirus-related enteric disease. Antibody assay cut-offs were selected to provide 100% diagnostic specificity for each target (S1 non S-INDEL, S1 S-INDEL, N, M, E, and WV). The earliest IgG antibody response was detected at days 7–10 post-infection, mainly directed against S1 polypeptides. With the exception of non-reactive protein E, we observed similar antibody ontogeny and pattern of seroconversion for S1 (non-S INDEL, S INDEL), N, M, and WV antigens. Recombinant S1 provided the best diagnostic sensitivity, regardless of PEDV strain, with no cross-reactivity detected against TGEV, PRCV, or PDCoV pig antisera. The WV particles showed some cross-reactivity against TGEV Miller and TGEV Purdue antisera, while N protein presented some cross-reactivity against TGEV Miller. The M protein was highly cross-reactive against TGEV and PRCV antisera. This study demonstrated that variations in the antibody response against different PEDV structural proteins may have important implications in the diagnosis of PEDV infection. We also successfully identified targets of interest (e.g., S1) for the diagnosis of PEDV, providing a truly molecular immunological view of antigenic distribution and a complete antibody cross-reactivity profile between PEDV and other porcine enteric coronaviruses.

◊ USAHA Paper

Poster Sun-35

Adaptation of the porcine epidemic diarrhea virus (PEDV) indirect immunofluorescent antibody (IFA) assay to a high-throughput format using imaging cytometry ◊

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Porcine epidemic diarrhea virus (PEDV) is responsible for significant economic losses in the swine industry due an elevated morbidity and mortality in neonatal pigs. For this reason, the diagnosis of the disease in the herds is of highly importance, in order to prevent and/or control the spread of the virus among pigs. Nowadays, there are several techniques to diagnosis PED, such as, RT-PCR, ELISA, FFN, and Immunofluorescence antibody (IFA) assay. IFA identifies the presence of antibodies bound to specific antibodies using a fluorescent dye. Although this test is useful for screening samples for PEDV antibody, the technique is labor intensive because it requires technicians to individually read and interpret each reaction on slides or plates using an inverted UV light microscope. The reliance on individual technician also introduces subjectivity into the test and raises repeatability/ reproducibility issues when comparing results produced by different technicians and laboratories. In addition, because the samples are run in two-fold dilutions, the results are semi-quantitative rather than exact estimates. The objective of this study was to convert a PEDV IFA standard procedure into a high-throughput format based on a SpectraMax MiniMax 300 imaging cytometer (Molecular Devices, Sunnydale, CA) which is able to measure fluorescence intensity yet with imaging and analyzing cells capabilities. For this purpose, 96-well black plates with clear flat bottom (cell bind surface) were seeded with 5 x 104 Vero 81 (ATCC® CCL-81TM) cells per well, incubated for 48 h (37 °C with 5% CO2), and subsequently infected with PEDV (Colorado strain P4; 0.2 MOI) and incubated for an additional 24 h. Then, plates were fixed with 80% acetone for 10 minutes, air dried for a minimum of 20 min, and stored at -20°C until use. IFA was made on sera collected from pigs of precisely known PEDV status, and read with the SpectraMax MiniMax 300 imaging cytometer. Results showed that the use of the imaging cytometer has the advantage of reducing the time for plate reading to < 3 min, improving the repeatability/ reproducibility of the test, and the precision of the antibody response estimates, resulting in an improvement over the classic IFA approach.

