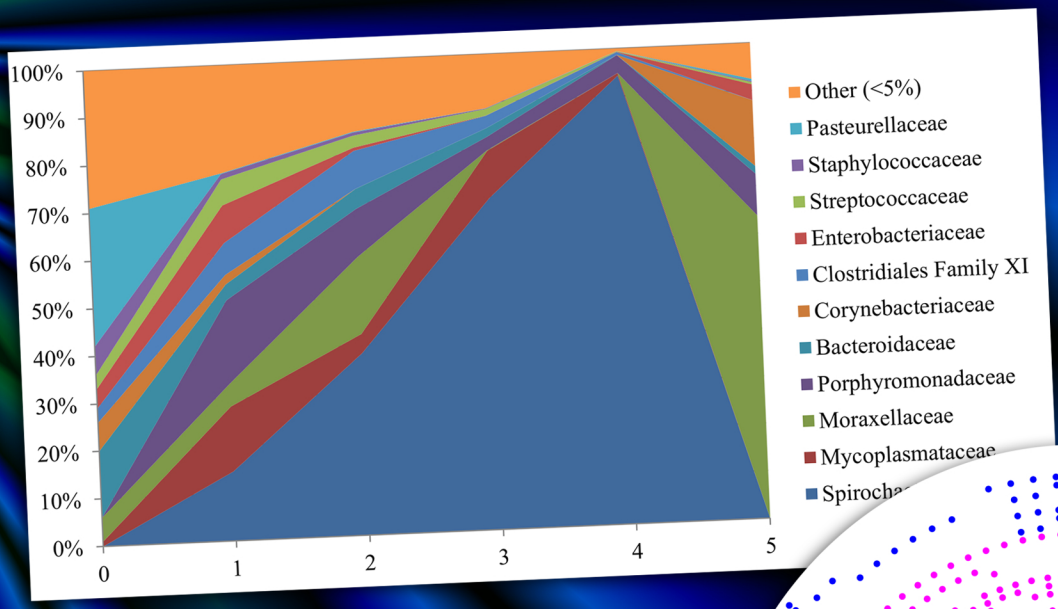
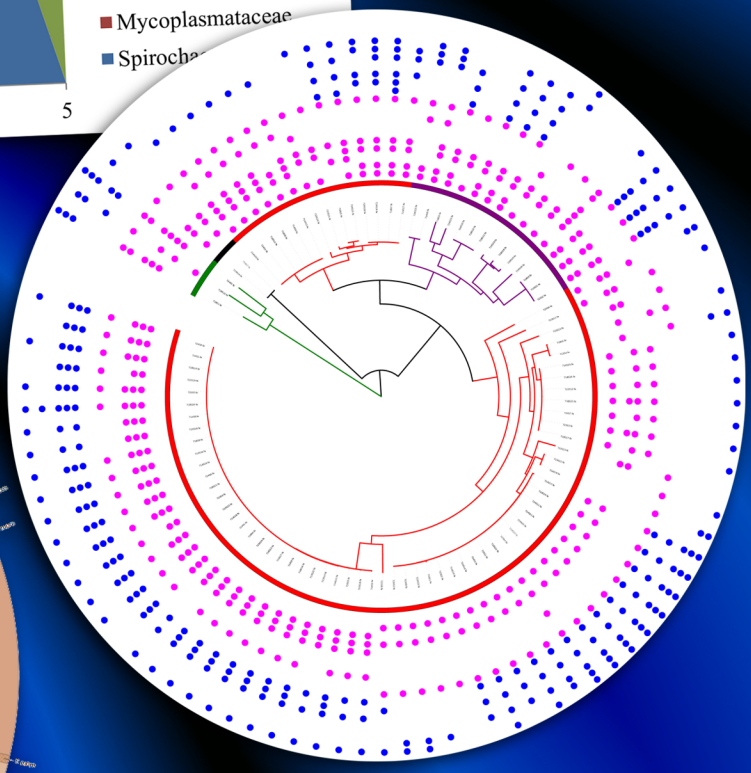
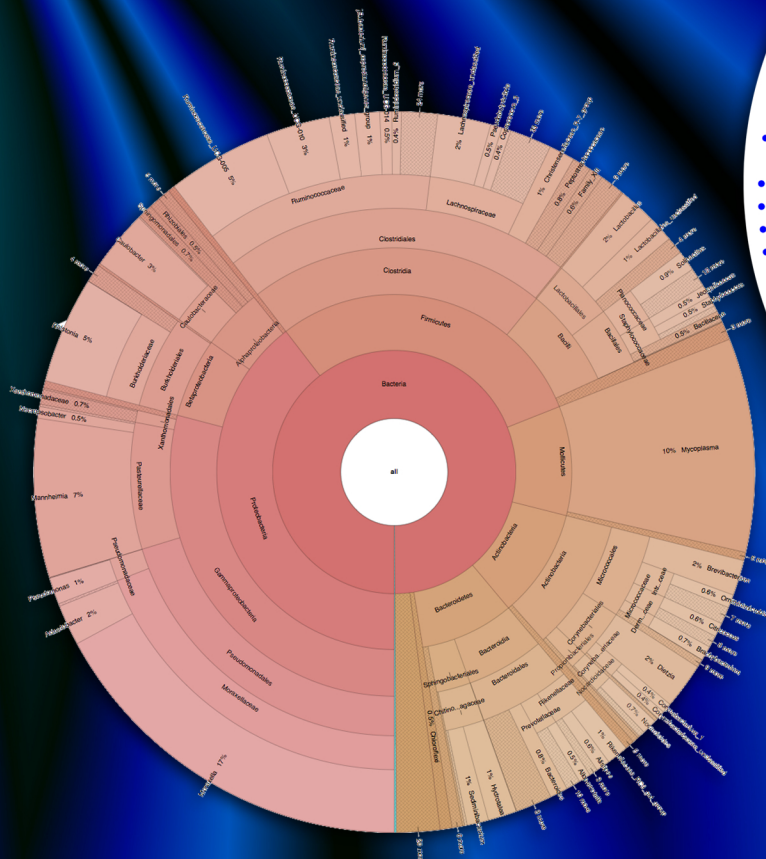
Proceedings of the American Association of Veterinary Laboratory Diagnosticians







59th Annual Conference

Sheraton Hotel
Greensboro, NC
October 13-19, 2016

On the cover: Upper left image of the microbiota associated with different stages of bovine digital dermatitis courtesy of Paul Plummer. Middle right image of genomic analysis of 95 hemolytic *E. coli* isolates from pigs courtesy of Ganwu Li. Lower left image of metagenomics analysis of a case of bovine pinkeye courtesy of Ganwu Li. Iowa State University College of Veterinary Medicine.

AWARDS

2016 Trainee Travel Awardees

Franco Matias Ferreyra	Iowa State University
Shubhada Chothe	Pennsylvania Animal Diagnostic Laboratory System
Noah Hull	University of Wyoming
Jaimie Strickland	Michigan State University
Nanny Wenzlow	University of Florida
Tessa LeCuyer	Washington State University
Erica Noland	Michigan State University
Arya Sobhakumari	University of California- Davis
Laila Akhter	Bangladesh Agricultural University
Jessica Rodriguez	Texas A&M University
Mathew Abraham	University of Georgia
Colleen Monahan	Michigan State University
Rahul Dange (Pathology)	Michigan State University

2016 Staff Travel Awardees

Kyriakos Deriziotis	Cornell University
Robert Bowden	University of Florida
Niesa Kettler	Michigan State University
Kevin Lin	Iowa State University
Eric Lee	Cornell University
Feng (Julie) Sun	Texas A&M TVMDL

2016 ACVP/AAVLD Award

Katie Barnes

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 function of the hard work, dedication and creativity of many people. A special thank you to all who present their data and conclusions, to all exhibitors and sponsors, and to everyone who attends our meeting. 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 been a win-win.

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 and Rhonda Bardsley from the Planning Connection, Inc., 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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Please note: Abstracts published in these proceedings were peer reviewed by the members of the Program Committee for data supporting conclusions to be presented, and were edited into a consistent format. Full manuscripts were not evaluated and readers should contact the author for referral to a full presentation of data or for permission to use, copy, or distribute data contained in an abstract.

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Imperial F

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AAVLD / USAHA Joint Plenary Session
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Precision Diagnostic Medicine: Game Changing Technology...Is Your Lab Ready?
Saturday, October 15, 2016
Imperial D

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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

The National Microbiome Initiative: Opportunities for diagnostic medicine and beyond

Jo Handelsman

Office of Science and Technology Policy, Executive Office of the President, Washington, DC

The microbiome is the community of microorganisms that inhabits an environment such as the human body, animals, plants, soil, oceans, and every other ecosystem on Earth. The last decade has witnessed an explosion of knowledge about microbiomes and the realization that microorganisms control the health of virtually every ecosystem. Recent findings show that the human microbiome is associated with chronic diseases such as obesity, diabetes, depression, and asthma, health conditions that were long assumed to be due to other causes. In the environment, the microbiome influences crop and livestock productivity, climate change, and ocean health. There is tremendous potential for managing microbiomes to achieve beneficial outcomes for human health, agricultural productivity, clean energy supply, environmental health, and the economy, but a new approach to microbiome research is needed. The National Microbiome Initiative was launched to spur new interdisciplinary research on fundamental questions about diverse microbiomes, development of platform technologies for probing and changing microbiomes, and computational tools to manage massive data sets, and a new workforce through citizen science, public engagement, and education opportunities.

Speaker Biography: Dr. Jo Handelsman is the Associate Director for Science at the White House Office of Science and Technology Policy, appointed by President Obama and confirmed by the Senate in June of 2014. Dr. Handelsman helps to advise President Obama on the implications of science for the Nation, ways in which science can inform U.S. policy, and on Federal efforts in support of scientific research. Prior to joining OSTP, Dr. Handelsman was the Howard Hughes Medical Institute Professor and Frederick Phineas Rose Professor in the Department of Molecular, Cellular and Developmental Biology at Yale University. She previously served on the University of Wisconsin-Madison faculty as a Professor in Plant Pathology from 1985 to 2009 and as Professor and Chair of the Department of Bacteriology from 2007 to 2009. In 2013, she served as President of the American Society for Microbiology. Dr. Handelsman is an expert in communication among bacteria that associate with soil, plants, and insects and helped pioneer the field of metagenomics, bridging agricultural and medical sciences. Dr. Handelsman received a B.S. from Cornell University and a Ph.D. in Molecular Biology from the University of Wisconsin-Madison.

Progress on the food animal microbiome and relevance to veterinary diagnostic medicine...using next generation sequencing to unravel complex infectious diseases

Paul Plummer

Iowa State University, Ames, IA

Recent advances in next generation sequencing has led to the rapidly developing field of metagenomics. Metagenomic approaches leverage the power of new genomic technologies to study entire microbial communities (bacterial, viral and fungal) using culture independent methodologies. In their joint summary report entitled "Understanding Our Microbial Plant: The New Science of Metagenomics", the National Academy of Sciences, National Academy of Engineering, Institute of Medicine and the National Research Council argue that "metagenomics represents a quantum leap forward in microbiology analogous to the revolutionary new view offered by [the] first microscope, and it provides new insights into a previously inaccessible living universe." This universe is assembled into highly specialized communities depending upon their ecological niche, including such diverse environments as the rumen, mammalian gastrointestinal tract, urinary tract and mammary gland. The study of the microbial composition of these niches, collectively referred to as the microbiome, has revealed several paradigm-changing insights for veterinary diagnostic medicine. These insights are revolutionizing the way that we research infectious disease of livestock and concurrently making us rethink the significance and interpretation of traditional diagnostic results. In this session we will briefly review several key lessons that we have learned to date, with specific examples of how the use of metagenomic approaches has altered our diagnostic approach or perspective.

Lesson 1: "Sterile" biological samples rarely, if ever, exist. Traditional microbial culture has been a mainstay of veterinary diagnostic medicine for decades, and dogma has suggested that many biological samples (ie. milk, urine, uterine swabs, bile) are normally sterile. It is now clear that each of these sample matrices contains highly complex microbial populations composed of hundreds of organisms that fail to grow with routine methodology.

Lesson 2: Simple cases may not be as simple as we thought! When we culture a single known pathogenic organism it is easy to assume that the pathophysiology is due to that organism. However, deeper evaluation of some of these cases reveals that the single cultured isolate is only a biomarker for a much more complex shift in microbiota that we have missed due to our inability to culture most of these organisms.

Lesson 3: We need to be transitioning to thinking polymicrobial as the norm. While there will always be diseases that are driven by a single organism (Brucellosis, FMD, TB etc), our understanding if infectious disease is transitioning to a population based perspective as opposed to single organism.

Lesson 4: Relative abundance of organisms does not tell the whole story. It is clear that pathogenicity is not driven by bacterial abundance, but this is a constant pitfall for folks new to metagenomics. Low abundance organisms can cause disease and high abundance does not assure pathogenicity.

Lesson 5: How relevant is an antibiogram in the age of metagenomics? Clinicians often rely heavily on antibiograms generated against organisms that grow in the laboratory following routine submission. As it becomes increasingly clear that there are a large number of normal flora in a variety of samples, and that organisms that are cultured may only represent a minor fraction of a much larger dysbiosis, the relevance and utility of single isolate antibiograms needs to be considered. In an age of prudent antimicrobial use, is it prudent to base treatment decisions off of a single organism or do we need to rethink our approach to the process?

Speaker Biography: Paul J Plummer, DVM PhD, Diplomate ACVIM (Large Animal Internal Medicine), Diplomate European College of Small Ruminant Health Management (ECSRHM). Dr. Plummer is an associate professor at the Iowa State University College of Veterinary Medicine. He attended veterinary school at the University of Tennessee. After graduation he did a large animal medicine and surgery internship at Texas A&M followed by a residency in large animal internal medicine at the University of Tennessee. He was boarded as a Diplomate of ACVIM (Large Animal Medicine) in 2004. At that time, he moved to the College of Veterinary Medicine at Iowa State University where he completed a PhD in Veterinary Microbiology and currently serves on faculty. Dr. Plummer's laboratory researches a variety of infectious and zoonotic diseases of importance to livestock and human health. He is also very interested in the human-wildlife-livestock interface and the mechanisms of environmental maintenance and transmission of zoonotic disease at this interface. Current projects in his laboratory focus on polybacterial infection dynamics, *Campylobacter jejuni* and *Coxiella burnetii*. In addition, he has international research projects in Ethiopia and South Africa that focus on disease transmission between livestock and humans.

Progress on the companion animal microbiome and relevance to veterinary diagnostic medicine

Jan Suchodolski

Texas A&M University, College Station, TX

GI microbiota

Intestinal microbiota is the set of microorganisms (bacteria, fungi, archaea, protozoa, and viruses) within the gut. Bacteria are of fundamental importance, because they help in maintaining gut homeostasis by competing with pathogens, and by producing short-chain fatty acids and other immunomodulatory metabolites. Various studies have reported changes in microbial communities in acute and chronic gastrointestinal diseases. Most commonly observed are decreases in the bacterial phyla *Firmicutes* (i.e., *Lachnospiraceae, Ruminococcaceae, Faecalibacterium*) and *Bacteroidetes*, with concurrent increases in *Proteobacteria*. Changes in the microbiota result in functional and immunological consequences for the host. It is important to better understand the functional consequences of microbial dysbiosis. For example, changes in bile acid metabolism, short-chain fatty acid concentrations, and tryptophan pathways have recently been reported in dogs using metabolomic analyses. This depletion of commensal groups and their respective immunoregulatory metabolites (e.g., indoles, and secondary bile acids) may impair the ability of the host to down-regulate the aberrant intestinal immune response, making dysbiosis an integral part of the pathogenesis of chronic GI disease. Of interest is that these changes did not correlate with clinical activity, suggesting the presence of ongoing inflammation.

Better characterization of dysbiosis may guide treatment decisions (need of antimicrobials vs. dietary, probiotics, and immunosuppression). Routine bacterial culture has no utility for characterization of the many anaerobes in the GI tract. The best method to characterize microbiota is through molecular methods. We have identified bacterial groups that are consistently altered in dogs with chronic enteropathies (CE). A mathematical algorithm is used to report these changes as a dysbiosis index (DI), and results can be reported in 24 hours. A negative DI indicates normobiosis, whereas a positive DI indicates dysbiosis. The DI was trained to diagnose the dysbiosis in CE, and an increased DI in dogs with CE provides additional information during the diagnostic work-up of these dogs. Another potential use of the DI is diagnosing primary dysbiosis. It would be reasonable to treat dogs with chronic intermittent diarrhea, which are not systemically ill, and have an increased DI, with probiotics and/or prebiotics.

Microbiota of skin and other organ systems

The intestinal microbiota has been studied for more than a decade, and the first diagnostic tests are available for better assessment of dysbiosis and treatment. The microbiota of other organ systems has been just recently reported in dogs and cats, with most data being mostly descriptive at this point. Initial studies evaluating the oral, nasal and the lung microbiota of dogs and cats have revealed a high diversity of microorganisms living in these ecosystems, especially when compared to bacterial culture results. Several studies have compared the bacterial and fungal microbiota of healthy dogs to dogs with atopic dermatitis. These studies suggest that the skin microbiota is also important in regulation of immunity and pathogen exclusion, but more mechanistic studies are required to link these microbiota changes to disease initiation and progression, and to diagnostic utility.

Speaker Biography: Jan S. Suchodolski graduated with a veterinary degree from the University of Veterinary Medicine in Vienna, Austria in 1997. After working for several years in a small animal specialty clinic he returned to academia and received his Dr. med. vet. degree from the University of Vienna, Austria. In 2005 Dr. Suchodolski received his PhD in Veterinary Microbiology from Texas A&M University for his work on molecular markers for the assessment of the intestinal microbiota. He is board certified in immunology by the American College of Veterinary Microbiologists (ACVM). He currently serves as Associate Professor and Associate Director of the GI Lab. His research is focused on gastrointestinal function testing, gastrointestinal pathogens, and intestinal microbial ecology with an emphasis on probiotics and prebiotics and how intestinal pathogens lead to disturbances in the intestinal microbiome and metabolome of companion animals.

Precision Medicine: An opportunity for a paradigm shift in veterinary diagnostic medicine

K.C. Kent Lloyd

University of California-Davis, Davis, CA

Precision medicine is a technology-driven approach to making clinical care decisions and processes more accurate, cost-effective, and egalitarian. The guiding principle of precision medicine is that patient-specific megadata and information will be key to precisely defining a new taxonomy of disease. This approach to clinical medicine relies on an informatics-based assessment of molecular and other disease-specific biomarkers, environmental data, and behavioral information to guide diagnoses, deliver targeted therapies, and implement reliable preventive strategies. Put simply, the goal of precision medicine is to identify clinically-actionable findings influenced by lifestyle features in the context of environmental factors. With this, precision medicine promises to deliver the "*right drug in the right amount to the right patient at the right time under the right conditions*". In the human medical field, precision medicine emphasizes the statistical analysis of electronic health record data from very large numbers of patients sharing common disease features, complemented by additional sources of information found in the published literature, clinical studies (e.g., GWAS), and human databases (eg., ClinVar). The need for such an approach is significant…on average, every person harbors knockout variants in ~20 genes, yet we know the genetic basis for only half (3500) of the approximately 7000 inherited human disease syndromes. Although already well-advanced in human medicine, precision medicine has the potential for making a similarly comprehensive impact on the delivery of clinical care to animals. This includes development of screening and confirmatory diagnostic tests and identification of molecularly targeted treatments. For example, tumors are now increasingly being classified on the basis of specific genetic mutations rather than tissue of origin, which in in turn informs targeted molecular therapy. In fact, there have already been significant advances in using molecular-guided approaches to diagnosis and therapeutics in veterinary cancer care. As evidence, note that BRAF V600E mutations have recently been identified with high frequency in primary canine prostatic and urothelial carcinoma. Further, molecularly-guided kinase inhibitors tocreranib (Palladia©) and masitinib (Kinavet©) are now FDA-approved treatments for mast cell tumors in dogs. In addition, considering the diversity of known and unknown disease mechanisms, and the variability in disease processes over time, relying solely on the accumulation of patient-derived measurements and observations that achieve statistical significance will not reveal clinically actionable findings in a timely fashion. Therefore, defining the molecular pathogenesis of commonly shared diseases can be facilitated by clinical studies in animals. Indeed, naturally occurring diseases with common pathogenic mechanisms between animals and humans enable a critical role for precision veterinary medicine to inform precision medicine in both persons and pets. Correlating patient-specific information with phenotype ontologies across multiple animal species will enable rapid identification of known determinants of disease. A comprehensive human-animal knowledge network populated by data derived from patient-guided precision disease models will inform evidence-based decisions on fewer numbers of individuals with commonly shared disease characteristics within a reasonable timeline. However, this new paradigm will only be realized when clinicians, medical informaticians, and data scientists work synergistically to thoroughly integrate diagnostic laboratory and –omic profiles with clinical patient information and metadata to inform precision veterinary medicine.

Speaker Biography: Dr. Kent Lloyd is a Professor in the Department of Surgery in the School of Medicine at the University of California-Davis. Dr. Lloyd earned his DVM from UC Davis and a PhD in Physiology from the University of California Los Angeles. His primary research focuses on development of mouse models of human disease, genome editing, and preservation and resuscitation of genetically altered mice. Dr. Lloyd serves as the Director of the UC Davis Mouse Biology Program where he is engaged in technology development to enhance the resources, services, products, and procedures available through the Mouse Biology Program and the associated NIH-funded projects, including the Mutant Mouse Resource and Research Center (MMRRC), the Knockout Mouse Production and Phenotyping (KOMP2) projects, and the Mouse Metabolic Phenotyping Center.

Advances in the use of molecular techniques to detect and monitor antimicrobial resistance

Nicole Ricker

NADC-ARS, USDA, Ames, IA

Antimicrobial resistance (AMR) is increasing in prevalence with potentially devastating results. This issue must be addressed simultaneously by all government and industry sectors currently using antibiotics if there is to be any success in halting the progression of resistance development. In veterinary diagnostics, there are two separate, yet complimentary, purposes for monitoring of antimicrobial resistance genes. First, it is preferable to determine the susceptibility of the pathogen to a particular antibiotic prior to administration in order to maximize the success of initial treatment. This is beneficial in reducing the likelihood of multiple rounds of treatment for infected animals. However, in addition to susceptibility testing, resistance monitoring is also important for surveillance of resistance gene carriage and dissemination. AMR can be an intrinsic feature of individual strains (since many antibiotics only target specific bacterial types) but can also be acquired through either mutation or horizontal transfer. Monitoring the emergence of new resistance is essential for understanding the limitations of currently available treatment options, and the potential for new AMR combinations to transfer into pathogens.

High throughput screening for diagnostic and monitoring purposes

Culture techniques have been the gold standard for detecting AMR, however they are typically restricted to a small number of bacteria and require 16-20 hours for bacterial growth. In order to have a meaningful impact on antibiotic use, point-of-care diagnostic tools would be most beneficial. Methods have been developed for pathogen detection based on a number of biomarkers including host cell responses, and polymerase chain reaction (PCR) based methods for pathogen related gene detection. Some of the diagnostic methods specific to antimicrobial resistance include single cell monitoring, rapid antibiotic susceptibility testing and PCR based testing for antibiotic resistant genes. Many of these methods can be performed in 3-4 hours, allowing for informative results prior to drug administration. This represents a substantial improvement in available information for individuals prescribing antibiotics and will hopefully facilitate the incorporation of antibiotic susceptibility into treatment decisions. There are limitations to each of these tests, including being restricted to a small number of known pathogens or genes. One substantial improvement in AMR monitoring is the development of high capacity quantitative PCR (qPCR) on microfuidic chips. This method can simultaneously detect and quantify hundreds of resistance genes from multiple samples. However, this technology is limited in that it cannot detect novel resistance mechanisms, and also that gene detection does not necessarily correlate with phenotypic resistance or treatment outcome in the host.

Full genome sequencing for novel pathogens or new combinations of resistance genes

Monitoring of resistance profiles provides important information for gene abundance and treatment decisions, however genetic context of these AMR genes is essential to fully understand the evolution of pathogens and predict the emergence of new resistance issues. Resistance genes that are intrinsic to particular pathogens, or that are long established in those strains, are of limited informative value and need not be investigated further. However, new combinations of resistance genes or increased mobility of the genes merit higher concern and can only be fully understood through whole genome sequencing (WGS). The recent discovery of plasmid-mediated colistin resistance highlights the importance of fully understanding the genetic context of resistance genes. Inclusion of WGS as a standard method for analysis is now being incorporated into most of the national monitoring agencies, aided by the development of low-cost, high-throughput next generation sequencing. The recent development of long-read next generation sequencing (over 20 kb reads), effectively removes the barriers to complete assembly of individual genomes, facilitating comprehensive understanding of the mobility and co-occurrence of important determinants of resistance.

Speaker Biography: ORISE Post-doctoral Microbiologist, Food Safety and Enteric Pathogen Research Unit, NADC-ARS, United States Department of Agriculture, Ames, IA 50010. Dr. Ricker received her Ph.D. in environmental science from the University of Toronto, Canada, with a specialization in bacterial genetics. Nicole is now a post-doctoral research microbiologist with Dr. Heather Allen at the USDA's National Animal Disease Center in Ames, Iowa, and a collaborating assistant professor at Iowa State University. She is an expert in mobile genetic elements that are associated with resistance genes and disseminate among different bacterial strains. Her current research is on developing methods for monitoring antibiotic resistance genes in animal and environmental samples.

Use of metabolomics in veterinary diagnostic medicine

Elizabeth Ryan

Colorado State University, Fort Collins, CO

Research utilizing global, non-targeted metabolomics technologies has increased substantially over the past decade and merits greater attention in veterinary science and medicine across the fields of infectious and chronic diseases, pharmacology, nutrition and diagnostics. This high throughput and sensitive tool may help us better understand veterinary disease etiology and progression, while developing strategic prevention, control and treatment measures. The databases for metabolites detected across a suite of biological and environmental matrices are expanding for plants and humans, such as within Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB). The development of a database relevant for the practicing and research-oriented veterinarian merits rigorous, detailed attention across wild, livestock, exotic and companion veterinary species. The clinical utility of metabolomics and metabolic profiling in health and disease will be presented alongside the integrated metabolic pathway networks in which metabolites are organized and should be better understood. Metabolite profiles from companion animal studies were reported to display widespread inter- and intra- individual variations that are likely due to a host of factors, including, but not limited to the influence of diet, breed, gut microbiota, living conditions and other environmental exposures. Evidence for dietary modulation of the companion canine metabolome in healthy, overweight and obese dogs strongly supports the need for this emerging metabolite profiling technology in the clinic. Taking into account lifestyle factors that can be detected via use of metabolomics approaches may improve our diagnostic and prognostic capability in the near future and for the long-term in veterinary medicine.

Speaker Biography: Elizabeth Ryan is an Associate professor of Toxicology in the College of Veterinary Medicine and Biomedical sciences at CSU. Her Toxicology and Nutrition laboratory utilizes translational and 'one-health' research approaches for investigating functional, sustainable foods such as whole grains and legumes for their gastrointestinal health and disease fighting properties across the lifespan. Her published and recent work explores the complex metabolism by microbial communities including native gut probiotics, enteric pathogens and antimicrobial resistant bacteria. She holds joint appointments with the Colorado School of Public Health where she is co-leader of the Animals, People and Environment concentration. Dr. Ryan's global health research program also includes developing innovative solutions to problems of malnutrition and environmental enteric dysfunction. Ryan has ~60 peer-reviewed publications and a research program that is currently funded by the National Institutes of Health, National Institute of Food and Agriculture, and the Bill and Melinda Gates Foundation.

Bacteriology 1

Saturday, October 15, 2016

Imperial B

Moderators: John Dustin Loy and Claire Miller

1:00 PM **Antimicrobial Susceptibility Testing in Veterinary Diagnostic Laboratories in the United States**
Beth Harris, David Dargatz, Matthew M. Erdman, Sarah Tomlinson *35*

1:15 PM **Increased frequency of isolation of multi-drug resistant *Salmonella* I 4,[5],12:i:- from swine with histologic lesions consistent with salmonellosis**
Adam Krull, Bailey Lauren Arruda, Kent L. Schwartz, Eric Burrough, Orhan Sahin, Amanda Kreuder *36*

1:30 PM **Culture and Sensitivity Results from Canine and Feline Cystocentesis Samples (2008-2014)**
Emily Knebel, Stephen Cole, Shelley C. Rankin *37*

1:45 PM **Antimicrobial susceptibility of *Streptococcus suis* isolated from diseased pigs from a veterinary diagnostic laboratory in the Midwest United States: Association between isolation site, serotype and resistance**
Orhan Sahin, Curt Thompson, Lei Dai, Adam Krull, Eric Burrough *38*

2:00 PM **Monitoring and source tracking of multiple antimicrobials resistant enteropathogenic *E.coli* and resistant genes in a dairy farm †**
El Bably M. A., Asmaa N. Mohammed, Manar B. Mohamed, Hanan A. Fahmy *39*

2:15 PM **Creation of a MALDI-TOF Library to Identify *Aeromonas salmonicida* # * †**
Tessa LeCuyer, Timberly Maddox, Dubraska Vanessa Diaz-Campos, Kevin R. Snekvik *40*

2:30 PM **Validation and optimization of the NIH Mold Database for MALDI-TOF MS in veterinary diagnostic setting §**
Niesa Kettler, Karen Parlor, Rinosh Joshua Mani *41*

2:45 PM **MALDI-TOF as a novel detection method for *Clostridium difficile* toxins**
Kenitra Hammac, Dian Dian Lin, Kelly Ray, Christina Wilson *42*

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

Antimicrobial Susceptibility Testing in Veterinary Diagnostic Laboratories in the United States

Beth Harris[2], David Dargatz[3], Matthew M. Erdman[1], Sarah Tomlinson[2]

[1]NVSL, Ames, IA; [2]National Animal Health Laboratory Network, USDA-APHIS-VS-STAS-NVSL, Ames, IA; [3]CEAH, APHIS-VS, Ft. Collins, CO

Antimicrobial resistance is a serious threat to animal and human health worldwide, requiring a collaborative approach to holistically address this issue. The U.S. Government has developed a national strategy to address antimicrobial resistance. One component of this national strategy is to monitor antibiotic susceptibilities in agricultural settings, including data generated by the veterinary community. A survey was used to collect information related to antimicrobial susceptibility testing (AST) from the veterinary diagnostic community in the U.S. The survey was developed in collaboration with AAVLD veterinary diagnostic laboratories, USDA, the Food and Drug Administration, and the Clinical and Laboratory Standards Institute (CLSI), and was administered by AAVLD in September 2015. The objectives were to assess current practices and technologies for detecting resistance in bacterial pathogens of veterinary significance and to determine how laboratories share information on antimicrobial resistance.

Fifty-two laboratories completed the survey. Agricultural animals (cattle, swine, sheep/goats, poultry and horses) were the most common sources of bacterial pathogens, followed by dogs and cats. Overall, *Escherichia coli* was the most common pathogen tested across all animal species. The most frequently employed AST methods were the disk diffusion method and the Sensititre® broth microdilution system. DNA sequencing is expanding as a diagnostic tool; over 1/3 of laboratories reported some in-house sequencing capabilities, used primarily for bacterial identification or identifying a specific gene/genome segment.

Laboratories largely use CLSI standards or the automatically programmed interpretations by commercial AST systems for interpreting AST results. About one-third of the laboratories use the CLSI standards developed for human isolates.

Half of the laboratories routinely archive at least some bacterial isolates, while nearly all archive AST results. Laboratories generally maintain AST data for at least 3 years and in some cases indefinitely. Most laboratories also maintain qualitative data regarding AST interpretation and at least some epidemiological data about the isolate, such as animal species and isolation date. Only a few laboratories compile and present or publish their AST data on a periodic basis, most commonly via annual reports through their laboratory's web site or through peer-reviewed journals focusing on specific pathogens.

Our results confirm that disk diffusion and broth microdilution remain the standard AST testing methods employed by veterinary diagnostic laboratories in the U.S., and that CLSI standards are commonly used for interpreting AST results. This information will be useful for determining the most efficient standardized methodology for future surveillance. Furthermore, evidence of an infrastructure within laboratories, once harmonized, will help provide a mechanism for conducting national surveillance programs.

Increased frequency of isolation of multi-drug resistant *Salmonella* I 4,[5],12:i:- from swine with histologic lesions consistent with salmonellosis

Adam Krull[1], Bailey Lauren Arruda[1], Kent L. Schwartz[1], Eric Burrough[1], Orhan Sahin[1], Amanda Kreuder[2]

[1]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA; [2]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA

Salmonella serotype I 4,[5],12:i:- has emerged over the last decade as a leading cause of human disease, both in Europe and more recently in the United States. Data from swine diagnostic samples submitted to the Iowa State University Veterinary Diagnostic Laboratory reveals a substantial increase in the relative frequency of isolation of this serotype over the past 5 years, from <3% in 2011 to >15% of all *Salmonella* isolated in 2015. While a few serotypes of *Salmonella* are demonstrated as primary pathogens in swine (such as Typhimurium), most serotypes are considered to be less pathogenic and can be frequently isolated from otherwise healthy pigs (such as Derby). In a review of case data from clinical submissions to the ISU-VDL, there is a consistent association between enteric disease with concurrent histologic lesions consistent with salmonellosis and the isolation of *Salmonella* I 4,[5],12:i:-, particularly during the post-weaning nursery phase of production. Of the serotypes identified in 2015, 76% of the *Salmonella* I 4,[5],12:i:- isolates were associated with microscopic lesions consistent with salmonellosis, compared to 94% of Typhimurium and 14% of Derby isolates. In cases from which *Salmonella* I 4,[5],12:i:- was isolated, a fibrinonecrotic colitis was commonly noted on gross examination. Histologic changes included multifocal superficial to deep ulceration of the large and less commonly the small intestinal mucosa, expansion of the lamina propria by neutrophils, and luminal accumulation of fibrin and cellular debris. Multi-drug resistance (MDR) in human isolates of I 4,[5],12:i:- is particularly concerning when it comes to ASSuT (ampicillin, streptomycin, sulfonamide and tetracycline) resistance phenotype. Antibiotic susceptibility testing data on the ISU-VDL swine isolates of I 4,[5],12:i- also confirms a rapid increase in the identification of ASSuT MDR isolates (from 3.5% in 2012 to 36.8% of isolates identified thus far in 2016); however, the rapid increase in resistant isolates from cases of human illness pre-dates the rise observed in swine isolates. In comparison, the percentage of Typhimurium isolates exhibiting the same resistance profile has remained relatively steady at approximately 10-11% since 2007. Importantly, 11 of 663 isolates of I 4,[5],12:i:- had resistance to all antibiotic classes tested including beta-lactams, cephalosporins, pleuromutilins, tetracyclines, aminoglycosides, amphenicols, fluoroquinolones, lincosomides, macrolides, and folate pathway inhibitors. In contrast, however, no isolates of Typhimurium with this profile have been identified to date. The increased isolation of *Salmonella* I 4,[5],12:i:- in association with clinical disease in swine along with increasing identification of the ASSuT MDR resistance profile in this serotype may represent an emerging threat to the swine industry and warrant increased awareness in diagnostic labs nationwide.

Culture and Sensitivity Results from Canine and Feline Cystocentesis Samples (2008-2014)

Emily Knebel, Stephen Cole, Shelley C. Rankin

UPenn, Philadelphia, PA

Urinary tract infections (UTI) are one of the most common problems in companion animals. The veterinary clinical microbiology lab plays a key role in the diagnosis of UTI and it also plays a role in understanding the epidemiology of antimicrobial resistance in common pathogens. This study analyzed the results obtained from 1267 bacterial isolates obtained from cystocentesis samples. 77% of isolates were from dogs and 23% were from cats. Biochemical identification and antimicrobial susceptibility testing were performed on a Microscan 40 Walkaway using PC20 and NC31 panels. The most common organism isolated was *E. coli* (715/1267, 56.4%), followed *Enterococcus faecalis* (142/1267, 11.2%), *Proteus mirabilis* (96/1267, 7.6%) and *Staphylococcus pseudintermedius* (82/1267, 6.5%). When host species was considered the distribution varied. In dogs, 551/959 (57.5%) of isolates were E. coli, *Proteus mirabilis* (86/959, 8.9%) and *Enterococcus faecalis* (83/959, 8.6%) were equally represented and there were 57/959 (5.9%) *S. pseudintermedius* isolates. In cats, 156/288 (54.2%) were E. coli. *E.faecalis* (58/288 20.14%) was second most common *S. pseudintermedius* (24/288, 8.3%) was slightly more common from cats and *Proteus mirabilis* (9/288, 3.1%) was less common. This study analyzed the susceptibility results of first line antimicrobials (ampicillin and TMS), as well as fluoroquinolones and other clinically important antimicrobials. 350/904 (38.71%) of all *Enterobacteriaceae* (E. coli, Klebsiella spp., Proteus spp. and Enterobacter spp.) were resistant to ampicillin. TMS (142/904, 15.70%) and fluoroquinolones (172/904, 19.03%). Isolates resistant to an aminoglycoside (gentamicin or tobramycin) and a fluoroquinolone were considered MDR. MDR isolates represented 4.2% of all *Enterobacteriaceae*. *Enterococcus* spp. are inherently resistant to TMS and the activity of fluoroquinolones are unreliable against this genus. Only 1 isolate of *E. faecalis* was resistant to ampicillin. As was expected, E. faecium was commonly resistant to ampicillin (49/53, 92.45%). Resistance to alternative therapies for *E. faecium* such as chloramphenicol (3/53, 5.66%) was far less common than resistance to others (i.e. tetracyclines). *Staphylococcus* spp. showed moderate resistance rates for TMS (26/118, 22.03%), fluoroquinolones (24/118, 20.33%) and Oxacillin/Methicillin (30/118, 25.42%). It will be lower for the general practice population. The data presented show that resistance to important antimicrobials is common and supports the critical role culture plays in managing small animal UTI.

Antimicrobial susceptibility of *Streptococcus suis* isolated from diseased pigs from a veterinary diagnostic laboratory in the Midwest United States: Association between isolation site, serotype and resistance

Orhan Sahin[1], Curt Thompson[1], Lei Dai[2], Adam Krull[1], Eric Burrough[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA;
[2]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA

Streptococcus suis, an emerging zoonotic agent, is a major bacterial pathogen in pigs causing a wide range of diseases including pneumonia, meningitis, arthritis, endocarditis, polyserositis, septicemia, abortion and sudden death. Since antimicrobials are commonly used to treat bacterial infection in pigs, up-to-date information on antimicrobial resistance in major disease causing bacteria is critical for informing therapeutic options as well as for monitoring resistance trends. The aim of this study was to determine the antimicrobial susceptibilities of *S. suis* isolates cultured from various tissues of diseased pigs at the Iowa State University Veterinary Diagnostic Laboratory during 2010-2016. The minimum inhibitory concentrations (MICs) of 18 antibiotics used in veterinary medicine for a total of 7,645 *S. suis* isolates were determined using a standard broth microdilution method by means of commercially available Sensititre plates. Using the available breakpoints established by CLSI for veterinary pathogens, the majority *S. suis* strains showed resistance to chlortetracycline (50.5%), clindamycin (78.3%), oxytetracycline (95.44%), sulfadimethoxine (68.48%), and tilmicosin (75.47%). A moderate level of resistance was observed for neomycin (28.07%), spectinomycin (11.85%) and tiamulin (14.84%). However, the isolates exhibited only a low level resistance to ampicillin (0.22%), ceftiofur (1.29%), enrofloxacin (4.0%), florfenicol (0.17%), gentamicin (3.54%), penicillin (2.9%), and trimethoprim/sulphamethozazole (3.27%). MIC90 values for danofloxacin, tulathromycin, and tylosin were 0.5, >64, and >32 μg/ml, respectively. The multiple drug resistance (≥3 different antimicrobial classes) was observed in 77.7% of the isolates while 1.7% of the strains were susceptible to all antibiotics tested. Comparison of susceptibility by specimen type (lung vs. brain) indicated that *S. suis* isolates from brain usually displayed a slightly elevated resistance level to several antibiotics than those from lung. Preliminary data also suggested the existence of serotype-related differences in resistance to certain antimicrobials. These results indicate that, while a high level of resistance to some antimicrobials was observed, the vast majority of recently isolated *S. suis* strains from the Midwest U.S. remain susceptible to penicillins and cephalosporins, which are commonly used antibiotics against these infections in swine production.

Monitoring and source tracking of multiple antimicrobials resistant enteropathogenic *E.coli* and resistant genes in a dairy farm †

El Bably M. A.[1], Asmaa N. Mohammed[1], Manar B. Mohamed[1], Hanan A. Fahmy[2]

[1]Department of Hygiene, Management and Zoonoses, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt; [2]Department of Biotechnology, Animal Health Research Institute, Dokki, Giza

Problem: Antimicrobial resistance (AMR) threats in Egypt are a snapshot of the complex problem today and the potentially catastrophic consequences of inaction.

Objective: The overriding purpose of this study was to screen and analyze the antimicrobial resistant profile of enteropathogenic *E. coli* isolated from calves and track their source.

Methodology: A cross sectional study was conducted between October 2014 and April 2016 in a dairy farm in Beni-suef Governorate. Data on types, sources, trends of antimicrobials usage and resistance was collected. Fecal samples (n=86) were collected from both apparently normal and diarrheic calves besides surrounding environment (n= 65) including milk, water, flies and soil samples in the examined farm. A total of 107 isolates of *E. coli* recovered from calves and environment were analyzed for antimicrobial susceptibilities against four classes of antibiotics and three disinfectants (TH[4], Virkon®S, and Iodine). Forty eight *E. coli* isolates showed resistance to nine or more antimicrobial agents were selected and investigated for resistance genes tetA (A), sul1, dfrA, floR, bla_{TEM}, bla_{SHV} bla OXA-1 and qac ED1 using PCR

Results: *E. coli* was detected in 65(75.5%), 11 (52.3%) and 31 (51.6%) samples from apparently healthy, diarrheic calves and environment respectively. Antimicrobial sensitivity test showed that *E. coli* isolates were resistance to all *tested* antibiotics (100 %) meanwhile, TH [4] followed by Virkon®S had higher germicidal activity against tested isolates (65 %) compared to iodine (40 %) depending on chemical composition of disinfectant and contact time. Distribution of antibiotic resistance genes in *E. coli* isolates included tetA (A) (50%), drfA (66.6%), sul1 (50%), floR (16.6%), bla_{TEM} (100%), bla_{SHV} (33.3%) and bla OXA-1 (16.6%), meanwhile, qac ED1 (83.3%) respectively.

Conclusion: The high prevalence of multidrug-resistant *E. coli* in this study provide insights that dairy farms may act as potential source for antimicrobial resistance genes as drA in the environment and may pose great health risks for human and animals.

† Graduate Student Oral Presentation Award Applicant

Creation of a MALDI-TOF Library to Identify *Aeromonas salmonicida* # * †

Tessa LeCuyer, Timberly Maddox, Dubraska Vanessa Diaz-Campos, Kevin R. Snekvik

Washington State University, Pullman, WA

Aeromonas salmonicida is a significant pathogen of fish that can cause severe disease and death. Additionally, the organism is of regulatory concern which expands its economic impact and the importance of proper detection. There are well-described biochemical methods for identification of typical strains of *A. salmonicida* ssp. *salmonicida*, but atypical strains can be quite variable and may be a diagnostic challenge.

Goal: In an attempt to find a more timely and efficient method for identification of *A. salmonicida*, we tested isolates using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF), but observed poor sensitivity using the Bruker database. The goal of this study was to enhance the MALDI-TOF library by creating a custom database including spectra of *A. salmonicida* type strains (ATCC 49385, 27013, 33658, 10801) and three well-characterized field strains.

Methods: We tested 30 isolates: 17 typical *A. salmonicida* ssp. *salmonicida*, 6 atypical isolates most consistent with *A. salmonicida*, three *A. sobria* isolates, two *A. veronii* isolates and one *A. hydrophila* isolate. The typical *A. salmonicida* isolates had biochemical results consistent with *A. salmonicida* ssp. *salmonicida* and were positive on an *A. salmonicida* ssp. *salmonicida*-specific PCR. The atypical and non-*salmonicida* isolates had biochemical test results consistent with *Aeromonas* sp. and were negative on the *A. salmonicida* ssp. *salmonicida*-specific PCR test.

Results: Using the commercial database, all non-s*almonicida* isolates were correctly identified to the species-level with scores between 2 and 2.299, indicating a secure genus and probable species identification. Four typical *A. salmonicida* ssp. *salmonicida* isolates were incorrectly identified as *A. bestiarum* and one atypical isolate was identified as *A. eucrenophila* with scores >2 and <2.229. The remainder of the *A. salmonicida* isolates were correctly identified but none of the isolates achieved a score of 2.3 or greater, which would indicate highly probable species identification. After running the same spectra against our enhanced library, we found that the identification of the non-*salmonicida* isolates did not change but the previously misidentified typical *A. salmonicida* ssp. *salmonicida* were all correctly identified, three with scores greater than 2.3. More than half of the typical and atypical *A. salmonicida* isolates achieved scores of at least 2.3 with the enhanced library, showing a statistically significant difference in the number of isolates with a highly probable species identification using the enhanced library (McNemar's binomial exact test, p=0.002). All of the *A. salmonicida* isolates achieved scores >2 and the enhanced library did not appear to adversely affect identification of other *Aeromonas* species.

Conclusion: By using the customized library we were able to improve identification of typical and atypical *A. salmonicida* strains at the species level.

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

Validation and optimization of the NIH Mold Database for MALDI-TOF MS in veterinary diagnostic setting §

Niesa Kettler, Karen Parlor, Rinosh Joshua Mani

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

Matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) is now routinely used for identification of bacteria and yeast in veterinary diagnostic laboratories; however, the use of this technology for mold identification has been hampered by cumbersome protocols and inconsistent results. Diagnostic laboratories, therefore still rely on the traditional phonotypic method of identification of mold which requires extensive technical expertise and often ends up relying on confirmatory nucleic acid-based identification.
The National Institutes of Health (NIH) mold database for MALDI-TOF MS currently has 187 different entries including 367 main spectrum profiles (MSPs) which covers most of the common molds. The protocol involves protein extraction from mold grown in solid culture media and requires minimum deviation from the usual mycology workflow in the laboratory. We describe here the validation and optimization of the NIH mold database for our laboratory setting and also the in-house expansion of the database to include emerging veterinary fungal pathogens including *Pseudogymnoascus destructans*. The NIH mold database and protocol have the potential to be used as a routine diagnostic technique in veterinary diagnostic laboratories and will significantly improve the quality of veterinary mycology diagnosis.

§ AAVLD Laboratory Staff Travel Awardee

MALDI-TOF as a novel detection method for *Clostridium difficile* toxins

Kenitra Hammac[1,2], Dian Dian Lin[2], Kelly Ray[2], Christina Wilson[1,2]

[1]Comparative Pathobiology, Purdue University, West Lafayette, IN; [2]Indiana Animal Disease Diagnostic Laboratory, West Lafayette, IN

Toxigenic *Clostridium difficile* is an important cause of enterocolitis in equine, porcine and canine veterinary species and humans, and can be identified by the presence of one or both toxins: Toxin A, an enterotoxin, and Toxin B, a cytotoxin. Isolation of *C. difficile* from fecal samples is not enough to diagnose the cause of diarrhea, as non-toxigenic isolates may be present in the feces of clinically normal animals. Detection of toxins A and/or B is important to differentiate a disease-causing toxigenic strain from a non-toxigenic strain, and this is most commonly accomplished through use of a cytotoxicity assay (CTA) or an enzyme linked immunosorbent assay (ELISA). Because matrix assisted laser desorption ionization – time-of-flight mass spectrometry (MALDI-TOF) is becoming a common method for bacterial identification and accurately identifies *C. difficile* isolates, a MALDI-TOF-based proteolytic assay was evaluated in this study as a novel means for *C. difficile* toxin detection. The peptide masses detected from purified *C. difficile* toxins were compared with expected masses generated with the ExPASY online peptide mass tool, revealing a set of signature peaks for each toxin. ATCC isolates and clinical isolates from the archives of the Indiana Animal Disease Diagnostic Laboratory, with known toxin status based on CTA and ELISA, were also evaluated. Two methods for concentrating toxins from broth cultures were evaluated as pre-proteolysis processing steps: toxin isolation based on molecular weight using a centrifugal filter device and toxin-trapping via lectin bound agarose beads. MALDI-TOF shows promise as a novel means for *C. difficile* toxin detection.

Bacteriology 2

Sunday, October 16, 2016

Imperial B

Moderator: Kris A. Clothier

8:00 AM ***Streptococcus halichoeri*, an emerging zoonotic pathogen. §**
Eric W. Lee, Rebecca Franklin-Guild, Anil J. Thachil *45*

8:15 AM **Factors and causal organisms associated with bacterial abscesses in goats from cases submitted to the California Animal Health and Food Safety Lab System from 2007-2014**
Kris A. Clothier, Michelle Schack, Ashley E. Hill. *46*

8:30 AM **Phenotypic characteristics and virulence genotypes of *Trueperella pyogenes* strains isolated from ruminants**
Artem Rogovskyy, Sara Lawhon, Kay Dunkan, Chris Gillis, Helen Hurley, Kathryn Kuczmanski, Kranti Konganti, Jing Wu, Ching-Yuan Yang. *47*

8:45 AM **Assessing genetic diversity within *Moraxella* isolates from cattle; application of high resolution melt analysis for rapid sequence typing**
John Dustin Loy, Joshua Payne, Aaron Dickey, Michael L. Clawson *48*

9:00 AM **Validation of a 24-hour enrichment followed by quantitative PCR to decrease turnaround time and improve detection of *Salmonella* in clinical samples and environmental surveillance**
Adam Krull, Carly Kanipe, Karen Harmon, Laura Bradner, Amanda Kreuder *49*

9:15 AM **Genotypic and phenotypic characterization of *Salmonella enterica* serovar *Dublin* in cattle**
Milton Thomas, Anil J. Thachil, Sudeep Ghimire, Amy Glaser, Angela E. Pillatzki, Russ Daly, Eric A. Nelson, Jane Christopher-Hennings, Joy Scaria *50*

9:30 AM **International impact of invalid *Salmonella* laboratory testing methods on public health**
Megin Nichols *51*

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

Streptococcus halichoeri, an emerging zoonotic pathogen. §

Eric W. Lee, Rebecca Franklin-Guild, Anil J. Thachil

AHDC Bacteriology, Cornell University, Ithaca, NY

Streptococcus halichoeri was first described in grey harbor seals (Halichoerus grypus) from the United Kingdom in 2004, and since then the organism has been isolated from a stellar sea lion in South Korea, and a European badger with pyogranulomatous pleuropneumonia. Five human isolates of S. halichoeri from human septicemic cases were reported by Centers for Disease Control and Prevention, another human isolate was reported in Sweden and one from a fish handler from China. While in these cases the contact between the humans and marine animals was unknown, one report indicated that the individual had no known contact with marine life and only had a dog. Since 2013 the Animal Health Diagnostic Center at Cornell University has received over 20 canine cases and one porcupine case from which S. halichoeri were isolated. Nine of these cases were received in 2015 and an additional 5 have already been confirmed in 2016. Multiple, different body sites tested positive, and the animals originated from multiple states and Canada. S. halichoeri is most likely under-reported due to the difficulties of identifying this organism. We used MALDI-TOF, 16s rDNA sequencing, rpoB sequencing or a combination of these processes to confirm the species of these isolates. The phenotypic characteristics we noticed in our isolates where different from the ones reported from isolates of human and seal origin. Considering that this organism is showing up more frequently in clinical veterinary cases and with a possible cross over or implication to human cases of septicemia, exploration into the zoonotic potential of S. halichoeri in animals kept as pets may be warranted.

§ AAVLD Laboratory Staff Travel Awardee

Factors and causal organisms associated with bacterial abscesses in goats from cases submitted to the California Animal Health and Food Safety Lab System from 2007-2014

Kris A. Clothier, Michelle Schack, Ashley E. Hill

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Bacterial abscesses, particularly those due to *Corynebacterium pseudotuberculosis*, contribute to severe economic and health problems in goats. Infections due to *C. pseudotuberculosis* are considered life-long due to virulence factors that support bacterial dissemination and persistence in lymphoid tissues in the host. Animals with confirmed infections (caseous lymphadenitis [CL]) should be culled from the flock. Other bacterial organisms may cause abscesses which can be confused with CL, making accurate detection critical to management of this disease.
The purpose of the present study was to examine cases of caprine abscesses submitted to the California Animal Health & Food Safety Lab System (CAHFS) between 2007 and 2014 to determine if any factors could be identified as predictors of CL infection. Information collected included site sampled, internal or external abscess identified, bacterial culture results, collection by a veterinarian or owner, animal age, and production class. Results were evaluated on 389 samples from both external and internal locations. *C. pseudotuberculosis* was recovered from internal sites (including thorax, abdomen, retropharyngeal lymph node, and joint) and external abscesses (including head, chest, neck, abdomen, perineum, and limb). *C. pseudotuberculosis* was detected more frequently in young adult animals and in meat goats. Nearly half of all cases had bacterial organisms detected in pure or nearly-pure culture. *Trueperella pyogenes* was the most frequently-identified rule-out organism, recovered from internal sites, primarily the thorax, and external abscesses with most being located in the head. Other commonly recovered organisms included *Staphylococcus aureus*, coagulase-negative *Staphylococcus* spp., *Streptococcus sp*., and *Actinomyces sp. C. pseudotuberculosis* was not recovered from any axilla or mammary samples submitted.
While CL is an important health condition to be controlled in goat flocks, culture should be performed to establish the presence of *C. pseudotuberculosis* before management decisions are made, particularly from non-lymphoid sites.

Phenotypic characteristics and virulence genotypes of *Trueperella pyogenes* strains isolated from ruminants

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Trueperella pyogenes is an opportunistic animal pathogen that causes various suppurative infections in animals. Not much is known about the pathogenesis and virulence of this bacterium. Previous work has phenotypically and genotypically characterized *T. pyogenes* strains isolated from wildlife, European bison and white-tailed deer. However, to date, no study examined clinical isolates of *T. pyogenes* derived from domestic animals, particularly cattle, goats, and sheep. Thus, the objective of this study was to determine phenotypic properties and virulence genotypes of thirty-five *T. pyogenes* isolates recovered from diverse diagnostic specimens of bovine, caprine, and ovine origin; and to establish their phylogenetic relationship. Biochemical and CAMP tests were performed for phenotypic characterization. Further, genomic DNA of each isolate was PCR analyzed to screen for eight virulence factor genes: *cbpA*, *fimA*, *fimC*, *fimG*, *fimE*, *nanH*, *nanP*, and *plo*. To establish phylogenetic relationships analysis of 16S rRNA gene partial sequences was performed. The results showed that the isolates showed variable biochemical activity. All bacterial isolates possessed the *plo* gene; whereas *nanH* was detected in 54.3% of the isolates. Most isolates carried *fimA* (94.3%), *fimE* (91.4%), *fimC* (85.5%), and *nanP* (80%). In contrast, the *fimG* and *cbpA* genes were least represented and were only detected in 20% and 8.6% of *T. pyogenes* isolates, respectively. The 16S rRNA sequences of all isolates were identical to that of the *T. pyogenes* reference strain. Overall, the *T. pyogenes* isolates demonstrated varying phenotype and virulence genotypes in both small and large ruminants and were not associated with any particular diagnostic specimens from which the isolates were recovered. This study supports previous findings that virulence factors are not significant determinants of type and localization of infection caused by *T. pyogenes*.

Assessing genetic diversity within *Moraxella* isolates from cattle; application of high resolution melt analysis for rapid sequence typing

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Infectious bovine keratoconjunctivitis (IBK) is an economically significant disease of cattle that causes ocular pain, blindness and decreased performance. An etiologic agent of IBK is the gram negative bacterium, *Moraxella bovis*. A closely related species, *Moraxella bovoculi* possesses virulence factors and is the most frequently isolated species from eyes during IBK outbreaks. Whole genome sequencing has shown profound genetic differences between *M. bovoculi* isolated from the eye and the nasopharynx (NP) of cattle with and without IBK, respectively. Therefore, an understanding of the diversity amongst *M. bovoculi* may provide insight into potential pathogenicity, inform prevention strategies through targeted vaccination, and improve the ability to identify these organisms at the sub-species level. High resolution melting (HRM) analysis is inexpensive, rapid, simple and does not require probes, restriction enzymes, or gel electrophoresis. To evaluate this method, three *M. bovoculi* genomes from the eyes of IBK cases, and four NP genomes from cattle without signs of IBK were compared. Housekeeping genes encoding for ATP synthase F1 (*atpC*), Phospho-N-acetylmuramoyl pentapeptide transferase D (*mraY*), 3-Hydroxyacyl-CoA dehydrogenase (*HADH*), 16S Ribosomal rDNA (*rDNA*) and RNA polymerase subunit B (*rpoB*) were aligned and short regions (100-150 bp) with multiple polymorphisms were identified. HRM assays were evaluated on geographically diverse *M. bovoculi* isolated from eyes (n=16) and reference strains (n=7). For the rDNA assay, *M.bovis* ocular isolates were included (n=12) to determine if the target allowed for species discrimination between *M. bovis* and *M. bovoculi*. Melt curves were subjected to principal component analysis (ScreenClust HRM). For each gene, multiple clusters were identified including *HADH* (3), *atpC* (3), *mraY* (4), and *rpoB* (4) and *rDNA*(2). Cluster 1 *HADH* contained nearly all of the ocular isolates (93%) and Cluster 2 *HADH* contained all NP isolates tested. Cluster 1 *atpC* was found associated with all of the NP isolates and Cluster 2 and 3 *atpC* contained the majority (82%) of the ocular isolates. Cluster 1 *rDNA* contained all *M. bovoculi* isolates tested and Cluster 2 contained all *M. bovis* isolates tested. The remaining genes (*mraY* and *rpoB*) distributed in clusters with no discernible pattern between NP and ocular isolates. In conclusion, HRM appears to be a useful, rapid method to generate sequence-based typing data for the study of bovine *Moraxella* diversity. Ocular and NP isolates were represented by distinct HRM clusters for *HADH* and *atpC* genes, which correlates with whole genome sequencing findings. The *rDNA* assay was able to distinguish all tested *M. bovoculi*, including NP isolates, from *M. bovis,* which the current RFLP assay does not allow. Further work to expand the number, diversity, and targets of these assays will be needed to identify the most informative regions for typing subpecies of *M. bovoculi.*

Validation of a 24-hour enrichment followed by quantitative PCR to decrease turnaround time and improve detection of *Salmonella* in clinical samples and environmental surveillance

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Rapid detection of *Salmonella*-shedding animals, along with environmental surveillance testing, is of utmost importance in avoiding disease outbreaks. *Salmonella* testing has traditionally relied on culture with various enrichment broths with an average turnaround time of 5 to 7 days. The use of PCR for detection of *Salmonella* can decrease turnaround time, but the detection of residual DNA from non-viable organisms in environmental samples following cleaning has led to positive results that are not a reflection of the status of the samples in terms of presence of viable organisms. Our studies aimed to determine whether an overnight enrichment followed by quantitative PCR (qPCR) could be as highly sensitive and specific as enrichment culture from a variety of sample types in identifying the presence of viable *Salmonella*. Experiments varied the type of enrichment media, the starting concentration of *Salmonella*, and the effects of disinfectants and residual *Salmonella* DNA on the assay. From these experiments, we were able to calculate correlation coefficients between culture CFU/mL and Ct values for each sample type and enrichment broth as well as determine the limit of detection for each methodology. Buffered peptone water (BPW) was found to be a superior enrichment media to tetrathionate broth, effectively amplifying as little as 1 colony-forming unit of starting *Salmonella* per 50 mL of media to detectable levels in less than 18 hours. The correlation coefficient (R^2) between Ct values and culture CFU/mL utilizing this method was 0.98 indicating a very strong correlation. Additional testing using killed *Salmonella* showed that the 24-hour enrichment step also allowed us to differentiate between viable and non-viable organisms. Based on known starting concentrations of bacteria, interpretive criteria were able to be assigned to ranges of Ct values to categorize viable *Salmonella* versus dead and demonstrated that by utilizing enrichment, the presence of live bacteria could clearly be differentiated from residual DNA. Our interpretive criteria were then compared to standard culture techniques and shown to be highly correlated. The use of this assay in *Salmonella* surveillance samples from the Iowa State University Veterinary Teaching Hospital has shown to be as accurate as traditional culture methods with a decrease in turn-around time. Results show that the use of qPCR following an overnight enrichment in BPW has sensitivity and specificity equivalent to enrichment culture over a much shorter time frame.

Genotypic and phenotypic characterization of *Salmonella enterica* serovar *Dublin* in cattle

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Salmonella enterica subsp. *enterica* serovar *Dublin* predominantly infects cattle, however these organisms could infect other species of animals as well as humans. The objective of this study was to a) Evaluate association between genomic features and phenotypic characters such as host tissue invasiveness, antibiotic sensitivity and acid tolerance for isolates from the USA b) Analyze the evolutionary pattern using genomic approach for the isolates obtained from North-east and Midwest regions of the USA and isolates from Europe. The host tissue invasiveness of individual isolates of *S. dublin* were estimated using Caco-2 cell line invasion assay. To evaluate the acid- tolerance, individual isolates were grown in Luria Broth medium with varying pH for 24h and OD_{600} was measured. There was no significant difference between the Midwest and Northeast isolates in cell invasiveness. All the isolates tolerated acidity and could grow at pH between 4 and 7. However, there was no clear correlation between cell invasiveness and ability to survive in acidic environment. Antibiotic sensitivity assay revealed that isolates from Midwest were susceptible only to fluoroquinolone and aminoglycoside classes of antibiotics. We also sequenced the genomes of 23 isolates from Midwest and 57 isolates from Northeast United States. The comparison of these genomes with 92 isolates of *S. dublin* from Europe which are available in NCBI SRA database is in progress. We hypothesize that comparative genomic analysis could reveal the underlying genomic features that are responsible for host tissue invasiveness, acid tolerance and antibiotic sensitivity. Additionally, population based genomic analysis would provide information on the evolutionary differences between the isolates from Europe and the USA

International impact of invalid *Salmonella* laboratory testing methods on public health

Megin Nichols

Centers for Disease Control and Prevention, Atlanta, GA

In 2015, the Centers for Disease Control and Prevention received a report of a young child who underwent removal of the appendix and gallbladder after developing a *Salmonella* abscess following contact with a small turtle (a shell length <4 inches) purchased at a flea market; sale of small turtles is banned in the US. Federal regulators visited the flea market; the turtle vendor reported that turtles were obtained from Turtle Farm X in Louisiana (LA). Farm X was previously investigated as a source of turtles during a 2011–2013 *Salmonella* outbreak which resulted in 473 *Salmonella* infections from 41 states.

Turtle Farm X, along with other LA turtle farms, routinely export turtles internationally. LA state law required *Salmonella*-negative test results before turtle export. In addition, some countries require a *Salmonella*-negative test result and a signed US Department of Agriculture (USDA) health certificate to import turtles. However, *Salmonella* illnesses linked to small pet turtles were reported in 2015 to the US by countries receiving exports from Turtle Farm X. Therefore, we investigated *Salmonella* testing practices and results for turtles exported from Farm X.

Turtle Farm X utilized Lab Z exclusively for *Salmonella* testing. Upon further examination of *Salmonella* laboratory test reports from Lab Z, the laboratory name in the heading of the official lab report was misspelled; additionally, Lab Z was not registered as a business in LA.

The authority overseeing the LA turtle industry reported that Lab Z existed; however, the testing methods utilized were outdated and inadequate to detect *Salmonella*. Lab Z had not been certified or inspected by the State authority for many years. This finding was reported to the USDA-Animal Plant Inspection Service and to the US Fish and Wildlife Service, as test results from Lab Z constituted part of the official health certificate process and for regulatory purposes before export of turtles to countries outside the US.

When valid testing methods were employed at accredited veterinary diagnostic laboratories, turtles tested positive for *Salmonella spp*. Therefore, per LA state law which required negative test results before/as a condition of export, turtles could not be exported. In 2016, <6 months after the investigation was initiated, an emergency change of LA administrative rules was issued to no longer require testing of turtles for *Salmonella* as a condition of exporting unless the importing country required it.

Invalid laboratory reports or methods used to certify that animals have tested negative for *Salmonella* can put the public in the US and in other countries at risk of severe illness and death. Test results used to certify that animals are disease free upon import or export should be performed at laboratories that are accredited and reputable.

Epidemiology 1

Saturday, October 15, 2016

Imperial C

Moderators: Michael Martin and Craig N. Carter

1:00 PM **An evaluation of the performance of pre-movement active surveillance testing protocol options for moving pullets during an outbreak of highly pathogenic avian influenza**
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1:15 PM **Small flock poultry diagnostics and veterinary training: a new approach to a capacity and emergency preparedness issue**
Melanie K. Barham, Marina Louise Brash, Csaba Varga, Leonardo Susta, Lloyd Weber, Al Dam, Elizabeth Black, Michael Petrik, Michele Guerin 56

1:30 PM **Evaluating the role of distance in the 2015 HPAI outbreak in Minnesota via a spatial transmission kernel**
Peter Bonney, Sasidhar Malladi, Todd Weaver, Amos Ssematimba, David Halvorson, Carol Cardona 57

1:45 PM **A comparison of modeling approaches for estimating within-flock disease transmission parameters for the 2015 H5N2 highly pathogenic avian influenza virus outbreak in the United States ◊**
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2:00 PM **Influenza receptor distribution in little brown bats (*Myotis lucifugus)* – uncovering the missing link in the influenza virus evolution # * †**
Shubhada Krishna Chothe, Ruth Nissly, Gitanjali Bhushan, Yin-Ting Yeh, Miranda Sill, Justin Brown, Gregory Turner, Jenny Fisher, Mauricio Terrones, Bhushan Jayarao, Suresh V. Kuchipudi 59

2:15 PM **Spatial autocorrelation and implications for oral fluid-based PRRSV surveillance * †**
Marisa Rotolo, Monica Haddad, Yaxuan Sun, Luis Gabriel Gimenez-Lirola, Sarah Bade, Chong Wang, Dave Baum, Phillip Gauger, Marlin Hoogland, Rodger Main, Jeff Zimmerman 60

2:30 PM **Epidemiology and management of endemic CWD in farmed elk through antemortem rectal biopsy testing † ◊**
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2:45 PM **Twenty-four year retrospective study of adenovirus hemorrhagic disease in California deer**
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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

An evaluation of the performance of pre-movement active surveillance testing protocol options for moving pullets during an outbreak of highly pathogenic avian influenza

Sasidhar Malladi[1], Peter Bonney[1], Todd Weaver[2], Amos Ssematimba[1], David Halvorson[1], Carol Cardona[1]

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In the United States, highly pathogenic avian influenza (HPAI) emergency response plans include provisions for the managed movement of non-infected animals and non-contaminated animal products to stabilize animal agriculture, the food supply, and the economy. Moving pullets to egg-layer facilities to ensure a constant supply of eggs to consumers is one such movement. However, risk managers have to consider the possibility of missed HPAI detections that would result in direct outbreak spread when making decisions to permit the movement of live birds. Pre-movement active surveillance using the influenza-A matrix gene real-time reverse transcriptase polymerase chain reaction test (RRT-PCR) is a key risk mitigation measure to ensure that HPAI infected flocks are not moved. We used within-flock HPAI disease transmission models and simulations models of active surveillance to evaluate the performance of active surveillance protocols for moving live pullets. The simulation models were modified to reflect pullet production systems using data provided by industry stakeholders. Specifically, we predict the likelihood of HPAI detection immediately prior to movement for 1) four separate 11-bird active surveillance sampling protocol options, 2) daily testing for either 5 or 10 days before pullet movement, and 3) sampling protocols where both tracheal and cloacal swabs from the same bird are tested. During the 2015 H5N2 HPAI outbreak in the Upper Midwestern United States, mortality patterns indicative of slow within-flock spread were observed in some pullet flocks. In these outbreaks, there was either no increase in mortality or the onset of increased mortality was considerably delayed, occurring after detection by diagnostic testing. We performed scenario analyses with a baseline contact rate estimated from past outbreak studies and a lower adequate contact rate where the reproductive number is between to 1 to 2, to ensure that surveillance protocols are robust to variability in the rate of HPAI spread. The analysis presented here will inform further risk assessment and risk management decisions for pullet movement during an HPAI outbreak.

Small flock poultry diagnostics and veterinary training: a new approach to a capacity and emergency preparedness issue

Melanie K. Barham[1], Marina Louise Brash[1], Csaba Varga[2], Leonardo Susta[3], Lloyd Weber[6], Al Dam[2], Elizabeth Black[5], Michael Petrik[4], Michele Guerin[3]

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During the avian influenza outbreak of 2015 in Canada, the need for increased numbers of veterinarians with small flock poultry training was apparent, as was a line of communication between these professionals, and producers. In Ontario, during the outbreak, the issue of information dissemination was overcome in a variety of methods (email of resources and disease notification to all veterinarians in Ontario via the provincial licensing body, targeted disease update conference calls, infosheets and factsheets, radio appearances and podcasts), but the need for additional knowledge of small flock disease issues and increased small flock veterinary capacity/knowledge provided a clear catalyst for change as emergency preparedness factors for foreign animal disease. As a result, 3 initiatives were started:

- Small flock veterinary training day, using live streaming, live tweeting, and a secure website to host the PowerPoint presentations, reference materials, and videos as a legacy project.

- A private small flock veterinary listserve for veterinarians in Ontario wishing to obtain and share information related to small flock disease and management issues.

- A disease surveillance project for non-quota, non-commercial poultry flocks in Ontario, which was launched October 1, 2015, and which involves government-subsidized post mortem diagnosis for cases submitted by flock owners and their veterinarians, coupled with comprehensive diagnostic testing for viral, bacterial, parasitic, and fungal pathogens, and collection of data on flock husbandry and biosecurity.

These initiatives had very specific outcomes: 1)increased capacity for small flock veterinary care, 2)improved and consistent communication with veterinarians with small flock training, 3)surveillance of small flock poultry disease and the management practices of their owners/producers, and 4) strengthening the relationship between small flock producers/owners and veterinarians. The small flock surveillance project is ongoing until Sept 2017, and continues to yield interesting results. The small flock training day took place with excellent attendance (37 veterinarians), and the veterinary listserv was established spring 2016.

Evaluating the role of distance in the 2015 HPAI outbreak in Minnesota via a spatial transmission kernel

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The 2015 HPAI H5N2 outbreak had considerable adverse impact on the poultry industry in Minnesota with 110 infected premises. Understanding the role of different transmission mechanisms from past outbreak data is critical to inform future preparedness efforts. Spatial transmission kernels are useful in predicting the probability that a virus-free poultry flock becomes infected over time, given that it is some distance away from a premises with infected poultry. Spatial transmission kernels have been previously used to analyze HPAI outbreaks, and in this paper, a transmission kernel was fitted to 2015 outbreak data from Minnesota. The kernel for this study is an enhanced version of that proposed in the analysis of the 2003 HPAI epidemic in the Netherlands and the 1999 epidemic in Italy. The kernel is adapted to assess the relative impact of distance-dependent mechanisms (e.g., local equipment sharing) vs. distance-independent exposures (e.g., wild birds, long distance movements). The transmission kernel parameters were estimated using a maximum likelihood approach from data on premises location and infection status from the Minnesota outbreak. The parameterized spatial kernel is then applied to generate risk maps that apportion the State into high and low risk areas of between-farm HPAI spread. Furthermore, the effectiveness of pre-emptive or early processing as an outbreak control strategy to reduce the susceptible population in an area to limit HPAI spread is evaluated based on a high density county in Minnesota. Finally, we illustrate the application of the spatial transmission kernel to predict the overall likelihood that a poultry flock under consideration for movement is in an infected and undetected disease state, given the implementation of RRT-PCR pre-movement active surveillance testing. The analysis presented here will be used to inform risk management decisions related to the managed movement of poultry from an HPAI Control Area.

A comparison of modeling approaches for estimating within-flock disease transmission parameters for the 2015 H5N2 highly pathogenic avian influenza virus outbreak in the United States ◊

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Developing mitigation measures to minimize the devastating effects of highly pathogenic avian influenza (HPAI) epizootics requires a better understanding of within-flock HPAI virus transmission dynamics. Within-flock transmission parameters inform a wide range of quantitative models including those used in risk assessment for the design and evaluation of active surveillance protocols, and between- premises disease spread models used to evaluate HPAI outbreak control strategies. The within-flock model components are used to predict outcomes such as the disease mortality and the number of infectious birds over time which are used in further analysis. However, transmission characteristics may vary between outbreaks, depending on the poultry species, the HPAI virus strain, and flock management practices. This variability makes extrapolations from past outbreaks less reliable. In this study, daily mortality data from the 2015 H5N2 HPAI virus epizootic in the United States is used to estimate the within-flock transmission rate parameter (β) in turkeys and the basic reproduction number (R_0) obtained from the product of the estimated transmission parameter and the deterministic (fixed) bird infectious period. We first use back-calculation from mortality data in combination with Generalized Linear Model-based epidemic modeling techniques and then compare the results from this approach to those obtained from forward simulation and curve fitting approaches. The back-calculation process followed a Susceptible-Exposed-Infectious-Recovered (SEIR) epidemic model formulation and involved correcting the recorded mortality data for normal daily mortality and subsequently assuming that the remaining mortality was HPAI-induced. Birds that succumbed to infection were assumed to have been infected five days earlier- with a one-day latent period and a four-day infectious period. The latent and infectious periods were estimated from inoculation studies using the Eurasian/American HPAI H5N2 virus turkey field isolate. We validated these methods by comparing our results with output from simulated outbreaks with known transmission parameters. Finally, we illustrate the application of our parameter estimates in an evaluation of pre-movement active surveillance testing protocol options for moving turkeys to processing.

◊ USAHA Paper

Influenza receptor distribution in little brown bats (*Myotis lucifugus)* – uncovering the missing link in the influenza virus evolution # * †

Shubhada Krishna Chothe[1], Ruth Nissly[1], Gitanjali Bhushan[1], Yin-Ting Yeh[2], Miranda Sill[1], Justin Brown[1,3], Gregory Turner[3], Jenny Fisher[1], Mauricio Terrones[2], Bhushan Jayarao[1], Suresh V. Kuchipudi[1]

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Influenza A viruses (IAVs) continue to threaten animal and human health globally. IAVs can infect a wide range of hosts with constant emergence of novel variants that can cross the species barrier. Several influenza pandemics have occurred in the past and the exact mechanisms underlying the origin of these viruses is yet to be fully understood. Bats serve as asymptomatic natural hosts and reservoirs for a number of zoonotic viruses. Two novel influenza like virus sequences have recently been identified in fruit bats and there is serological evidence of avian influenza H9 infection in frugivorous bats. There are more than 1300 bat species globally and the ability of different bats to be naturally infected by IAVs is unknown. A key determinant of the ability of IAVs to infect a host is the expression of glycosylated oligosaccharides that terminate in sialic acid (SA) residues on the host cells. Avian influenza viruses preferentially bind to the SA receptors bound to galactose with an α2,3 linkage, whereas human and swine influenza viruses bind to SA receptors bound to galactose with an α2,6 linkage. Till date there are no reports of influenza virus receptors in any bat species. We investigated SA receptors in little brown bats, the most common bat species of North America and found widespread presence of both avian and mammalian influenza virus receptors in little brown bats. The receptor distribution pattern was distinctly different from species such as humans, pigs, chicken and ducks. SA α2,3-Gal receptor expression was predominant on the non-ciliated tracheal mucosa lining whereas SA α2,6-Gal receptors were predominant in the alveolar spaces of the lung. The luminal surface of intestine had predominant SA α2,3-Gal receptor type expression whereas the SA α2,6-Gal receptor type was dominant in the lamina propia and muscularis mucosa of the intestine. The functional relevance of the SA receptors in bat tissues was demonstrated by virus binding assay. We showed for the first time that little brown bats express abundant SA receptors raising a possibility that they could be infected by a range of avian and mammalian influenza viruses. Predominance of SAα2,3-Gal receptor type over SAα2,6-Gal receptor type infers that these bats are more susceptible to avian influenza viruses which corroborates with the recent serological evidence of avian influenza infection in bats. Our data strongly suggests that a thorough epidemiological screening of influenza viruses in bats could be important to fully understand the ecology and evolution of IAVs.

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

Spatial autocorrelation and implications for oral fluid-based PRRSV surveillance * †

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INTRODUCTION

Oral fluids (OF) are a convenient surveillance sample because they are easily collected and can be tested for nucleic acids and/or antibodies for PRRSV and a variety of pathogens. In addition, this approach facilitates the ability to study infectious diseases in the field. Two questions were addressed in this project: 1) Does PRRSV infection in the field exhibit spatial autocorrelation? 2) Is "spatially based" sampling (as opposed to random allocation of samples) a viable option for routine PRRSV surveillance?

MATERIALS AND METHODS

In 3 wean-to-finish barns on one finishing site, OF samples were collected weekly from every occupied pen (108 pens; ~25 pigs per pen) for 8 weeks for a total of 972 OF samples. OF samples were completely randomized and then tested for PRRSV by RT-PCR.

1. PRRSV RT-PCR results were used to test for the presence of spatial autocorrelation of PRRSV infection on the site, both globally and locally, using threshold distance as the spatial weight matrix. Moran's *I* was used to test for global spatial autocorrelation, giving indication of clustering of PRRSV virus concentration within the barns. LISA (Local Indicators of Spatial Association) were used to test for local spatial autocorrelation, identifying clusters of high PRRSV virus concentration within the barns.

2. Statistical analyses were used to compare the probability of detection based on spatial vs random sampling.

RESULTS

1. Moran's *I* and LISA were computed and supported the presence positive global spatial autocorrelation in the distribution of PRRSV virus in the swine barns. Additionally, clusters of high levels of PRRSV virus were identified at the local level. Further analyses showed that the disease status of a pen in a barn was highly influenced by the disease status of other pens in the same barn, e.g., the presence of ≥ 1 positive pens increased the probability of detecting another positive pen. For most swine veterinarians, this is a statement of the obvious, but spatial autocorrelation has previously not been quantified at the barn level.

2. Analyses comparing the probability of detection (spatial vs random) showed that spatially-based sampling was as good as, or better than, random sampling for the detection of PRRSV.

DISCUSSION

The results of the analyses showed that PRRSV (and probably other infectious diseases of swine) exhibit spatial autocorrelation. This finding has important implications for surveillance and may help explain why spatial sampling is an effective strategy. Overall, the results suggest that a simple, but reliable, oral fluid-based sampling strategy can be developed.

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

Epidemiology and management of endemic CWD in farmed elk through antemortem rectal biopsy testing † ◊

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) affecting members of the cervid family which has been reported in 24 states and 2 Canadian provinces, as well as the Republic of South Korea and most recently Norway. The disease has been found with increasing frequency in both farmed and free ranging cervids – transmitting freely and frequently within both groups. Management has historically involved depopulation in the case of farmed animals and herd reduction in the case of wild deer and elk, the latter with limited success. In CWD endemic areas, where prevalence rates in farmed deer and elk mirror those found in wild cervids, the appropriateness of alternative management strategies for farmed animals has not been examined. We sought to evaluate the practicality and sustainability of managing CWD in a closed elk herd, where CWD prevalence rates approach 20%, using a test and cull strategy relying on rectal biopsies and conventional and experimental diagnostic approaches. We have correlated our findings with genetic background, pregnancy status and progesterone levels, sex, and age to further our understanding of the epidemiology of CWD. We will continue to monitor these correlations over the length of the study to identify the effects of our strategy on CWD prevalence, herd genetics, and reproductive success. This project represents a unique opportunity to collect valuable information on CWD diagnostics, epidemiology, and resistance.

† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Twenty-four year retrospective study of adenovirus hemorrhagic disease in California deer

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A newly recognized cervid adenovirus was the cause of an epizootic which caused high mortality in California mule deer in 1993 and 1994 (Woods et al., 1996). Gross lesions of adenovirus hemorrhagic disease in mule deer typically mimic the *Orbivirus* hemorrhagic diseases in white-tailed deer. Case records from the California Animal Health and Food Safety Laboratory System and the California Department of Fish and Wildlife spanning 24 years were reviewed for all deer accessions, with and without a diagnosis of adenovirus infection in order to examine the prevalence of adenovirus hemorrhagic disease and determine its impact on the California deer herd populations. Of 483 deer submitted to the laboratory for diagnostic testing during a 24 year period, 23.1% were diagnosed with adenovirus hemorrhagic disease. In most Northern California counties, seropositive animals were detected in years with large epizootics (1986/87 and 1993/94) or small mortality events. In general, few serum samples were positive for antibodies to adenovirus (34/446) indicating the disease is not ubiquitous with high asymptomatic infection rate and seroprevalence, antibodies wane quickly and/or only few animals survive exposure and infection. Black-tailed deer, and fawns and juveniles were most frequently affected. Of 1038 lymph node/tonsil pools tested, four from known infected counties were positive for cervid adenovirus by PCR. Squirrels and mice were tested as possible wildlife reservoirs for the virus and determined as not likely reservoirs. Bluetongue and epizootic hemorrhagic disease cases were rare in mule deer species and when diagnosed as the cause of death, did not produce similar lesions to the adenovirus hemorrhagic disease. This retrospective study demonstrated the most common cause of hemorrhagic disease in California mule deer species is cervid adenovirus.

Epidemiology 2

Sunday, October 16, 2016

Imperial C

Moderators: Albert Rovira and Ashley E. Hill

8:00 AM **Agricultural animal population database and case study for the DTRA BSVE ◊**
Jamie L. Barnabei, Anna M. Dixon, Danielle S. Fields, Catharine Weber, Shawn S. Jackson, Erin T. Lauer, Eric Hess, Margaret A. Rush 65

8:15 AM **Achieving efficiency: Systems for receiving case submissions in a high throughput veterinary diagnostic laboratory**
Katie Woodard, Michelle Grabosch, Kelly Boesenberg, Wendy R. Stensland, Dave Baum, Rodger Main 66

8:30 AM **Attaining document control compliance using the I.D.E.A.S. framework**
Susan L. Martin, Thomas James Reilly, Timothy Evans, Shuping Zhang 67

8:45 AM **Podcasts as a tool to enhance communications, lab sample quality, continuing education, and emergency preparedness with practicing veterinarians**
Melanie K. Barham, Andrew Vince, Michael Deane 68

9:00 AM **U.S. survey of AAVLD veterinary dagnostic laboratory Leptospirosis diagnostic capabilities**
Gloria Gellin, Craig N. Carter, Jackie Smith, Erdal Erol 69

9:15 AM **Break**

10:00 AM **Holstein single nucleotide polymorphisms analyzed by genome wide association study for associations with mastitis resistance and susceptibility**
David J. Wilson 70

10:15 AM **Frequency of detection and serotype distribution of Salmonella in backyard poultry flocks in California**
Kris A. Clothier, Asli Mete, Ashley E. Hill 71

10:30 AM **Vesicular stomatitis virus in Colorado horses: seroprevalence and associated risk factors * †**
Anna Claire Fagre, Kristy Pabilonia, Gabriele Landolt, Christie Mayo 72

10:45 AM **A deterministic model to quantify risk and guide mitigation strategies to reduce bluetongue virus transmission in California dairy cattle**
Christie Mayo, Courtney Shelley, N. James MacLachlan, Ian Gardner, David Hartley, Christopher Barker 73

11:00 AM **Estimation of sensitivity of pooled sample testing in surveillance for dwarf gourami iridovirus (*Infectious spleen and kidney necrosis virus*)**
Paul M. Hick, Sophia Johnson, Andrew Robinson, Alison Tweedie, Anneke Rimmer, Joy A. Becker *74*

11:15 AM **A qualitative risk assessment of likelihood of introduction of Brucella serotypes into Egypt from Sudan via illegal camel trade † ◊**
El Bably M. A., Asmaa N. Mohammed *75*

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

Agricultural animal population database and case study for the DTRA BSVE ◊

Jamie L. Barnabei[1], Anna M. Dixon[1], Danielle S. Fields[1], Catharine Weber[2], Shawn S. Jackson[1], Erin T. Lauer[1], Eric Hess[2], Margaret A. Rush[1]

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The Defense Threat Reduction Agency's (DTRA) Chemical and Biological Technologies Directorate (CB) is tasked with safeguarding the United States from chemical and biological threats. In support of this mission, DTRA CB is developing a Biosurveillance Ecosystem (BSVE) that aims to accelerate 'detect – identify – respond' capabilities for biological threats. Since more than 60% of all emerging infectious disease events are characterized as zoonoses, as are many established diseases of public health concern, any comprehensive system for biosurveillance for human disease should include capabilities for monitoring animal populations and disease trends to inform both potential zoonotic and emerging disease surveillance.