◊ USAHA Paper

Poster Sun-36

Virus Isolation: The gold standard in virology veterinary diagnostic methods §

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For decades, virus isolation has been held as the gold standard in diagnostic methods for detecting viral agents in clinical samples. The question today is: With all of the new, modern, faster technology is virus isolation still the gold standard or even relevant in current diagnostic systems? Polymerase chain reaction (PCR) assays have moved to the fore front of veterinary diagnostics with next generation sequencing becoming more routinely available. PCR assays have an extremely high sensitivity and a quick turnaround time. However, PCR's are geared to only look for one specific virus type and might miss unanticipated agents or mixed infections. Virus isolation is generally a non-targeted test which allows for the isolation of more than one virus from a sample or the isolation of an agent unanticipated by clinical history. Virus isolation is still needed in order to discover new emerging viruses. Virus isolation also proves both the presence and viability of the virus, therefore, allowing for the differentiation of viable from nonviable virus. This differentiation is not made by most nucleic acid detection methods. Having access to viable virus may also be necessary for animal studies be it pathogenicity studies or vaccine efficacy trials or for rapid development of serological assays. Three examples of the value of virus isolation are presented 1) In 2011, an aborted canine fetus from Texas was submitted to the AHDC. No significant lesions were noted on histopathology. Virus isolation testing identified *Bluetongue virus* as a cytopathic agent in the fetal tissue. Bacterial cultures only grew a few environmental contaminants and fluorescent antibody staining for leptospira was negative. Nearly a year later, BTV-11 was isolated from an aborted fetus from Kansas; 2) In 2014, two Great Dane females aborted litters of puppies two weeks apart. Fetus from second abortion submitted for abortion work-up. Histopathology provided no useful information. Tissue submitted for virus isolation yielded Jamestown Canyon virus. Virus neutralization tests using the clinical isolate demonstrated high Ab titers in both females; 3) In 2015, a canine from Virginia died with symptoms of hemorrhagic enteritis. PCR assays for standard enteric agents, bacterial cultures and toxicology diagnostic testing all resulted in negative findings. Tissue samples submitted to the AHDC were tested using the virus isolation test method. Inoculating a canine kidney cell culture with filtered gastrointestinal extract resulted in the identification of a novel canine *Calicivirus*. This isolate was sequenced, showing that this virus is most related to a bioreactor contaminant of CHO cells in Germany in 2003.

§ AAVLD Laboratory Staff Travel Awardee

Poster Sun-37

Outbreak of canine distemper virus in Mississippi

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Canine distemper virus (CDV) is a negative sense, single stranded RNA virus in the genus Morbillivirus of the family Paramyxoviridae. CDV is a highly contagious virus, and is one of the most important diseases of domestic and wild canid species, other wild carnivore and omnivore species, and non-human primates. CDV has a worldwide mortality rate second only to rabies in dogs. CDV is a pantropic morbillivirus with a worldwide distribution and has affinity for lymphoid and epithelial tissues (i.e. lung, gastrointestinal tract, urinary tract, skin) and CNS (including the optic nerve and eye). From 2013 to the end of May 2017, positive cases detected from submissions to MVRDL increased from 6 in 2013 to 50 in the first five months of 2017. Within the past four and half years (Jan 2013 to May 2017), a total of 115 positive cases have been detected in MVRDL from domestic dogs, raccoons, foxes, and red pandas. Fifty positive cases were detected in the first five months of 2017. CDV was detected from different tissue (lung, urinary bladder, brain), swab, blood, and urine samples using conventional PCR. Whole genomic sequencing was accomplished for eight positive CDV cases, and more will done within a few months. Sequences from some samples group with America-3 and some samples group with a sequence in GenBank from an outbreak in a breeding facility in Wyoming in 2010. Different samples from each positive case with more than one sample were compared. Based on our current data, urine appears to be more sensitive than mixed tissues, and swabs (10 nasal swab, 8 conjunctival swab, 5 unknown) are more sensitive than blood samples. However, with the limited data available, more samples should be collected for comparison.

Poster Sun-37

Serological survey for antibodies against pestiviruses in sheep in Wyoming

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Pestiviruses including *Bovine Viral Diarrhea Virus type 1* (BVDV1), BVDV-2 and *Border Disease Virus* (BDV) have been reported in sheep populations worldwide. These viruses are not strictly host specific and can also infect cattle, goats, swine and wild ruminants. In sheep, clinical signs are related to reproductive problems, respiratory disease, neurological signs, abnormal body conformation and congenital disorders characterized by hairy shaker disease. Despite the importance of pestiviruses for animal health and its economic impact, little is known regarding the prevalence of exposure to pestiviruses in the U.S. sheep population as determined by seroprevalence. The aim of this study was to perform a serological surveillance of pestivirus infection in domestic sheep from Wyoming, which is one of the most important states in sheep industry of Unites States. Pools of sera from 500 sheep were examined by virus neutralization assay against four species of pestiviruses: BVDV-1, BVDV-2, BDV and an emerging species of virus known variously as HoBi-like virus, atypical bovine pestivirus or BVDV3. The overall pestivirus prevalence of exposure rate was 5.6%. Antibodies were most frequently detected against BVDV-1 (4%). Overall the highest titers detected were also against BVDV-1. The seropositive rates against HoBi-like virus, BVDV-2 and BDV were 3.8%, 3% and 2%, respectively. This study suggests that BVDV-1 may be the major ruminant pestivirus infecting sheep in Wyoming. However, the seroprevalence for sheep in other regions of the US may differ due to variation in interspecies contact and transmission. Interspecies pestivirus transmission is important to the design of a successful BVDV control program for the US.