Recognizing the importance of animal species in the transmission of many human diseases, Gryphon Scientific and SES, Inc. have initiated a project to bring agricultural animal population and production practice data into the BSVE and to perform a case study to explore the utility of these data to inform BSVE surveillance. In addition to informing zoonotic disease prediction, the collected animal population data have the potential to be a useful decision support tool for State Animal Health Officials in planning for and responding to animal disease outbreaks.

We have developed a methodology to estimate seasonal, county-level commercial animal populations using data from the United States Department of Agriculture (USDA) Census of Agriculture (CoA) and USDA surveys. Additionally, we have re-classified data from the CoA to estimate the frequency of specific production types (such as feedlots, dairies, or cow-calf operations). Drawing upon USDA Animal and Plant Health Inspection Service (APHIS) National Animal Health Monitoring System (NAHMS) reports, animal population data have been augmented with production practice data describing the frequency of human-animal contact and general biosecurity characterizations for each production or farm type. These data are informative for their relevance to disease spread and their ability to help human-health specialists understand the specific risk characteristics of each type of production.

Using historical human and animal disease incidence data, we are performing a case study to test the utility of these animal population and production practice data for predicting outbreaks in human populations of the zoonotic diseases brucellosis, novel influenza A virus, and Q fever. We are developing hypotheses to identify predictors of human zoonotic disease and testing them using simple and multivariate regression with the aim of pinpointing those data in both the existing BSVE and our animal population and production practice database that are most useful for predicting human zoonotic disease. These data can then be incorporated into the BSVE as a data source for analysts or other developers interested in building predictive epidemiological models.

Approved for public release, distribution is unlimited.

◊ USAHA Paper

Achieving efficiency: Systems for receiving case submissions in a high throughput veterinary diagnostic laboratory

Katie Woodard, Michelle Grabosch, Kelly Boesenberg, Wendy R. Stensland, Dave Baum, Rodger Main

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Streamlined, quality assured systems for receiving, inventorying, accessioning, processing, and distributing case material to the necropsy floor and appropriate ancillary labs has long been recognized as an important component of diagnostic laboratory operations. The need for a well-coordinated, multidisciplinary system of receiving, accessioning, and distributing diagnostic case material becomes paramount when caseloads are high and expectations for same-day results are standard. Recent experiences in providing diagnostics during large-scale emerging disease epidemics (PEDV and HPAI) have reinforced the importance and need for streamlined, scalable procedures on the front-end of the diagnostic process. Receiving, accessioning, and entering the myriad of case and specimen level information have commonly been identified as bottlenecks in diagnostic laboratories during emergency disease response situations. The ISU VDL has recently heavily invested (facilities, equipment, human resources, and information technology) in an effort to expand the capabilities and proficiencies of its receiving, accessioning, sample distribution, and case entry functions. Although opportunities for improvement are ever-present, the ISU VDL's system for receiving, processing, and entering, the 300 to 400 cases received each day, has evolved to a reasonably sophisticated and bio-secure pipeline of work. Improving facility design, creating a singular flow of case receipt and sample distribution, increasing the total space available, establishing one cohesive, responsible unit, and developing a suite of focused information management technologies have all played a role in the continuous improvement and scalability of our procedures and services.

Attaining document control compliance using the I.D.E.A.S. framework

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Background:

Document control serves to manage documents throughout their life cycle to provide document integrity and to insure only current versions are in use and available to staff. Faced with a wide-ranging series of document control non-conformances, we sought to establish a systematic process to achieve and maintain compliance.

Methods:

The myriad of deficiencies identified along with the variety of document types in use, made it imperative to adopt a systematic approach to attaining compliance. The I.D.E.A.S. framework, a quality assurance approach to creating, testing, and standardizing processes was employed. The framework consists of five phases: Identify the problem, Design a plan, Execute the plan, Analyze the outcome, and Standardize and monitor the process. When the outcome does not meet expectations, the I.D.E.A.S. framework cycles back to the design phase.

After identifying the problem, an initial plan compared new or revised documents to the designated document template and recorded the observations on a printed copy of the template for each document evaluated; after a one-month trial period, the method was abandoned as it was unable to address all aspects of the problem. Going back to the design phase, a more robust process was developed to systematically examine and record compliance with the template type used, specific elements of document format, revision history, and total number of versions. This process closed the loop of the document's life cycle by tracking the retrieval of former documents. A key component of the plan was a form designed specifically for tracking these data. The process was gradually refined, the form underwent several revisions, and after three months, the entire process was incorporated into an existing Quality Assurance standard operating procedure (SOP) in order to standardize it. An incident log to capture adverse events related to document control and monthly trend analysis of incidents serve to monitor the process.

Results:

Utilization of the I.D.E.A.S. framework enabled correction of identified deficiencies in document control, and the new process serves as an ongoing mechanism to maintain and monitor document control compliance. Compliance levels improved substantially over a four-month time frame. Specific elements and their respective compliance levels before and after establishing the process are as follows: 1) revision history in SOPs (71%, 98%), 2) comportment to approved SOP templates (46%, 99%), no duplicate document versions in the electronic content management system (92%, 100%).

Podcasts as a tool to enhance communications, lab sample quality, continuing education, and emergency preparedness with practicing veterinarians

Melanie K. Barham, Andrew Vince, Michael Deane

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Podcasts are downloadable digital audio files that can be listened to at any time, on any digital device, like a radio show that is available at any time. They allow for the unidirectional transfer of information and can be developed and produced in an inexpensive fashion. While podcasts from various sources vary in length, the recording of brief podcasts, < 20 mins in length, can allow for easy and palatable delivery of practically useful information. Such technology has been shown to enhance traditional methods of teaching in medical schools[1], and has improved the accumulation and retention of novel curriculum material[2].

The Ontario Animal Health Network (OAHN) is a program funded by the Disease Surveillance Plan under the auspices of the Ontario Ministry of Agriculture, Food, and Rural Affairs, with the task of improving animal health and disease surveillance within the province, and has successfully used podcasting methods as an effective manner of communicating with practicing veterinarians and, at times, producers, within the province on many topics. Because podcasts can be accessed hands-free, veterinarians can listen while driving between calls or between appointments. Recordings are generally interview style, usually not more than 20 minutes in length, and are tailored to a species-specific issue, or diagnostic area. The OAHN podcasts were piloted in September 2014 at our annual stakeholder meeting. The early podcasts featured a species specific diagnostic topic, and had an average of 300 listens to each of the 3 recordings. Following this success, a podcast per month was instituted, increasing to 2 per month where possible. Since September 2014, OAHN has produced 39 podcasts (May 2016), with 13,000+ listens. Podcasts have also proven effective vehicles of communicating during an emergency: during the avian influenza outbreak in Ontario 2015, an avian influenza podcast for small flock producers was created featuring practical information tailored to small flock owners/producers. There have been over 1,000 listens to this specific podcast. We have tried several methods of podcasting, including a series of lectures divided into small chunks, single topic interviews, promotional topics, summaries, and emergency podcasts. The field is evolving, and we are learning about veterinary behavior as we develop the use of this medium.

Further reading:

Sandars J. Twelve tips for using podcasts in medical education. Med Teach. 2009 May;31(5):387-9.

References:

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2. Pilarski PP, Johnstone A, Pettepher CC, Osheroff N. From music to macromolecules: using rich media/podcast lecture recordings to enhance the preclinical educational experience. Med Teach 2008;30:630–632.

U.S. survey of AAVLD veterinary dagnostic laboratory Leptospirosis diagnostic capabilities

Gloria Gellin, Craig N. Carter, Jackie Smith, Erdal Erol

University of Kentucky, Lexington, KY

Leptospirosis is a worldwide, re-emerging zoonotic disease. A 2015 report estimates 1 million human infections with 59,000 deaths worldwide annually. *Leptospira spp*. has been detected in over 160 mammals worldwide. Approved vaccines are only available for dogs, swine, cattle and most recently, horses (Zoetis, 2015) making infection in other mammals, including humans, a major health concern in many countries. In most of the US, leptospirosis is rarely reported in domestic animal populations, yet leptospirosis is routinely diagnosed annually in Kentucky every year. One explanation might be that many states do not have laboratories with the capability of diagnosing leptospirosis in animals. Secondly, practicing veterinarians may lack clinical suspicion for the disease. In 2011, a national serological study was conducted by our laboratory involving 29 AAVLD laboratories and one Canadian laboratory (University of Guelph, Ontario) to assess the prevalence of positive titers in 1495 healthy horses. Leptospirosis microscopic agglutination titers (MAT) were all run at the University of Kentucky. The study revealed seropositivity (i.e. evidence of exposure and/or infection) ranging from 10% (Northeast) to 52% (Southeast). The purpose of this current survey is to attempt to determine how many AAVLD laboratories offer leptospirosis testing to their clients. A survey was sent to all AAVLD/NAHLN laboratory directors on the AAVLD listserv in early October 2015, requesting information on any leptospirosis testing offered by their laboratory, including types of tests offered (e.g. MAT, PCR, FA) and test results (e.g. seropositivity, abortions, uveitis, chronic renal or systemic leptospirosis). Seventeen of the 60 AAVLD/NAHLN laboratories (28%) responded, of those responding, only 13/60 (22%) laboratories are offering one or more tests for leptospirosis. One laboratory had discontinued testing in the past year due to lack of submissions. Thirteen of the responding laboratories test for serovars Pomona, Hardjo, Icterohaemorrhagiae, Grippotyphosa, Canicola, and Bratislava by MAT. Additionally, three laboratories also test for Autumnalis, two for Sejroe and one for Copenhageni by MAT. To determine whether *Leptospira* was the cause of an abortion 10/17 (77%) utilize polymerase chain reaction (PCR) while 4/17 (31%) labs run FA tests. Regarding the diagnosis of uveitis in the horse, 7/17 (41%) use PCR and/or MAT tests. Ten of the seventeen responders (59%) do not test eyes for the presence of leptospires by any method. The results of this survey and results from the serological survey in 2011 suggest that the lack of reporting of positive titers and/or diagnoses for clinical leptospirosis around the US may be due to inaccessibility of testing and/or lack of clinical suspicion by laboratory staff and/or practitioners for the disease.

Holstein single nucleotide polymorphisms analyzed by genome wide association study for associations with mastitis resistance and susceptibility

David J. Wilson

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Bovine mastitis resistance is partly associated with genetics. This study evaluated single nucleotide polymorphisms (SNPs) in Holstein genes using genome wide association study (GWAS) and compared SNP distribution among highly mastitis-resistant vs. mastitis-susceptible cows. Milking cows in a 224-cow Holstein herd were evaluated at 2 visits/mo for 1 yr, once/mo for whole-herd milk bacterial culture and once/mo to detect clinical mastitis (CM) and culture all mastitic cows. Milk from other CM cases was sampled by farm staff and frozen for culture; each cow's somatic cell count (SCC) was measured monthly. Case definitions: mastitis Resistant: 4+ months sampled, Lact 2+, no CM, all milk cultures no growth, all SCC < 200,000/ml; Susceptible: 4+ months sampled, all parity groups, 3+ qtrs with CM, and/or 3+ times culture-pos. Genomic DNA was isolated from ear notches and purified with the Gentra Puregene Tissue Kit. The DNA was tested for 777,000 SNPs using the Illumina BovineHD BeadChip, scanned with Illumina iScan. A GWAS multi-locus mixed model and then Golden Helix SVS software tested SNPs' associations with mastitis Resistance or Susceptibility, and calculated statistical significance. SNPs categorized: DD (both alleles minority of Holstein population), Dd heterozygous, or dd (both alleles majority of pop.) Resistant (R) (n=19) cows: mean 5.4 mo studied, SCC 93,000/ml. Susceptible (S) (n=28): mean 6.3 mo studied, SCC 257,000/ml, 172 mastitis cases by culture and/or CM, mean 6.1 cases/cow. 253 SNPs $P<0.0001$, 1402 SNPs $P<0.001$ were significantly associated with R/S cow phenotypes. E.g. chromosome 15 (7 linked SNPs): DD 90% (9/10 cows) R, SCC 106,000, CM 0.6/cow; Dd 55% (11/20) S, SCC 161,000, CM 4.3/cow; dd 94% (16/17) S, SCC 291,000, CM 4.8/cow. Chromosome 12, *Bos taurus* claudin 10 (may protect mammary epithelial cells from bacteria): DD 78% (7/9) R, SCC 81,000, CM 1.2/cow; Dd 52% (11/21) R, SCC 151,000, CM 2.8/cow; dd 94% (16/17) S, SCC 311,000, CM 6.0/cow. Some variant (minority of Holsteins) SNPs (D) were associated with susceptibility, e.g. Chromosome 10, *Bos taurus* integrin α 11 (muscle protein affects skin, and possibly teat sphincter): DD 100% (7/7) S, Dd 74% (17/23) S, dd 82% (14/17) R. Increased mastitis susceptibility was strongly evident in number of mastitis cases/mo, defined by culture and/or clinical signs, more than by SCC. More herds, cows, and years of study are needed. Characterization of mastitis by more than SCC alone is essential. Bioinformatics, including GWAS interpretation of results, and use of P values for this science, is also evolving. This genetic information applies to Holsteins only; Jerseys, crosses, other breeds need to be studied also. It is also critical that all SNPs be evaluated to see whether an advantage conferred in mastitis resistance may also be associated with increased susceptibility to other diseases. Nevertheless, the practicality of bovine whole genome testing is increasing markedly at present.

Frequency of detection and serotype distribution of Salmonella in backyard poultry flocks in California

Kris A. Clothier, Asli Mete, Ashley E. Hill

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Backyard poultry operations are increasingly popular and commonplace in both rural and suburban locations. Birds are kept for a variety of reasons, from home consumption to niche market production. While Salmonella surveillance and intervention strategies have been well-established for large commercial poultry systems, information on smaller operations is lacking. The purposes of this study were to identify the occurrence and serotype distribution of *Salmonella* spp. recovered from backyard poultry flock cases submitted to the California Animal Health and Food Safety Laboratory System, Davis (CAHFS) from 2012-2015 for diagnostic investigations, and to evaluate pathology associated with Salmonella in these cases. Records of 2165 backyard poultry flock (defined as flocks with fewer than 1000 birds including chickens, turkeys, ducks, geese, squabs, and swans) cases on 2425 animals were reviewed for recovery of *Salmonella sp.,* serotype identification, antimicrobial susceptibility, and the presence of pathology associated with these organisms. Samples collected for Salmonella testing included fecal material, small intestine, large intestine, liver, bursa of fabricius, cerebellum, and coelomic cavity/oviduct. Birds were submitted from 44 counties throughout the state. *Salmonella sp.* was recovered in 1.4% of cases. Recovered serotypes included *S*. Agona, *S*. Braenderup, *S*. Enteritidis, *S*. Heidelberg, *S*. Infantis, *S*. Kentucky, *S*. Montevideo, *S.* Meunchen, and *S.* Ohio, *S*. Saint Paul, *S*. Seftenbertg, *S*. Thompson, untypable *Salmonella sp.,* and *Salmonella* 4,5,12:i:-. Pathology associated with *Salmonella* spp. was infrequently identified in birds positive for this organism. Backyard poultry flocks represent a risk for zoonotic transmission of a diverse range of Salmonella via environmental contact or food product contamination.

Vesicular stomatitis virus in Colorado horses: seroprevalence and associated risk factors * †

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[1]Department of Microbiology, Immunology, and Pathology, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO; [2]Department of Clinical Sciences, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO

Vesicular stomatitis virus (VSV) is an economically important disease of ruminants, horses, and pigs, occasionally causing human disease. It results in low mortality, high morbidity, and significant financial losses due to testing and quarantine measures as it is clinically indistinguishable from foot-and-mouth disease. During a statewide VSV outbreak in 2014, 495 horses in the state of Colorado tested positive for VSV via qRT-PCR. In 2015, 231 equine samples were tested for VSV at Colorado State University's Veterinary Diagnostic Laboratory (CSU VDL). The results demonstrated that 153/231 (66.2%) were qRT- PCR-positive and 101/257 (39.3%) were positive as determined by complement fixation assay, indicating that many horses in Colorado have been exposed to VSV.

The objectives of this study were to: 1. characterize the seroprevalence of VSV in the state of Colorado and 2. spatially assess the distribution of seropositive as compared to seronegative animals. Banked serum samples previously submitted for equine infectious anemia virus testing (May - September 2015) were acquired from the CSU VDL and branch laboratories.

All sera were tested for exposure to VSV utilizing a serum neutralization assay against both the New Jersey and Indiana strains. Of the 124 samples tested, 26 (21.0%) were positive for at least one strain, 24 (19.4%) for only the New Jersey strain, 1 (0.8%) for the Indiana strain alone, and 1 for both strains (0.8%). In mapping animals with VSV titers compared to those without, it appeared that the locations of positive cases were grouped similarly to clinical cases identified in 2014. There were also several seropositive animals in the same regions as 2015 cases, though these data are difficult to interpret due to unknown duration of titer persistence.

Several risk factors for VSV exposure and infection have been cited in past studies, including travel, lack of shelter, access to pasture, greater than normal insect populations, and access to running water. Upon receiving approval from the submitting veterinarian, the owners of the horses were contacted and asked to complete an online questionnaire regarding risk factors for exposure to VSV.

Evaluation of VSV seroprevalence among horses in Colorado indicated the proportion of horses exposed to VSV was geographically similar to the incidence of horses demonstrating clinical signs in 2014 and 2015. Seroprevalence rates were remarkably lower amongst horses on the Eastern Plains region as compared to the Front Range and Western Slope. This might suggest that there are geographic and environmental components influencing distribution of associated risk factors. In summary, findings from this study confirm the importance of using the combination of incidence and serosurveillance data for VSV detection, especially factors critical to informing the development of mathematical models to predict the occurrence of VSV infection of livestock and horses.

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

A deterministic model to quantify risk and guide mitigation strategies to reduce bluetongue virus transmission in California dairy cattle

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Bluetongue virus (BTV) is the cause of bluetongue (BT), an economically important, arboviral disease of ruminants transmitted by various species of hematophagous *Culicoides* midges. In North America, *Culicoides sonorensis (C. sonorensis)* is the predominant, if not exclusive, vector of BTV. Recent changes in the epidemiology of *Culicoides*-transmitted viruses, particularly the emergence of previously exotic viruses in Europe, have highlighted the dynamic nature of host-vector-pathogen interactions and implicated multiple environmental and anthropogenic factors as potential drivers of virus emergence and spread, including changes in climate, land use, trade and animal husbandry.

Historically, statistical models have been developed to identify seasonal environmental predictors of BTV infection within endemic region; however, mechanistic models that estimate the basic reproduction number (R_0) have not been utilized to quantify the risk BTV strains within endemic regions such as California. Recent epidemiological investigations in California have screened dairy cattle and *Culicoides* vectors for BTV infections. These studies have provided detailed field data for estimation of the host and vector state variables and transmission parameters utilized in mathematical models. Therefore, the goals of the present study were to 1) use mathematical modeling and field surveillance data to better characterize BTV transmission dynamics among intensively-managed dairy cattle in California, and 2) determine if an endemic equilibrium alters risk predictions of a standard model for BTV infection.

The deterministic model developed in this study assessed the risk of BTV infection among a population utilizing a quantitative framework by calculating the basic reproduction number (R_0) derived from vector, host, and virus parameters. A defining feature of this model, in comparison with other vector-borne disease models, was the incorporation of temperature dependence and real-world surveillance observations to define parameters for biting rate, extrinsic incubation period (EIP), vector mortality, and carrying capacity. Utilizing parameters obtained from sentinel surveillance program, this model demonstrated the greatest risk of BTV transmission was within the Central Valley and southeastern deserts of California. Temperature and non-temperature- dependent parameters (carrying capacity of the vector and probability of transmission from vector to host) had the greatest effect on BTV's basic reproduction number, R_0. In combination with the sensitivity analysis, the spatial and seasonal results indicate that simple and cost-effective strategies to reduce vector abundance (i.e. disrupting larval habitat) might be the most efficacious mitigation strategy to decrease BTV transmission among dairy cattle within an endemic region such as California.

Estimation of sensitivity of pooled sample testing in surveillance for dwarf gourami iridovirus (*Infectious spleen and kidney necrosis virus*)

Paul M. Hick[1], Sophia Johnson[1], Andrew Robinson[2], Alison Tweedie[1], Anneke Rimmer[1], Joy A. Becker[1]

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Nearly 18 million ornamental fish are imported to Australia annually. Despite the biosecurity measures in place, this pathway has resulted in several incidents of exotic pathogens affecting wild and farmed fish populations. These include atypical *Aeromonas salmonicida* and *Cyprinid herpesvirus 2* (CyHV2). Particularly lethal was dwarf gourami iridovirus (DGIV), a genotype of *Infectious spleen and kidney necrosis virus* (ISKNV), genus *Megalocytivirus*. This pathogen is still considered exotic to Australia and poses a high risk to aquaculture and conservation. Infection with DGIV and ISKNV-like viruses is listed as a reportable disease in Australia and the related but distinct red sea-bream iridovirus is listed by the OIE. Changes to biosecurity effective from March 2016 required health certification for all fish from cichlid, gourami and poeciliid families including freedom from infection with megalocytiviruses (MCV). Surveillance testing for freedom from infection commonly employs pooling of samples without knowledge of the effect of pooling on the diagnostic characteristics of the test. The OIE provides a general recommendation that no more than 10 fish per pool be used for detecting subclinical virus infection.

The aim of the present study was to evaluate pooling strategies to test for freedom from infection with DGIV at a confidence consistent with the revised import risk analysis. The analytical sensitivity and specificity for pooled samples was determined for a previously validated real-time PCR assay for MCV. The limit of detection was defined using a sample matrix derived from fish tissue samples with known quantities of DGIV with pooling of tissues, homogenates and purified nucleic acids. Pooled diagnostic sensitivity was determined using 630 pools prepared by random selection from a library of gourami tissues. Pools of 5 and 10 were prepared according to the probability distribution for the number of positive fish when the prevalence was 2, 5 or 10%. Simulation modeling for different prevalence scenarios predicted the number of fish samples and optimal pool size to most efficiently test for freedom from infection with 95% confidence at a minimum expected prevalence of 2%. The sensitivity for detection of a single positive sample in a pool of 3 was 75% (95% CI: 59-87%) for a preliminary selection of samples with 4.66 +/- 1.7 log10 DGIV genome copies per mg. For pool sizes of 5 and 10, the sensitivity was 68% (51%-81%) and 60% (43%-75%), respectively. All of the negative pools were prepared with samples containing < 103 copies of DGIV. Samples stored at -80°C for 6 years were not appropriate for the study because 51.2% of stored nucleic acid samples that tested positive were negative when stored tissue homogenates were tested ($n=630$, $p<0.01$).

Acknowledgment: This study was funded by the Australian Government Fisheries Research and Development Corporation.

A qualitative risk assessment of likelihood of introduction of Brucella serotypes into Egypt from Sudan via illegal camel trade † ◊

El Bably M. A., Asmaa N. Mohammed

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Abstract

Brucellosis is considered endemic in animals and humans in most parts of Egypt leading to an estimated yearly economic loss of 60 million Egyptian pounds. Several studies have attempted to determine the incidence of brucellosis in ruminants and humans in Egypt leading to a high variability of estimates depending on the analyzed host species, geographic localization, and the serological technique used. A qualitative risk assessment to determine the likelihood and consequences that the Brucella serotypes are introduced into Egypt from Sudan via *illegal camel trade at southern Egyptian boundary in 2016.* The OIE recommended methodology (OIE, 2004) for qualitative risk assessments has been adopted. Camel brucellosis was recorded in Egypt by many authors with variable incidence ranged from 7.9% to 10.92%.B. melitensis biovar 3 is the most commonly isolated species from animals in Egypt, B. abortus biovar 1 was reported in Egypt. A pilot study revealed high genetic heterogeneity of Brucella spp. isolates recovered from domestic ruminants in different governorates of Egypt suggesting a complex underlying epidemiological situation in Egypt. In Sudan, the overall seroprevalence of brucellosis in camels (milk and serum samples) was 37.5%. Brucella abortus biovar 6 was isolated from two camels and three cows. From this qualitative risk assessment it can be concluded that; the risk of introduction of endemic Brucella serotypes or / new serotypes into Egypt via illegal camel trade is high and the consequence of introduction including socio-economic and public health impacts are high moreover, study revealed a major gaps in epidemiological data, diagnostics and misconceptions surrounding brucellosis. There is a need for implementing a plan for control of animal movement at Egypt boundaries and increasing public awareness in the prevention methods of brucellosis.

Keywords. Camel, Brucellosis, risk assessment, Egypt, prevalence, Sudan, endemicity, control

† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Molecular Diagnostics and Bioinformatics 1

Saturday, October 15, 2016
Imperial H

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1:30 PM **Evaluation of targeted next generation sequencing for detection of bovine pathogens in clinical samples**
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1:45 PM **High-throughput whole genome sequencing of porcine reproductive and respiratory syndrome virus from cell culture materials and clinical specimens using next-generation sequencing technology**
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2:00 PM **Detecting porcine coronaviruses PEDV, PDCoV, and TGEV by real-time reverse transcriptase PCR**
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2:15 PM **Application of digital PCR for the detection and association of major Shiga toxigenic *Escherichia coli* serogroups and key virulence genes**
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2:30 PM **Evaluation of a multispecies nanoscale PCR array for detection of enteric pathogens**
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2:45 PM **Validation and implementation of a 48-hour CWD test in hunter and diagnostic veterinary submitted samples**
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* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant
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Rapid microbiome profiling using high performance bioinformatic tools and curated genomic databases

Nur A. Hasan[1], Poorani Subramanian[1], Richard Isom[1], Manoj Dadlani[1], Karl Nestor[2], Steve Lerner[2], Rita Colwell[1]

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Increasing trends of nosocomial and mixed microbial infections and the rising association of the microbial community in animal health and wellness have led to the strong necessity to identify the entire microbial community rather than targeted pathogens to understand health and the dynamics of infection. Whole genome shotgun (WGS) metagenomics is the method of choice for such unbiased, culture-independent, and high-resolution characterization of microbial communities. We developed a rapid metagenomics analysis platform, MetaGenID, for accurate shotgun metagenomics and microbiome analysis for infectious disease, food safety, animal health, and environmental applications. The platform utilizes high performance data mining algorithms and phylogenetically organized curated genome databases and operates through an intuitive cloud interface. It offers rapid profiling of microbial communities at subspecies and/or strain level from WGS data and facilitates accurate measurement of their relative abundance. MetaGenID also identifies antibiotic resistance and virulence genes from the shotgun data and offers comparative analyses of datasets employing heat maps and principal component analysis. Its performance is validated using a large number of benchmarking samples (*in silico*, laboratory-constructed, as well as orthogonally validated biological samples, derived from various sources including a commercial sequencing laboratory) encompassing varying levels of microbial diversity and complexity. On a dataset consisting of cow manure samples derived from animals fed with probiotic feed, traditional feed, and spiked manure samples, MetaGenID was able to successfully identify probiotic strains in appropriate samples, and demonstrate the over and under abundances of different bacterial taxa across different sample groups. By using principle component analysis different groups were distinguished based on their community composition. The diversity measures using different methods indicated a similar trend of diversity across samples and/or groups. Blast analysis of a subset of reads resulted in matches against unclassified livestock microbiomes, indicating the extent of cow gut microbiome diversity yet to be uncovered. In summary, the versatility and reduced cost of metagenomics and superior speed, specificity, and precision of the MetaGenID microbiome analysis solution make this technology attractive and powerful for many animal health applications. In particular, MetaGenID can support rapid microbiome profiling, disease diagnostics and outbreak surveillance.

Identification of multiple pathogens in clinical samples using Kraken algorithm-based bioinformatics analysis pipeline ◊

Ying Zheng, Qi Chen, Baoqing Guo, Jianqiang Zhang, Phillip Gauger, Kyoung-Jin Yoon, Sarah Bade, Wendy R. Stensland, Karen Harmon, Rodger Main, Ganwu Li

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The obvious advantage of next-generation sequencing (NGS) technology is that the hypothesis-free metagenomics strategy enables NGS to simultaneously detect the presence of multiple microorganisms in samples and identify uncharacterized pathogens directly from clinical samples without prior knowledge. However, identifying '*a viral needle in a metagenomics haystack*' extensively relies on bioinformatics to tackle the huge amounts of sequence data involved. Here we describe a Kraken algorithm-based bioinformatics analysis pipeline to identify mixed infections in swine. We performed metagenomics sequencing on 217 PEDV-positive swine fecal swab samples and found that 52 samples (24.00%) were PEDV positive only. The presence of diverse RNA viruses along with PEDV such as deltacoronavirus (8.8%), astrovirus (53.9%), enterovirus G (32.7%), sapovirus (12.9%), kobuvirus (28.5%), posavirus (32.3%), pasivirus (6.9%), and sapelovirus (31.8%) was successfully identified. Among them, whole genome sequences of porcine sapelovirus type 1 (PSV-1), also known as sapelovirus A, present in US swine had not been previously reported. In the current study, the entire genome of a US PSV-1 strain was determined and characterized. This US PSV-1 strain (USA/IA33375/2015) had a genome of 7565 nucleotides in length; it had 87.8%-83.9% nucleotide identities at the whole genome level compared to the other 7 global PSV-1 strains (1 from United Kingdom, 3 from China, and 3 from South Korea) with whole genome sequences available in GenBank thus far. Phylogenetic analysis based on whole genome sequences revealed that global PSV-1 isolates formed two clusters and the PSV-1 USA/IA33375/2015 strain together with Korean (GenBank Accession numbers: KJ821021, KJ821020, KJ821019) and Chinese (GenBank Accession numbers: JX286666, HQ875059, KF539414) PSV-1 strains formed a separate cluster from UK PSV-1 V13 strain (GenBank Accession number: AF406813). Partial or complete genome sequences of some viruses such as kobuvirus and Posavirus present in the PEDV-positive samples were also determined and sequence analysis data will be presented. In summary, we have established a NGS-based metagenomics pipeline to successfully identify multiple pathogens present in clinical samples. This provides a powerful and cost-effective tool to determine the prevalence rate of each pathogen or co-infected microorganisms in animals.

◊ USAHA Paper

Evaluation of targeted next generation sequencing for detection of bovine pathogens in clinical samples

Eman Anis[1,2], Ian K. Hawkins[3], Marcia Ilha[3], Moges Woldemeskel Woldemariam[3], Jeremiah T. Saliki[1], Rebecca P. Wilkes[1]

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The diagnosis of bovine infectious diseases, especially those caused by multiple pathogens, can pose a significant challenge to the clinician and the diagnostic laboratory. Historically, diagnosis of infectious diseases has been based on detection of organisms by bacterial culture, virus isolation, or parasite identification. These techniques suffer a number of limitations, including the need for a dedicated specialized staff, the intrinsic inefficiency in the propagation of fastidious bacteria and viruses, and the need for proper sample handling to maintain viable organisms. These techniques have been progressively complemented and even replaced by nucleic acid-based tests like PCR. The advantages of PCR are numerous: speed, sensitivity, and specificity. The main drawback of PCR is that it is limited by the number of pathogens that can be targeted in a single reaction. Recent advances in sequence-targeted next generation sequencing (NGS) have provided the opportunity for development of new research and diagnostic techniques. This study describes the use of target-specific primers for PCR-mediated amplification with the NGS technology, in which pathogen genomic regions of interest are enriched and selectively sequenced from clinical samples. In the study 183 primers were designed to target the most common bovine bacterial, viral, and parasitic pathogens (41 pathogens) and a bioinformatics tool was specifically constructed for the detection of the targeted pathogens. The targets included important bacterial toxin genes and subtypes of some viruses. In effect, this is a very large multiplex molecular panel for infectious disease testing. The primers used in the NGS method were confirmed to detect the intended pathogens by testing known bacterial cultures and parasites and virus isolates. The method was then validated using bovine clinical samples (including tissues and swabs) that were also tested with other diagnostic techniques, including bacterial culture, Fluorescent antibody test (FA), and PCR. Detection limits of the targeted NGS method were evaluated with samples that were also tested by real-time PCR. The method was successful for the detection of multiple pathogens in the clinical samples, including some additional ones missed by the routine techniques because the specific tests needed for these particular organisms were not performed. These results demonstrate the general feasibility of the approach and indicate that it is possible to incorporate next generation sequencing in a cost effective manner into the veterinary diagnostic laboratory.

High-throughput whole genome sequencing of porcine reproductive and respiratory syndrome virus from cell culture materials and clinical specimens using next-generation sequencing technology

Jianqiang Zhang, Ying Zheng, Qi Chen, Sarah Bade, Kyoung-Jin Yoon, Karen Harmon, Phillip Gauger, Rodger Main, Ganwu Li

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Next-generation sequencing (NGS) technologies have increasingly played crucial roles in biological and medical research, but are not yet in routine use in veterinary diagnostic laboratories. In this study, a procedure for high-throughput sequencing was developed and applied to determine whole genome sequences of porcine reproductive and respiratory syndrome virus (PRRSV) from cell culture derived isolates and clinical specimens. Ten PRRSV isolates with known sequences, 2 mixtures each with 2 different PRRSV isolates, and 51 clinical specimens (19 sera, 16 lungs, and 16 oral fluids) with various PCR cycle threshold (Ct) values were subject to nucleic acid extraction, cDNA library preparation (24-plexed), and sequencing. Whole genome sequences were obtained from 10 reference isolates; the nucleotide identities between the NGS sequences and the previously determined sequences (Sanger method) ranged from 99.3-99.9% for all PRRSV reference isolates evaluated. For mixtures with type-1 and type-2 isolates which shared 57.8% nucleotide identity at the whole genome level, NGS was able to distinguish them with their respective genome sequences obtained. For mixtures with two type-2 isolates which shared 92.4% nucleotide identity at the whole genome level, sequence reads with nucleotide ambiguity at numerous sites were observed indicating the presence of more than one PRRSV strain; however, individual virus sequences could only be separated when one isolate identity and sequence in the mixture was known. For clinical samples, using NGS, full-length genomic sequences were successfully obtained from serum samples having PRRSV real-time RT-PCR Ct values 15.1-23.6, lung tissues with Ct 16.4-21, and oral fluid samples with Ct 18.7-20.6. When Ct values were greater than 24.3, 21.3, and 22.0 for sera, lungs, and oral fluids, respectively, full-length sequences could not be obtained, with assembly of no or only partial contigs possible. It must be noted that, in the present study, 24 samples were multiplexed and sequenced each time in Illumina MiSeq system. If fewer samples are multiplexed per run, the success rate of obtaining the PRRSV whole genome sequences from clinical specimens using NGS MiSeq system could be higher, but the cost per sample would increase accordingly. Use of Illumina HiSeq systems can be an option for a better success rate of whole genome sequencing with high throughput and cost saving, but turnaround time would be substantially extended in labs with low caseload. In the summary, the NGS approach on the MiSeq system described in this study offers the prospect of high-throughput sequencing and could be adapted to routine workflows in veterinary diagnostic laboratories, although further improvement of sequencing outcomes from clinical specimens with higher Ct values remains to be investigated.

Detecting porcine coronaviruses PEDV, PDCoV, and TGEV by real-time reverse transcriptase PCR

Robert Sterling Tebbs, Angela Burrell, Adam Allred, Michelle Swimley, Quoc Hoang, Johnny Callahan, Richard Conrad

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Porcine epidemic diarrhea virus (PEDV), porcine delta coronavirus (PDCoV), and transmisible gastroenteritis virus (TGEV) cause gastrointestinal diseases in pigs. Clinical signs can include diarrhea, vomiting, dehydration and anorexia. The diseases are often associated with villous atrophy. The emergence of PEDV and PDCoV in the US in May 2013 and January 2014, respectively, caused the deaths of approximately 7 million pigs in the first year, mainly among piglets (1). We designed a real-time, reverse transcriptase PCR (RT-PCR) assay to detect all three coronoaviruses in a single reaction. The multiplex RT-PCR assay uses a different fluorescent dye for each target for pathogen ID, and includes an internal positive control. The assay was designed to detect all PEDV, PDCoV and TGEV sequences found in GenBank and tested on field samples to validate sensitivity and specificity. The assay showed an analytical sensitivity of ≤10 copies for all three targets when present individually, and ≤80 copies in complex samples when the concentration of the other two targets were $>10^6$ copies.

The sensitivity and specificity of the multiplex coronavirus assay was determined on field samples consisting of oral fluids, feces, and environmental samples. All field samples were purchased from Iowa State University, the University of Minnesota, or Rural Technologies. Over 400 field samples were tested. The workflow for testing field samples includes RNA isolation, RT-PCR, and data analysis. Total RNA was isolated from field samples using the MagMAX™ Pathogen RNA/DNA Kit. The RT-PCR reaction was prepared by combining the TaqMan® Fast Virus 1-Step Master Mix, the coronavirus primer/probe mix, water, and 8 uL of the isolated RNA sample (20 uL total reaction volume). The samples were run on the Applied Biosystems 7500 Fast real-time PCR system using standard run mode with the following cycling conditions: 48C for 10 min, 95C for 10 min, followed by 40 reps at 95C for 15 seconds and 60C for 45 seconds, The data was analyzed by setting the threshold as a percentage of the positive control reactions (control-based threshold). Results with a Ct value ≤37 is classified positive, results between 37 and 40 are suspect and require repeat testing, and undetected results (≥40 Ct) are classified negative. Results from testing field samples showed a sensitivity of ≥97% and a specificity of 100% for all three targets.

In conclusion, a multiplex RT-PCR assay was designed for detecting three enteric coronaviruses. Here we show a workflow that is simple, sensitive, and accurate for detecting PEDV, PDCoV, and TGEV in diverse biological and environmental samples.

Reference

United States Government Accountability Office (2015). USDA Response to Emerging Animal Diseases, GAO-16-132. Website: http://www.gao.gov/assets/680/674174.pdf.

Application of digital PCR for the detection and association of major Shiga toxigenic *Escherichia coli* serogroups and key virulence genes

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Shiga toxin-producing *E. coli* (STEC) are major foodborne pathogens and seven major serogroups, O157, O26, O45, O103, O111, O121, and O145 that carry *stx*1 and/or *stx*2 and *eae* genes have been declared as adulterants in ground beef by the USDA-FSIS. The USDA-FSIS recommended detection method involves culture-based isolation that takes at least a week, and it does not fit high throughput settings. A number of PCR detection assays have been developed; none is capable of associating virulence genes with the STEC O-groups. Some multi-channel digital PCR systems disseminate a PCR reaction into hundreds or thousands of tiny reactions. Each reaction chamber can only hold a single to a couple of templates, providing the opportunity to differentiate if 2 or 3 genes are from a single genome or multiple genomes. Using Thermo Fisher QuantStudio 3D system (20K chambers/chip), we demonstrated, with both pure culture (n=8) and culture-spiked cattle feces (n=4), that the gene association rates (percentage chambers that have dual signals) of *rfb*E-O157 and *stx*2 from the same genome (69.9±8.5) was significantly higher than that from two separate genomes (24.8±5.0). The Fluidigm Biomark 37K chip (48 samples/chip, 770 chambers/sample) allowed us to associate each of the seven major O-groups with one of the *stx* genes at a higher sample throughput setting. Gene association rates for strains that carry two genes were 67.3 to 90.5, with an average of 77.3 for culture and 74.6 to 88.0 with an average of 81.1 for culture-spiked fecal samples. The gene association rates for the two genes carried by separate genomes were 8.7 to 28.8, with an average of 21.4 for culture, and 8.3 to 30.9, with an average of 13.9 for culture-spiked fecal samples. In addition, we were able to associate three genes, *rfb*E-O157, *stx*2 and *eae* from the same *E. coli* genome. We identified three O103 strains that carried *eae*, and three O45 strains that carried *stx*1 from cattle feces, and the results were confirmed by the traditional IMS-based isolation, followed by PCR confirmation. Our simplified method involves an overnight enrichment and digital PCR detection, which can be done within two days. To our knowledge, this is the first report of the application of digital PCR system to detect and associate major virulence genes with O-groups of STEC.

Evaluation of a multispecies nanoscale PCR array for detection of enteric pathogens

Laura B. Goodman, Renee R. Anderson, Rebecca Franklin-Guild, James R. Ryan, Anil J. Thachil, Amy Glaser

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Nanoliter scale real-time PCR uses spatial multiplexing to allow multiple assays to be run in parallel on a single plate without the typical drawbacks of combining reactions together. We designed and evaluated a panel based on this principle to rapidly identify the presence of common enteric pathogens in veterinary fecal and tissue samples in addition to environmental swabs. The pathogens detected included Betacoronavirus, *Campylobacter* Spp., *Clostridium Difficile*, *Clostridium perfringens*, *Cryptosporidium* spp., *Giardia lamblia*, *Listeria* Spp., *Salmonella* Spp., and Rotavirus. Twelve *E. Coli* virulence markers and an internal control (MS2) assay were also included. The 28 total assays were each printed in duplicate on a customized OpenArray plate, accommodating up to 48 samples per plate. A universal extraction and pre-amplification workflow was optimized for high-throughput sample preparation to accommodate multiple matrices and DNA and RNA based pathogens in a cost-effective manner. A total of 164 previously characterized clinical samples consisting of fecal samples, tissues, and bacterial cultures from multiple species were tested. Pooled amplification controls and diagnostic samples were then used to assess repeatability, reproducibility, limit of detection, diagnostic accuracy, range, and linearity for each assay. Cycle threshold (Ct) values were approximately 10-20 cycles lower than observed with microliter scale real-time PCR platforms. Overall, all analytic parameters met expected performance characteristics for microliter-scale reactions with the exception of inter-assay variability, which was elevated for some targets. Representative summary results from the Betacoronavirus assay were 100% agreement for clinical samples, efficiency of 103%, R^2 of 0.99, intra-assay/inter-assay coefficients of variation of 2.0% and 1.1%, and 100% detection at 100 copies. The ability to quickly and accurately test for this comprehensive group of pathogens is a valuable tool for improving efficiency and ergonomics of testing and for enteric disease diagnosis and management. This study was funded and performed in collaboration with the Food and Drug Administration's Veterinary Laboratory Investigation and Response Network (FDA Vet-LIRN).

Validation and implementation of a 48-hour CWD test in hunter and diagnostic veterinary submitted samples

Davin Henderson[1], Nicholas James Haley[2], Edward Hoover[1]

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Chronic wasting disease (CWD) is an emerging prion disease affecting cervid species in the United States, Canada, South Korea and, recently, Norway. CWD is efficiently transmitted through wild and captive cervid populations and poses significant economic, environmental and food safety risks. To promote CWD surveillance we propose the formulation of a new diagnostic paradigm based on the real-time quaking induced conversion assay (RT-QuIC). The RT-QuIC assay platform is currently being adopted as a diagnostic assay for Creutzfeldt Jakob disease in humans and has shown up to 99% specificity and 99% sensitivity. Our laboratory has pioneered numerous advances in the detection of CWD prion seeding activity in deer or elk saliva, urine, CSF, retropharyngeal lymph node, RAMALT, spleen and many other tissues. Recent technological advances in CWD detection have increased sensitivity and specificity as well as enabled the potential for higher-throughput and lower cost. The RT-QuIC assay utilizes a recombinant form of the PrP^C protein to amplify and detect low levels of disease specific CWD prions using a 96-well plate format. A recent analysis of 388 Elk rectal biopsies found that 24 hour RT-QuIC analysis detected 62 positive and 22 suspect positive samples where IHC had detected 34 total positive samples. Only one IHC positive sample was not positive by RT-QuIC but there were only 2 follicles in the sample and one was positive suggesting a possible anatomical sampling error. Biopsies were shipped overnight and analyzed in less than 48 hours from sampling in order to minimize the time the Elk spent in smaller enclosures. Moreover, we have recently published a new technique to increase sensitivity utilizing iron oxide particles to extract CWD prions form tissue sources, which increases sensitivity up to 100-fold.

To increase the through-put and public compliance of a CWD test we envision setup and assay reaction time to be approximately 32 hours, allowing the identification of negative samples in a 48-hour time window. Submitted samples where CWD is suspected in the initial screening will be more thoroughly analyzed and results may take a week. A preliminary cost analysis predicts that the final assay cost would likely be less than currently available test such as immunohistochemistry identification and antibody based methods such as western blot or enzyme-linked immunosorbent assay.

Molecular Diagnostics and Bioinformatics 2

Sunday, October 16, 2016

Imperial F

Moderator: Karen Harmon

10:15 AM **Development and validation of the VetMAX™-Gold MAP detection kit**
Angela Burrell, Ivan Leyva Baca, Rohan Shah, Daniel Kephart 89

10:30 AM **Comparison of four DNA extraction methods for the detection of *Mycobacterium avium* subspecies *paratuberculosis* from the VersaTrek broth cultures by polymerase chain reaction**
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10:45 AM **Improved performance and turnaround time of PRRSV PCR using optimized TaqMan® Fast Virus 1-Step Master Mix**
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11:00 AM **Prevalence of Porcine Parainfluenza Virus Type 1 (PPIV-1) in diagnostic specimens §**
Kevin Lin, Sarah Bade, Karen Harmon, Pablo E. Pineyro, Jianqiang Zhang, Phillip Gauger 92

11:15 AM **Survey of inhibitor resistance in qPCR/qRTPCR master mixes ◊**
Derek Grillo, Sarah Read, Sharon Matheny, Richard Conrad 93

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◊ USAHA Paper

Development and validation of the VetMAX™-Gold MAP detection kit

Angela Burrell, Ivan Leyva Baca, Rohan Shah, Daniel Kephart

Thermo Fisher Scientific, Austin, TX

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent for Johne's disease in cattle and causes severe economic losses in the cattle industry due to reduced productivity, reproductive losses, and the eventual death or culling of the infected animal.

The VetMAX™-Gold MAP Detection Kit is a real-time PCR assay for the rapid *in vitro* detection of MAP DNA purified from bovine feces. The assay targets a unique sequence element in the MAP genome to provide highly sensitive and specific results. The purpose of this study is to determine the performance characteristics of the VetMAX™-Gold MAP Detection Kit in detecting MAP DNA from nucleic acid extracted from individual and pooled bovine fecal samples.

The VetMAX™-Gold MAP Detection Kit was evaluated with 126 individual MAP-positive and 134 individual MAP-negative bovine fecal samples. The MAP status of each sample was confirmed with culture prior to the start of the study. MAP samples were sourced from diverse geographic regions and represented a range of MAP infectivity including heavy, moderate, and light shedders.

The feasibility of pooling up to 5 bovine fecal samples into a single nucleic acid extraction and detection test was evaluated by testing 51 MAP-positive pools and 24 MAP-negative pools. All pools consisted of 5 individual fecal samples. 49 positive pools were created by combining 1 MAP-positive sample with 4 MAP-negative samples. 2 positive pools were created by combining 2 MAP-positive samples with 3 MAP-negative samples.

A collaborator laboratory purified nucleic acid from bovine fecal samples using the MagMAX™ Total Nucleic Acid Isolation Kit. MAP bacterium was physically and chemically lysed by homogenizing the fecal supernatant samples using the FastPrep®-24 homogenizer in the presence of lysis solution. 5,000 copies/reaction of Xeno™DNA Control was spiked into the lysis solution of each purification to monitor for inhibition. Samples were processed using the MagMAX™ Express-96 Deep Well Magnetic Particle Processor. 8 µL of extracted nucleic acid was tested with using the VetMAX™-Gold MAP Detection Kit on the 7500 Fast Real-Time PCR system according to the Instructions for Use.

The results of testing show the VetMAX™-Gold MAP Detection Kit produced diagnostic sensitivity and specificity values of 96.2% and 96.4%, respectively, when testing individual fecal samples as compared to culture. Pooled sample testing with the VetMAX™-Gold MAP Detection Kit resulted in diagnostic sensitivity and specificity values of 96.2% and 100%, respectively as compared to culture. This study indicates that DNA isolated from diagnostic bovine fecal sample, tested with the VetMAX™-Gold MAP Detection Kit, provides an economical and rapid solution for MAP detection from both individual and pooled fecal samples. The results of this study are under review by APHIS' Center for Veterinary Biologics in support of a Biological Product License application.

Comparison of four DNA extraction methods for the detection of *Mycobacterium avium* subspecies *paratuberculosis* from the VersaTrek broth cultures by polymerase chain reaction

Nagaraja Thirumalapura, Willard Feria, Deepanker Tewari

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Johne's disease (paratuberculosis), a chronic enteric disease of cattle and several other domestic and wild animals, is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Culture and subsequent identification of MAP from diagnostic specimens such as feces and tissue has been considered the "gold standard" for the diagnosis of Johne's disease. Liquid culture systems facilitate faster diagnosis of Johne's disease in about 6 weeks compared to more than 16 weeks needed with use of solid media. In addition, liquid culture systems allow monitoring of bacterial growth based on changes in the pressure, production of carbon dioxide or consumption of oxygen in the culture bottles. The presence of MAP in liquid cultures is confirmed by PCR in conjunction with acid-fast staining. The complex cell wall structure of MAP in addition to presence of PCR inhibitors in the egg yolk-rich, dye containing liquid culture medium makes efficient extraction and subsequent detection of MAP DNA by PCR difficult. A simple, reliable and cost effective method of DNA extraction that fits into workflow of diagnostic laboratories handling a large number of samples is highly desirable. In the present study, we evaluated four different DNA extraction reagents for isolation of DNA from MAP culture using a panel of 36 fecal samples that included 25 individual- and 5 pooled-Johne's disease proficiency test samples obtained from the National Veterinary Services Laboratories and 5 diagnostic specimens submitted to Pennsylvania Veterinary Laboratory. The reagents evaluated included: (1) InstaGene Matrix (6% w/v Chelex resin), (2) InstaGene Matrix containing 1% IGEPAL CA-630 and 1% Tween 20, (3) DNAzol Direct (alkaline polyethylene glycol) and (4) DNA Extract All Reagent. All samples were cultured using a VersaTREK Automated Microbial Detection System. After six weeks of incubation, 24 samples out of 36 samples yielded acid-fast bacilli. DNA was extracted from all samples using the four different reagents following the manufacturers' instructions and subjected to MAP IS900 real-time PCR. Of the 24 acid-fast bacilli positive samples tested, MAP DNA was detected in 6 (25%), 16 (66%), 3 (12%) and 24 (100%) samples after extraction using InstaGene Matrix, InstaGene Matrix containing 1% IGEPAL CA-630 and 1% Tween 20, DNAzol Direct and DNA Extract All Reagent, respectively. All eight acid-fast bacilli negative samples were also negative for MAP DNA. Furthermore, of the 74 fecal samples submitted for Johne's disease testing, 21 samples yielded acid-fast bacilli and MAP DNA was detected in all 21 acid-fast bacilli positive samples after extraction using DNA Extract All Reagent. In summary, of the 4 reagents evaluated, DNA Extract All Reagent provided reliable results, is technically simple and less time-consuming making it an ideal reagent for extraction of MAP DNA from the VersaTrek broth cultures.

Improved performance and turnaround time of PRRSV PCR using optimized TaqMan® Fast Virus 1-Step Master Mix

Kelly Smith, Sarah Bade, Phillip Gauger, Karen Harmon

VDL, Iowa State University, Ames, IA

Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded positive-sense RNA virus causing reproductive failure in sows and respiratory problems in piglets and growing pigs. Because of the high economic impact of this agent, the need to accurately and rapidly assess the presence of PRRSV in clinical specimens is crucial. Consequently, PRRSV has consistently been the most requested PCR test at the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL). The ISUVDL previously converted the majority of real-time PCR (qPCR) testing to the ThermoFisher® TaqMan® Fast Virus 1-Step Master Mix (FVMM), after confirming equivalency of results compared to the former assays. This change resulted in thermal cycling time for most agents being reduced from over an hour to around 44 minutes. We were unable to transition our PRRSV PCR assay to this format because those reagents are obtained as an agent-specific assay from ThermoFisher (VetMAX® NA and EU PRRSV Reagents), with its own mastermix (VMMM). Through assistance from ThermoFisher®, the goal of this study was to optimize PRRSV PCR testing with the FVMM to improve overall efficiency without compromising the quality of results. Three hundred each of known positive and negative specimens (100 of each status of lung, oral fluid and serum) were selected for the FVMM and VMMM comparison. In the first trial, the PCR reaction formulation was consistent with the other assays and the manufacturer's recommendation. Results were comparable between FVMM and VMMM on serum and lung samples; however, numerous discrepancies were observed with late Ct (>35) oral fluid samples. Further comparisons were conducted to evaluate of buffer and template volumes, as well as additional enzyme. The formulation which performed best on all sample types included a 30% increase in the amount of buffer, a 40% increase in template volume, and supplemental enzyme mix. With this enhanced FVMM formulation, of the 600 samples tested, all but 20 weak positives agreed with the VMMM results. These 20 discrepant samples were tested in triplicate with the VetMax® and Tetracore® PRRSV PCR assays. The retest results were inconsistent within and between the respective assays, which is common in samples with Ct values >35, where the virus is close to the limit of detection, regardless of sample type or assay. The integration of this PRRS Fast PCR assay will increase efficiency and throughput of testing, as this assay is now conducted with the same thermal cycling profile as the majority of the other qPCR tests at ISUVDL. This provides the option of combining PRRSV with other agents on the same PCR plate, if necessary. The decreased time for thermal cycling also allows for faster reporting of results to clients, and increases testing capacity without requiring additional instrumentation.

Prevalence of Porcine Parainfluenza Virus Type 1 (PPIV-1) in diagnostic specimens §

Kevin Lin, Sarah Bade, Karen Harmon, Pablo E. Pineyro, Jianqiang Zhang, Phillip Gauger

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

Porcine parainfluenza virus 1 (PPIV-1) was first discovered in slaughter swine in China in 2013. Recently, PPIV-1 has been detected in diagnostic submissions from US swine demonstrating respiratory disease without evidence of viral co-infections. Although serology suggests PPIV-1 is widespread in US swine, the prevalence of PPIV-1 in different sample matrices is uncertain as the virus may exist as a subclinical infection.

A total of 1,240 samples including porcine lungs (n=293), nasal swabs (n=176), oral fluids (n=727), and environmental samples (n=44), submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) were tested for the presence of PPIV-1 using real-time reverse transcription PCR (qRT-PCR). RNA was extracted using the Ambion® 5X MagMax™- 96 Viral RNA Isolation Kit (Thermo Fisher Scientific). The qRT-PCR primers and probes targeting the PPIV-1 nucleocapsid (N) gene were developed at Kansas State University. The PCR utilizes the Ambion® Path-ID™ Multiplex One-Step RT-PCR Kit (Thermo Fisher Scientific). PPIV-1 qRT-PCR thermocycler profile is identical to the Influenza A Virus (IAV) Screen PCR (Thermo Fisher Scientific) performed at ISU-VDL using the Applied Biosystems® 7500 Fast Real-Time PCR instrument. Co-infection with IAV was evaluated by qRT-PCR in a subset of the same sample matrices used for PPIV-1 testing.

The highest percentage of PPIV-1 positive samples were detected in oral fluids at 47% [95% CI 43.37-50.63], followed by 36% of nasal swabs [95% CI 28.91-43.09], 12% of lungs [95% CI 7.42-14.58], and 25% of environmental samples [95% CI 12.21-37.79]. PPIV-1 was detected in samples from 21 pork producing states. The largest proportion of positive samples across all matrices evaluated was observed in Illinois 42% (75/180), North Carolina 40% (96/243), and Iowa 33% (119/356). Of the sample matrices tested under both PPIV-1 and IAV qRT-PCR, only 20% of oral fluids (110/544), 3.0% of lungs (8/267), and 9.7% of nasal swabs (17/176) had dual infections with both PPIV-1 and IAV.

The 1st (Q_1) quartile and 3rd quartile (Q_3) cycle threshold (C_T) of PPIV-1 positive oral fluids were 30.3 and 35.4, respectively; however the lowest C_T observed in oral fluids was 20.4. Nasal swabs and lung C_T ranges were similar to oral fluids values: nasal swab Q_1-Q_3 C_T = 30.0 and 34.9, and lung Q_1-Q_3 C_T = 31.2 and 36.4. The large proportion of positive samples reported in oral fluids may suggest PPIV-1 is widespread in U.S. swine. PPIV-1 positive environmental samples recorded higher overall C_T compared to other specimen types (Q_1-Q_3 C_T = 34.7 and 37.5), and suggest the virus may be transmitted through contaminated fomites. Although PPIV-1 may be highly prevalent in swine, clinical significance remains unknown and requires further evaluation under experimental conditions.

§ AAVLD Laboratory Staff Travel Awardee

Survey of inhibitor resistance in qPCR/qRTPCR master mixes ◊

Derek Grillo, Sarah Read, Sharon Matheny, Richard Conrad

Thermo Fisher Scientific, Austin, TX

qPCR/qRT-PCR are the most sensitive high-throughput technologies for the diagnosis of infections in food animals. A common reason for false negatives is molecular inhibitors in the biological sample. Inhibition is mitigated by chemical characteristics of the reaction mastermix. Consequently, animal health (AH) customers are vigilant about the performance of mastermixes. To address this need, we conducted a performance study of 10 mastermixes in the Thermo Fisher portfolio alongside 10 of our leading competitors.

We extracted five common biological sample types encountered by our customers. Into each extract, we spiked a dilution series of positive control and assay to detect a porcine virus (for qRT-PCR) or a bovine bacterium (for qPCR). Mastermix performance was measured by detection sensitivity. For both qPCR and qRT-PCR, PathID brand mastermixes performed the best overall out of the Thermo Fisher products and equal to or better than external competitors. However, the most interesting results were individual performance by sample type; for example, a mastermix that is superior for blood samples is not necessarily one to use for saliva samples.

This study directly benefits our customers by providing guidelines for mastermix use for specific sample types, as well as for overall performance.

◊ USAHA Paper

Pathology 1

Saturday, October 15, 2016

Imperial A

Moderators: Jamie Henningson and Pablo E. Pineyro

1:00 PM **Pathology findings in veterinary pharmacovigilance cases at the Oklahoma Animal Disease Diagnostic Laboratory: 2013-2015**
Keith L. Bailey, Yoko Nagamori, Akhilesh Ramachandran, Grant Rezabek *97*

1:15 PM **A retrospective study of inflammatory conditions of the large intestine in race horses in California : 1990 – 2015 +**
Karina Cecilia Fresneda, Luis Hoyos, Ashley E. Hill, Francisco R. Carvallo, Santiago Diab, Francisco Uzal *98*

1:30 PM **Ribonucleic acid (RNA) decay and the estimation of the postmortem interval (PMI) in horses # + † ◊**
Nanny Wenzlow *99*

1:45 PM **Detection of Bovine Viral Diarrhea virus (BVDV) in a Holstein heifer with mucosal disease**
Melissa Behr, Kathy L. Toohey-Kurth, Sheila McGuirk, Sarah Jacob *100*

2:00 PM **Hyperplastic goiter in adult dairy cattle**
Scott D. Fitzgerald, Chee Bing Ong, Thomas H. Herdt *101*

2:15 PM **An influenza D virus vaccine protects cattle from respiratory disease caused by homologous challenge**
Jamie Henningson, Ben Hause, Lucas Huntimer, Shollie Falkenberg, Jodi McGill, Tom Halbur *102*

2:30 PM **Documentation for a suspect animal cruelty case in a miniature horse**
Doris Marie Miller *103*

2:45 PM **Clinical and pathologic characterization of an outbreak of highly pathogenic avian influenza H7N8 in commercial turkeys in southern Indiana**
Grant N. Burcham, Jose A. Ramos-Vara, Duane A. Murphy *104*

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

Pathology findings in veterinary pharmacovigilance cases at the Oklahoma Animal Disease Diagnostic Laboratory: 2013-2015

Keith L. Bailey, Yoko Nagamori, Akhilesh Ramachandran, Grant Rezabek

OADDL, Oklahoma State University, Stillwater, OK

Veterinary pathologists play a critical role in ensuring that pharmaceutical products are safe and efficacious. While most of these efforts are concentrated on drug development and safety testing, veterinary diagnostic laboratories are uniquely positioned, yet generally underutilized, to contribute to these important activities. From 2013 to 2015, 48 companion animals (representing 42 cases) were submitted to the Oklahoma Animal Disease Diagnostic Laboratory for postmortem examination in support of pharmacovigilance efforts. Thirty-four (81%) of the cases involved dogs and 8 (19%) involved cats. Of the 42 cases investigated, the cause of illness and/or death was identified in 35 (83%) and was not directly attributed to a pharmaceutical product. No definitive diagnosis was determined in 7 cases (17%). These data underscore the need for continued pharmacovigilance efforts to better understand potential adverse events associated with pharmaceutical products and the role that veterinary pathologists can play in the investigation of such cases.

A retrospective study of inflammatory conditions of the large intestine in race horses in California : 1990 – 2015 +

Karina Cecilia Fresneda[1], Luis Hoyos[2], Ashley E. Hill[1], Francisco R. Carvallo[1], Santiago Diab[1], Francisco Uzal[1]

[1]CAHFS, UCDavis, San Bernardino, CA; [2]Universidad Nacional Mayor de San Marcos, Lima, Peru

Inflammatory conditions of the large intestine are amongst the most important alimentary diseases of horses. A variety of etiologies, including bacterial, viral, parasitic, toxic and mechanical are responsible for them. However, the etiology of a significant percentage of these cases remains undetermined. One hundred and seventy three cases of inflammatory disease of the large intestine were identified amongst > 6,500 racehorse necropsies performed at CAHFS laboratories from 1990-2015, under the post-mortem program for the California Horse Racing Board. One hundred and one cases (58.3%) were between 2 and 3-year-old. The most common diagnosis was colitis (122 horses, 70.52%), followed by typhlocolitis (47 horses, 27.17%) and enterocolitis (4 horses, 2.31%). Lesions were acute in 100 horses (57.80%), while in 50 horses (28.90%) they were chronic and in 22 cases sub-acute (12.72%). The most frequently observed distribution was diffuse (n = 114; 65.90%); less common were multifocal, focal or multifocal to coalescing lesions, which were present in 24 (13.87%), 17 (9.83%) and 13 (7.51%) horses, respectively. In 100 (57.80%) horses the cause was undetermined, while 66 (38.15%) had bacterial etiology and 7 (4.05%) cases had mechanical causes (displacements). In cases with bacterial etiology, identified pathogens included *Clostridium difficile* in 32 horses (18.50%) and *Clostridium perfringens* (not typed) in 20 horses (11.56%). In addition, other clostridial species (such as *Clostridium sordellii* or *Clostridium terminale*), *Bacteroides fragilis, Bacteroides uniformis, Listeria spp., Prevotella spp., Pseudomonas aeruginosa, Streptococcus equi* subspecies *zooepidemicus*, Salmonella *spp*., *Staphylococcus aureus* and *Actinobacillus spp.* were detected in 4 or less cases, each. Septicemia was infrequent and it occurred in 14 cases (8.09%). In the majority of the cases in which a final etiology was established, this was bacterial, with *Clostridium difficile* being the most frequent cause. Despite recent significant progress in the development of diagnostic tests for enteropathogens in horses, etiology in many cases (57.80%) was undetermined. Better understanding of the equine intestinal microbiome, along with screening for infectious or toxic enteropathogens using traditional and newer diagnostic techniques may improve identification of causes of intestinal inflammation in horses.

+ AAVLD/ACVP Pathology Award Applicant

Ribonucleic acid (RNA) decay and the estimation of the postmortem interval (PMI) in horses # + † ◊

Nanny Wenzlow

Infectious Diseases and Pathology, University of Florida, Gainesville, FL

The goal of this study was to investigate the RNA decay in equine tissues in order to determine the feasibility of this data for the aid in estimating the PMI in horses and to determine the morphological changes of autolysis in the same equine tissues during the first 72 h after death. Currently, no field applied methods exist to accurately estimate the PMI in animal or humans. The PMI determination capability would be of central importance for forensic investigations of suspicious death in horses. The hypothesis investigated is that RNA degrades in a predictable and step-wise fashion in post-mortem tissues and provides a decay profile for the estimation of the PMI in horses.

Material and Methods: Brain, liver, and skeletal muscle from 12 freshly euthanized horses, were held at 22°C and 8°C. The RNA decay was assessed at T0h, T1h, T2h, T4h, T6h, T12h, T24h, T36h, T48h, T60h, and T72h. The RNA degradation was determined by microfluidic analysis and the decay of the mRNA (cDNA) of β-actin, histone and β-tubulin was assessed by conventional PCR.

Results: In liver tissue, RIN (RNA integrity number) and 28S showed the most predictable decay rate over time with significant differences for temperature. Muscle RIN and 28S were the most stable and brain showed the most unpredictable decay rates and was the only tissue affected by the storage time. The decay of β-actin mRNA (cDNA) was the most representative in all tissues and the most predictable in liver.

Conclusion: Horse liver tissue showed the most predictable decay rate over 72h after death by both methods. Results for horse liver RIN or 28S taken together with results from conventional PCR for liver β- grouped into a clinical index could estimate the PMI in horses.

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

Detection of Bovine Viral Diarrhea virus (BVDV) in a Holstein heifer with mucosal disease

Melissa Behr[1,2], Kathy L. Toohey-Kurth[1,2], Sheila McGuirk[3], Sarah Jacob[3]

[1]Wisconsin Veterinary Diagnostic Lab, Madison, WI; [2]Department of Pathobiology, School of Veterinary Medicine, Madison, WI; [3]Department of Medical Sciences, School of Veterinary Medicine, Madison, WI

A 4-month-old, embryo transfer (ET) Holstein heifer was presented to UW Veterinary Care, with a 3-day history of fever 105 degrees Fahrenheit (F), responsive to antibiotics. After treatment, she was moved into a new group, in which another heifer then died with bronchopneumonia, subsequently attributed to *Mannheimia hemolytica* but negative for BVD by polymerase chain reaction (PCR). The ET heifer became ill again, with fever 104 F, and evidence of pneumonia. She had received 3 bags of colostrum at birth, as well as Calf Guard, Equalizer + C, Enforce 3, and BoSe; her recipient dam tested negative for BVDV (test method unknown), but the ET calf was not tested. At two months of age, she was vaccinated with Bovishield Gold, and again 3 weeks later.

Upon presentation, the heifer was dull and depressed. She was mildly pyrexic 102.2 F, and tachycardic with a normal respiratory rate. She passed a large volume of malodorous diarrhea. Blood count showed neutropenia (1310/uL) with left shift (998/uL). A fecal sample was PCR-positive for BVD at a Cycle threshold (Ct) of 22.4; gram stain revealed mixed fecal flora; *C. difficile* ELISA was negative. The calf made no significant progress and continued to pass watery diarrhea with blood clots; she was euthanized two days after presentation. Necropsy revealed diffuse, severe fibrinous enterocolitis, and cranioventral bronchopneumonia. BVD PCR was strongly positive on ear (Ct 25.3), lung (26.3) and ileum (19.3); ileal content was culture-negative for *Salmonella* and negative for *C. difficile* by ELISA. Histopathology revealed severe, diffuse, full-thickness necrosis of intestinal mucosae. The final diagnosis was BVD.

Bovine Viral Diarrhea virus (BVDV) continues to be a major pathogen in dairy and beef cattle operations in Wisconsin and the USA. Reliable detection of persistent infection is critical for herd health. At the WVDL, a transition to PCR took place in 2008, as cumulative data showed it to be more reliable for detection of persistently infected animals at any age. The accepted definitive diagnosis is two positive results 3-4 weeks apart. Follow-up PCR is unavailable for this case, but most animals with Ct <25 (ear notch sample) are persistently infected (PI). This case demonstrates the continual need to test both dam and calf in order to detect PI animals promptly.

Hyperplastic goiter in adult dairy cattle

Scott D. Fitzgerald[1], Chee Bing Ong[3], Thomas H. Herdt[2]

[1]Diagnostic Center for Population & Animal Health, Michigan State University, Lansing, MI; [2]Diagnostic Center for Population & Animal Health, Michigan State University, Lansing, MI; [3]Institute of Molecular and Cell Biology, AMPL, Proteus, Singapore

Iodine excess and resulting hyperplastic goiter are well recognized in neonatal ruminants, but infrequently encountered in adult cattle. A Michigan dairy had lost 10 out of 300 adult cattle for a variety of problems over a 3 month period, so two adult Holstein cows were submitted to the DCPAH for diagnostic necropsy. One cow had grossly and markedly enlarged thyroid glands consistent with cystic hyperplastic goiter; the second had normal size thyroids. Histologically, the thyroid lesions varied from cystic nodular hyperplasia to follicular atrophy with fibrosis. Iodine concentrations in both animals were moderately to markedly elevated; 1,065ug/g and 394ug/g respectively. Nutritional evaluation of total mixed rations from the farm revealed mildly elevated levels of iodine, but well below the reported toxic values for adult dairy cattle of 12-100mg/100kg body weight reported in the literature. However, upon further investigation it was discovered that supplementation with top dressed mineral supplement was routinely added, and that free choice trace mineralized salt was also available to cattle on this facility. Serum levels of iodine were measured in 3 normal herd mates; levels ranged from 322 to 913 ng/ml. Once the owner stopped using top-dressed and free choice trace mineralized salt, a second set of serum levels were measured in the same 3 cows; values had returned to normal levels of 46, 48 and 71 ng/ml. Due to public safety concerns regarding excess iodine in the milk from this farm, 3 bulk tank milk samples were analyzed. Iodine concentrations were 359, 423 and 503 ng/ml; and were deemed within acceptable limits. This case illustrates that adult cattle are susceptible to excess iodine supplementation and can develop hyperplastic goiter. It further indicates that correction of the iodine excess can result in rapid decline of serum iodine to normal levels. Ancillary tests including serum iodine measurements can be a useful tool in documenting and correcting excess iodine levels in cattle feeds.

An influenza D virus vaccine protects cattle from respiratory disease caused by homologous challenge

Jamie Henningson[1], Ben Hause[1], Lucas Huntimer[2], Shollie Falkenberg[3], Jodi McGill[4], Tom Halbur[2]

[1]Kansas State Veterinary Diagnostic Lab, Manhattan, KS; [2]Elanco, Larchwood, IA; [3]USDA-ARS, Ames, IA; [4]Department of Diagnostic Medicine/Pathobiology, Kansas State, Manhattan, KS

Originally isolated from swine, the proposed influenza D virus has since been shown to be common in cattle. Inoculation of IDV into naïve calves resulted in mild respiratory disease histologically characterized by tracheitis. As several studies have associated the presence of IDV with acute bovine respiratory disease (BRD), we sought to investigate the efficacy of an inactivated IDV vaccine. Vaccinated calves seroconverted with hemagglutination inhibition titers 137-169 following two doses. Groups challenged with a homologous virus exhibited signs of mild respiratory disease from day 4-10 post challenge which was significantly different than negative controls at day 9 post challenge. Peak viral shedding of approximately 5 $TCID_{50}$/mL was measured in nasal and tracheal swabs and bronchoalveolar lavage fluids 4-6 days post challenge which was significantly (P<0.05) decreased 1.0-1.6 $TCID_{50}$/mL, 3.6 $TCID_{50}$/mL and 3.8 $TCID_{50}$/mL, respectively, in the aforementioned samples collected from vaccinated animals. On histopathology, inflammation characterized by neutrophils was observed in the nasal turbinate and trachea. Lesions included neutrophils in the submucosa and the respiratory epithelium of the nasal turbinates and trachea. One calf did have a suppurative bronchitis and bronchiolitis in the lung. Viral antigen deposition was detected as cytoplasmic staining in the respiratory epithelium of the nasal turbinates and trachea by immunohistochemistry from all unvaccinated calves and significantly fewer vaccinates. Two calves had weak positive staining in the airway epithelium of the lungs. IDV transmitted to all calves exposed via direct contact and displayed similar replication and shedding. The results in this study demonstrate that influenza D can play a role in BRD and in stressed cattle may replicate in the lung and contribute to pneumonia. Together these results support an etiologic role for IDV in BRD and demonstrate that partial protection is afforded by an inactivated vaccine. Examination of retrospective diagnostic lung samples from BRD cases is in progress.

Documentation for a suspect animal cruelty case in a miniature horse

Doris Marie Miller

Athens Veterinary Diagnostic Lab, University of Georgia College of Veterinary Medicine, Watkinsville, GA

An adult male miniature horse was submitted for a necropsy as a suspect cruelty case. The history submitted indicated the horse had been found dead on the side of the road wearing a blue halter secured to a tree. The animal was in poor body condition with multiple healing and non-healed rib fractures, parasites, pyoderma, thyroid tumor, myositis, and wavemouth. The horse had been owned for over one year by one owner and only one week by the second owner. Before charging one or both owners animal control officers wanted to know an estimation of time for the animal to "get to the condition it was in". Discussion of time of development and documentation of bone healing, parasites, starvation, and wear pattern of teeth will be discussed in reference to case reporting and forensic cases.

Clinical and pathologic characterization of an outbreak of highly pathogenic avian influenza H7N8 in commercial turkeys in southern Indiana

Grant N. Burcham[1], Jose A. Ramos-Vara[1], Duane A. Murphy[2]

[1]Animal Disease Diagnostic Laboratory, Purdue University, Dubois, IN; [2]Farbest Foods, Jasper, IN

Highly pathogenic avian influenza (HPAI) is a systemic lethal disease of poultry caused by several subtypes of influenza A viruses and classified based on serologic reactions to hemagglutinin and neuraminidase surface glycoproteins. In January 2016, a novel subtype of HPAI—H7N8—was diagnosed in a commercial turkey flock in southern Indiana. The affected birds were 135-day-old tom turkeys located in a single barn of 5400 turkeys. The farm had 3 other barns of the same age and size and 2 brooder barns with 25-day-old poults; all other barns were clinically unaffected. Clinical signs/history included increased mortality (800 birds overnight), dyspnea, head tremors, recumbency, and somnolent or unaware birds. Autopsy of six recently dead birds showed red-tinged mucous in the choana and trachea, and marked pulmonary edema. Histologic lesions in the brain included severe, multifocal lymphohistiocytic meningoencephalitis with foci of malacia, neuronal necrosis, and neuronophagia. Heterophils were common in some regions of meninges. All anatomic locations of the brain were affected, although histologic changes in the cerebellum were considered mild. Other histologic lesions included pulmonary congestion and edema, splenic congestion and lymphoid depletion, and fibrinoid necrosis of vessels within the spleen. Immunohistochemistry was weakly positive for influenza A in the brain; IHC was negative in other tissues tested. Histologic lesions and immunoreactivity were notably absent from the heart, a tissue commonly affected in previous cases of HPAI. In addition to IHC, a commercial immunochromatographic assay for influenza A was positive on two sets of pooled oropharyngeal swabs, as was influenza A PCR.

Subtyping and pathotyping of the detected virus was completed at the National Veterinary Services Laboratory in Ames, IA, and showed infection with a highly pathogenic strain of H7N8. Surveillance of the surrounding 65 commercial and 100 backyard poultry farms within the 10-km control area by company and state officials detected 8 commercial turkey premises infected with low pathogenic H7N8. Other premises, including commercial laying houses and backyard flocks were negative. The clinical and pathologic characteristics of this case match previously published material concerning HPAI, and add to instances of known or suspected mutation of a low pathogenic virus to a highly pathogenic virus.

Pathology 2

Sunday, October 16, 2016

Imperial A

Moderators: Dodd Gray Sledge and Rachel Derscheid

8:00 AM **Nutritional steatitis in salmonids from the Western United States ◊**
Danielle Darracq Nelson, Bethany Frances Balmer, Kevin R. Snekvik *107*

8:15 AM **Canine ocular melanocytic neoplasms: morphologic and immunohistochemical evaluation # + †**
Erica Noland, Megan Climans, Matti Kiupel, Dodd Gray Sledge *108*

8:30 AM **Proliferative thrombovascular necrosis of the pinnae in dogs # +**
Rahul Babulal Dange, Katherine Barnes, Barbara Steficek, Matti Kiupel *109*

8:45 AM **Concurrent ocular T cell lymphoma with lineage infidelity and histiocytic sarcoma with B cell receptor IGH gene clonality in a cat (felis catus) †**
Katie Jean Barnes, Matti Kiupel, Jean Stiles, Madison Operacz, Dodd Gray Sledge *110*

9:00 AM **Chromatophoromas in bearded dragons # + †**
Colleen F. Monahan, Michael Garner, Anne Meyer, Kristen Phair, Gary West, Matti Kiupel *111*

9:15 AM **Evaluation of pathogenicity and viral quasispecies diversity of genetically distinct strains of Rift Valley Fever virus in a mice model**
Vinay Shivanna, Aaron Balogh, Chester McDowell, Anne Sally Davis, William C. Wilson, Juergen Richt. *112*

9:30 AM **Break**

10:15 AM **Development of posterior ataxia, paralysis and myelitis in cesarean-derived colostrum-deprived pigs following experimental inoculation with either *Teschovirus A* serotype 2 or serotype 11 #**
Franco Sebastian Matias Ferreyra, Bailey Lauren Arruda, Darin Madson, Kent L. Schwartz, Gregory Stevenson, Jianqiang Zhang, Qi Chen, Kyoung-Jin Yoon, Paulo Arruda *113*

10:30 AM **An outbreak of mycoplasmosis causing arthritis, pneumonia and meningitis in dairy goat kids**
Gayle C. Johnson, William H. Fales, Thomas James Reilly, Brian M. Shoemake, Pamela R.F. Adkins, John R. Middleton, Fred Williams, W. Jefferson Mitchell, Michael Calcutt. *114*

10:45 AM **Arsenic toxicosis in dairy calves**
Alexander D. Hamberg, Lisa A. Murphy, Lore Boger *115*

11:00 AM **Morphological correlation of gammaherpesvirus-5 cellular replication with immune and inflammatory responses in equine multinodular pulmonary fibrosis**
Brieuc Cossic, Matthew Pennington, Gerlinde Van de Walle, Amy Glaser, Gerald E. Duhamel *116*

11:15 AM **Pathologic findings in horses experimentally infected with Equine herpesvirus-1 and mutants of differing neuropathogenic potential**
Dodd Gray Sledge, Matti Kiupel, Carine Holz, M. Wilson, Lila Marek Zarski, Rahul K. Nelli, Anthony Pease, Walid Azab, Klaus Osterrieder, Lutz S. Goehring, Gisela Soboll Hussey 117

11:30 AM **Diagnostic challenges in the first confirmation of sporadic bovine encephalomyelitis (*Chlamydia pecorum* encephalitis) in New Zealand**
Kelly Buckle, Hayley Hunt, John Munday, Geoff Orbell, Hye-Jeong Ha. 118

Symbols at the end of titles indicate the following designations:

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

Nutritional steatitis in salmonids from the Western United States ◊

Danielle Darracq Nelson[1,2], Bethany Frances Balmer[1,2], Kevin R. Snekvik[1,2]

[1]Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [2]Washington Animal Disease Diagnostic Laboratory, Pullman, WA

Nutritional steatitis occurs in many species when diets are deficient in active antioxidants and/or when diets contain excessive oxidized lipids. Both antioxidants and polyunsaturated lipids in feeds are highly labile when exposed to oxygen, heat, or ultraviolet light during transport or storage. With prolonged storage of feeds and supplements, such nutrient damage is inevitable. In animals ingesting excessive oxidized lipids and/or deficient antioxidants such as vitamin E, free radical cell membrane damage causes degeneration and necrosis in multiple tissues, particularly adipose tissue. Reportedly affected species include farmed salmonids, catfish, mink, cats, pigs, poultry, and wild fish-eating birds. This retrospective study of diagnostic cases seen at the Washington Animal Disease Diagnostic Laboratory during 2014 and 2015 includes farmed juvenile Spring Chinook salmon and rainbow trout juveniles and adults from Colorado, Arizona, and Idaho. Some cases revealed clear evidence of excessive heat during feed storage. One case involved wild Coho salmon fingerlings from Washington State in association with a prolonged heat wave and elevated water temperatures. The histological changes included sterile granulomatous steatitis with intralesional acicular clefts, and commonly affected sites included the dorsal fat pads and coelomic adipose tissue. Grossly, the skin can darken, the swim bladder may appear thickened, and cachexia is often observed. It is likely that younger, faster growing fish have increased susceptibility over adults, and associated debilitation can lead to opportunistic infections. Preventative measures such as avoiding excessive heat and light when transporting and storing feeds and avoiding feeding expired feeds are critical for preventing this debilitating disease.

◊ USAHA Paper

Canine ocular melanocytic neoplasms: morphologic and immunohistochemical evaluation # + †

Erica Noland, Megan Climans, Matti Kiupel, Dodd Gray Sledge

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

Ocular melanocytic neoplasms are comprised of variable mixtures of two morphologically distinct cell populations, large discrete round cells with abundant intracytoplasmic melanin and variably pigmented spindloid cells. The significance of the variability in morphology is unclear, but may suggest differing origins or degrees of differentiation. Given morphology, pigmented round cells may represent melanocytes or melanomacrophages. Suggested prognostic indicators for these tumors include cell morphology, mitotic index (>4 mitoses per 10 high power fields), nuclear atypia, and degree of pigmentation; however, the exact significance of such indices with respect to outcome is unclear. While similar prognostic indices have been suggested in oral and cutaneous melanocytic neoplasms, growth fraction as evaluated by Ki67 has been shown to be a superior prognostic indicator. The goals of this study were to immunophenotype the pigmented cell populations of ocular melanocytic neoplasms in order to confirm that pigmented round and spindle cell populations are of melanocytic origin, and to contrast the expression of markers of melanocytic differentiation between cell types. Further, this study aimed to compare the growth fraction of these tumors to mitotic index and cell morphology. 19 archived cases were categorized according to spindle cell percentage and included 5 with <25% spindloid cells, 4 with 25-50% spindloid cells, 6 with >75% spindloid cells and ≤4 mitoses, and 4 with >75% spindloid cells and >4 mitoses. Immunohistochemistry for Melan-A, HMB45, PNL2, CD18, CD204, CD179a, CD163, and Ki67 was performed on tissue microarray sections that included cores from the 19 cases along with cores from normal, but inflamed iris. Pigmented round cells within the inflamed iris stroma were CD18+/HMB45+/CD204-/CD179a-/CD163-. In tumors with <25% spindle cells, large heavily pigmented round cells were diffusely CD18+, variably HMB45+, and CD204-/CD179a-/CD163-, consistent with being melanocytes not melanomacrophages. In tumors containing greater percentages of spindloid cells, up to 50% of the pigmented round cells were CD18+/CD204+ consistent with proportions being melanomacrophages. Pigmented spindle cells within the inflamed iris were MelanA+/PNL2-; however, PNL2 was strongly expressed in spindloid neoplastic cells of some tumors and coexpression with Melan-A was not mutually exclusive. Spindle cell percentage and number of mitoses were not consistently correlated with overall growth fraction as determined by Ki67. These findings indicate: 1) densely pigmented discrete round cells at least in round cell predominant ocular melanocytic neoplasms represent melanocytes that are immunophenotypically distinct from spindloid melanocytes; 2) due to variable expression of Melan-A and PNL2 in spindloid cells, multiple markers should be used to improve diagnostic sensitivity; and 3) evaluation of the Ki67 index is a better indicator of growth fraction than mitotic index.