Poster Sun-39

Comparison of virus isolation and PCR assays for detection of avian diseases §

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The poultry industry is an important part of agriculture in Western Kentucky, therefore testing for avian diseases is a significant responsibility of Breathitt Veterinary Center (BVC). Among the significant avian viral diseases in this region are: avian influenza virus (AI), Newcastle disease virus (NDV), infectious laryngotracheitis virus (ILT), infectious bronchitis virus (IBV), avian reovirus (Reo), infectious bursal disease (IBD), and chicken anemia virus (CAV). Traditionally, avian viral disease diagnoses were based on viral isolation in embryonated chicken eggs, followed by viral identification through transmission electron microscopy (TEM). Breathitt Veterinary Center is one of few diagnostic laboratories that continues to have the capacity to offer this method of testing. This method takes a minimum of one week to produce results, and the high cost associated with the use of specific pathogen free (SPF) eggs and maintenance of the electron microscope make it an expensive test for both the laboratory and the client. Conventional and real-time PCR assays are widely utilized as diagnostic tools for many diseases. PCR assays are excellent method choices for detection of avian viral diseases due to their rapid turn-around times and high sensivities. While detection of virus after viral isolation in embryontated eggs by PCR assay can be more sensitive than identification by TEM, direct testing of tissue samples by PCR assay can save time and cost.

At BVC, the PCR assays developed by NAHLN for the detection of AI and NDV are used for routine diagnosis of these two viruses. As we pursued new PCR assays for various avian viruses, we conducted a comparison study of the PCR assay versus traditional viral isolation. Known stock virus and/or field samples were propagated in SPF embryonated chicken eggs following standard protocol. Serial dilutions of virus were used in side-by-side testing by the TEM method and PCR assays. Direct PCR testing of field samples was also evaluated. Test sensitivity, testing cost, testing time, turn-around time and other logistics of performing each method were analyzed. Viral isolation is proven to be more tedious, more time consuming and less sensitive than PCR assays, but it does allow detection of multiple viruses in a single test, including potential unknown viral agents. Testing for multiple viruses using several separate PCR assays can quickly elevate testing cost. Also, it can be extremely challenging to detect unknown viral agents by PCR assay. If a specific viral disease is suspected, detection by direct PCR is highly preferred due to lower cost and quicker turn-around time. However, if multiple or unknown agents are in the differential diagnosis, it is preferable to use the standard isolation/TEM method. Depending on testing needs, both methods still have a place as valuable diagnostic tools.

§ AAVLD Laboratory Staff Travel Awardee

Poster Sun-40

A duplex TaqMan real-time PCR differentiating PRRSV MLV- and ATP-like vaccine strains from field strains

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most important economic diseases worldwide. Virus eradication programs frequently use mass vaccination with modified live vaccines. The Ingelvac PRRS MLV and ATP vaccines (Boehringer Ingelheim) are widely used in North America. The progress of eradication programs relies on regular PCR testing of samples such as serum or oral fluid. PCR results interpretation is seriously complicated by the use of vaccines since positive results may be due to field or vaccine virus. This results in additional costs and time to sequence positive cases in order to differentiate field and vaccine virus. Moreover sequencing is not always successful as the virus load may be below the sensitivity level of sequencing. The objective of this study was to develop a real-time multiplex TaqMan qPCR assay allowing differentiating the ATP- and MLV-like vaccine strains from field strains in PRRSV PCR positive samples. PRRS MLV and Ingelvac PRRS ATP, specific primers and probes were designed to target MLV and ATP strains. Different fluorophores, FAM for MLV and HEX for ATP, were used to label the probe in order to run the assay in duplex. Primers and probes were synthetized and optimized for SensiFast one-step RT-qPCR mix (Bioline). Then, samples from sequenced cases were tested with the new assay and our regular ORF7-based PRRSV screening RT-qPCR. The homology of the MLV- and ATP-like cases with the MLV and ATP strains at the ORF5 level varied from 97.1 to 99.8%. So far we have tested a total of 96 sequenced samples (20 ATP, 30 MLV, 6 Fostera and 40 wild-type) with the new qPCR assay. All MLV and ATP strains were detected by the new assay (sensitivity: 50/50 = 100%). Moreover the Ct were closed to those obtained with the regular ORF7-based screening test. None of the wild-type strains reacted in the new assay (specificity: 100%). However, some Fostera strains (4/6), another vaccine strain (Zoetis) demonstrated some cross-reactions with MLV primers/probe pair. However the Ct difference between the new assay and the regular ORF7-based assay was greater than 4 Ct. Based on these results, we suggest classifying the samples with a Ct difference between the vaccine specific and the screening assays greater than 3 as wild-type or others as vaccine strains. Using this cut-off, only 2 out 20 ATP strains and 1 out of 30 MLV strain would have been falsely declared negative.