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

Proliferative thrombovascular necrosis of the pinnae in dogs # +

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Proliferative thrombovascular necrosis of the pinnae (PTNP) is a fairly uncommon condition that affects the canine ear. The condition presents as bilateral, or occasionally unilateral, ear lesions that are histologically characterized by alterations of small arteriole walls in the pinnae, with progression to thrombus formation and subsequent ischemic necrosis of the ear tips. Dogs with PTNP lack systemic disease and the lesions are limited to the ears. Clinical differentials for such cases may include septicemia, frostbite, disseminated intravascular coagulation (DIC) or cold agglutinin disease. The aim of this retrospective study was to characterize the histologic lesions associated with PTNP in dogs and to review the diagnostic criteria to accurately diagnose PTNP. Surgical biopsy specimens from the pinnae of 58 dogs with a presumptive clinical diagnosis of PTNP were selected from the archives of the Diagnostic Center for Population and Animal Health at Michigan State University over a 10 year period. Ischemic pinnal necrosis and epidermal ulceration with associated serocellular crusting and neutrophilic and lymphoplasmacytic dermal inflammation were consistent findings in all 58 dogs. The diagnosis of PTNP was confirmed in 10 dogs (17%) based upon the characteristic histologic lesions of thickening of the wall of small arterioles and luminal occlusion, thrombosis, and ischemic necrosis. Additional histologic features observed in dogs with confirmed PTNP included chronic hemorrhage with hemosiderin laden macrophages (8/10), hyaline degeneration and fibrinoid vascular necrosis (3/10), vascular sclerosis (2/10), thrombosis with recanalization (2/10) and focal necrosis of pinnal cartilage (2/10). A presumptive diagnosis of PTNP was made in 46 dogs (79%) as the lack of sufficient healthy tissue adjacent to the areas of necrosis did not allow detection of proliferating arterioles or thrombosis in the remaining cases. The lesions in 2 dogs (3%) were not histologically consistent for PTNP despite similar proliferative vascular changes due the presence of vasculitis. PTNP should be the considered as a primary differential for dogs with lesions limited to pinnal necrosis in the absence of systemic clinical disease. To accurately diagnose PTNP, multiple biopsies of adequate size and depth from the leading edge of the lesion that include sufficient non-necrotic tissue are optimal. Educating practitioners on proper sample collection and the necessity of a detailed clinical history for suspected PTNP cases will be essential to more consistently diagnosis this syndrome.

AAVLD Trainee Travel Awardee
+ AAVLD/ACVP Pathology Award Applicant

Concurrent ocular T cell lymphoma with lineage infidelity and histiocytic sarcoma with B cell receptor IGH gene clonality in a cat (felis catus) †

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Enucleation was elected in a 17-year-old cat with a five-year history of suspected uveitis in the right eye that progressed to include corneal opacity obscuring intraocular structures. The globe was expanded and ablated by two morphologically and immunophenotypically distinct neoplastic cell populations. Expanding and infiltrating the cornea and uvea was a smaller, monomorphic neoplastic round cell population that had immunoreactivity for CD3 and not CD20 or CD204, consistent with T cell lymphoma. Regionally expanding the stroma of the anterior uvea was a larger, anaplastic cell population that had immunoreactivity for CD204 and not CD3 or CD20, consistent with histiocytic sarcoma. PCR assessing clonal rearrangement of T and B cell receptor genes was performed on the histiocytic and lymphocytic populations separately following gross dissection from the formalin-fixed, paraffin-embedded tissue and laser capture microdissection. Clonal rearrangement was only confirmed for the B cell receptor IGH gene in both cell populations; however, the length of the clonal PCR products varied by 20 base pairs between the histiocytic and lymphocytic populations. Demonstration of clonality of the B cell receptor IGH gene in lymphocytes that are phenotypically T cell in origin is consistent with lineage infidelity. Clonality of the B cell receptor IGH gene in a histiocytic sarcoma may be due to transdifferentiation from a concurrent or previous lymphoma or sporadic inheritance of a B cell genotype. The different clonal peaks in the electropherograms support two clonally unrelated lymphocytic and histiocytic neoplastic cell populations and therefore suggest a unique histogenesis for each neoplastic population.

† Graduate Student Oral Presentation Award Applicant

Chromatophoromas in bearded dragons # + †

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Chromatophores are pigment-bearing and light-reflecting cells in the skin of reptiles, fish, and amphibians. Neoplasms arising from chromatophores are called chromatophoromas and have further been classified based on the type of pigment they contain as xanthophoromas, iridophoromas, and melanocytic neoplasms. This study characterizes 17 cases of chromatophoromas in bearded dragons (*Pogona vitticeps*).

The majority of chromatophoromas were located in the dermis, but commonly extended into the subcutis and muscular layers. Most neoplasms were poorly demarcated, non-encapsulated, and moderate to highly cellular. Neoplastic cells were most commonly spindloid (12/17), but 5 neoplasms were composed of mixed polygonal and spindloid cells. Pigment varied from amber and birefringent (3 iridophoromas) to dark brown/black (5 melanocytic neoplasms). Some tumors contained both types of pigments and were classified as mixed chromatophoromas (3/17). The degree of pigmentation was low for 7, moderate for 2, and high for 2 cases. Six cases had no visible degree of pigmentation and were classified as nonpigmented chromatophoromas. Nuclear atypia was mild in 7, moderate in 7, and marked in 3 neoplasms. The mitotic count varied from 0 to 26 mitoses in 10 high power fields, but was low in most neoplasms. A few neoplasms (6/17: 4 melanophoromas, 1 mixed chromatophoroma, 1 nonpigmented chromatophoroma) had abundant, PAS-positive, mucinous stroma. Two of these 6 cases had heavy melanin pigmentation and one had bone and cartilage metaplasia. There was no evidence of lymphatic invasion, junctional activity, lentiginous spread, or intranuclear pseudoinclusions in any of the neoplasms examined. In general, microscopic examination alone was adequate to diagnose chromatophoromas. Immunohistochemistry was of limited use in aiding the diagnosis as all cases were negative for Melan A, 3 neoplasms were positive for PNL-2, and all, but one chromatophoroma had at least partial labelling for S-100.

Follow up information was only available for 6 cases. None of these neoplasms recurred after initial excision or caused mortality. The clinical behavior of the neoplasms in our study was similar to that of 15 previously reported cases. However, 2 (1 melanophoroma, 1 iridophoroma) of these 15 cases had visceral metastases, but both neoplasms had significantly greater pleomorphism and mitoses compared to the chromatophoromas with no evidence of malignancy in either study. This is in contrast to chromatophoromas in snakes that have been reported with a much higher incidence of malignancy. Marked nuclear atypia was the only factor associated with a high risk of metastasis in snake chromatophoromas. In this study, while there was no evidence of intravascular invasion or visceral metastasis there were 3 neoplasms that exhibited marked nuclear atypia and high mitotic count. However, follow up data was not available for these cases, but no metastases were noted at the time of excision.

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

Evaluation of pathogenicity and viral quasispecies diversity of genetically distinct strains of Rift Valley Fever virus in a mice model

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Rift Valley fever virus (RVFV, *Bunyaviridae*: *Phlebovirus*) is a mosquito-borne zoonotic virus with tripartite, single stranded negative sense RNA genome. RVFV primarily infects sheep, goats and cattle causing abortion and high mortality in young animals. Human infections exhibit clinical signs ranging from acute febrile illness to fatal complications. Although currently endemic to sub-Saharan Africa and the Arabian Peninsula, the virus has high potential for transboundary spread due to the existence of competent vectors in many non-endemic countries. The objective of the present study was (1) to characterize a mouse model for RVFV infection, (2) to analyze the pathogenicity of the two genetically distinct RVFV strains obtained from separate RVF outbreaks and (3) to characterize the RVFV quasispecies diversity generated during infection of mice. The two RVFV strains used in the study were Kenya 128B-15 and SA01-1322 (isolated from outbreaks in Kenya 2006-07 and Saudi Arabia 2000, respectively). The nucleotide homology between the two strains was 99.8, 98.5 and 98.5% at the nucleotide level and 99.4, 98.9, and 98.1% at the amino acid level for the L, M and S segments, respectively. Groups of six to eight week old female BALB/c mice (N=5) were infected subcutaneously with 10-fold serial dilutions (0.1-1000 PFU) of the viruses propagated in Vero cells. Mortality was recorded and LD_{50} values were calculated. Liver and spleen samples were collected from dead or moribund mice for sequencing and histopathological analysis. The observed LD_{50} of 7.9 PFU was similar for both strains of viruses, indicating that both virus strains were equally virulent to BALB/c mice. Histopathology and RVFV nucleoprotein immunohistochemistry of liver and spleen of mice inoculated with 1000 PFU revealed that both virus strains caused remarkable lesions. In the liver, there were multifocal areas of coagulative necrosis, with karyorrhectic debris and mild inflammation, predominantly neutrophils and lesser numbers of lymphocytes. These lesions were positive for RVFV antigen. In the spleen there was marked multifocal to diffuse lymphocytolysis around periarteriolar sheath and marginal zones of the white pulp. There was multifocal to coalescing red pulp necrosis, again lymphocyte loss was the main characteristic. Spleens were also positive for RVFV antigen, in particular the marginal zones and red pulp. Deep sequencing of the virus from the liver and spleen of mice inoculated with 1000 PFU showed a low degree of quasispecies diversity for both strains. The nucleotide variants (quasispecies) span all three genome segments including their non-coding regions. The present study shows that both outbreak RVFV strains, although genetically distinct, possess equivalent pathogenicity in BALB/c mice and do not generate extensive amounts of genetic variants after replication in mice. We concluded that both RVFV strains can be used for studies on pathogenesis and vaccine development for RVF.

Development of posterior ataxia, paralysis and myelitis in cesarean-derived colostrum-deprived pigs following experimental inoculation with either *Teschovirus A* serotype 2 or serotype 11 #

Franco Sebastian Matias Ferreyra, Bailey Lauren Arruda, Darin Madson, Kent L. Schwartz, Gregory Stevenson, Jianqiang Zhang, Qi Chen, Kyoung-Jin Yoon, Paulo Arruda

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In the US, sporadic outbreaks of posterior paresis and/or paralysis (Talfan-like disease) have been associated with *Teschovirus A* (PTV), predominantly serotype 1 (PTV-1). Over the past several years, the Iowa State University Veterinary Diagnostic Laboratory has had an increased number of submissions from multiple states of growing swine presenting with posterior paralysis, and less commonly, abnormal mentation. Clinical histories from submitting veterinarians suggest that disease is affecting more pigs over wider age ranges with clinical disease persisting in the herd for greater duration than was observed in previous decades. Polioencephalitis and/or poliomyelitis are consistently observed histologically and PTV is detected in neural tissue by PCR from these cases. Sequencing frequently identifies the PTV as serotype 2 or 11. No previous reports of PTV-2 or PTV-11 in cases of Talfan-like disease in North America are found in peer-reviewed literature. To confirm the neuropathogenicity of PTV-2 and PTV-11, eighteen, 5-week-old cesarean-derived and colostrum-deprived pigs were randomly assigned to three groups: negative control (n=4), PTV-2-inoculated (n=7), and PTV-11-inoculated (n=7). Negative control animals were intravenously inoculated with 3ml of cell culture media; animals in PTV-2 and PTV-11-inoculated groups were intravenously inoculated with 3 ml of 10^6 $TCID_{50}$/ml of PTV-2 and 3 ml of 10^6 $TCID_{50}$/ml of PTV-11, respectively. Neither clinical signs nor microscopic lesions in spinal cord were detected in negative control animals. Three of seven animals in the PTV-2-inoculated group developed mild incoordination of the hind limbs. Clinical signs consistent with Talfan-like disease, specifically posterior ataxia and paralysis, were observed in six out of seven animals in the PTV-11-inoculated group. Diarrhea was consistently observed two to three days prior to development of central nervous system signs in those clinically affected pigs. All pigs in the virus-inoculated groups, both with and without clinical signs, had mild to severe lymphoplasmacytic myelitis with multifocal areas of gliosis, neuron degeneration and satellitosis. To our knowledge this is the first experimental study demonstrating the neuropathogenicity of PTV-2 and PTV-11.

AAVLD Trainee Travel Awardee

An outbreak of mycoplasmosis causing arthritis, pneumonia and meningitis in dairy goat kids

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Over a period of two years, an owner submitted seven 1-3 week old goat kids of both sexes and several breeds for examination, with a clinical complaint of joint pain. The kids came only from that portion of the flock that was bottle-raised and removed from their dams and disease did not occur in kids left with their dams. Kids raised independently of their dams in this herd were fed from bottles that were sanitized only every other day. There were no clinical signs in adult animals. Florfenicol therapy did not alleviate the condition. Approximately 14 of 55 kids at risk became ill during the second year. Kids were uniformly negative when tested for CAEV antibody, and the VMDL mycoplasma PCR testing was also uniformly negative. Postmortem examination revealed low viscosity, transparent to cloudy joint fluid, which was usually present in multiple joints of each animal. Microscopic findings demonstrated necrotizing and suppurative to granulomatous synovitis that frequently extended into surrounding tissues. In addition, interstitial pneumonia and mild meningoencephalitis (each in 3/7 goats) were present. One goat had fibrinous pleuritis. Eventually, an alpha hemolytic organism was recovered on blood agar from 4 synovial fluid samples from 4 goats, and from multiple samples from 1 goat. The organisms were Gram positive, but were amorphous, without a distinct cellular morphology. The organism was not identified on MALDI-TOF, but 16S rRNA sequencing identified the isolates as *Myoplasma mycoides* subspecies *capri*. Although the published protocol for mycoplasma rRNA intergenic region amplification is appropriate for most species of mycoplasmas from cattle, birds and swine, the reverse primer is not sufficiently complementary to *M. mycoides* cluster taxa to be used for identification. Further verification of the *Mycoplasma mycoides* subspecies *capri* identificationwas obtained by sequencing PCR amplicons of the *rpoB* and *metS* housekeeping genes. This report indicates the potential long term persistence of *Mycoplasma* infections among young goats under certain husbandry conditions, and underscores significant differences between the mycoides subgroup and other *Mycoplasma* species. It also is a first report of meningitis associated with this organism, although experimental infection with *Mycoplasma capricolum* produced severe CNS inflammation.

Arsenic toxicosis in dairy calves

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Two dairy heifers and tissues from an additional 2 dairy heifers, all approximately 2 months of age, presented with a history of staggering and jaundice. The calves submitted to the Pennsylvania Veterinary Laboratory were the fourth and fifth calves to die within a short period of time on this farm. All four calves exhibited marked jaundice and three had severe abomasal ulcers. The two calves autopsied in house had large, firm, orange livers, pigmenturia and the primary ruminal content was abundant woody material. Microscopic examination revealed hepatocellular necrosis with lipidosis, periportal to bridging fibrosis and bile duct hyperplasia, abomasal congestion, edema and ulceration/necrosis and acute renal tubular necrosis. High concentrations of arsenic were found in the rumen contents, woody material from the rumen, and kidney. Toxic levels of copper were identified in the liver of one calf and the kidneys of both calves. Also noted were deficient levels of iron, manganese and zinc in the liver of one calf. Thirteen of twenty whole blood samples drawn from co-housed calves had toxic levels of arsenic. Further investigation revealed that these calves were housed on high-moisture, small-sized wood chips produced from pressure treated wood. The wood chips were obtained from a commercial supplier of wood chips for animal bedding.

Morphological correlation of gammaherpesvirus-5 cellular replication with immune and inflammatory responses in equine multinodular pulmonary fibrosis

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An association between equine multinodular pulmonary fibrosis (EMPF) and equine gammaherpesvirus-5 (EHV-5) is well established; however, the cellular target of EHV-5 within the lung parenchyma and associated host immune and inflammatory responses are incompletely characterized. The objective of this study was to assess the immune and inflammatory environments in relation to EHV-5 replication in the lungs of horses with naturally-occurring EMPF. Infection with EHV-5 was confirmed by PCR assays, while EHV-5 cellular replication was characterized by using a virus-specific in situ hybridization (ISH) method. Some of the key immune and inflammatory cells in the lungs of EHV-5 positive EMPF horses were assessed by immunohistochemical (IHC) staining with a panel of cell markers and compared with control EHV-5 negative horses with pulmonary interstitial fibrosis or normal lungs. When compared with controls, the lungs of horses with EMPF had massive infiltration predominantly by CD3+ T cells together with monocytes/macrophages (Iba-1, pan-macrophage/dendritic cell; MAC387; recently infiltrating M1-like pro-inflammatory monocyte/macrophage). When the distribution of immunopositive cells was compared with ISH assays for EHV-5, we found that viral replication in horses with EMPF was restricted to pulmonary alveolar macrophages (PAM) within areas of pulmonary fibrosis. Moreover, the population of PAM within areas of fibrosis was heterogeneous with productive EHV-5 infection corresponding to MAC387- cells, presumably M2-like anti-inflammatory macrophages. These findings will assist in the development of improved protocols for the diagnosis and control of EMPF associated with EHV-5 infection.

Pathologic findings in horses experimentally infected with Equine herpesvirus-1 and mutants of differing neuropathogenic potential

Dodd Gray Sledge[1], Matti Kiupel[1,2], Carine Holz[2], M. Wilson[2], Lila Marek Zarski[2], Rahul K. Nelli[2], Anthony Pease[3], Walid Azab[4], Klaus Osterrieder[4], Lutz S. Goehring[5], Gisela Soboll Hussey[2]

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Manifestations of equine herpesvirus type 1 (EHV-1) infection can range from subclinical infection to overt debilitating disease including encephalomyelopathy. Understanding of the pathogenesis of EHV-1-associated disease and the factors that result in variable clinical presentation is limited. The current study aimed to evaluate differences in response to infection with EHV-1 strains with differing neuropathogenic potential. Age-matched, mixed sex groups of horses were infected with wild type EHV-1 (Ab4WT), EHV-1 with mutation in the polymerase gene (Ab4N752), or mutant EHV-1 expressing glycoprotein D of EHV-4 (Ab4gD4). At regular intervals post infection, animals were clinically monitored, viremia and nasal shedding were assessed by real time PCR, and serum neutralization (SN) titers were determined. Two female Ab4WT infected horses developed signs of equine herpesvirus myeloencephalopathy (EHM) and were euthanized within 14 days of infection. One male Ab4WT infected horse showed mild neurological disease but recovered. Other horses were sacrificed at 10 weeks post infection. Ab4WT infected horses developed a classical bi-phasic fever, Ab4N752 infected horses had only primary fevers and Ab4gD4 infected horses had only late secondary fevers. All groups showed respiratory tract disease signs with Ab4N752 infected horses having the most severe clinical signs followed by Ab4 infected horses and Ab4gD4 infected horses. Nasal shedding and viremia was most pronounced in Ab4WT infected horses, while EHV-1 SN increased in Ab4WT and Ab4N752 infected horses to comparable levels but was significantly lower in Ab4gD4 infected horses. On histologic examination, animals that developed signs of EHM had multifocal lymphohistiocytic vasculitis in which EHV1 antigen was detected by immunohistochemistry in the choroid of the eyes, endometrium, and spinal cord. In 2/4 of the remaining Ab4WT infected horses, 4/9 Ab4N752 infected horses, and 8/8 Ab4gD4 infected horses, there were mild multifocal perivascular and scattered interstitial infiltrates of lymphocytes and plasma cells within the choroid of the eyes. All animals had mild infiltrates of lymphocytes, plasma cells and histiocytes surrounding terminal bronchioles and vasculature of the lungs. Males from all groups had marked testicular atrophy associated with occasional interstitial lymphoplasmacytic and/or histiocytic infiltrates. In conclusion, there were differences in respiratory and neurological disease as well as nasal shedding and viremia between horses infected with EHV-1 strains of differing neuropathogenic potential. Only animals that were sacrificed while having clinical manifestations of disease associated with infection with Ab4WT had overt vasculitis, but mild choroiditis in the eyes persisted in many animals infected with all EHV-1 strains. The exact significance of the marked testicular changes is unclear, and to our knowledge such changes have not been previously reported with EHV-1 infections.

Diagnostic challenges in the first confirmation of sporadic bovine encephalomyelitis (*Chlamydia pecorum* encephalitis) in New Zealand

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In 2013, severe neurological signs developed in forty of 150 crossbred 1-3 month old Friesian dairy calves in New Zealand. Affected calves exhibited a range of neurological signs that included generalised depression, hind limb ataxia with a stiff gait, and knuckling of the fetlocks. In advanced cases, calves became recumbent with opisthotonus, and died. Over a four-week period, a total of thirteen calves died or were euthanased and a complete necropsy was performed on seven of these calves. Necropsy findings included fibrinous peritonitis, pleuritis and pericarditis, with no gross abnormalities in the brain. Formalin-fixed brain was obtained for seven of the affected calves, and histopathology showed extensive inflammatory lesions ranging from lymphocytic and histiocytic vasculitis and meningoencephalitis, to extensive thrombosis and neutrophilic inflammation. Because of the variability of brain lesions, a broad range of possible bacterial causes was considered including *Pasteurella multocida*, *Histophilus somni*, and *Chlamydia pecorum*. *C. pecorum* has been previously isolated from goats in New Zealand, but had never before been identified in cattle. PCR for *C. pecorum* was negative for six formalin-fixed samples. Conversely, immunohistochemistry using an anti-Chlamydial LPS antibody revealed immunostaining of endothelial cells and inflammatory lesions in all seven cases, with no brain samples exhibiting immunostaining for *Histophilus somni*. Following these results, DNA was extracted from the only sample of fresh brain obtained, and chlamydial DNA sequences were amplified by PCR and found to be homologous to *Chlamydia pecorum*, the causative agent of sporadic bovine encephalomyelitis. This is the first time that *C. pecorum* has been confirmed as a cause of clinical disease in New Zealand. We found that histologic lesions of the disease sporadic bovine encephalomyelitis are more variable than previously reported in the literature, and suggest that pathologists should be aware that histologic features may overlap with those traditionally ascribed to other organisms, such as *Histophilus somni* and *Pasteurella multocida.* Furthermore, optimal rule-out of *C. pecorum* encephalitis requires fresh brain for PCR in addition to routine formalin-fixed samples.

Serology

Saturday, October 15, 2016

Imperial G

Moderators: Dave Baum and Devi P. Patnayak

1:00 PM **Statistical process control (SPC) charts: The operational definition for ELISA quality**
Dave Baum, Kelly Boesenberg, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola, Rodger Main, Chong Wang, Calista Koeneke *121*

1:15 PM **Assessment of ELISA discrepant Equine Infectious Anemia (EIA) samples submitted to the National Veterinary Services Laboratories ◊**
Tiffany Palmer, Kevin Lake, Tracy L. Sturgill *122*

1:30 PM **Diagnosis of *Brucella canis* infection using serologic assays**
Yan Zhang, Jing Cui, Mary Beth Weisner, Anne Parkinson, Jeffrey R. Hayes, Beverly Byrum *123*

1:45 PM **Detecting circulating antigens in dogs naturally infected with *Heterobilharzia americana* # * †**
Jessica Yvonne Rodriguez, Govert J. Van Dam, Karen F. Snowden *124*

2:00 PM **Serological responses to Senecavirus A infection in pigs**
Lok R. Joshi, Steven R. Lawson, Maureen H. V. Fernandes, Jane Christopher-Hennings, Eric A. Nelson, Diego G. Diel *125*

2:15 PM **Rapid, simple and innovative diagnosis of Classical Swine Fever**
Carsten Schroeder, Stefanie Fritsche, Claudia Engemann, Alexander Postel, Paul Becher, Denise Meyer *126*

2:30 PM **Serologic detection of equine antibodies to vaccine and field strains of rabies virus using a multiplex microsphere-based assay**
Susan M. Moore, Kaitlin Haukos, Kelley Black, Beth Davis, Melinda Wilkerson *127*

2:45 PM **Duration of serum antibody response to rabies vaccination in horses**
Alison Harvey, Johanna Watson, Stephanie Brault, Judy Edman, Susan M. Moore, Philip Kass, W. David Wilson *128*

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

Statistical process control (SPC) charts: The operational definition for ELISA quality

Dave Baum, Kelly Boesenberg, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola, Rodger Main, Chong Wang, Calista Koeneke

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

"The question that is addressed by control charts is somewhat different from the questions which are commonly considered by statistical procedures: instead of seeking a theoretical model for data obtained from some well-defined phenomenon, the control chart seeks to determine if a sequence of data may be used for prediction of what will occur in the future," Wheeler and Chambers, (1992, Understanding Statistical Process Control, SPC Press, p. 37). An operational definition consists of 1) a criterion to be applied, 2) a way to determine whether the criterion is satisfied, and 3) a way to interpret the results of the test (WE Deming, *Out of the Crisis*). Ultimately, this discipline enables the distinction between normal (random) variation and special cause (non-random) variation (WE Shewhart, 1931, Economic Control of Quality of Manufactured Product). This distinction is fundamental for studying a process and its output prior to recommending and implementing changes. The porcine reproductive and respiratory syndrome virus (PRRSV) ELISA contains positive and negative control sera used to determine each ELISA plate's validity and to calculate a result for each sample tested. Their optical density (OD) values are then used to calculate the sample result known as the sample-to-positive (S/P) ratio. This presentation will demonstrate SPC (process behavior) charts used to describe PRRSX3 ELISA output.

Materials and Methods. The two negative control values of each plate were used to create an average and range (R) chart for 1,162 PRRSX3 ELISA plates from January 1, 2015 through May 23, 2016. The chart's limits were calculated globally and graphed as described previously by Wheeler and Chambers (1992).

Results and Discussion. The average for all 1,162 negative control sera OD's was 0.062, and average range was 0.003. The upper and lower limits for the OD's were 0.067 and 0.056, respectively; the upper and lower limits for the OD's were 0.009 and 0.000, respectively. Non-random variation was identified by average OD values exceeding the upper 3-s limit. The assignable causes were associated with the test process itself where the control sera passed the assay's validation specification, a plate that failed to meet the assay's specification, and a clogged plate washer pre-filter. Thus, demonstrating why SPC charts are the operational definition for ELISA quality.

Assessment of ELISA discrepant Equine Infectious Anemia (EIA) samples submitted to the National Veterinary Services Laboratories ◊

Tiffany Palmer, Kevin Lake, Tracy L. Sturgill

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In 2015, there were approximately 1.3 million equine infectious anemia (EIA) tests performed in the United States. The majority of these tests are routine, for transport or surveillance purposes. A small percentage test positive on any one of the four commercially available enzyme-linked immunosorbent assays (ELISA). A discrepant sample is forwarded to the National Veterinary Services Laboratories (NVSL) for confirmatory testing. A discrepant sample typically includes a positive or equivocal result on ELISA but negative on any other test. An equivocal result is a result that is questionable or ambiguous. NVSL tests the sample by all commercially available ELISAs and by agar gel immunodiffusion (AGID). The NVSL algorithm indicates that when a sample is positive or equivocal on two or more ELISAs and negative on AGID, a Western blot (WB) is performed. For calendar year 2015, NVSL received 252 discrepant samples that resulted in a negative EIA status of the horse following confirmatory testing. Of those, 22 were positive on ELISA kit "A", 35 were positive with 3 equivocal on ELISA kit "B", 102 were positive with 6 equivocal on ELISA kit "C", and 33 were positive with 2 equivocal on ELISA kit "D". Fifty-six of the 252 samples tested positive on two or more ELISAs. For these 56 samples, WB was performed. Ninety-three of the 252 samples were negative on all tests performed. While ELISA tests are highly sensitive, they are not specific enough to eliminate false positive reactions observed during routine EIA testing, thus requiring additional testing to verify the EIA status of the animal. Testing of each sample by all commercially available tests allows identification of potential performance issues with the test kits.

◊ USAHA Paper

Diagnosis of *Brucella canis* infection using serologic assays

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Canine Brucellosis, caused by *Brucella canis*, is a significant reproductive disease of dogs in breeding kennels throughout the United States. It is also a zoonotic organism that can infect humans. In Ohio, the Commercial Dog Breeders Act law (OAC 901:1-6) became effective on October 10, 2013. In the law, any adult dog or animal known to be positive for *B. canis* shall not be for sale, trade, or removed from the premises. Currently, diagnosis of *B. canis* is based on serology, culture and identification, or a molecular assay such as PCR. Culture or molecular assay for ante-mortem animals is usually carried out using whole blood and is only reliable to detect animals with bacteremia. We have adapted a testing scheme for *B.canis* using two serologic assays for screening using an indirect florescent antibody test followed by a tube agglutination test for confirmation. Various tissues were used for bacterial isolation and identification for animals that were positive by the serologic methods. Animals that were positive by the serologic assays were confirmed to be infected with *B. canis*, indicating that the two-assay testing scheme is highly specific for detecting *B. canis* infected dogs.

Detecting circulating antigens in dogs naturally infected with *Heterobilharzia americana* # * †

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Diagnosing intestinal schistosome parasites is a challenge in many host species. Egg shedding can be low and intermittent, leading to false negative results by fecal examination. Molecular diagnostics are also available; however, fecal inhibitors can cause false negative results. In addition, because residual egg shedding can occur after parasite clearance, both of these methods may not definitively determine active infection. Circulating worm gut antigens, circulating cathodic antigen (CCA) and circulating anodic antigen (CAA), have been detected from the serum and urine of humans infected with *Schistosoma mansoni*. Immunochromatographic lateral flow assays (POC-CCA, POC-CAA; Rapid Medical Diagnostics, Pretoria, RSA) have been developed to rapidly diagnose infection in human populations. These highly glycosylated antigens have been identified in other schistosome species. We evaluated the utility of this test in diagnosing *Heterobilharzia americana,* a related schistosome parasite in dogs in a comparative diagnostics study. Veterinarians submitted urine, serum, and feces from suspected cases (n=57). Antigen in both urine (POC-CCA) and serum (POC-CAA) was detected in 5 out of 7 dogs that were positive by fecal examination and fecal PCR. Thirteen additional dogs were diagnosed by fecal PCR only, and all were antigen negative. Antigen was not detected in 37 dogs negative by fecal examination and PCR. The POC-CCA and POC-CAA tests became negative in 3 of 4 dogs that were retested after at least one treatment. Failure to detect antigen in dogs that were diagnosed by fecal exam or PCR could be due to low worm burdens. Concentrating samples and using other immunodiagnostic test modalities could improve the ability to detect these antigens in dogs infected with *H. americana*.

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

Serological responses to Senecavirus A infection in pigs

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Senecavirus A (SVA), a member of the genus *Senecavirus* of the family *Picornaviridae*, has recently re-emerged in the US causing neonatal mortality and vesicular disease in swine. Here we evaluated the dynamics of antibody responses to SVA infection in pigs. Serum samples collected from experimentally infected animals on days 0, 3, 5, 7, 10, 14, 21, 28, 35 and 38 post-infection (pi) were tested for SVA-specific antibodies by virus neutralization (VN), immunofluorescence (IFA) and VP1, VP2 and VP3 fluorescent microsphere immunoassays (FMIA). Notably, pigs infected with SVA presented an early and robust neutralizing antibody (NA) response to SVA, with NAs being first detected on day 5 pi and peaking on day 10 pi. High levels of NAs were still detected on day 38 pi. SVA specific IgG antibodies were first detected by IFA on day 10 pi, peaked on day 14 pi and presented a slight decline on days 35 and 38 pi. The dynamics of antibody responses detected by VP2 IgG FMIA were similar to those detected by IFA. Interestingly, while VP1- and VP3- specific IgG antibodies were detected on day 10 and peaked on day 14 or 21 pi, respectively, antibodies against these proteins declined to pre-infection levels by day 35 p.i. Results here provide important information regarding the dynamics of the antibody responses to SVA infection in pigs.

Rapid, simple and innovative diagnosis of Classical Swine Fever

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Classical swine fever (CSF) is a highly contagious disease, affecting domestic swine and wild boar. Due to its highly variable clinical symptoms, CSF surveillance is mostly based on CSF Virus (CSFV) antibody ELISAs. QIAGEN's new double-antigen ELISA *pigtype*® CSFV E^{rns} Ab can be used as a CSF screening test but also for verification of results obtained with CSFV ELISAs based on E2 antibody detection.

In outbreak situations, emergency vaccination with live marker vaccines is an alternative to 'stamping out' strategy. Marker vaccines are considered a general control strategy against CSF in the European Union. Successful marker vaccine use requires a reliable diagnostic test to differentiate infected from vaccinated animals (DIVA strategy). A promising DIVA strategy is based on the recently in the EU approved Suvaxyn® CSF Marker vaccine, which does not induce an immune response to CSFV E^{rns}. The *pigtype* CSFV E^{rns} Ab detects antibodies to CSFV E^{rns} and can therefore be used as accompanying DIVA with suitable CSFV marker vaccines.

Validation data for *pigtype* CSFV E^{rns} Ab ELISA as CSF screening test as well as DIVA test in combination with a marker vaccine will be presented.

Materials and Methods

Sensitivity of *pigtype* CSFV E^{rns} Ab was evaluated by testing a serum sample panel comprising 293 CSFV antibody positive sera as well as altogether 135 serially-derived sera from 24 CSFV-infected animals in comparison to a commercially available E2-specific antibody ELISA. Moreover, 1053 CSFV antibody negative field sera as well as 99 samples from specific pathogen-free pigs were tested to assess specificity. In addition, application as a discriminatory test was validated using 95 sera taken from animals vaccinated with the marker vaccine Suvaxyn CSF Marker as well as 123 sera from vaccinated and subsequently CSFV challenged pigs.

Results

The *pigtype* CSFV E^{rns} Ab proved highly specific (99.7/99.9% for cut-off values 0.3/0.5) and sensitive in comprehensive validation with CSFV antibody positive and negative sera. Compared to a commercial E2-specific antibody ELISA, *pigtype* CSFV E^{rns} Ab was more sensitive to sera obtained very soon after infection and detected all infection samples within 15-21 days post infection. The novel test reliably detected E^{rns} antibodies to a variety of isolates belonging to various CSFV genotypes. *pigtype* CSFV E^{rns} Ab showed comparable specificity for sera obtained after vaccination with the marker vaccine when compared to the only other commercial E^{rns}-specific antibody ELISA, but demonstrated to be more sensitive (90%/98% with *pigtype* CSFV E^{rns} Ab for cut-off values 0.5/0.3 compared to 78-89% for the other commercial E^{rns} antibody ELISA [1]) for CSFV "challenge" sera.

Conclusion

The *pigtype* CSFV E^{rns} Ab ELISA is a novel CSF screening test, but can also be employed as an accompanying differentiation test with suitable CSFV subunit or marker vaccines.

References

[1] Pannhorst et al., J. Vet. Diagn. Invest. 27, 449

Serologic detection of equine antibodies to vaccine and field strains of rabies virus using a multiplex microsphere-based assay

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Rabies is a fatal neurological disease caused by an RNA virus in the family Rhabdoviridae. Horses are susceptible to rabid wildlife. The majority of viral strains isolated from rabies positive horses in Kansas are skunk; however several bat strains have also been identified. The hypothesis that horses vaccinated with laboratory adapted rabies strains have weaker antibody titers to virus variants occurring in nature was investigated. A multiplex-bead-based indirect immunoassay was developed to screen sera obtained from adult horses against rabies antigens isolated from seven rabies virus isolates. This multi-analyte technology (xMap) was designed to quantify equine IgG binding to viral antigens derived from the seven different rabies virus strains, simultaneously. Characterization of the dominant viral proteins in the antigen preparations was performed by silver stain of SDS-PAGE. Coupling of G and N viral proteins derived from three laboratory rabies strains to three sets of xMag beads was confirmed. The 7-plex set of rabies antigen coated xMap beads was tested against serum samples obtained from 18 horses before and after rabies vaccination. Results were compared to ELISA and RFFIT results from the same serum samples. Analysis of the antibody response measurements indicate that all horses increased their vaccine response following vaccination, but a variety of responses were detected to rabies strains that differ from the vaccine strain.

Duration of serum antibody response to rabies vaccination in horses

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The objective of this study was to investigate the impact of age and inferred prior vaccination history on the persistence of vaccine-induced antibody against rabies in horses.

Horses (48 horses with an undocumented vaccination history) were vaccinated against rabies once. Blood samples were collected prior to vaccination, 3 to 7 weeks after vaccination, and at 6 month intervals for 2 to 3 years. Serum rabies virus neutralizing antibody (RVNA) values were measured. An RVNA value of ≥ 0.5 IU/mL was used to define a predicted protective immune response on the basis of WHO recommendations for humans. Values were compared between horses < 20 and ≥ 20 years of age and among horses inferred to have been previously vaccinated and those inferred to be immunologically naïve.

A protective RVNA value (≥ 0.5 IU/mL) was maintained for 2 to 3 years in horses inferred to have been previously vaccinated on the basis of prevaccination RVNA values. No significant difference was evident in response to rabies vaccination or duration of protective RVNA values between horses < 20 and ≥ 20 years of age. Seven horses were poor responders to vaccination. Significant differences were identified between horses inferred to be previously vaccinated and horses inferred to be naïve prior to the study.

A rabies vaccination interval > 1 year may be appropriate for previously vaccinated horses but not for horses vaccinated only once. Additional research is required to confirm this finding and to identify the optimal frequency of primary vaccine administration.

Toxicology
Sunday, October 16, 2016
Imperial G

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8:00 AM **Metabolomics tools…is your lab ready?**
David Borts *131*

8:15 AM **A metabolomics study for detection of novel serum biomarkers of microcystin LR intoxication**
Ann Perera, Lucas Showman, Elisiane Camana, Belinda Mahama, Poojya Vellareddy Anantharam, Wilson Kiiza Rumbeiha *132*

8:30 AM **Translational studies on efficacy of cobinamide or thiamine for treatment of hydrogen sulfide-induced neurodegeneration**
Poojya Vellareddy Anantharam, Elizabeth Whitley, Belinda Mahama, Dongsuk Kim, Dwayne Edward Schrunk, Paula Martin Imerman, Gerard Boss, Wilson Kiiza Rumbeiha *133*

8:45 AM **Xylitol detection in meat and dog food by fourier transform infrared spectroscopy**
Deon Van der Merwe, Keith B. Byers *134*

9:00 AM **Paint ball toxicosis: A case review ◊**
Dwayne Edward Schrunk, Steve M. Ensley, Laura Vander Stelt *135*

9:15 AM **Break**

10:00 AM **Eaglet Poisoning: A case review**
Dwayne Edward Schrunk, Steve M. Ensley, Pat Schlarbaum *136*

10:15 AM **Update of the inter-laboratory evaluation of a high performance liquid chromatography-fluorescence method for detection and quantification of aflatoxins B_1 and M_1 in animal liver**
Xiangwei Du, Dahai Shao, Dwayne Edward Schrunk, Paula Martin Imerman, Chong Wang, Steve M. Ensley, Elizabeth R. Tor, John Tahara, Cynthia Gaskill, Lori Smith, Wilson Kiiza Rumbeiha *137*

10:30 AM **Intra-laboratory evaluation of an extended urine-based quantitative diagnostic method for aflatoxicosis**
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10:45 AM **Lead contamination in backyard chicken flocks - Incidence and exposure assessment in positive cases #**
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11:00 AM **Manganese deficiency implicated in a case of angular limb deformities in yearling ewes # * †**
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11:15 AM Organophosphate/carbamate poisoning: Is acetylcholinesterase inactivation a biomarker of interpretation or misinterpretation?
Ramesh C. Gupta, Michelle Lasher, Robin Doss *141*

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

Metabolomics tools…is your lab ready?

David Borts

VDPAM, Iowa State University, Ames, IA

Metabolomics is a technique that has grown rapidly over the past 15 years thanks to its promise and potential to identify biomarkers, reveal mechanism of disease pathogenesis, and to elucidate pathways of metabolic regulation. Metabolomics has recently seen increasing application in the area of veterinary medicine.

Metabolomic workflows are comprised of a complex series of steps including: study design, sample collection, metabolism quenching, sample extraction, chromatographic separation, mass spectrometry analysis, data processing, statistical analysis, selection of features of interest, database searching, and metabolite identification. For workflows intended to yield clinically applicable biomarkers, additional steps including multivariate model construction, model validation, and targeted quantitative assay development and validation are required. For laboratories considering establishing a metabolomics capability, the number of tools, available options, and decisions required for each step of the metabolomics workflow can be bewildering.

This presentation will provide a survey of the most commonly applied technologies and processes for each step of the metabolomics workflow, a brief summary of the strengths, weaknesses, and tradeoffs involved with each technology and process, and general recommendations for each decision point, dependent upon the objectives of the metabolomics workflow.

For example, nuclear magnetic resonance (NMR) and gas chromatography/mass spectrometry (GC/MS) are instrumentation platforms that are sometimes used as part of metabolomics workflows. However, for a variety of reasons (including metabolome coverage), liquid chromatography/mass spectrometry (LC/MS) has emerged as the most commonly applied analytical instrumentation platform for metabolomics. And within the realm of LC/MS, ultra performance liquid chromatography (UPLC) coupled to quadrupole – time of flight (Q-TOF) and quadrupole – orbitrap (“Q-Exactive”) mass analyzers have emerged as the dominant instrument configurations.

In addition, an overview of the recent initiative undertaken by the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) to establish a metabolomics capability will be provided. The selection of tools by the ISU VDL for each step of the metabolomics workflow will be discussed. Finally, preliminary data generated by the ISU VDL metabolomics platform will be presented.

A metabolomics study for detection of novel serum biomarkers of microcystin LR intoxication

Ann Perera[2], Lucas Showman[2], Elisiane Camana[1], Belinda Mahama[1], Poojya Vellareddy Anantharam[1], Wilson Kiiza Rumbeiha[1]

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Microcystins are common water-borne toxins produced by harmful algal blooms. Although there are > 80 microcystins congers, microcystin-LR (MCLR) is the most common and most potent. The liver is the primary target organ of microcystins. MCLR is selectively taken up by a bile acid carrier in hepatocytes where it inhibits the activity of protein phosphatases 1 and 2A. This causes disruption of cellular microtubules leading to massive intrahepatic bleeding, the cause of death under acute exposure conditions. Chronic MCLR intoxication follows prolonged ingestion of water contaminated with low to medium levels of MCLR. Diagnosis of MCLR intoxication in live animals currently relies detection of elevated serum liver enzymes. Unfortunately, elevated serum enzymes are not sensitive biomarkers of early liver injury. The objective of this study was to identify early sensitive biomarkers of MCLR of effect using metabolomics in a mouse model. We chose the mouse because limited amount of toxinprecluded a large animal study. Mice (5 male per group) were randomly divided and distributed into one of the following groups: Negative control received 0.9% normal saline; Low dose 40 ug/kg (LD); Medium dose 200 ug/kg (MD); and High dose 1000 ug/kg (HD) bw for 4, 8, or 13 weeks by oral gavage. Mice were decapitated and trunk blood was allowed to clot, and serum was stored at -80^{o}C untill analysis. 50 µl of serum was mixed with 10 µl of internal standards (ribitol) in water and nonadecanoic acid e (1mg/ml) before adding to 900 µl of extraction solvent [cold methanol:water (8:2)]. Supernatants were dried under nitrogen and resuspended in 200 µl of acetonitrile. 50 µl of this extraction was used for GC-MS analysis. Methoximation was carried out with 50 µl of methoxiamine hydrochloride at 60^{o}C for 90 min, to protect active moieties in sugars, followed by silation reaction prior to GC-MS run with 50 µl of BSTFA +1% TMS at 60^{o}C for 30 min. The Agilent GC-MS model 7890 A/5975C was used with HP-5MSI 30m x 0.250 mm ID column and temperature gradient was programmed from 120 to 325°C at 5°C/min with Helium flow rate at 1.0 mL/min. The GC-MS data files were deconvoluted by NIST AMDIS software, and searched against an in-house compound library as well as the NIST 2014 Mass Spectral Library. Thirty nine compounds were found to be statistically significantly modulated in serum of MCLR dosed mice compared to control ($p \leq 0.05$). Of these, 5 compound (galactopyranoside, methyl galactoside derivatives 1 and 2, and the allyl alcohol of exo-Norbornanol) were significantly elevated; while glyceric acid, glucose, glycine, ethanolamine, the gamma lactone of gluconic acid and an unidentified compound (unknown 1) were significantly decreased in a dose and time dependent manner. This research has yielded novel biomarkers of MCLR intoxication. The correlation of these novel biomarkers with established serum enzyme biomarkers is ongoing.

Translational studies on efficacy of cobinamide or thiamine for treatment of hydrogen sulfide-induced neurodegeneration

Poojya Vellareddy Anantharam[1], Elizabeth Whitley[2], Belinda Mahama[1], Dongsuk Kim[1], Dwayne Edward Schrunk[1], Paula Martin Imerman[1], Gerard Boss[3], Wilson Kiiza Rumbeiha[1]

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Hydrogen sulfide (H_2S) is a highly neurotoxic gas most often encountered in high concentrations as an environmental toxicant. H_2S exposure in animals is by inhalation or the oral route through ingestion of feeds and water with high sulfur content. Acute exposure by inhalation is a common cause of massive deaths in confinement-raised pigs. Currently, there is no ideal antidote for treatment of H2S-induced neurotoxicity. Polioencephalomalacia is a H_2S-induced and thiamine-responsive neurodegenerative disease of ruminants. The objective of this study was to evaluate the efficacy of cobinamide (Cob) or thiamine for prevention and treatment of H_2S-induced neurotoxicity using a mouse model. Test drugs (Cob 100mg/kg BW IM or thiamine hydrochloride or sulbutiamine, a source of thiamine, 100 mg/kg IM) were given either 5-10 minutes prior to or 2 mins after H_2S exposure by whole body inhalation daily for 7 days. Control mice were exposed to H_2S without Cob or thiamine. Negative control mice received 0.9% saline IM. A battery of functional observations was used to assess clinical signs during H_2S exposure. Behavioral and histopathological changes were additional endpoints measured. Cobinamide significantly reduced the number of mice with seizures and "knockdowns" during H_2S exposure and reversed weight loss after H_2S exposure ($p<0.05$) compared with mice exposed to H_2S and treated with saline. Cobinamide-treated mice performed better on the behavioral tests, increased time on the Rotarod, and improved activity in open field tests, compared with H_2S-exposed mice treated with saline (all $p<0.05$). Histological evaluation of the brain revealed that Cob significantly protected mice against H_2S-induced neurodegeneration ($p<0.05$). In contrast to Cob, thiamine treatment of H_2S-exposed mice was not efficacious in this model. Overall, these results suggest that Cob is a promising novel antidote for prevention and treatment of H2S-induced neurotoxicity and neurodegeneration.

Xylitol detection in meat and dog food by fourier transform infrared spectroscopy

Deon Van der Merwe, Keith B. Byers

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Xylitol is a sugar alcohol that is often used in place of sucrose and fructose due to its lower caloric value and lower glycemic index. Additional benefits may include reduced incidence of tooth decay and middle ear infections in children. Xylitol ingestion is relatively safe in humans, but it produces a dose-related insulin release in dogs. Toxic effects in dogs include hypoglycemia and liver failure. Its popularity as a human food ingredient occasionally results in accidental exposure in dogs, but there are increasing concerns about its intentional use for the poisoning of dogs and related canine species. Several methods for detection of xylitol are available, including gas chromatography coupled mass spectroscopy (GCMS) and high pressure liquid chromatography (HPLC). These methods are, however, associated with relatively complex or time-consuming procedures such as derivatization (GCMS), and the need for specialized detectors such as refractive index and mass chromatography detectors (HPLC). In comparison, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) requires less expensive equipment, limited use of solvents, simple and rapid sample preparation, and no derivatization. The viability of ATR-FTIR as a practical xylitol detection method in dog food and meat products was investigated. A simple water extraction method was developed, followed by xylitol crystallization onto a glass slide. The first derivative at wavenumbers from 1502 cm^{-1} to 484 cm^{-1} was used for identification, while the absorption peak centered at 940 cm^{-1} was used for quantification. The limit of xylitol detection was 1 % (weight/weight). The calibration curve followed a linear model ($y = 0.0047x – 0.0001$) with an R^2 value of 0.9997. Xylitol could be differentiated from aspartame, saccharin, sucralose and cane sugar. ATR-FTIR provides a relatively fast, inexpensive and simple procedure for the detection of xylitol in relevant matrices.

Paint ball toxicosis: A case review ◊

Dwayne Edward Schrunk[1], Steve M. Ensley[1], Laura Vander Stelt[2]

[1]Toxicology and Nutrition, Iowa State University, Ames, IA; [2]Orange City Vet Clinic, Orange City, IA

The Iowa State University Veterinary Diagnostic Laboratory received stomach content from a German Short Hair for toxicological analysis on the 31st of August 2015. Owners returned to their residence to find the dog seizing and covered in bright pink vomitus. Treatment was given unsuccessfully and the dog was eventually humanely euthanized. The dog was not known to have been exposed to any potential toxins, however it had been outside for a brief time in the morning. The submitting veterinarian requested testing for ethylene glycol as the initial testing option. A negative result for ethylene glycol resulted in testing for anticoagulant rodenticides. This analysis was also negative. The pink color of the stomach content prompted screening for strychnine by GC/MS, which although negative for strychnine indicated the presence of multiple poly ethylene glycol compounds. With this information the owners went through their home again and found that the dog had gotten into and consumed a large portion of a box of paint balls.

◊ USAHA Paper

Eaglet Poisoning: A case review

Dwayne Edward Schrunk[1], Steve M. Ensley[1], Pat Schlarbaum[2]

[1]Toxicology and Nutrition, Iowa State University, Ames, IA; [2]Iowa Department of Natural Resources, Boone, IA

The Iowa Department of Natural Resources (DNR) routinely observes a pair of American bald eagle nests in northeast Iowa. Both nests are observed using a webcam, one nest is linked to a website that allows the public to view the hatching and raising of the eaglets. The non-website nest consisted of an adult female and male, along with two eaglets. It was observed that one of the eaglets was acting abnormal and the adult female became ill, was wobbling, but then recovered. A six-week-old, 5 pound eaglet was received at the Iowa State University Veterinary Diagnostic Laboratory (ISUVLD) on the 27th of May 2016. Necropsy was performed and the following was observed; mild to moderate autolysis of all organs, diffuse edema with multifocal areas of hemorrhage in the lungs. Microspcopic examination of the brain, lung, heart, liver, kidney, spleen, ventriculus and skeletal muscle revealed no remarkable findings. The ISU VDL Toxicology and Nutrition Section determined that the cholinesterase activity in the eaglet brain was inhibited. A screen of the proventriculus content by GC/MS indicated the presence of methomyl and caffeine. It is likely that a common fly bait that contains methomyl was mixed with a soft drink to make a bait for a pest animal like a raccoon or skunk, and that the female eagle brought some of the remains back to the nest to feed the eaglets.

Update of the inter-laboratory evaluation of a high performance liquid chromatography-fluorescence method for detection and quantification of aflatoxins B_1 and M_1 in animal liver

Xiangwei Du[1], Dahai Shao[1], Dwayne Edward Schrunk[1], Paula Martin Imerman[1], Chong Wang[1], Steve M. Ensley[1], Elizabeth R. Tor[2], John Tahara[2], Cynthia Gaskill[3], Lori Smith[3], Wilson Kiiza Rumbeiha[1]

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In collaboration with FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN), we have performed one round of inter-laboratory and one round of single laboratory evaluation of a liver-based diagnostic method for aflatoxicosis. In Round I of inter-laboratory evaluation conducted by 3 laboratories, control liver samples were fortified with aflatoxin (AF) B_1 at three levels of 0.20, 1.0, and 7.0 ng/g, with AFM_1 at 0.20, 1.0, and 4.0 ng/g. Inter-lab reproducibilities were 19.7% and 28.5% for AFM_1 at 1.0 and 4.0 ng g^{-1}, respectively; 27.3% and 25.8% for AFB_1 at 1.0 and 7.0 ng g^{-1}, respectively. Inter-laboratory accuracies were 95.5% and 87.6% for AFM_1 at 1.0 and 4.0 ng g^{-1}, respectively; 97.0% and 100% for AFB1 at 1.0 and 7.0 ng g^{-1}, respectively. This indicates satisfactory results for the upper two levels for both AFB_1 and AFM_1 among all three laboratories. There were some questions regarding the method performance at the lowest spike level of 0.20 ng/g for both AFB_1 and AFM_1, which is the lowest level of method quantification. Consequently, performance at lower concentrations was evaluated in a single laboratory using an adjusted standard curve in a blinded Round II. Livers were fortified with AFB_1 and AFM_1 at four levels 0.10, 0.15, 0.20, and 0.30 ng/g. The correlation coefficients for standard curves for each aflatoxin were > 0.99. Test results for each aflatoxin were consistently higher than expected levels, however, results were within 95% confidence interval., indicating method performance was at the lower level was excellent. A third and final round of inter-laboratory evaluation is currently ongoing. Completion of this study will yield a robust tissue-based method for the diagnosis of aflatoxicosis in animals which can be shared by the Vet-LIRN network laboratories.

Intra-laboratory evaluation of an extended urine-based quantitative diagnostic method for aflatoxicosis

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Aflatoxins B_1 (AFB_1) is a common mycotoxin produced by various fungal species including *Aspergillus flavus* and *Aspergillus parasiticus* as a secondary metabolite. It has been found in a wide variety of seed and grains used as raw materials in manufacture of animal feeds. It is the most potent aflatoxin and a common cause of pet food recalls. Aflatoxicosis can either be acute or chronic, depending on concentration in feed and frequency of ingestion. AFB_1 metabolites include M_1, P_1, and Q_1 depending on species. In live animals, especially pets, diagnosis of aflatoxicosis is very challenging, primarily because of a lack of urine-based assays for etiological confirmation of AFB_1 exposure. We have developed a urine-based HPLC fluorescence method for detection and quantitation of AFB_1 and its metabolites AFM_1 and AFQ_1 using canine, feline, and porcine urine samples. The limits of detection for AFB_1, AFM_1, and AFQ_1 are 0.20, 0.27, and 1.02 pg respectively. Lower limits of quantification are 0.30 pg for AFB_1, 0.50 pg for AFM_1, and 2.5 pg for AFQ_1 respectively, indicating high sensitivity. Intra-run repeatability was 4%, 7%, and 5% for AFB_1, AFM_1, and AFQ_1 respectively at 2 ng/mL. Inter-run repeatability was 3%, 8%, and 20% for AFB_1, AFM_1, and AFQ_1 respectively at 5 ng/mL. Recoveries were 84%, 85%, and 81% for AFB_1, AFM_1, and AFQ_1 respectively. Recoveries for feline and porcine urine were comparable to those of canine urine. These results indicate that the method is suitable for the detection and quantification of AFB_1, AFM_1, and AFQ_1 in animal urine. This noninvasive method will be very helpful in diagnosis of aflatoxin exposure in live animals, which is currently lacking. The next step is inter-laboratory evaluation of the method before it can be shared among the FDA Vet-LIRN network laboratories for routine diagnostic applications.

Lead contamination in backyard chicken flocks - Incidence and exposure assessment in positive cases #

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Backyard flock chickens are submitted to the California Animal Health and Food Safety Laboratory System for routine diagnostic work up as a part of California's avian influenza surveillance program. Some of these birds were tested for heavy metals in the liver and occasionally found to be positive for lead when no clinical signs of lead toxicoses were noted. The potential public health concerns associated with lead exposure prompted us to begin to systematically screen liver samples from every back yard chicken submitted to CAHFS to find the true incidence of lead exposure. Since October 2015, we have performed heavy metal testing in 1002 birds of which 29 cases were positive (2.9%). Highest incidence was from the submissions to the Davis Branch location (36.6%) followed by San Bernardino (24.3%), Tulare (2.3%) and Turlock (0.8%). Liver lead concentrations ranged from 1.1 to 41 ppm. Positive cases were further investigated by follow-up phone calls with the owners of the birds. The owners were asked a pre-determined set of questions including details of the premise (location, presence of old buildings), information about family members (number, gender, and age group), egg consumption and general management of the birds. They were asked to submit eggs for lead determinations so that a conservative estimate of daily exposure of family members to lead could be made. They were also encouraged to consult their physician for possible blood testing and to submit feed, water, soil and other environmental samples to identify a lead source. Lead was measured by ICP/AES and the measured concentrations varied from below the method reporting limit of 0.095 to 0.26 micrograms per gram of egg.

To determine the lead intake, the weight of one egg was estimated to be 50 grams. The maximum amount of lead in one 50 gram egg was determined to be 13 micrograms based upon the highest lead concentration of 0.26 micrograms per gram of egg. The current FDA guidelines suggest that the maximum allowable intake of lead by children from all sources should not exceed 6 micrograms per day. Thus at the highest lead concentration detected, ingestion of one egg per day would approximately double the allowable lead intake. In 4 cases (13.7%), soil, feed and water samples were also tested for lead to determine possible sources but a definitive source of lead was not identified. Additionally, only 34% of owners submitted eggs and environmental samples or eggs alone indicating a lack of interest or financial concerns. Altogether, this study highlights the importance of the need for backyard chicken owners, veterinarians and public health personnel to be aware of the risk and undertake proper surveillance measures. In most of these birds neither the case history nor necropsy findings were indicative of lead exposure. In this scenario, without systematically testing all birds, some cases would likely have been missed.

AAVLD Trainee Travel Awardee

Manganese deficiency implicated in a case of angular limb deformities in yearling ewes # * †

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A flock of Polypay sheep in Northwestern Michigan experienced an increased rate of angular limb deformities and poor reproductive performance in ewe lambs. Of the 365 lambs in the group, 158 were affected. Changes in the limbs became apparent at ~6 months of age, increased in severity over time, and included lateral or medial deviation of the fore limbs at the carpus or metacarpus and swelling of carpi. Conception rates were 62% compared to previous on farm averages of 80% in yearling ewes. Otherwise, lambs had moderate growth rates (0.275-0.513 pounds/day), were in good body condition, and had no other clinical signs. Total mixed ration (TMR) and water samples; serum samples from 11 affected lambs; and fresh samples of liver, heart, 11th rib, forelimb distal to the elbow, and hindlimb distal to the fetlock from 5 lambs taken at slaughter were submitted for analysis. Radiographs of two of the forelimbs showed lesions typical of physeal dysplasia. On histologic examination of the distal radius and ulna, metacarpal and metatarsal bones, and ribs, there were chondrodystrophic changes that varied in severity between animals, between examined bones, and within regions of the physes of individual bones. These changes included flaring of the metaphyses, variation in thickness of the physes, separation and disorganization of chondrocytes, pallor and degeneration of epiphyseal cartilage matrix, segmental retainment of cartilage cores, growth arrest lines, and microfracture or broad physeal fracture. Serum manganese concentrations were below the detection limit of 0.5 ng/ml (reference: 0.5-2.0 ng/ml). Other serum trace nutrient aberrations included elevated molybdenum with a mean of 260.55 ng/ml (reference: 12-30 ng/ml) and elevated selenium concentrations with a mean of 245.91 ng/ml (reference: 110-160 ng/ml). Bone ash manganese concentrations ranged from 2556 to 10790 ng/g, had a mean of 5597.4 ng/g, a median of 3495 ng/g and a standard deviation of 3816.95 ng/g. Concentrations of trace nutrients in hearts, livers, and TMR and water samples were within expected ranges, as was rib bone ash concentration (g ash/g fat-free dry bone; expected range: 53.82-60.62%). On further investigation, TMR had been supplemented with a commercially produced mineral mix that included manganous oxide as the manganese source, which is not as absorbable as other sources, such as manganous sulfate. Given these findings, limb deformities and low conception rates were considered to be associated with manganese deficiency. Manganese, an essential trace element, plays a critical role in enzymes such as glycosyltransferases that affect cartilage synthesis and formation of epiphyseal plates, and deficiency has been proposed as a cause of osteodystrophy in neonatal ruminants. To our knowledge, development of limb deformities in this age of animals has not previously been reported.

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

Organophosphate/carbamate poisoning: Is acetylcholinesterase inactivation a biomarker of interpretation or misinterpretation?

Ramesh C. Gupta, Michelle Lasher, Robin Doss

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Organophosphate (OP)/carbamate (CM) poisoning is commonly encountered in animals around the world. Most animal poisonings are acute and occur as a result of accidental or malicious activity. Often, acetylcholinesterase (AChE) inhibition in brain/blood or presence of an insecticide's residue in GI content is used as a criterion of diagnosis. In acute OP/CM toxicity, a three phase mechanistic model is involved (1. Excitotoxicity due to accumulation of acetylcholine, 2. NMDA neurotransmitter involvement within 5 min of seizure onset, and 3. Dominance of NMDA system within 40 min). Therefore, OP/CM toxicity involves multiple neurotransmitters, receptors, and enzymes, thereby affecting many organs and systems (nervous, skeletal, cardiovascular, respiratory, immune, etc). In the case of OP/CM compounds, the framework of the adverse outcome pathway (AOP) explains multiple cholinergic and non-cholinergic molecular initiating events (MIE), key events (KEs), key events relationships (KEs), and adverse outcome (AO) that can be toxic or lethal. Evidently, quantifiable biomarkers suggest that in OP/CM induced neuroinflammation, neurotoxicity, and neuronal death, noncholinergic mechanisms play a greater role than cholinergic mechanisms. Furthermore, published evidence suggests that AChE activity can be inactivated not only by OPs or CMs, but also by other classes of pesticides, metals, mycotoxins, plant alkaloids, and synthetic drugs. Some compounds directly interact with muscarinic and/or nicotinic ACh receptors and complicate the diagnosis of OP/CM poisoning. Taking all findings into consideration, inactivation of AChE can be used as an indicator of OP/CM exposure, but the diagnosis of poisoning should not be solely based on AChE inactivation.

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Virology 1

Saturday, October 15, 2016

Imperial F

Moderators: Jianqiang Zhang and Douglas Marthaler

1:00 PM **PEDV shedding patterns and antibody kinetics in commercial growing pigs * †**
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1:15 PM **Serum and mammary secretion antibody responses in PEDV-exposed gilts following PEDV vaccination * †**
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1:30 PM **Quantifying the effect of lactogenic antibody on porcine epidemic diarrhea virus infection in neonatal piglets * †**
Korakrit Poonsuk, Jianqiang Zhang, Qi Chen, Wendy Gonzalez, Lucas Correa da Silva Carrion, Yaxuan Sun, Chong Wang, Rodger Main, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola *147*

1:45 PM **Pathogenesis and antibody responses of porcine deltacoronavirus in weaned pigs †**
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2:00 PM **Pathogenesis of Senecavirus A in swine**
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2:30 PM **Outbreak of a pandemic H1N1 influenza virus in a swine herd**
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2:45 PM **Experimental infection of U.S. swine with HeN1 variant Pseudorabies virus of Chinese origin: Coverage of diagnostic assays and commercial vaccines**
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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

PEDV shedding patterns and antibody kinetics in commercial growing pigs * †

Jordan Bjustrom Kraft[1], Katie Woodard[1], Luis Gabriel Gimenez-Lirola[1], Marisa Rotolo[1], Chong Wang[2], Yaxuan Sun[2], Pete Lasley[3], Jianqiang Zhang[1], Dave Baum[1], Phillip Gauger[1], Rodger Main[1], Jeff Zimmerman[1]

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Longitudinal samples collected from two production sites (one PEDV positive; one PEDV negative) were used to 1) describe the pattern of PEDV shedding (RT-PCR) in individual pig fecal swabs, pen fecal samples, and pen oral fluids (OF); 2) describe the kinetics of PEDV antibody by ELISA (IgA, IgG) testing of pig serum and pen OF samples; and 3) establish cutoffs and performance estimates for PEDV "whole virus" IgA and IgG ELISAs (PEDV WV ELISA).

Materials and Methods

Farm 1 was a 52-pen wean-to-finish (WTF) barn stocked with 800 pigs. Pen samples (feces and OFs) and individual pig samples (fecal swabs and sera) were collected from the same 6 pens and a convenience sample of 5 pigs in each of the 6 pens at placement and at 2-week intervals for 27 weeks. At 13 weeks of age, the population was exposed to PEDV using standard field exposure methods.

Farm 2 consisted of 3 identical 40-pen WTF barns, each stocked with 900 pigs. Pen OF samples were collected from 36 pens in each of the 3 barns and serum samples were collected from a convenience sample of 20 pigs in 2 pens (10 pigs per pen) in each barn. Sampling began at placement and was done weekly for a total of 9 samplings.

Pen feces, pen OFs, and individual fecal swabs were tested by PEDV RT-PCR; OF and sera were tested by PEDV WV ELISA (IgG, IgA) at the ISU VDL.

Results

On Farm 1, PEDV was detected by RT-PCR at the first sampling post inoculation (DPI 6) in individual fecal swabs, pen fecal samples, and pen OF. The last RT-PCR positives were detected in fecal swabs and OFs on 69 DPI. Overall, the highest percent of positive samples was observed in OF. Anti-PEDV IgG and IgA was detected in OF and serum samples collected at 13 DPI. The OF IgA response increased through 97 DPI, while serum IgA responses peaked at 27 DPI.

Farm 2 remained RT-PCR negative throughout the monitoring period. These samples provided a source of negative samples for calculating cutoffs and performance estimates for the PEDV WV IgA and IgG ELISAs.

Conclusions

The purpose of surveillance is to provide timely information on pathogen exposure and immune responses in swine populations in order to optimize health and prevent disease. Well-validated, reproducible, high-throughput nucleic acid and antibody assays are necessary to achieve this purpose. This study showed that oral fluid-based testing could provide an easy and "animal-friendly" approach to nucleic acid and/or antibody-based surveillance of PEDV in swine populations. In particular, the exceptional strength and duration of the PEDV IgA antibody response in oral fluids warrants further research to investigate whether OF IgA could serve as an indicator of protective immunity.

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

Serum and mammary secretion antibody responses in PEDV-exposed gilts following PEDV vaccination * †

Jordan Bjustrom Kraft[1], Katie Woodard[1], Luis Gabriel Gimenez-Lirola[1], Blake Setness[2], Ju Ji[2], Pete Lasley[3], Eric A. Nelson[4], Jianqiang Zhang[1], Dave Baum[1], Phillip Gauger[1], Jeff Zimmerman[1], Rodger Main[1]

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Since there is little possibility that PEDV will be eradicated in the near future, we need to identify methods to prevent and/or control its effects. In the sow herd, this implies monitoring/maintaining levels of immunity sufficient to protect neonatal pigs. The objective of this study was to compare gilt responses to vaccination using two commercially available PEDV vaccines, HarrisVaccines™ (Vaccine A) and Zoetis (Vaccine B).

Materials and Methods

PEDV antibody-positive gilts (n = 36) in a commercial production system were randomly assigned to one of five vaccination protocols: 1) no vaccine (controls); 2) PEDV vaccine A (2 weeks pre-farrow); 3) PEDV vaccine A (5 and 2 weeks pre-farrow); 4) PEDV vaccine B (2 weeks pre-farrow); and 5) PEDV vaccine B (5 and 2 weeks pre-farrow).

Colostrum, serum, and fecal swab samples were collected within 12 hours of farrowing. Milk samples were collected at 3, 10, and 21 days post farrowing (DPF). Serum, colostrum, and milk samples were tested by PEDV whole virus (WV) IgG and IgA ELISAs and for neutralizing antibody by PEDV fluorescent focus neutralization assay (FFN). Thirty-three gilts completed the study, i.e., farrowed viable litters and provided a full complement of samples.

Statistical analyses were performed using SAS® (Version 9.4). A nonparametric one-way ANOVA was used to test for differences among treatment groups for IgG, IgA, and FFN by sample type (serum, colostrum, milk). A mixed-effects repeated measures model (Proc GLIMMIX) was used to analyze the difference between treatment groups for IgG, IgA, and FFN by sample type.

Results

Gilt serum antibody levels (IgG, IgA, FFN) at 5 weeks pre-farrow, i.e., pre-vaccination, were not different. In gilts with prior exposure to PEDV, vaccination significantly ($p < 0.05$) increased IgG, IgA, and neutralizing antibody levels in serum, colostrum, and milk samples (3 DPF).

Conclusions

Vaccination can be an effective approach to management of anti-PEDV maternal immunity in PEDV endemically-infected sow herds.

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

Quantifying the effect of lactogenic antibody on porcine epidemic diarrhea virus infection in neonatal piglets * †

Korakrit Poonsuk, Jianqiang Zhang, Qi Chen, Wendy Gonzalez, Lucas Correa da Silva Carrion, Yaxuan Sun, Chong Wang, Rodger Main, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola

Iowa State University, Ames, IA

The objective of this experiment was to assess the role of lactogenic immunity in protecting piglets against the effects of PEDV by quantifying virus shedding in feces and piglet growth, thermoregulation, and survival in the presence or absence of PEDV antibody in colostrum and milk.

Methods

Sixteen sows and their litters were allocated to one of 3 treatment groups: Group 1 - 6 PEDV antibody-negative sows and 11 piglets. Group 2 - 8 PEDV antibody-positive sows and 91 piglets. Group 3 - 2 PEDV antibody-negative sows and 22 piglets. Group 1 and 2 piglets were orally inoculated with PEDV at 4 or 2 days of age, respectively. Group 3 (not inoculated with PEDV) were included to provide a baseline for piglet survivability and growth rate.

All piglets were closely observed through day post-inoculation (DPI) 12 (Groups 2 and 3) or 14 (Group 1) or until humane euthanasia was necessary. Body weight & body temperature measurements were taken daily. Samples collected for analysis included sow milk and piglet fecal samples (daily) and serum from sows (Groups 2 and 3 - DPI 12, Group 1 - DPI 14). Serum, colostrum, and milk were tested for PEDV IgG, IgA, & FFN. Feces were tested by PEDV rRT-PCR. Data were analyzed for the effect of maternal PEDV antibody levels in colostrum and milk on piglet PEDV serum antibody levels, PEDV fecal shedding, body temperature, weight gain, and mortality.

Results

Analysis showed differences (ANOVA, $p < 0.0001$) in lactogenic antibody in Groups 1 and 2 at all sampling points for all tests with the exception of the WV IgG ELISA on DPIs ≥ 4. The level of PEDV fecal shedding was significantly lower (ANOVA, $p = 0.0025$) in Group 2 piglets on DPIs 1 to 5. A significant difference in body temperature was detected between Group 1 and 2 piglets at all sampling points (ANOVA, $p = 0.0001$) except DPIs 9, 11, and 12. The rate of growth between Group 1 and 2 piglets differed at all time points (ANOVA, $p = 0.002$) except DPIs 2 to 4. All uninoculated control piglets (Group 3, n = 22) survived to the end of the observation period. In contrast, 10 piglets (91%) in Group 1 died (DPIs 4 to 10) and 14 piglets (15%) in Group 2 died (DPIs 1 to 9). Proportional hazard regression analysis of Groups 1 and 2 found a significant difference in piglet survivability ($p < 0.0001$).

Conclusions

In this study, the presence of lactogenic antibody markedly affected the outcome of PEDV infection in neonates, including less PEDV shedding in feces, better thermostability (p = 0.0001), higher rate of growth, and higher rate of survivability. Therefore, maintenance of sufficient levels of lactogenic immunity will be the cornerstone for the prevention of PED in endemically-infected herds.

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

Pathogenesis and antibody responses of porcine deltacoronavirus in weaned pigs †

Qi Chen[2], Phillip Gauger[2], Joseph Thomas[2], Michael Welch[2], Priscilla Gerber[1], Tanja Opriessnig[2,1], Paulo Arruda[2], Drew Magstadt[2], Luis Gabriel Gimenez-Lirola[2], Jianqiang Zhang[2]

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Porcine deltacoronavirus (PDCoV) was first reported in the U.S. in February 2014. Koch's postulates have been recently fulfilled and pathogenesis has been studied in neonatal and pre-weaned pigs. However, pathogenicity and kinetics of antibody response of PDCoV in weaned pigs have not been investigated. The objectives of this study were to 1) assess the pathogenicity of PDCoV in 21-day-old pigs and the effect of rechallenge; and 2) evaluate the appropriateness of different specimen types for PDCoV nucleic acid and antibody (Ab) detection.

Thirty PDCoV negative, 3-week-old pigs were randomly divided into 2 groups with 15 pigs per group. On Day 0 (D0, pigs were 3 weeks old), group 1 was orogastrically inoculated with PDCoV USA/IL/2014 isolate (10^5 $TCID_{50}$/pig) and group 2 was inoculated with virus-negative medium. Five pigs/group were necropsied on D7 for pathogenesis evaluation. The remaining 10 pigs/group were kept through D42. On D35 (pigs were 8 weeks old), both groups were challenged with PDCoV (10^5 $TCID_{50}$/pig). Clinical observations were collected. Individual fecal swabs and sera, and pen-based feces and oral fluids were collected. The intestinal tissues were evaluated for gross and microscopic lesions and also examined by PDCoV immunohistochemistry (IHC). Fecal swabs, feces, and oral fluids were tested by PDCoV real-time RT-PCR. Sera were tested for PDCoV Ab by indirect fluorescent antibody (IFA) and virus neutralization (VN) assays. Sera, feces and oral fluids were tested for PDCoV Ab by a S1-based ELISA.

PDCoV-inoculated pigs developed soft to semi-watery diarrhea during D4-10. Viral RNA shedding peaked on D7 and gradually decreased thereafter in individual fecal swabs, pen-based feces, or pen-based oral fluids. Specifically, PDCoV RNA was detected through at least D14 (negative on D21) in pen-based feces, at least D28 (negative on D35) in individual fecal swabs, and at least D35 (negative on D42) in pen-based oral fluids. After 2nd challenge on D35, no diarrhea was observed in either group, and PDCoV RNA was not detected in fecal swabs, feces and oral fluids during D36-D42 from either group.

On D7, 3 out of 5 necropsied PDCoV-inoculated pigs had soft to semi-watery intestine content, and 2 of them had gross lesions in intestines. PDCoV-inoculated pigs had significantly lower villus height to crypt depth ratio in middle- and distal-jejunums and ileum compared to the negative control pigs. No significant gross lesions were observed from pigs necropsied on D42. IHC results are in progress.

In PDCoV-inoculated pigs, serum IFA IgG Ab was detected from D14, peaked on D21-28 and started to decrease from D35. Serum VN Ab appeared on D7 and maintained at high level during D14-42. As tested by PDCoV S1-ELISA, serum IgG and IgA Ab were detected from D14 through D42; IgG and IgA Ab were detected in pen-based feces from D21-42; and IgG and IgA Ab were detected in pen-based oral fluids from D14-42.

† Graduate Student Oral Presentation Award Applicant

Pathogenesis of Senecavirus A in swine

Lok R. Joshi[1], Maureen H. V. Fernandes[1], Travis Clement[1], Steven R. Lawson[1], Fabio Vanucci[2], Eric A. Nelson[1], Diego G. Diel[1]

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Senecavirus A (SVA), a picornavirus of the genus *Senecavirus*, has been recently associated with vesicular disease and neonatal diarrhea and mortality in swine the US and in Brazil. Many aspects of SVA infection biology and pathogenesis remain unknown. In this study, the infection dynamics and pathogenesis of SVA were investigated in pigs. Twelve finishing pigs (~100 lb) were randomly allocated into two experimental groups as follows: Group 1: Mock-control group (*n = 4*) and Group 2: SVA-inoculated group (*n = 8*). Animals were inoculated intranasally and orally with a contemporary SVA strain and monitored daily for characteristic clinical signs and lesions associated with SVA infection. Viremia was assessed in serum and virus shedding was monitored in oral and nasal secretions and feces. Samples collected on days 0, 3, 5, 7, 10, 14, 21, 28 and 35 post-inoculation (pi) were tested by SVA real-time reverse transcriptase PCR (rRT-PCR) and virus isolation. Clinical signs were first observed on day 4 pi, and were characterized by lameness and lethargy. Vesicular lesions were observed on the snout, coronary bands, dewclaws and sole of the hooves of inoculated animals. Lameness and vesicular lesions were observed between days 4 and 14 pi. Viremia was detected between days 3 and 10 pi, whereas virus shedding was detected between days 1 and 28 pi in oral and nasal secretions and feces. Notably, rRT-PCR and *in situ* hybridization performed on tissues collected on day 38 pi revealed the presence of viral RNA on the tonsil of all SVA infected animals. These results demonstrate the pathogenicity of a contemporary SVA strain and provide significant insights on the infection dynamics, pathogenesis and shedding patterns of SVA in swine.

Serological and molecular detection of Senecavirus A associated with an outbreak of swine idiopathic vesicular disease and neonatal mortality ◊

Luis Gabriel Gimenez-Lirola[1], Christopher Rademacher[2], Daniel Correia-Lima-Linhares[2], Karen Harmon[2], Marisa Rotolo[2], Yaxuan Sun[2,3], Dave Baum[2], Jeff Zimmerman[1], Pablo E. Pineyro[2]

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We performed a longitudinal field study in a swine breeding herd that presented with an outbreak of vesicular disease (VD) associated with an increase in neonatal mortality. Initially, a USDA Foreign Animal Disease (FAD) investigation confirmed the presence of Senecavirus A (SVA) and ruled out the presence of exotic agents that produce vesicular lesions, e.g., foot-and-mouth disease virus and others. Subsequently, serum samples, tonsil swabs, and feces were collected from sows (n = 22) and their piglets (n = 33) beginning one week after the onset of the clinical outbreak and weekly for 6 weeks. The presence of SVA RNA was evaluated in all specimens collected by RT-qPCR targeting a conserved region of the 5' untranslated region (5'UTR). The serological response (IgG) to SVA was evaluated by weekly testing sow and piglet serum samples on a SVA VP1 recombinant protein (rVP1) indirect ELISA. The rVP1 ELISA detected seroconversion against SVA in both clinically affected and non-clinically affected sows at early stages of the outbreak, as well as maternal SVA antibodies in offspring. Overall, the absence of vesicles (gross lesions) in SVA-infected animals and the variability of RT-qPCR results among specimen type demonstrated that a diagnostic algorithm based on the combination of clinical observations, RT-qPCR in multiple diagnostic specimens, and serology is essential to ensure an accurate diagnosis of SVA.

◊ USAHA Paper

Outbreak of a pandemic H1N1 influenza virus in a swine herd

Yan Zhang, Leyi Wang

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A closed swine sow herd was reported to have high fever, respiratory disease, and abortions. Porcine reproductive and respiratory syndrome virus (PRRSV) was suspected. Nasal swab samples were sent to the Ohio Animal Disease Diagnostic Laboratory. All five samples were negative for PRRSV. Four out of the five samples were positive for H1N1 influenza virus type A. Several employees working on the farm also developed fever and respiratory disease before and during the swine outbreak. A human nasal sample was collected and tested to be positive for the 2009 pandemic H1N1 virus by the Ohio Public Health Laboratory and later confirmed by CDC. Whole genome sequencing was performed for the swine and the human viruses. Sequence analysis demonstrated that both viruses are identical. In addition to the pandemic NP gene, an additional swine lineage NP gene was identified in both swine and human viruses, indicating a mixed infection caused the outbreak in the pig farm. These results strongly suggest that a bi-directional transmission of type A influenza viruses between humans and pigs occurs in the fields.

Experimental infection of U.S. swine with HeN1 variant Pseudorabies virus of Chinese origin: Coverage of diagnostic assays and commercial vaccines

Rachel M. Tell, Sabrina L. Swenson, Tracy L. Sturgill, Richard Clayton, Katie Mozingo, Leo G. Koster, Melinda Jenkins-Moore, Tamara J. Beach, Ann Predgen, Dawn R. Toms

NVSL, USDA, Ames, IA

Pseudorabies virus (PRV) causes pseudorabies (Aujeszky's disease) in swine and can lead to significant economic losses for the producer. Infection can induce neurologic, respiratory, and reproductive signs, and death. PRV was eradicated from the U.S. domestic swine population in 2004 through the use of gene-deleted vaccines and serologic assays to identify infected herds for depopulation. In late 2011, severe pseudorabies (PR) with over 50% mortality in piglets was reported in PRV vaccinated herds in China. Recent U.S. introductions of porcine epidemic diarrhea virus and porcine deltacoronavirus, both of which had close homology to viruses circulating in China, has led to questions about our preparedness for potential introduction of other pathogens, such as this Chinese variant (HeN1) PRV. Most importantly: will our diagnostic assays detect infection with HeN1 PRV and will our vaccines protect against it? Two trials were performed to address these questions. Through collaboration between the Chinese government and USDA, the HeN1 strain was obtained from China. All work was done under BSL-3 and BSL-3Ag conditions. The first study was a viral dose titration evaluating the ability of current PRV diagnostic assays to detect infection with HeN1 PRV. Five- to six-week old pigs were challenged intranasally with one of four virus dilutions and monitored for 23 days. Blood samples and nasal and oral swabs were collected at specific time points for testing. Swine exhibiting signs of PR were euthanized; brain, lung, tonsil, and spleen were collected. Viral shedding was detected in nasal swabs at first collection on day two post-challenge in the two highest viral dose groups. Real-time PCR detected viral DNA from oral and nasal swabs as well as from post-mortem tissues from animals found dead or euthanized due to clinical signs of PR. Seroconversion was detected by all three U.S. licensed, commercially available PRV serological assays. The second study was a preliminary assessment of the ability of two U.S. licensed, commercially available, gI-deleted, modified live vaccines against PRV to protect pigs from HeN1 PRV. Pigs were housed together until vaccination when each vaccine group was separated. After 14 days, all pigs were housed together and challenged intranasally with the HeN1 virus. All nonvaccinated pigs were euthanized between days 4 and 9 post-challenge due to severity of clinical signs. During that same time period, half of the vaccinates, spread between both vaccine groups, exhibited transient clinical signs consistent with PR. All vaccinates seroconverted on the gB ELISA and Autolex assays pre-challenge. All pigs shed HeN1 virus post-challenge and had seroconverted on the gI ELISA when they were euthanized (either for clinical signs or at end of study). Neither full disease protection nor prevention of viral shedding were achieved; however, both vaccines appear to reduce clinical signs in 5-6 week old pigs under current study conditions.