Poster Sun-41

Detection of the porcine *Circovirus* type 3 using a real-time TaqMan qPCR

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Porcine *Circovirus* type 3 (PCV3) is a new *Circovirus*, different from the PCV2, which has been recently identified in the USA using the metagenomics approach. The virus has been detected in cases of reproductive disorders (abortions), Porcine dermatitis and nephropathy syndrome (PDNS) in sows, and lymphohistiocytic inflammations of multiple organs in piglets. More recently the virus was also reported in China and it appears to be frequent and widely distributed in both countries. In order to evaluate the presence of the virus in the Quebec swine population we have developed a TaqMan qPCR assay. PCV3 specific primers and probe were designed to target ORF2 of the newly discovered PCV3. Primers and probe were synthetized and optimized for SensiFast probes qPCR mix (Bioline). A total of 252 randomly selected samples (100 oral fluids, 100 lung tissues and 52 serums) and submitted for various reasons were tested with the new qPCR assay. Oral fluid samples were also tested for presence of porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV) and PCV2. Lung tissues were tested for all the above pathogens as well as for *Mycoplasma hyopneumoniae* (Mh). Finally, serum samples were also tested for PRRSV and PCV2.

Results showed that 66% (mean Ct of 30.3±3.9), 46.2% (mean Ct of 33.3±2.8) and 16% (mean Ct of 28±4) of oral fluids, serum and lung samples respectively were positive for the presence of PCV3 DNA. PCV3 was the only detected pathogens from all tested pathogens in 53, 50 and 43.8% of positive oral fluids, serum and lung samples respectively. PCV2 (34.9%) and SIV (27.8%) were also founded in some PCV3 positive oral fluids. No PRRSV were detected in PCV3 positive oral fluids. In PCV3 positive lung tissues, PCV2 (37.5%), SIV (31.3%), PRRSV (12.5%) and Mh (12.5%) were also detected. Finally, PCV2 (33.3%) and PRRSV (16.7%) were present in PCV3 positive serums.

To our knowledge, this is the first time that PCV3 is reported in Canada. Our results showed that PCV3 is widely circulating in the province of Quebec. PCV3 was the only detected pathogen in approximatively the half of the samples. PCV2 and SIV were the most frequently co-detected pathogen in the tested samples.

Poster Sun-42

A competitive ELISA for the detection of antibodies to *Senecavirus A* in swine sera

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Senecavirus A (SVA), also called Seneca Valley Virus (SVV) belongs to the same family, *Picornaviridae*, as foot and mouth disease virus and swine vesicular disease virus. SVA has been recently associated with idiopathic vesicular disease in swine in Brazil, United States, Canada, and China. It has also been involved in systemic diseases in piglets in Brazil and USA. Several serological assays such as the virus neutralization test (VNT) and the immunofluorescence antibody test (IFAT) are being used in order to detect SVA antibodies in serum samples. However the availability of a rapid and automated immunoassay such an ELISA would greatly facilitate the surveillance of the virus. We have modified a competitive enzyme-linked immunosorbent assay (cELISA) previously described by Goolia et al, JVDI, 2017, 29 (2): 250-253, making it simpler, cheaper and safer by replacing some of the reagents. The modified assay was then validated as a screening test for SVA antibodies in swine serum samples. Microplates are coated with purified inactivated SVA. Diluted serum samples and an SVA monoclonal antibody (mAb) are added to the antigen coated microplate and incubated at 37^0C for 1 hour. The competition between the sample SVA antibodies when present and the mAb is then revealed by incubating the plates at 37^0C for 1 hour with a peroxidase conjugated anti-mouse IgG followed by a chromogenic substrate (TMB). The signal emitted is inversely proportional to the amount and/or the strength of the SVA antibodies in the test sample. Serum samples originating from 100 pigs naturally and experimentally infected with SVA and 400 pigs from Canadian farms with no history of SVA infection were examined using the cELISA and the VNT. The cutoff percent inhibition was temporarily established at ≥ 60 for positive samples. At this cut off the relative sensitivity and specificity compared to the VNT are 97.5% and 96.1% respectively. Detailed results will be presented at the meeting.

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