Virology 2

Sunday, October 16, 2016

Imperial F

Moderator: Diego G. Diel

8:00 AM **Complete genome sequencing and phylogenic analysis of cervid adenovirus from naturally occurring cases in Wyoming, Washington, and Colorado**
Myrna M. Miller, Todd Cornish, Jennifer McKenna, Marce Vasquez *155*

8:15 AM **Identification of a novel virus causing mass mortalities in an endangered species of freshwater turtle**
Jing Zhang, Melinda Frost, Andrew J. Read, Mukesh Srivastava, Kate Parrish, Deborah S. Finlaison, Sarah Gestier, Xingnian Gu, Jane Hall, Karrie Rose, Peter Daniel Kirkland *156*

8:30 AM **Observations on embryo mortality during avian influenza virus propagation from wild birds**
Beate Crossley, Munashe Chigerwe, Kathy L. Toohey-Kurth, Hon Ip, Mia Kim Torchetti, John Baroch *157*

8:45 AM **Evaluation of oral swabs as a sample for FMDV surveillance**
Peter Daniel Kirkland, Rodney J. Davis, Bernd Haas, Kerstin Wernike, Martin Beer *158*

9:00 AM **Detection of Foot and Mouth Disease virus serotypes and persistence of infection induced antibody against FMD in naturally infected cattle # † ◊**
Laila Akhter *159*

9:15 AM ***In vivo* bioluminescent imaging of J Paramyxovirus (JPV) infection # †**
Mathew Abraham, Zhuo Li, Biao He *160*

9:30 AM **Break**

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

Complete genome sequencing and phylogenic analysis of cervid adenovirus from naturally occurring cases in Wyoming, Washington, and Colorado

Myrna M. Miller, Todd Cornish, Jennifer McKenna, Marce Vasquez

Veterinary Sciences, Wyoming State Veterinary laboratory, Laramie, WY

Odocoileus hemionus deer adenovirus (OdAdV) has been shown to be a cause of hemorrhagic disease in cervids, characterized by systemic vasculitis in mule deer, black-tailed deer, and moose. Lesions include pulmonary edema and hemorrhagic enteritis and may be similar to hemorrhagic disease caused by the orbirviruses bluetongue and epizootic hemorrhagic disease virus. In California, large epizootics with high fatality have been attributed to OdAdV (Woods *et al.*, 1996, 1999; Sroden *et al.*, 2000). In Wyoming, individual or small clusters of cases of hemorrhagic disease attributed to adenovirus have been diagnosed in moose, mule deer, and white-tailed deer, but large epizootics have not been recognized. The incidence and distribution of OdAdV of cervid populations and virus related factors contributing to disease outbreaks are largely unknown. Previously, only partial OdAdV sequences have been published (Zakhartchouk *et al.*, 2002). In the present study, we obtained whole genome sequences from naturally occurring adenovirus infections (N=9) in mule deer, white-tailed deer, moose, and elk that were used to estimate phylogenic relationship with other related adenoviruses. Cases were from Wyoming, Washington, and Colorado, and occurred between the years 1999 and 2015. Alignment of the 30,645-30,697 bp genomes found genetic sequence was highly conserved between species and years with 99% identity. However, within a highly variable, noncoding, AT-rich region, specific genotypes were identified that differed between adenoviruses recovered from elk and moose and those from mule deer and white-tailed deer. Transcription units and predicted proteins had homology and genome organization typical of members of the genus *Atadenovirus*, but had more than 15% phylogenetic divergence from other species based on amino acid sequence of the conserved hexon and polymerase proteins. Our data shows that OdAdV is well established in these wildlife species and supports the classification of OdAdV as a new species within the genus *Atadenovirus.* Based on the complete genome sequence, PCR-based diagnostic and genotyping assays have been developed to support epidemiology and pathogenesis studies, and to test for genetic variations that may be associated with outbreaks and severe disease.

Identification of a novel virus causing mass mortalities in an endangered species of freshwater turtle

Jing Zhang[1], Melinda Frost[1], Andrew J. Read[1], Mukesh Srivastava[1], Kate Parrish[1], Deborah S. Finlaison[1], Sarah Gestier[1], Xingnian Gu[1], Jane Hall[2], Karrie Rose[2], Peter Daniel Kirkland[1]

[1]Virology Laboratory, Elizabeth Macarthur Agriculture Institute, Camden, NSW, Australia; [2]Australian Registry of Wildlife Health, Taronga Conservation Society Australia, Taronga Zoo, Sydney, NSW, Australia

In February 2015 a large number of sick and dead Bellinger River snapping turtles (*Myuchlys georgesi*) were found in the Bellinger River in New South Wales, Australia. Prior to the mortality event the population of these turtles was geographically restricted to a 47 km stretch of the Bellinger River, but was considered stable with estimates of between 1,500 – 4,500 individuals. Subsequent to the mortalities the species has been listed as critically endangered. No other terrestrial or aquatic animal species were affected, with healthy fish and other reptiles found in and around the river. Almost all of the sick turtles that were collected died within a few hours to days. Clinical signs observed in sick turtles included blindness, aimless wandering, severe conjunctivitis and emaciation. At post mortem examination there was evidence of severe fibrino-necrotising conjunctivitis, keratitis, stomatitis, multifocal epidermal necrosis, variable sinusitis and variable miliary to diffuse pale foci throughout the splenic and renal parenchyma. Histopathology revealed fibro-necrotising and granulocytic splenitis, nephritis and multi-systemic fibrinoid vasculopathy. All affected turtles had acute lesions and it was considered that the emaciation was likely to have occurred before the disease process. Preceding the outbreak there had been an increase in both environmental and water temperature and river levels were lower than usual.

An extensive range of toxicological tests were undertaken with negative results. Bacterial cultures were attempted for a many different organisms but only aquatic commensals were detected. Similarly, PCR assays were completed for wide range of aquatic and reptilian pathogens with negative results. Virus isolation using pooled lung and spleen tissues was attempted on a range of cell lines. There was evidence of vague cytopathology after 6-7 days on the second passage in one cell line which gave reproducible cytopathology on subsequent passages. Electron microscopy examination of cell culture fluid detected virus particles with a rhabdovirus-like morphology. Next generation nucleic acid sequencing was utilized to establish the sequence of this virus and allowed the rapid development of real time PCR assays.

The complete characterization of this novel virus, application of qRT-PCR assays to implicate this virus as the aetiological agent and later the use of these qRT-PCR assays for large scale screening during epidemiological studies will be discussed.

Observations on embryo mortality during avian influenza virus propagation from wild birds

Beate Crossley[1], Munashe Chigerwe[2], Kathy L. Toohey-Kurth[3], Hon Ip[4], Mia Kim Torchetti[5], John Baroch[6]

[1]UC Davis, California Animal Health and Food Safety Laboratory, Davis, CA; [2]UC Davis, Department of Medicine and Epidemiology, Davis, CA; [3]University of Wisconsin, Madison, Wisconsin Veterinary Diagnostic Laboratory, Madison, WI; [4]National Wildlife Health Center, United States Geological Survey, Madison, WI; [5]National Veterinary Services Laboratory, Avian Virology, Ames, IA; [6]Wildlife Service/APHIS/USDA, National Wildlife Research Center, Fort Collins, CO

Surveillance and disease outbreak response are important missions of veterinary diagnostic laboratories nationally. Membership in the National Animal Health Laboratory Network (NAHLN) allows approved state laboratories to use standardized federal protocols and assays for the detection of avian influenza virus (AIV) via real-time PCR. The Wildlife Services contracted with the NAHLN laboratories to test regionally collected swab samples for AIV. Samples testing positive for the Avian Influenza matrix gene during wildlife surveillance testing in 2015 - 2016 in the Pacific Flyway were inoculated into specific pathogen free (SPF) chicken eggs for virus propagation. Wild bird viruses are considered challenging for virus propagation, because they might not be adapted to poultry cells and therefore need adjustment periods for successful propagation.

A total of 488 positive Matrix samples were inoculated into SPF eggs at CAHFS. Of these 488 samples, 99 (20.29%) were propagated during the first passage. Confirmatory testing of the AIV was performed using real-time PCR. No virus was propagated during a second passage. Each sample was inoculated into 4 eggs. Embryo mortality was assessed by the total number of eggs dying during the 5-day incubation period, by mortality pattern and by virus strain. Mortality patterns were evaluated to assess a potential adaptation time for wildlife isolates.

Positive allantoic fluids were typed by sequencing fragments of the hemagglutinin (HA) and neuraminidase (NA) genes. The most commonly propagated HA types were H6 (n=15) and H7 (n=15), and for the NA types N2 (n=20) and N8 (n=20). In 3% of positive virus isolation samples (n=3) a dual infection was found. Out of the 99 isolates 23 caused embryo mortality of all inoculated eggs, 42 isolates led to a 75% mortality rate. In 78.57% (33/42) of cases a 75% mortality was reached by day 3, whereas only 19.4% (8/42) showed an evenly distributed pattern throughout the 5-day incubation period. The highest mortality rate with regards to numbers of embryos killed during the incubation period were associated with H10 and H7 (with 87.5 and 70% embryo mortality, respectively), whereas H2, H8 and H12 showed a comparable low cumulative embryo mortality of 25%. For NA subtypes, N6 had the highest rate of embryo mortality (70%), whereas N5 isolates showed an overall embryo mortality of 33.3%. A total of 12 isolates propagated without causing embryo mortality.

Propagating viruses from wildlife samples collected by trained Wildlife Services personnel and others allowed us to determine that PCR is a successful screening method for allantoic fluids and can eliminate the need of a second viral passage. Based upon mortality patterns observed, the majority of viruses isolated did not require an adjustment period for successful propagation.

Evaluation of oral swabs as a sample for FMDV surveillance

Peter Daniel Kirkland[1], Rodney J. Davis[1], Bernd Haas[2], Kerstin Wernike[2], Martin Beer[2]

[1]Elizabeth Macarthur Agriculture Institute, Camden, NSW, Australia; [2]Friederich Loeffler Institute, Griefswald, Germany

Of all exotic diseases, foot and mouth disease (FMD) presents the greatest threat to Australia's livestock industries. An outbreak of FMD would have a severe impact through loss of both productivity and exports. This virus can be difficult to detect in sheep and sometimes there has been significant dispersal of FMD virus (FMDV) through the movement of sheep. Consequently, there is a need for a laboratory based method for rapid testing to support large scale surveillance.

FMDV has been detected in oral fluids/oral swabs for lengthy periods using virus isolation indicating that there is merit in using oral swabs for FMDV surveillance because of the ease of sample collection and capacity to detect virus for longer than from blood. Virus isolation is also labour intensive, expensive and relatively slow and amplifies infectious virus. In contrast, quantitative 'real time' reverse transcription polymerase chain reaction (qRT-PCR) technology is very sensitive and allows rapid detection of viral nucleic acid (RNA) without amplifying infectious virus. A combination of qRT-PCR assays to detect FMDV RNA in oral swabs would provide an efficient option for large scale FMDV surveillance – either during an outbreak or during 'proof of freedom' testing. A large collection of plasma and oral swab samples from experimentally infected sheep were available for use in this study. These were invaluable because the time of infection and stages of infection were accurately defined and virus isolation, a 'gold standard against which to evaluate the high throughput qRT-PCR assay, had been completed on each sample.

The objectives of this project were to confirm the capacity of qRT-PCR to detect FMDV in oral swabs from sheep; to define the onset and duration of detection of FMDV in oral fluids and plasma and to estimate the level of pooling of samples that may be achieved without compromising the capacity to detect FMDV in a flock

The qRT-PCR assay detected FMDV in almost all oral swabs collected in the first 7-10 days after infection and 65% of samples were still positive at 30 days post infection. In contrast virus was only detected in plasma for a short time. Virus isolation was also less sensitive than the qRT-PCR when testing either oral swabs or plasma. The results of sample pooling studies showed that it was possible to detect a single positive sample in a pool of ten samples during the early stages of infection and to detect one positive in a pool of at least 5 samples one month after animals had been exposed to FMDV.

The combination of testing of oral swabs by high throughput qRT-PCR adds a new dimension to the detection of FMDV in sheep and will facilitate surveillance in the event of a disease outbreak. As the virus levels in sheep are low compared to cattle and pigs, it is expected that this sampling strategy can also be readily applied to monitoring of these species.

Detection of Foot and Mouth Disease virus serotypes and persistence of infection induced antibody against FMD in naturally infected cattle # † ◊

Laila Akhter[1,2,3]

[1]Department of Medicine, Bangladesh Agricultural University, Dhaka, Bangladesh; [2]Ministry of fisheries and Livestock, Department of Livestock services, Dhaka, Bangladesh; [3]Ministry of fisheries and livestock, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh

Background: FMDV is endemic in Bangladesh and causes huge loss declining productivity of cattle. To efficiently control the disease in endemic countries like Bangladesh, vaccination of animal is the most conventional and effective way. Naturally infected animals develop some innate antibody against the disease and it persists for a time period. For effective vaccination program it is essential to know how long the natural immunity persists and protect the animal against circulating FMD serotypes.

Objective: The proposed study was conducted for identification and serotyping of Foot and Mouth Disease virus from clinically infected cattle and detection of persistence of antibody induced by infection in their serum.

Methodology: FMD infection was confirmed by DIVA ELISA with sera samples and uniplex one-step RT-PCR with clinical samples using universal primer pair 1F and 1R. After initial confirmation of FMD virus, multiplex RT-PCR (mRT-PCR) was employed using serotype specific primers (P38:P40:P74-77:P110) to confirm the FMD virus serotypes. The serological responses of cattle to natural FMD infection were measured with collected sera samples by performing the Liquid Phase Blocking ELISA.

Result: All twenty five samples showed positive result by DIVA ELISA for FMDV infection whereas RT-PCR of clinical samples with universal primer pair (1F, 1R) revealed sixteen samples positive for FMDV. Out of the 16 FMD positive samples 7 samples (43.75%) were positive for FMD O type followed by 5 samples (31.25%) positive for FMD A type, and 2 samples (12.5%) were positive for FMD Asia-1 type. There were 2 cases (12.5%) of mixed infection with synchronized presence of O and Asia-1 serotype. The antibody evoked by natural FMD infection was evaluated by LPB ELISA. The antibody titers arose at high level (PI value>80) at the first week of infection and reached at its peak (PI value >90) on the 70-75 days post infection (dpi) for serotypes A, O and Asia 1. After that a steep fall was observed for all serotypes. But the antibodies remained above the protective level (PI value >50) up to 140 -145 dpi for all three serotypes. The protective antibody titer was recorded against only that serotype with which the animal was infected only.

Conclusion: Three serotypes of FMD virus O, A and Asia 1 were circulating in the study area and type O was dominating among these serotypes followed by A and Asia 1. There were also occurrence of mixed infection with serotype O and Asia 1. Antibody induced by natural FMD infection remains at protective level (according to test interpretation, PI value >50) up to 145 days post infection (dpi). The protective antibody titers were serotype specific.

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† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

In vivo bioluminescent imaging of J Paramyxovirus (JPV) infection # †

Mathew Abraham, Zhuo Li, Biao He

Infectious Diseases, The University of Georgia, Athens, GA

J Paramyxovirus (JPV) was first isolated from moribund mice with hemorrhagic lung lesions in Australia in 1972. It is a rodent paramyxovirus classified under genus *Jeilongvirus*. JPV has a large genome of 18,954 nucleotides consisting of eight genes in the order 3'-N-P/V/C-M-F-SH-TM-G-L-5'. To understand the real-time infection dynamics, we have created a replication-competent JPV reporter virus suitable for *in vivo* bioluminescent imaging in mouse model. We have used a small and bright NanoLuc luciferase (nL) gene to create the reporter virus, rJPV-nL, using the already established reverse genetics system of JPV. A full length sequencing of the cDNA of a single virus clone of rJPV-nL confirmed the identity of a mutation-free reporter virus. Infection of vero cells with rJPV-nL revealed that the luciferase activity is in correlation with the concentration and growth kinetics of rJPV-nL. A sublethal intranasal challenge of mice with rJPV-nL enables the visualization of virus spread and clearance on anaesthetized mice using IVIS (In vivo Imaging System) Lumina XR imaging system. These findings provide a novel approach to study JPV replication and spread.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

AAVLD / USAHA Joint Plenary Session
Challenges, High Tech Solutions and Success Strategies in Animal Agriculture

Monday, October 17, 2016
Imperial DEFGHI

Moderator: Max Armstrong

8:00 AM **Opening Remarks**
Boyd Parr, Pat Halbur

8:10 AM **Initial Remarks from Moderator**
Max Armstrong *163*

8:25 AM **Economic challenges and opportunities facing U.S. animal agriculture**
David Kohl *164*

9:10 AM **Challenges and Opportunities for US Animal Agriculture: Meeting the demands of global and domestic markets while fighting burdensome regulation**
Dale Moore *165*

9:55 AM **Panel Discussion**

10:05 AM **Break**

10:20 AM **Experiences with Precision Livestock Farming in Europe**
Daniel Berckmans *166*

11:05 AM **Precision breeding to advance animal health and welfare**
Randall Prather *167*

11:50 AM **Panel Discussion**

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

Initial Remarks from Moderator

Max Armstrong

Farm Progress America, St. Charles, IL

Abstract pending.

Speaker Biography: Max Armstrong, The Voice of American Agriculture, anchors the Penton Agriculture broadcast group that includes television, radio, enhanced Web content, custom video, and custom programming.

Millions of farmers, ranchers and consumers have viewed Max's TV programs and heard his radio broadcasts during his more than 30 years of industry experience. He is one of the most widely recognized and highly regarded agricultural journalists in America.

You can hear him on radio stations throughout the country with weekday broadcasts of his ag perspectives on "Farm Progress America" programs and his wit, wisdom and observations in "Max Armstrong's Midwest Digest" segments; and weekly co-hosting the "Saturday Morning Show" on the legendary radio powerhouse, WGN radio.

He is co-founder and co-host of "This Week In AgriBusiness," broadcast on the popular RFD-TV satellite and cable channel that is carried on more than 120 additional local television stations throughout the nation's best ag areas. Max and Orion Samuelson host this highly regarded weekly ag business and news program 52 times each year.

In pursuit of the news of agriculture, Max has originated broadcasts from every U.S. state and more than 30 nations. His work has earned dozens of honors from agriculture groups, trade associations and professional organizations.

From his boyhood of growing up on a farm near Owensville, Indiana, to his years in Chicago radio and television, Max's background and experience have developed to give him the perspectives and industry access to produce his insightful broadcasts. He maintains close ties with agriculture and proudly displays his boyhood 1953 Farmall Super H tractor at parades, fairs and festivals.

Economic challenges and opportunities facing U.S. animal agriculture

David Kohl

Dept. of Agricultural and Applied Economics, Virginia Polytechnic Institute and State University, Blacksburg, VA

Abstract pending.

Speaker Biography: David Kohl received his M.S. and Ph.D. degrees in Agricultural Economics from Cornell University. For 25 years, Kohl was Professor of Agricultural Finance and Small Business Management and Entrepreneurship in the Department of Agricultural and Applied Economics at Virginia Tech, Blacksburg, Virginia. He was on special leave with the Royal Bank of Canada working on advanced initiatives for two years, and also assisted in the launch of the successful entrepreneurship program at Cornell University. Kohl is Professor Emeritus in the Agricultural and Applied Economics Department at Virginia Tech.

Kohl has traveled almost 9 million miles throughout his professional career! He has conducted more than 6,000 workshops and seminars for agricultural groups such as bankers, Farm Credit, FSA, and regulators, as well as producer and agribusiness groups. He has published four books and over 1,500 articles on financial and business-related topics in journals, extension, and other popular publications. Kohl regularly writes for *Corn and Soybean Digest*, and other ag lending publications.

He has received 11 major teaching awards while teaching over 10,000 students, and 18 major Extension and Public Service awards from Virginia Tech, Cornell University, and state and national organizations. Kohl is a two-time recipient of the prestigious American Agricultural Economics Association's Outstanding Teaching Award. Kohl is one of only five professors in the nearly 100-year history of the Association to receive the award twice. He received the Governor's award for his distinguished service to Virginia agriculture, the youngest recipient to receive this award. Kohl was recognized as one of 30 leaders who are the brains behind innovative business management and economic information that agriculture uses today. He also has been named one of 7 economists and bankers who challenge the status quo.

Kohl has addressed the American Bankers Agricultural Conference for more than 35 consecutive years, and has appeared before numerous state bankers' schools and conferences throughout the U.S., Canada, Mexico, and the world. He has also been one of the top rated instructors at the LSU and Colorado Graduate Schools of Banking, and is Chancellor of Farm Credit University, which has trained over 2,000 lenders using an online and face-to-face educational approach.

As facilitator of the United States Farm Financial Standards Task Force and member of the Canadian Agricultural Financial Standards Task Force, Dr. Kohl was one of the leaders in establishing guidelines for the standardized reporting and analysis of agricultural producers' financial information on a national and international basis. The Kohl Agribusiness Centre has been established in the College of Agriculture and Life Sciences at Virginia Tech in honor of his long-term commitment to practical teaching, research, and extension.

Kohl is currently President of AgriVisions, LLC, a knowledge-based consulting business providing cutting-edge programs to leading agricultural organizations worldwide. He is also a business coach and part owner of Homestead Creamery, a value added dairy business in the Blue Ridge Mountains.

On a more personal note, Dave enjoys playing basketball and likes most sports, farms, dogs, and covered bridges; he dislikes lazy students, administrative bureaucracies, and paperwork!

Challenges and Opportunities for US Animal Agriculture: Meeting the demands of global and domestic markets while fighting burdensome regulation

Dale Moore

American Farm Bureau Federation, Washington, DC

Abstract pending

Speaker Biography: Dale is the Executive Director for Public Policy at American Farm Bureau Federation.

Dale joined AFBF in November 2011 as deputy executive director for Public Policy and as manager of the agriculture and trade policy team. Dale brings 30 years of experience in public policy and communications to Farm Bureau.

In 2001, Dale was appointed by President Bush as USDA's chief of staff and served all four individuals appointed as Secretary of Agriculture by the President (Ann M. Veneman, Mike Johanns, Chuck Conner (acting) and Ed Schafer). He also provided transition assistance to President Obama's agricultural advisors.

He also spent over a dozen years on Capitol Hill, working in various positions for the House Agriculture Committee and in the personal office of (now) Sen. Pat Roberts (KS) when he served in the House of Representatives.

Just prior to joining Farm Bureau, he worked for Policy Directions Inc. as a lobbyist, providing strategic planning and representation for a variety of agricultural and food industry clients. Dale has also worked for two livestock associations. He was executive director for legislative affairs of the National Cattlemen's Beef Association in the late '90's, and in the early "80's as the communication director for the Kansas Pork Producers Council. He also has worked as a writer and photographer for Blue Cross and Blue Shield.

Dale received a bachelor of science degree in animal science from Fort Hays State University (Hays, Kansas) and grew up in southwest Kansas on a livestock, hay and grain farm.

He and his wife, Faith, have two grown sons and are the proud grandparents of three grandkids.

Experiences with Precision Livestock Farming in Europe

Daniel Berckmans

Division M3-BIORES, Dept. of Biosystems, Heverlee, Belgium

The worldwide demand for meat and animal products might increase by 40% in the next 20 years. A question is how to achieve high-quality, sustainable and safe meat production that can meet this demand. At the same time, livestock production is currently facing serious problems such as animal health in relation to food safety and human health. Europe wants improved animal welfare and has made a significant investment in it. At the same time, the environmental impact of the livestock sector is far from being solved. Finally, we must ask how the farmer, who is the central stakeholder in this process, will make a living from more sustainable livestock production.

One tool that might provide real opportunities and solutions to make farmers more competitive is Precision Livestock Farming (PLF). PLF systems aim to offer a real time monitoring and managing system for the farmer. This is fundamentally different from all approaches that aim to offer a monitoring tool without improving the life of the animal under consideration on that moment in the process. The idea of PLF is to provide a real-time warning when something goes wrong so that immediate action can be taken by the farmer. Continuous, fully automated monitoring and improvement of animal health, welfare, yields and the environmental impact will become possible.

In this paper several examples are given of PLF systems that are operational today in about 60 compartments all over Europe for fattening pigs and broilers. We give details of which variables these systems measure in real time in a fully automated way. Moreover, we show how in the running EU-PLF project we analyse how these data can generate added value for the farmer. PLF systems can replace the ears and the eyes of the farmer and work 24 h a day and 7 days a week. The challenge now is to show how the farmer gets an advantage from these systems as we start to see in the EU-PLF project.

Collaboration between “animal people” (physiologists, veterinarians, ethologists, etc.) and technical people is needed to make these systems become real support systems for farmers.

Speaker Biography: Professor Daniel Berckmans, Department of Biosystems, Division M3-BIORES: Measure, Model & Manage Bio responses, KU Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium. Daniel.Berckmans@biw.kuleuven.be. Daniel Berckmans obtained a Masters in Bio-engineering and received his PhD (1986) in Agricultural Sciences from Katholieke Universiteit Leuven, Belgium. Since 1998, he is Full Professor at the K.U. Leuven and Head of the Division M3-Biores (Measure, Model & Manage Bio-responses). Daniel Berckmans is also Coordinator of the European Committee for Precision Livestock Farming (since 2003). During the last 20 years, the research group M3-Biores, under the guidance of Daniel Berckmans, expanded and for the last 15 years continuously counts more than 20 researchers who prepare their PhD. The main field of research consists of real time signal analysis of humans, animals and plants including bio-environmental monitoring and management. The focus of the research team lies on the development of real time algorithms to monitor and control Complex, Individual and Time varying Dynamic (CITD) living organisms. The group is doing research and pioneering with the approach of Precision Livestock Farming since 1991. The team has over 230 journal publications, 14 patents and generated 15 products in collaboration with industry, including stress monitoring in race car driving, lameness detection in dairy cows and a pig cough monitor.

Precision breeding to advance animal health and welfare

Randall Prather

Division of Animal Science, University of Missouri, Columbia, MO

Genetic selection and breeding programs have resulted in remarkable improvements in almost every aspect of production. Improvements have been achieved for litter size and feed efficiency, and there have been improvements in carcass quality, etc. Unfortunately, in many cases the genes responsible for the traits are known, but breeding and selection programs have not addressed concerns.

Since the genome provides the blueprints for making the cell and the animal, if we alter the blueprints, then we will alter the animal. At a simplistic level the genome is composed of individual genes. These genes code for proteins. Proteins are the tools, and parts of the tools, that makeup and comprise the cell. An analogy may be a hammer; which is composed of two parts, the handle and the head. In this analogy, each may be coded for by a different gene; one for the hammer and one for the handle. The two proteins/parts must self-assemble and fit together or they cannot function. Similarly, the head has at least two functions, 1. To hammer nails, and 2. To pull nails.

New technologies have recently been introduced that permit quick and efficient editing of the genome. Altering individual genes/blueprints in the genome will result in altering individual proteins. To continue the hammer analogy, we could change the blueprints/genome and make the handle longer/shorter, and/or fatter/thinner. Similarly, we could make the head larger/smaller and the prongs longer/shorter. Such changes could be brought about by, for example, adding a longer coding region that adds additional amino acids; thus making the protein longer. Alternatively, the gene encoding the handle could be disrupted so that a functional handle is not made, and then you could neither drive nails, nor pull them out; this would be a knockout. In some cases, a knockout can be made by changing a single base (letter) of the genome. The pig genome contains some 3,000,000,000 bases (letters). Alternatively, longer sequences of a gene can be moved between breeds of cattle, or even between species.

One area of application of genome editing is resistance to disease; e.g. porcine reproductive and respiratory syndrome virus (PRRSV) and African Swine Fever virus (ASFV). For PRRSV the protein that the virus uses to infect the cell has been identified and knocked out. Pigs that have this gene knocked out are resistant to North American and European strains of PRRSV. A genetic element from warthogs that confers resistance to ASFV has been introduced into domestic pigs; it remains to be seen if they are resistant to ASFV. Genetic editing has been applied to other areas of production agriculture. The genetic sequence responsible for the polled trait in Angus has been identified and introduced into Holsteins; thus making them polled.

There are other diseases, and areas of production that could be more efficient, and opportunities to improve other traits, e.g. carcass quality growth, sex ratio. Genetic modification technology has also been used to create models of human diseases like cystic fibrosis, pharmaceutical factories, regenerative medicine and to create pig organs that can be transferred to humans. The most limiting factor to genetic modifications in pigs is our imagination.

Speaker Biography: Dr. Randall Prather is a Curators' Professor and Distinguished Professor of Reproductive Biotechnology at the University of Missouri, where he also serves as Associate Leader of the *Food for the 21st Century* Reproductive Biology Cluster. He earned his BS and MS from Kansas State University, and PhD and Postdoc from the University of Wisconsin-Madison. Since 2003, Dr. Prather has also provided leadership for UMC's National Swine Research and Resource Center as its Co-Director. Under his leadership the center has produced over 1000 cloned pigs representing wild types and over 40 different genetic modifications. Dr. Prather's research has focused on the early mammalian embryo. His group has been a leader in developing pig models for human diseases such as cystic fibrosis and advancing the potential for using genetically engineered pigs for human organ transplantation. In 2015, he led a team of researchers that utilized gene editing to produce pigs resistant to porcine reproductive and respiratory syndrome virus which costs the global swine industry over $800 million per year.

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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 1

Mycotic myocarditis in a dog attributed to infection with *Triadelphia sp*.

Ashleigh Hall[1], Scott Talent[1], Haley Bates[1], Rupika Desilva[1], Johnson Chris[2], Steven Hodges[2], Adrienne Hale[2], Akhilesh Ramachandran[1], Keith L. Bailey[1]

[1]Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, OK;
[2]Oklahoma Veterinary Specialists, Tulsa, OK

Triadelphia spp. are soilborne dematiaceous fungal organisms. Infections with *Triadelphia* spp. have been reported in humans. Here we report infection in a 2-year-old German shepherd dog. The patient had been on long-term prednisone and mycophenolate treatments for a perianal fistula when it presented to a private veterinary clinic following one week of lethargy and reduced appetite. The dog was diagnosed with pericardial effusion and underwent serial pericardiocentesis procedures, however its condition continued to deteriorate. Euthanasia was performed due to poor prognosis. Hemorrhagic pericardial fluid was submitted to the Oklahoma Animal Disease Diagnostic Laboratory for culture. On postmortem examination, the pericardium was thickened and the heart contained multiple granulomas. Histopathology revealed granulomatous and pyogranulomatous myocarditis with myriad intra-lesional fungal hyphae. Fungal colonies recovered from the pericardial fluid were identified as *Triadelphia* sp. by 28s rDNA sequencing. To our knowledge, this is the first report of myocarditis in a dog attributed to infection by *Triadelphia* sp.

Poster 2

Change in resistance of *Pasteurella multocida spp multocida* and *Mannheimia haemolytica* isolates obtained from bovine pneumonic lungs from 2008 through 2015 to selected antimicrobial agents

Arthur Hattel, Subhashinie Kariyawasam, Thomas Denagamage, Jason W. Brooks, Jenny Fisher

Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA

This retrospective study was conducted to determine if antibiotic resistance developed for seven antibiotics commonly used to treat bovine respiratory bacterial pathogens by comparing antibiotic resistance data of *Pasteurella multocida spp multocida* and *Mannheimia haemolytica.* Isolates were recovered during necropsy from the lungs of cattle with bronchopneumonia from 2008 through 2011 and compared with those similarly collected from 2012 through 2015. The minimal inhibitory concentration for each antibacterial agent obtained by a broth microdilution technique was used to determine if a bacterial isolate was resistant or sensitive to an antibiotic. Data comparison was performed on a percentage resistance to seven individual antibiotics. Isolates (49) of *P. multocida spp multocida* from pneumonic lungs obtained from 2012 through 2015 displayed less resistance to ceftiofur, chlortetracycline, danofloxacin, florfenicol, oxytetracycline and tilmicosin than those isolates (74) of *P. multocida spp multocida* obtained from 2008 through 2011. No change in the percentage resistance was noted for enrofloxacin. Isolates (36) of *M. haemolytica* from pneumonic lungs obtained from 2012 through 2015 displayed less resistance to chlortetracycline, danofloxacin, florfenicol, oxytetracycline and tilmicosin than those isolates (66) of *M. haemotycia* obtained from 2008 through 2011. An increase in resistance to enrofloxacin was noted. No change in the percentage resistance was noted for ceftiofur. In conclusion, there was no emergence of antibiotic resistance observed in the isolates of *P. multocida spp multocida* and *M. haemolytica* for the antibiotics commonly used to treat bovine respiratory infection with the exception of *M. haemolytica* for enrofloxacin.

Poster 3

Vet-LIRN proficiency test to detect *Listeria* in raw dog food

Christopher Powers[2], Sarah Nemser[1], Samantha Lindemann[3], Matthew Kmet[2], Andriy Tkachenko[1], Ravinder Reddy[3], Renate Reimschuessel[1]

[1]CVM, FDA, Laurel, MD; [2]Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL; [3]CFSAN, FDA, Bedford Park, IL

Listeria monocytogenes is a pervasive foodborne pathogen that is identified as a leading cause of death in connection with foodborne illness. In 2013, the CDC reported 13 deaths attributed to *Listeria*. Recent research demonstrates that *L. monocytogenes* in raw pet food can lead to illness in domesticated pets and subsequent transmission of *L. monocytogenes* from pets to humans. Heightened surveillance by the Food and Drug Administration in the past year has resulted in an increased number of pet food product recalls citing potential *L. monocytogenes* contamination. The aim of Vet-LIRN's proficiency test (PT) was to evaluate analyst capability of network laboratories to detect and identify *Listeria* species and *L. monocytogenes* at low levels in raw dog food. A total of 37 analysts from 26 network laboratories participated in the proficiency study. The matrix for the PT samples was composed of commercially available raw chicken dog food. The matrix was tested and verified as negative for *Listeria* species prior to inoculation. PT samples consisted of approximately 25 grams of product inoculated with *L. monocytogenes*, *L. welshimeri*, or *L. innocua* along with uninoculated samples. Prior to distributing PT samples, homogeneity and stability studies were completed. Target inoculation levels included 125cfu per sample as well as 10cfu, 5cfu, and 0.5cfu per sample to test the capabilities of the laboratories' methods near the limit of detection. The assigned values for PT samples were determined based on consensus according to ISO 13528:2005 (Statistical Methods for Use in Proficiency Testing by Inter-laboratory Comparisons) and ISO/IEC 17043:2010 (Conformity assessment – General requirements for proficiency testing). Following 42 CFR, consensus agreement is defined as ≥80% agreement among analyst results. If consensus is not met for a set of samples, those samples are not assigned scores. For example, 32% of analysts reported positive detection in samples spiked at 0.5 cfu per sample; therefore, those samples were excluded from scoring. Overall, 95% of analysts had satisfactory results. No false positives were reported by PCR methods and 5.5% of analysts reported false positives by culture methods. The study revealed that a majority of analysts are proficient to detect *L. monocytogenes* and other *Listeria* species at low levels in raw pet food.

Poster 4

Challenges in laboratory characterization of *Aeromonas salmonicida* §

Timberly Maddox, Tessa LeCuyer, Dubraska Vanessa Diaz-Campos, Kevin R. Snekvik

Washington State University, Pullman, WA

Aeromonas salmonicida, the causative agent of furunculosis, is an important pathogen of fish. Disease severity is variable, ranging from subclinical infection to acute mortality due to septicemia. Over the past 20 years, we have isolated typical strains from brook trout, cutthroat trout, Coho salmon, rainbow trout, Atlantic salmon, and arctic char and atypical strains from halibut, carp, burbot, koi, goldfish, Atlantic salmon, and sablefish from across the western United States. Testing for *A. salmonicida* is outlined in the USFWS/AFS-FHS Standard Procedures for Aquatic Animal Health Inspections "Blue Book". The identification of typical *A. salmonicida* ssp. *salmonicida* isolates is well described, but biochemical reactions associated with the atypical strains are variable. A 2008 USFWS Ring Test showed that many laboratories performed poorly at identifying *A. salmonicida* ssp. *achromogenes*, and our laboratory has also recognized the difficulty in identifying other atypical isolates. The aim of this study was to identify a set of biochemical tests that may help to identify typical and atypical *A. salmonicida* strains. To identify *A. salmonicida*, the Blue Book indicates that the isolate should be oxidase-positive, non-motile, and ferment glucose. If an organism does not ferment glucose, it is not considered to be *A. salmonicida*. Pigmented gelatinase-positive and indole-negative isolates are presumptively *A. salmonicida* ssp. *salmonicida*, and non-pigmented gelatinase-negative and indole-positive organisms are presumptively *A. salmonicida* ssp. *achromogenes*. Isolates that do not follow these patterns are considered non-*A. salmonicida*. Our lab isolated organisms from diseased fish that differ from the recommended biochemical profiles, but that were determined to be most likely *A. salmonicida* based on 99-100% sequence identity of the *rpoB* gene and/or the 16S RNA gene compared with *A. salmonicida* sequences in GenBank. Atypical isolates from our lab include a gelatinase and indole-positive isolate, as well as a glucose-negative isolate and a motile isolate; the latter two would have been ruled out as *A. salmonicida* based on the current identification scheme. Use of API test strips has been described in the Blue Book to make *A. salmonicida* identification, but we noted differences in biochemical reactions at 24 hours versus 48 hours incubation time. We observed biochemical variability over time such as a change from Voges–Proskauer negative to positive (2 isolates), motility from negative to positive (1), glucose fermentation negative to positive (1), and amylase fermentation negative to positive (4). The biochemical variability described here supports the need for more genetic and biochemical characterization of the full diversity of *A. salmonicida* subspecies in order for laboratories to correctly identify this important pathogen.

§ AAVLD Laboratory Staff Travel Awardee

Poster 5

When routine encounters a select agent: A lesson on *Burkholderia pseudomallei* and the importance of universal laboratory diagnostic precautions §

Kyriakos Deriziotis, Anil J. Thachil

Animal Health Diagnostic Center - Bacteriology, Cornell University College of Veterinary Medicine, Ithaca, NY

Kyriakos Deriziotis, Jennifer Moody, Rebecca Franklin-Guild, and Dr. Anil Thachil

Burkholderia pseudomallei is designated by the CDC as Tier 1 Select Agent that can infect both humans and animals. It is a Gram-negative, aerobic, motile, bipolar-staining rod-shaped bacterium. It is able to grow on a variety of culture media, and presents wrinkly, metallic-looking colonies with an earthy odor. It is rarely seen in the Western hemisphere, and as such, can be overlooked in the lab as a contaminant that is considered insignificant. A routine urine culture, to rule out a UTI for urinary incontinence, was requested from our lab for a 4 year old mixed breed canine which was in for a rehabilitory consult for paralysis. Aerobic bacterial culture revealed pinpoint-sized colonies at 24 hours. At 48 hours, it appeared as white, mucoid colonies on TSA blood agar, EMB, and CNA culture plates. The MALDI-TOF Biotyper identified it as *Burkholderia thailandensis*. As it was closely related to *Burkholderia pseudomallei,* further biosafety precautions were taken. The culture and sample were moved into the BSL-3 facility with limited access. Further in-house testing did not match the phenotypic profile of *Burkholderia pseudomallei.* The isolate was sent out to Wadsworth Center Biodefense Laboratory at the NYSDOH. It was reported at the time as 'Most closely resembling *Burkholderia sp.'* The Wadsworth Center shortly thereafter identified it as 'presumptive *Burkholderia pseudomallei'* by PCR, which was later confirmed by the CDC as an 'atypical strain of *Burkholderia pseudomallei.'* All personnel who handled the sample, as well as the owner of the dog, were notified and advised to take special precautions. Medical consultation, monitoring, and follow-up was provided for identified personnel based on risk analysis. Presumptive identification criteria for Burkholderia species in our laboratory was reestablished based on our experience with an atypical variant of *Burkholderia pseudomallei.*

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Poster 6

Comparison of the Clinical Presentation and Urinalysis Results of Companion Animals with *Staphylococcus spp.* and *E. coli* Bacteriruria (2008-2014)

Stephen Cole, Shelley C. Rankin

UPenn, Philadelphia, PA

Urinary tract infection (UTI) is a common clinical problem in companion animals and a leading indication for the administration of antimicrobials. The most common cause of bacterial UTI in dogs and cats is *E. coli;* however, it is not uncommon to isolate *Staphylococcus* from urine cultures. The goal of this study was to evaluate the clinical presentation and urinalysis results from dogs and cats with *Staphylococcus* urine cultures and compare to those of patients with *E. coli* bacteriuria. A total of 117 dogs and cats (representing 126 cultures) with staphylococcal bacteriuria were included in the study. Exclusion criteria included insufficient data in the medical record and submission of a non-cystocentesis sample (34/160 excluded). An equal number of *E. coli* urine cultures from the same time period, chosen using a random number generator, were used as a control group. The most common species isolated was *Staphylococcus pseudintermedius* (99/126, 78.5%). About 20% (26/126) of all *Staphylococcus spp.* isolates were methicillin-resistant (MR). All MR isolates were from dogs. *E. coli* (85/117) and *Staphylococcus spp* (93/126) were isolated from cats and dogs in similar proportions. The average age of patients with *Staphylococcus spp*. (7.86 years) was 1.5 years younger than patients with *E. coli* (9.36 years). About three times as many *Staphylococcus* isolates (45.7%, 42/92) were isolated from basic urine (pH>7) than *E. coli* isolates (16.7%, 15/90). Significantly more *Staphylococcus spp.* patients had concurrent urolithiasis (51.4%, 37/72) when compared to E. coli patients (2.6%, 2/78, P<0.001). Both of these findings are consistent with urease production by staphylococci and their subsequent role in urolith pathogenesis. Significantly less cases of staphylococci (11.1%, 8/72) were associated with chronic kidney disease when compared to *E. coli* (30.8%, 24/78, p=.003).
We hypothesized that concurrent dermatologic and gastrointestinal signs would be associated with *Staphylococcus spp.* and *E. coli* UTI, respectively. More than twice as many *E. coli* UTI (34/124) cases had concurrent GI signs, supporting our hypothesis (P=.01). However, no significant difference was found between dogs infected with *E. coli* and *Staphylococcus spp.* with regards to concurrent dermatologic lesions. This study highlights differences in the clinical presentation and urinalysis results for dogs and cats with bacteriuria. Empirical antibiotic selection can be informed by clinical differences identified in this study. Future directions include understanding the molecular epidemiologic relationship of *S. pseudintermedius* as a uropathogen and the underlying bacterial physiology.

Poster 7

Pseudomonas aeruginosa mastitis in two goats associated with contaminated essential-oil based teat dip

Jane Kelly[1,2], David J. Wilson[1,2]

[1]Animal Dairy and Veterinary Sciences, Utah State University, Springville, UT; [2]Utah Veterinary Diagnostic Lab, Logan, UT

Pseudomonas aeruginosa is an opportunistic pathogen that has been associated with mastitis in dairy animals including goats. Often, the environmental sources of the bacteria are water-related (such as hoses and muddy pastures). In the case reported here, *P. aeruginosa* mastitis in two dairy goats in a small herd was investigated to determine the source of infection. Multiple samples from the goats' environment were cultured including water from the trough, bedding, the hose used to wash udders, and the teat dip and teat dip containers. The bacterium was isolated from the teat dip and the teat dip container. The teat dip consisted of water, liquid soap, and several drops of essential oils (including tea tree, lavender, and peppermint). The owners culled the two goats and switched to a commercial teat dip and have not had any *P.aeruginosa* mastitis since. To the authors' knowledge, this is the first report of *P. aeruginosa* mastitis in small ruminants caused by contamination of teat dip made up of essential oils in water.

Poster 8

The Futures Laboratory: A virtualized collaborative space meant to foster communication and cooperation between DoD and non-DoD academic institutions in areas of common interest, including public health/One Health

Robert A. Norton[2], Stephanie Renee Ostrowski[1], James C. Wright[1]

[1]Pathobiology Department, College of Veterinary Medicine, Auburn University, Auburn, AL; [2]Poultry Science, College of Agriculture, Auburn University, Auburn, AL

The Futures Laboratory is a virtualized and physical collaborative space located in Montgomery, Alabama which is designed to foster communication, as well as research, teaching, and training collaboration between civilian academic institutions in the Southeastern United States with Department of Defense (DoD) entities located in this region (U.S. Air Force [Air University], U.S. Special Operations Command [USSOCOM] and U.S. Army [The Maneuver Center – Fort Benning, GA]) on topics of common interest. The Futures Laboratory was established by a group of academics who realized that DoD and non-DoD research interests are frequently the same, including--but not limited to--Public Health/One Health, yet often characterized by duplication of effort and inefficiency. Individuals in the DoD and non-DoD domains are frequently not even aware of those housed in institutions outside their domains. It was the founding academic member's sincere conviction that these "stove piped" efforts had to be modified so that communication and partnering could be developed in a timely manner. DoD is concerned about Public Health/One Health from multiple perspectives including, 1) Force Protection involving humans teamed with service animals [dogs and horses], 2) Military Force Projection and Operational Capability (involving service animals); 3) Civil Affairs and Humanitarian Assistance in war zones, Transnational Border Regions and "Gray Zones"; and 4) Emergency and Disaster Response (domestic and international). Civilian academic institutions are often involved in similar research, teaching and outreach efforts involving these same regions through the efforts of academics based in Colleges of Veterinary Medicine and Agriculture. The Futures Laboratory's Virtualized Collaborative Space (VCS) is located at: https://community.apan.org.wg/futures-lab/ within the DoD sponsored All Partners Access Network https://www.apan.org/ and sponsors monthly seminars on topics of mutual interest.

Poster 9

Current situation assessment of biosecurity measures in small scale broiler poultry farms and backyards in Egypt * ◊

Asmaa Nady Mohamed[1], Hassan E. A. Helal[2]

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A cross-sectional study was designed to assess the current situation of biosecurity measures in small scale commercial poultry farms and backyards (Sector III and IV). A total of (*n*=50) small commercial poultry farms and back yards were selected from each Giza, Beni-Suef and Fayoum province. A structured pre-tested questionnaire survey and observation were used for collecting data on risk indicators, bio-security measures and poultry health practices in both examined sectors. The respondents were farm owners, farm managers, veterinarians and workers. The results showed that the number of broiler birds reared per cycle of small commercial farms was from (*n*= 6000-20000) compared with (*n*=30- 150) birds in backyards, Small scale broiler farms had a higher level of biosecurity than the backyards. 89% of the farms practiced all in all out system when compared with 22.1 % of the backyards which had less secure boundaries; 31% of farms did not have fence when compared with 69% of the broiler farms. Only 6.1% of broiler farms had used disinfectants at gate. On the other hand, health practices in side small scale broiler farms were followed by veterinarian (74.3%). Mortality rate/cycle was (10%) in almost broiler farms. Newcastle, IB, Gumboro, marek's and HPAI-vaccination against H5N2 and H9N2 (100%) compared with no vaccination program held in backyards. The percentages of parasitic infestation (3.1%) in broiler farms compared with (30.4%) in backyards. Disinfections of farms in between cycles represented 86.5% by using virkon's, iodine and phenol meanwhile, in backyards, disinfection didn't apply. In broiler farms, landfills were used for carcasses disposal. In conclusion, the majority of the small scale broiler farms and all most backyards were far from the implementation of biosecurity measures. Many farm workers don't know how to maintain and improve biosecurity to protect the poultry and themselves from diseases risks. Biosecurity situation needs a combined effort from stakeholders, small breeders of hobby bird to improve biosecurity level for these sectors.

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster 10

Genotyping of *Mycobacterium bovis* from ruminants in Taiwan during 2014-2016

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Bovine tuberculosis (bTB) is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In Taiwan, the intradermal tuberculin test (ITT) is the routine method to test bTB every year in cattle, dairy goats, and domestic deers (Formosan Sambar, *Cervus unicolor swinhoei*, Sclater). The policy until now still is "ITT positive and slaughter", but we found bTB cases every year. From 2014 to 2016, twenty-four *M. bovis* strains from 13 cattle herds and 1 deer herd were isolated from ITT-positive animals. In this study, we used Spoligotyping and MIRU (8 different loci, ETRA, ETRB, ETRC, MIRU 4, MIRU 16, MIRU 20, MIRU 24, and MIRU 31) as genotyping methods which were commonly used for genotyping of *Mycobacterium tuberculosis* complex. Two spoligotypes and 5 MIRU types were identified and the discriminatory power was 0.73. The main type, type 1(SB0265-53333223), was the largest population (41.7%, 10/24) distributed across northern, middle and southern Taiwan, and the deer strain, the only one, was also belong to this type. Type 3 and 4 were the second and third most found respectively in southern and middle Taiwan. We need more epidemiologic researches to clarify the reasons why bTB persistent to occur in Taiwan.

Poster 11

Modeling condemnation cases in cattle slaughter plants in California ◊

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The cattle industry is the largest segment of US agriculture. In 2015 the US commercially slaughtered 28.74 million head with a total carcass weight of 23.69 billion pounds [1]. Based on data from USDA in 2015, 141,450 carcasses were condemned in the US, approximately 0.5% of the total cattle carcasses produced. Beef price in 2015 was $6.29/lb1 resulting in a cost of about $0.81 billion (0.5% x 23.69 billion lb x $6.29/lb) to US producers. California (CA) has one of the most important cattle industries in the US. A total of 21.3% of all condemnation cases from 2005-2015 in slaughter plants in US occurred in CA (USDA), which corresponds to approximately to $1.38 billion (307,966 head condemned x 714 lb/carcass x $6.29/lb) over the 10 years and $0.18 billion in 2015.

First aim was to calculate the slope of the reported condemnation reasons in CA and the US from 2005-2015 based on a smoothed random walk [2]. Second, to identify cattle condemnation cases that showing seasonality by fitting an auto regression spectrum model to the data, for which the model order was identified by the Akaike Information Criteria. Third, to evaluate if dynamic harmonic regression (DHR) model [2] can predict the number of those condemned cases that have seasonal component 3 month ahead from 2012-2015 to inform cattle producers and related stakeholders to prevent/ minimize carcasses condemnation in CA.

A USDA:FSIS database with detailed information on condemnation cases in the US from 2004 to 2015. The information corresponds to a total of 684 slaughter plants in the US (29 in CA). The majority of the condemned reasones in US and CA have a slope of zero which indicate no changes in the number of reported cases. Others, such as the number of condemnations due to Epithelioma, Malignant Lymphoma, Pericarditis and Emaciation in both CA and the US are decreasing (slope<0) from 2005-2015 but the decrease in the number of cases in the US is steeper than in CA. The number of condemnations due to Icterus, Pneumonia, Abscess/Pyemia, Misc. Inflammatory Diseases, Septicemia and Peritonitis shows clear evidence of increase (slope>0).

Based on the Auto-Regressive spectrum results four condemnation cases are showing seasonal components in CA, which are Abscess/Pyemia, Emaciation, Epithelioma and Malignant Lymphoma. The DHR model could predict the number of Abscess/Pyemia, Emaciation, Epithelioma and Malignant Lymphoma cases 3 month ahead with mean relative prediction error of %23, %32, %28 and %13 respectively.

These methods can be used in real-time to identify emerging reasons for condemnation cases and inform educational, syndromic and risk-based surveillance programs.

[1] National Cattlemen's Beef Association. Beef Industry Statistics. Available at: http://www.beefusa.org/beefindustrystatistics.aspx (2016).

[2] Taylor, C. J., Pedregal, D. J., Young, P. C. & Tych, W. Environmental time series analysis and forecasting with the Captain toolbox. Environ Modell Softw 22, 797-814, (2007).

◊ USAHA Paper

Poster 12

Devising a disease surveillance and reporting system using Orchard® Harvest™ LIS *

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Appropriate disease surveillance in a hospital setting paves the way for proactive outbreak control and mitigation, in addition to providing crucial information regarding changes in pathogen populations of the clientele. Virginia Tech Laboratory Animal Services (ViTALS) serves as the central diagnostic laboratory for the Virginia Teaching Hospital (VTH). This project incorporated utilities contained within the Orchard® Harvest™ laboratory information system (LIS) program to devise a disease surveillance and reporting system.

This project was implemented over the course of several phases. Reportable diseases were identified as defined by the hospital director, the Virginia Department of Agriculture and Consumer Services (VDACS), the United States Department of Agriculture (USDA) through the National Animal Health Reporting System (NAHRS), and the World Organisation for Animal Health (OIE). In-house diagnostic tests capable of screening for these select agents were identified. Using Orchard®'s Rules and Data Browser utilities, the results of the appropriate diagnostic tests are matched to the list of reportable diseases. Through the Rule utility, if a match is made, an e-mail is generated including patient identifiers and is automatically sent to defined hospital section heads. The Browser utility is implemented to monitor incidence of organisms of concern over a month long period.

Creation of this system identified minor underlying issues with data input in the Orchard® system as it is used in ViTALS. Some of the in-house diagnostic tests are capable of automatically importing results into Orchard®, however, this sometimes occurs in the form of a .pdf file; Orchard®'s Rules and Browser utilities do not currently have a method for scanning .pdf files for key terms. Other results are put into Orchard® by trained technologists in the diagnostic laboratory, however, some minor variations in data input contributed to Rules and Browsers failing to match reportable organisms. When the data is input correctly, Rules and Browsers are triggered as expected.

Although there are some issues that need to be addressed with data input and Rule and Browser triggering, this project lays the framework for a disease monitoring and reporting system using a common market LIS program, and may be adapted for use in other veterinary hospitals across the globe.

* Graduate Student Poster Presentation Award Applicant

Poster 13

The epidemiology of *Campylobacter jejuni* and *Campylobacter coli* in geese in Taiwan

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Campylobacteriosis is one of the most frequently occurring acute gastroenteritis in human worldwide and *Campylobacter jejuni* and *C. coli* are mostly related to human illness. Because the main carriers of *Campylobacter* spp. are domestic poultry such as chickens, geese and ducks, campylobacteriosis is mainly caused by consuming of undercooked and cross-contaminated poultry products. Moreover there are many other routes of human campylobacteriosis, so further epidemiological study is very important to control campylobacteriosis cases. Many reports indicate an increased multiple antimicrobial resistance of animal and human *Campylobacter* isolates worldwide that results in failure of treating severe patients of campylobacteriosis by fluoroquinolones. Therefore, it is necessary to understand the epidemiology and antimicrobial resistance of *Campylobacter* spp. from human and animal in Taiwan. We are going to survey the prevalence and antimicrobial resistance of *C. jejuni* and *C. coli* in geese in Taiwan. Then we will use the multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) to trace the genetic relatedness of the *Campylobacter* isolates from domestic geese. The results of these studies will contribute important information for the control campylobacteriosis outbreak by identifying the infection source and intervening the transmission route.

Poster 14

Field trial using a combined treatment of garlic and organic spray based formula for fly control and animal's defensive behaviour alleviation in cattle farms * ◊

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Background: The global problem of fly resistance to conventional insecticides has resulted in renewed interest in organic pesticides as alternative management tools for flies' control. The present study aimed to determine flies' activity in cattle environment and evaluate the efficacy and persistent of a combined treatment of garlic based formula, pour-on and organic spray on the suppression of fly population on animals and their environment respectively.

Materials and Methods: A cross sectional study was carried out in a cattle farm during the period from February to May, 2015. The daily average of microclimatic factors (ambient temperature (^{o}C), relative humidity (%) and airspeed (knots/hr.) were measured and recorded. Monitoring of flies' count pre- and post-treatment was done on both animals using (visual observation, photographic tool) whereas, six similar sites were selected on the animals body (the neck, shoulder, backline, abdomen, limbs and tail), which are particularly attractive to flies and in their environment, flies were collected using (sticky cards). The effectiveness of tested formulae was determined by calculating the percentage reduction in flies' attack rate and animal's defensive behaviour.

Results: The highest population of flies' activity on both dairy cows and their environment was recorded in April and May months (260.0±5.28, 253.0±4.30, 457.0±7.14, 485.0±7.32) respectively. Calves barn, stall corner and animal stall appeared as predilection sites for flies' activity (503.33±7.4, 473.0±5.3 and 383.66±4.81 respectively). The percentage reduction in average flies' count was significantly ($P< 0.05$) on both calves, beef cattle and their environment (31.1, 42.6, 43.2 and 47.9 % respectively). Animal's defensive behaviour decreased post-treatment especially for tail flicks (26.3, 23.5, 11.1 and 11.6% respectively) and skin twitching (81.6, 72.5, 90.8 and 65.1%).

Conclusions: a combined treatment of garlic based formula pour-on animals with organic spray on their environment are effective at time interval once/week in knocking down flies' population, its impacts on public health and alleviate animal's defensive behaviour.

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster 15

Creating a true quality system with an electronic QMS

Sarah Obenauer

Qualtrax, Blacksburg, VA

Brenda Jackson, Quality Systems Manager with North Carolina Department of Agriculture and Consumer Sciences will discuss how they moved to an electronic quality management system, how they use their system, how the system has improved audit preparation and the long-term value of the system. As the need to manage and maintain accreditation continues, efficient methods of managing quality-related records and documents in support of ongoing compliance to quality standards is essential. While many laboratories use paper systems, file directories on a shared computer drive, or a combination of these, numerous commercial quality management systems exist that are designed to help ease the burden.

Poster 16

Development of a diagnostic duplex real-time PCR for the detection of *Mycoplasma gallisepticum* and infectious laryngotracheitis in chickens *

Rachel Jude, Naola Ferguson-Noel

Population Health, University of Georgia, Athens, GA

Mycoplasma synoviae (MS), *Mycoplasma gallisepticum* (MG), avian influenza (AI), and infectious laryngotracheitis (ILT) are economically significant respiratory pathogens affecting chickens. To effectively control these pathogens, there is increasing need for rapid detection of all relevant respiratory pathogens in order to properly diagnose flocks suspected of respiratory disease. In the first step to creating a novel respiratory panel for detection of these pathogens, we have developed a duplex real-time PCR for simultaneous detection of ILT and MG, two pathogens that are commonly confused based on clinical signs alone. The duplex targets previously described and widely used genes, the MG cytadhesion encoding surface protein (*mgc2*) and the ILT glycoprotein C protein (*gC*). In anticipation of adding the viral RNA AI to the panel, this duplex also includes an RT step in the real-time protocol. As a stand-alone test, the MG/ILT duplex is already expected to lower costs and shorten wait time for diagnostic PCR results; eventual addition of targets for MS and AI to the respiratory panel will further improve these costs and wait times.

* Graduate Student Poster Presentation Award Applicant

Poster 17

Enable the right result the first time with xeno internal positive control

Michelle Swimley, Rohan Shah, Richard Conrad

R&D, Thermo Fisher Scientific, Austin, TX

Misdiagnosis of one sick animal can endanger the health of an entire herd, affecting the producer's livelihood, and potentially damage a lab's reputation. When using real-time PCR for molecular diagnostic testing, false negatives rarely result from a lack of sensitivity. Rather, the biological material used to test these animals can contain a variety of compounds that inhibit the actual PCR chemistry. A simple way to test for PCR inhibition is to include an internal positive control. Our newly released VetMAX™ Xeno™ Internal Positive Control (IPC) products, for both DNA and RNA testing, can be easily integrated into any real-time PCR workflow, greatly reducing the likelihood of costly false negatives and thus providing confidence in the accuracy of our customer's qPCR test results.

Although Xeno has been incorporated into many of our existing Animal Health diagnostic products, these new products, which include Xeno IPC templates and Xeno IPC assays, are now conveniently available as standalone products that are directly orderable from our catalog. The Xeno IPC assay is a highly specific primer/probe mix that detects the Xeno IPC and is available in VIC and LIZ dye channel formats for flexibility of multiplexing with a variety of diagnostic assays. The Xeno IPC template, available as either DNA or RNA, was designed as a unique synthetic sequence and was evaluated against a comprehensive nucleotide database to show no off-target alignments to sequences relevant to animal health. The Xeno IPC template serves as both an internal positive control for the recovery of nucleic acid during the isolation process, and as a positive control for qPCR. It is a very effective control for qPCR inhibition, which enables the customer to be easily alerted to problematic test results, reducing the likelihood of false-negatives. Xeno IPC performance was tested and shown to be compatible with a wide variety of veterinary sample matrices and qPCR master mixes. Nucleic acid isolation was performed using MagMAX™ Total Nucleic Acid Isolation Kit with an automated Applied Biosystems™ MagMAX Express 96 instrument. Real-time PCR was performed on a 7500 Fast real-time PCR system and on a QuantStudio 5 real-time PCR system.

Recommended by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and used in our USDA-licensed kits, Xeno IPC is a trusted product intended for use in a growing Animal Health diagnostic market seeking improved quality control.

Poster 18

Development and validation of a probe hybridization qPCR for rapid identification and quantification of *Pythium insidiosum* in clinical samples §

Robert Bowden[1], April Childress[1], Galaxia Cortes[1], Jackson Presser[2], Erica Goss[2], Justin Shmalberg[1], James Wellehan[1]

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Pythium insidiosum is an emerging oomycete pathogen of the tropics and subtropics, causing severe and often fatal infections in mammals and birds. Dogs and horses are most frequently affected, but a variety of species are susceptible, including humans. As clinical presentation may be initially mistaken for fungal or bacterial diseases or neoplasia, diagnosis may be delayed. Available diagnostics include culture, serological assays, and PCR.
A probe hybridization qPCR assay was developed to target the ITS region. A sample set of sequences from over 50 environmental and clinical isolates previously identified by conventional PCR, comprising all four known clades of *P. insidosum*, were used to construct primers. In validation of the assay, the standard curve demonstrated good efficiency and analytical sensitivity, reliably detecting 10 copies. No amplification resulted from the testing of non-target organisms, including other *Pythium* sp. and *Lagenidium* sp., or from known negative samples. This assay serves as a new tool to allow for rapid identification of *P. insidiosum* and presents several benefits over traditional identification methods. First is the ability to diagnose *P. insidosum* in samples that may lack viability for culture. Second, probe hybridization qPCR is more economical, less labor intensive and provides faster turnaround time than sequencing of conventional PCR products, and can reasonably be expected to be more specific than other common methods of PCR product identification. Finally, the quantitative results generated will allow for investigations into correlations between pathogen loads and clinical outcomes. This probe hybridization qPCR provides a cost-effective and rapid method for distinguishing *P. insidiosum* from infections due to fungi and other etiologies, allowing for appropriate treatment to be provided sooner.

§ AAVLD Laboratory Staff Travel Awardee

Poster 19

The benchtop and field validation of a novel qPCR assay for the detection of *Brucella abortus* field strain and vaccine strains # * † ◊

Noah Hull[2], Suelee Robbe-Austerman[4], Jon Miller[2], William Laegreid[1,2], David Berry[1], Christine Quance[4], Christine Casey[3], Brant Schumaker[1,2]

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Brucella abortus is the etiologic agent of brucellosis. In the United States, the sole remaining reservoir is the Greater Yellowstone Area, encompassing parts of Wyoming, Idaho, and Montana. Current diagnostics are not ideal for eradication efforts. Serology is plagued by false positive tests due to cross-reacting organisms. Furthermore, serology merely indicates exposure to *Brucella spp.*, not necessarily current infection. Meanwhile bacterial culture can take up to ten days, is labor and space intensive, and presents a high risk to personnel, as brucellosis is the most commonly laboratory acquired infection worldwide. Representing the most robust *in-silico* analysis to date, 95 whole genome sequences of all known biovariants of *B. abortus* in the United States were obtained from United States Department of Agriculture – National Veterinary Services Laboratory. Novel primer-primer and primer-probe targets containing informative single nucleotide polymorphisms were identified. Forty-seven candidate sets were screened with nine candidates moving on to full validation. No one set was able to differentiate field strain from both vaccine strains (RB51 and S19). A validation box was assembled containing tissues from 99 bison and 37 cattle that were all sero-positive for *B. abortus*. The primer-primer and primer-probe sets were validated on the known culture-positive tissues from the 99 bison and 37 cattle samples from the Designated Surveillance Areas of Wyoming and Montana and experimentally infected animals at United Stated Department of Agriculture – National Animal Disease Center. Additionally, we have tested our samples on culture-negative tissues from bison and cattle. Specificity was confirmed by NCBI BLAST and showed that primers and probes were specific to *B. abortus* only. This assay shows promise toward replacing culture as the "gold-standard" for the definitive diagnosis of *B. abortus*. Development of an ante-mortem qPCR for brucellosis in multiple species using these primer sets is underway.

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

Poster 20

Real-time PCR for porcine cytomegalovirus utilizing antemortem samples

Susan Schommer[1,2], Melissa Samuel[1,2], Sabrina Hammond[1,2], Ben Jacquin[1,2], Eric Walters[1,2], Randall Prather[1,2]

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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 xenotransplantation. PCMV has been shown to reactivate when tissues from positive pigs are transplanted to baboons in xenotransplant models. This reactivation significantly decreases the survival time of donor organs. At the National Swine Resource and Research Center clients using the pigs for xenotransplantation desired an antemortem test for PCMV, so that animals could be tested before shipping and transplantation preparation. Virus levels in blood have been shown to be below the limit of detection during latent infection, but the use of oral fluid samples has not been reported. A real-time PCR assay (Mueller, N.J. et al. 2002) was adapted to include an internal control for inhibition and PCR results from antemortem samples (blood, oral fluid) were compared to results from postmortem spleen. The goal of the project was to determine how predictive PCR results on oral fluid and blood are of an animal's true PCMV status. PCR and extraction conditions were optimized by utilizing a gblock (IDT) containing the amplified region of the PCMV DNA polymerase gene. The limit of detection was 10 copies per reaction with 5 copies detected 50% of the time in spiked blood and oral fluid samples. PCR reactions were run on an ABI 7500 Fast machine using commercial reagents (Qiagen QuantiFast Pathogen + IC). The internal control was added with the sample during extraction utilizing the Zymo Quick DNA universal kit. After analyzing 10 complete sample sets (blood, spleen, and oral fluid) from PCMV positive animals 50% of blood samples were positive, while 80% of oral fluid samples were positive. These preliminary results warrant further investigation into oral fluids as a sample for PCMV antemortem screening.

Poster 21

Detection of *Toxoplasma gondii* and *Neospora caninum* in ruminant abortions by real-time PCR § ◊

Feng (Julie) Sun, Gabriel Gomez, Megan Schroeder, Andres de la Concha-Bermejillo, R. Jay Hoffman, Guy Sheppard, Terry Hensley, Pamela J. Ferro

Texas A&M Vet Med Diag Lab, College Station, TX

Protozoal-associated abortions in ruminants often times represent a diagnostic challenge using conventional methods alone. *Toxoplasma* and *Neospora* are the most common and important protozoal pathogens associated with ruminant abortion and are characterized by their ability to produce lifelong infection of the dam and, thereby, representing a viable risk for *in-utero* infection of the fetus and placenta during pregnancy. Microscopically, these organisms are not commonly identified on tissue sections and the pathological changes they produce are often obscured by autolytic changes, however, identification of these organisms is very important in managing the diseases in a herd. The objective of the present study was to utilize a Taqman® real-time PCR (qPCR) assay, for the detection of *Toxoplasma gondii* and *Neospora caninum* DNA in fresh tissue and formalin-fixed, paraffin-embedded sections from clinical samples submitted to the Texas A&M Veterinary Medical Laboratory Diagnosis (TVMDL) from 2011 to 2016. A qPCR assay targeting a gene fragment of *T. gondii* and *N. caninum*, respectively, was evaluated and shown to be sensitive and specific for the detection of *T. gondii* and *N. caninum* in clinical samples. This assay, coupled with histologic evaluation, provides a method for identifying *T. gondii* and *N. caninum* as causative agents of ruminant abortion.

§ AAVLD Laboratory Staff Travel Awardee
◊ USAHA Paper

Poster 22

Peritonitis & necrotizing hepatitis in a Quarterhorse mare with *Clostridium haemolyticum*

Kelli Almes[1], Pankaj Kumar[1], Laurie Beard[2], Tanya Purvis[1], Brian Lubbers[1], Russell Ransburgh[1], Jianfa Bai[1]

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A 20 year old Quarterhorse mare was presented to the Veterinary Health Center at Kansas State with a 2 day history of fever, anorexia, and weakness. An abdominal ultrasound revealed free fluid in the peritoneal and pleural cavities. Analysis of the abdominal fluid revealed a markedly increased total cell count with 90% neutrophils along with intracellular and extracellular rod shaped bacteria. Aerobic and anaerobic culture of the abdominal fluid yielded no growth of organisms. A complete blood count revealed a neutrophilic leukocytosis and hyperfibrinogenemia. There were also marked increases in AST, SDH, bile acids, and total bilirubin. Despite supportive treatment the horse's condition continued to deteriorate and she was euthanized two days later. Gross necropsy revealed bicavitary effusion with fibrinous peritonitis and necrosis of the left liver lobe with multiple vascular thrombi in the liver. Histopathologic examination of the tissues revealed intrahepatic thrombosis with necrosis, serositis of multiple abdominal organs, and lymphoplasmacytic and eosinophilic enterocolitis. Fluorescent antibody testing of liver for *Clostridium chauvoei, C. novyi, C. septicum,* and *C. sordellii* was negative. Anaerobic culture of the liver yielded growth of a *Clostridium* sp. which was most consistent with *C. haemolyticum* on MALDI-TOF. The 16S region of the isolate was then sequenced but came back with a 100% positive read for both *C. haemolyticum* and *C. novyi* strains on two different runs. The sequenced segment of the two strains differ by one nucleotide and the result displayed both a C and T at that position. Additional amplification and sequencing was then performed on a portion of the flagellin gene. This sequencing attempt produced a definitive identification of *C. haemolyticum*.

Poster 23

Suspected fatal hypothermia in a dog with generalized demodicosis

Doris Marie Miller

Athens Veterinary Diagnostic Lab, University of Georgia College of Veterinary Medicine, Watkinsville, GA

A young Pitbull mix female dog was presented for necropsy with a history of being found dead while chained outside with no shelter during extreme cold temperatures. The body was found in a curled position, very cold to the touch and no recorded body temperature. The animal had hair loss and ulcerations of the skin with prominent enlarged lymph nodes. The animal had subcutaneous and internal fat present. Stomach contents consisted of numerous bone fragments and non-food items. Very little feces were present in the intestines. Subcutaneous, gall bladder and lymph node edema were present. Histopathology confirmed generalized demodicosis with folliculitis, furunculosis and granulomatous lymphadenitis.

Often dogs with generalized pustular demodicosis are depressed, febrile, debilitated and may die. Most gross and microscopic findings in cases of hypothermia are non-specific with the diagnosis of death due to hypothermia based on the environmental conditions in which the animal was found in conjunction with the necropsy/autopsy findings. The environmental conditions, necropsy/autopsy findings and histopathology will be discussed in reference to hypothermia and Demodicosis.

Poster 24

Post-surgical inflammatory neuropathy in a dog

Tuddow Thaiwong, Matti Kiupel

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Common postsurgical complications in dogs include seroma/hematoma, dehiscence, and wound infection. In humans, postsurgical neuropathy is an uncommon complication usually attributed to an inflammatory process secondary to mechanical force. Here we present a case of severe inflammatory neuropathy targeting peripheral nerves in a dog following surgical removal of a mast cell tumor.

A 10-year-old, male castrated Chihuahua developed a mass behind the right ear that increased in size over several weeks and spread as an irregular chain to below the right ear. A mast cell tumor over the right shoulder had been removed a year prior. The chain of masses was surgically removed and submitted for histopathology. Microscopically, the masses were characterized by severe multifocal to coalescing inflammation within the deep dermis and subcutis centered on peripheral, most likely sensory nerves. Large numbers of lymphocytes and fewer macrophages, plasma cells, and neutrophils caused perineurial thickening and focally infiltrated into nerve fascicles. Affected nerve fascicles had marked myelin loss and axonal degeneration with scattered deposition of small globular fragments in the endoneurium (digestion chambers). There was proliferation of small epi- and endoneurial vessels commonly exhibiting mild lymphocytic vasculitis and perivasculitis. Immunohistochemistry for CD45 with Masson trichrome counterstaining confirmed an inflammatory neuropathy. Disruption of muscle walls in these vessels, consistent with microvasculitis, was documented by smooth muscle actin immunolabeling counterstained with PAS. Immunohistochemical labeling for Iba-1 and neurofilament revealed scattered infiltrates of macrophages surrounding degenerate axons in the endoneurium.

The described lesions are most consistent with post-surgical inflammatory neuropathy. While it is difficult to establish a temporal relationship between the previous surgery and the subsequent neuropathy, the pathologic findings of chain-like lesions are characteristic for post-surgical inflammatory neuropathy in humans. This condition is poorly understood, but believed to be an idiopathic immune-mediated response to a physiologic stress amenable to prolonged immunosuppressive treatment. In dogs, post-operative injury of sciatic nerves has been reported during treatment of pelvic orthopedic diseases and has been associated with a poor prognosis. Whether a subset of these post-operative sciatic nerve neuropathies represent a post-surgical inflammatory neuropathy rather than a traumatic injury is difficult to determine due to the lack of nerve biopsies from affected dogs. It has been suspected that the incidence of post-surgical inflammatory neuropathies in humans is vastly underestimated, and the same might apply to dogs. Therefore, it is important to accurately diagnose postsurgical inflammatory neuropathies with surgical biopsies; so that appropriate immunotherapy can be initiated to potentially improve impairment.

Poster 25

Rhodococcus equi osteomyelitis in an Anglo-Nubian buck *

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Rhodococcus equi is a soil-borne, aerobic, Gram's-positive, facultative intracellular bacterium that is most commonly seen in veterinary medicine as a respiratory and enteric pathogen of foals and occasionally other domestic animals. It is increasingly being recognized as a zoonotic pathogen, particularly in immunocompromised individuals. The disease is rarely seen in goats but a few cases in yearlings have been reported. A 1-year-old Anglo-Nubian buck was presented with a 3-week-history of non-weight bearing lameness and a large "swelling" of the proximal tibia in the right hind limb. Radiographically, the "swelling" was a large lytic and productive lesion. Nodules/masses were also identified radiographically in lung. At necropsy, a round, 4 cm mass of tan, caseated exudate expanded the proximal tibial metaphysis. Adjacent cortical bone was sclerotic. Similar caseated lesions were also identified in the liver, caudal lung lobes, and parietal pleura. Numerous Gram's-positive coccobacilli were identified in macrophages. Aerobic culture isolated *Rhodococcus equi*.

* Graduate Student Poster Presentation Award Applicant

Poster 26

Clostridium haemolyticum infection in a horse

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Clostridium haemolyticum (*C. novyi* type D) is a soilborne anaerobe and the causative agent of bacillary haemoglobinuria/red water disease. Infection with *C. haemolyticum* is commonly seen in cattle and sheep, however other host species can also be affected. Here we report clinical disease in a 13-year-old Quarter horse that was attributed to infection with *C. haemolyticum*. The horse initially presented to Oklahoma State University veterinary teaching hospital with the complaint of recent weight loss, inappetance and lethargy. Serum chemistry analysis revealed markedly increased aspartate aminotransferase (AST) and bilirubin levels. During cytologic evaluation of abdominal fluid, intracellular bacteria were identified supporting the diagnosis of septic peritonitis. The horse was euthanized due to poor prognosis. Postmortem examination confirmed the presence of septic peritoneal effusion along with an intra-abdominal abscess involving the spleen and liver. Discrete regions of necrosis were identified in the liver; these foci contained small clear spaces and were bordered by a rim of red-black liver tissue. During microscopic examination of the liver, plump bacterial rods were present within the leading edges of the necrotic regions. Anaerobic culture of the abscess aspirate yielded *C. haemolyticum* in pure culture. No aerobic bacteria were isolated. Infection with *C. haemolyticum* should be considered as a differential diagnosis for abscessation in the abdominal cavity of horses, particularly when accompanied by liver necrosis.

Poster 27

Corynebacterium pseudotuberculosis and copper deficiency in a male Rocky Mountain bighorn sheep in Utah

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Corynebacterium pseudotuberculosis is a pleomorphic Gram-positive rod that has been associated with suppurative diseases and abscesses in many species worldwide. Disease caused by *C.pseudotuberculosis* is perhaps most well-known in veterinary medicine in domestic small ruminants and horses. In sheep and goats the bacterium causes caseous lymphadenitis and in horses abscessation in the pectoral and ventral abdominal region is known as pigeon fever. Copper deficiency in livestock is prevalent in many areas of Utah and has been associated with diminished immune function, poor growth rates, poor reproductive performance, and increased incidence of disease. A 66.8 kg, 7.5 year-old male Rocky Mountain bighorn sheep in poor body condition and good postmortem condition was submitted to the Utah Veterinary Diagnostic Laboratory for necropsy in November 2015. On postmortem examination, there were numerous thick-walled (0.25 cm to 0.5 cm) abscesses subcutaneously and internally ranging in size from 1 cm in diameter to the largest one in the pelvis that was almost 15 cm in diameter. *Corynebacterium pseudotuberculosis* was isolated in pure culture from one of the abscesses. In an attempt to identify factors that may have caused immunosuppression in this animal, a liver mineral analysis was performed. The animal was severely copper deficient (liver copper was 1.571 ppm, normal = 25 to 100ppm). Respiratory infection is the most frequent cause of disease-related bighorn sheep mortalities in Utah. This is an unusual case and further mineral analyses of animals that die will be necessary to determine the prevalence of copper deficiency in our Rocky Mountain bighorn sheep populations.

Poster 28

Pyelonephritis associated with *Aspergillus fumigatus* in a captive reindeer calf *(Rangifer tarandus*)

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Aspergillus spp. are ubiquitous, saprophytic fungi that are a well-documented cause of opportunistic local and disseminated infections in humans and animals. Renal involvement has been reported in a number of different species associated with disseminated infection and, occasionally ascending urinary tract infection. This report describes a case of fatal disseminated aspergillosis of suspect urinary tract origin in a ten-day-old, male reindeer calf. Grossly, multifocal fungal granulomas were detected. A severe, suppurative pyelonephritis with intratubular fungal elements morphologically consistent with *Aspergillus* spp. involving the renal pelvis and medulla was evident microscopically. Histopathologic examination of additional tissues revealed evidence of embolic dissemination to lung, tracheobronchial lymph nodes, and brain. *Aspergillus fumigatus* was isolated on fungal culture of lung. To the authors' knowledge, this is the first report in reindeer of pyelonephritis associated with ascending infection of the urinary tract by *Aspergillus* spp.

Poster 29

Escherichia fergusonii enteritis and septicemia in a 3 day old Holstein bull calf

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A 3-day old Holstein bull calf presented with a history of sudden death. Pathologic findings were acute suppurative and necrotizing enteritis, acute suppurative pneumonia and lymphadenitis. Samples from the small intestine were collected and pure growth of *Escherichia fergusonii* was obtained. Small intestine samples were negative for *Salmonella spp*. and *Clostridium perfringens*. *E. fergusonii* is a gram negative bacterium in the family Enterobacteriaceae and is closely related to *Escherichia coli.* Infections of *E. fergusonii* have been reported in adult cattle, horses, goats, sheep, ostriches and farmed tilapia causing sepsis, enteric disease, pneumonia, mastitis and abortion. The aim of this report is to highlight enteric *E. fergusonii* infection as a differential for sudden death and enteric disease in calves.

Poster 30

Retrospective study of *post-mortem* cases of pneumonia in racehorses of California

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Respiratory diseases have a major impact on racehorses in training and are often cited as the second most common reason of horses failing to perform in different countries. Literature on the pathology of pneumonia in horses is scant. The objectives of this study were: 1) determine the demographics of race horses with pneumonia, 2) characterize the pathological findings in race horses with post mortem diagnosis of respiratory infection and 3) determine the most significant pathogens associated with lung pneumonia. Data from 90 racehorses submitted for post-mortem examination to CAHFS between January 1st 2005 and December 31st 2014, which had a diagnosis of pulmonary abscess, pneumonia, bronchopneumonia and/or pleuropneumonia were analyzed. Horses were submitted from 8 different racetracks with similar frequencies in all seasons, and most of them were Thoroughbred (84.4%). A total of 41 horses (45.6%) were females, 36 (40%) were geldings and 13 (14.4 %) were colts. More than 50% of cases were in horses that were two or three years old. Pleuropneumonia (70%) and bronchopneumonia (17.8%) were the most frequent morphologic diagnoses. In 50% of all cases, one or multiple abscesses / areas of lytic necrosis was present, this finding was more frequent in the right caudal lung. The most commonly isolated bacterium was *Streptococcus equi* spp *zooepidemicus*, which was obtained from 57 cases (63.3%), followed by coliforms (16 cases, 17.8%), mixed bacterial flora (12 cases, 13.3%) and *Actinobacillus* spp (10 cases, 11.1%). No viral agents were detected. *Streptococcus equi* spp *zooepidemicus* was the most common cause of pneumonia in racehorses of California. This microorganism is a normal inhabitant of the upper respiratory tract of healthy horses. However, its presence in the pulmonary parenchyma is associated with severe and extensive damage of the lung, especially in young animals. Furthermore, this agent is zoonotic, which stresses the importance of early detection and correct management of cases of pneumonia in racehorses.

Poster 31

Diagnosis and within-flock seroprevalence of ovine Johne's disease caused by a sheep (type S) strain of *Mycobacterium avium* subsp. *paratuberculosis* in Uruguay

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Johne's disease (JD) is an economically-important disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which also infects other species including humans. Two major MAP strain types are currently recognized based on genetic differences: sheep (S) and cattle (C) types. The prevalence of JD and the MAP types present in Latin America are largely unknown. Although JD is widespread in Uruguayan cattle, to date it had not been recognized in sheep, and there are no descriptions of the MAP types in any species from this country. The aim of this study is to describe clinicopathological and microbiological findings in cases of ovine JD, and the within-flock seroprevalence, in a ~735-sheep operation managed under grazing conditions in Colonia Department, Uruguay. Five affected sheep and 1 ram showed chronic weight loss, emaciation, mucosal pallor (anemia), fecal softening, intermittent diarrhea, and/or subcutaneous submandibular edema. Postmortem examination and histology (hematoxylin-eosin and Ziehl-Neelsen stains) of 41 animals, including the 6 clinically affected ones, revealed lymphohistiocytic/granulomatous enteritis and/or mesenteric lymphadenitis in 11 animals, with moderate/severe multibacillary lesions in 6 cases, and minimal/mild paucibacillary lesions in 5 cases. Immunohistochemistry using a polyclonal primary antibody against *Mycobacterium bovis* (Bacille Calmette-Guerin strain) that cross-reacts with MAP (performed in the intestines of 2 cases), and transmission electron microscopy (performed in one case), revealed myriads of intrahistiocytic mycobacteria. MAP was isolated from the ileum in one case in M7H9C Middlebrook liquid medium supplemented with mycobactin J, and detected by PCR in 6 cases. The S type of MAP was identified using a multiplex PCR that distinguishes between S and C strains. Three (1.23%) of 244 serum samples obtained from sheep in the cohort flock tested positive for anti-MAP antibodies using a commercial ELISA kit (Paracheck 2, Life Technologies). Considering the variable values of test sensitivity and specificity for this assay reported in the literature, the estimated within-flock prevalence for this flock ranges from 0 to 2.3%. In conclusion, ovine paratuberculosis is present in Uruguay, which may have negative economic consequences for the Uruguayan livestock industry. Additional studies are required to better understand the disease prevalence, its geographic distribution, patterns and dynamics, and to characterize different genetic subtypes of MAP infecting small ruminants and cattle in the country.

Poster 32

A study on Marek's disease pathology and viral loads in backyard chickens with and without tumors

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Marek's disease (MD) is a major cause of mortality in backyard chickens. The diagnosis of MD is complex, however, and knowledge about Marek's disease virus (MDV) in spontaneous field cases such as in backyard chickens is largely unknown. Forty backyard chickens with presumptive MD diagnosis based on histological lymphoid infiltrations in peripheral nerves with and without lymphomas were investigated. Twenty-eight of the birds were submitted to the diagnostic laboratory for disease investigations, and 12 chickens were from a flock where some demonstrated anisocoria and pupil irregularities compatible with ocular MD. Histological scores were established for brain, peripheral nerves, heart, lung, liver, kidney and gonad sections, ranging from mild (+) to severe (+++) lymphoid infiltrations. Twelve chickens had gross lymphomas; all but two chickens had mild to severe peripheral nerve lymphoid infiltrates. There were no age or breed predispositions in the study group. Quantification of serotypes MDV-1, 2 and 3 performed using real-time PCR demonstrated no significant difference between fresh and formalin-fixed paraffin-embedded (FFPE) spleen specimens. MDV-2 DNA was detected in a portion of the chickens, likely consistent with naturally occurring virus, whereas the vaccine strain MDV-3 was hardly detected in any of the birds. Significant differences in MDV-1 viral loads between tumorous and non-tumorous chickens were observed, where MDV-1 gB/GAPDH copies ≥0.5 was suggestive of gross tumors in this study. These results suggest that either fresh or FFPE spleen tissues can be a valuable sample for supporting the diagnosis of Marek's disease in backyard chickens presenting with MD tumors.

Poster 33

Lamanema chavezi (Nematoda: Molineidae) hepatitis in 3 llamas (*Lama glama*) from California

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Lamanema chavezi is a nematode parasite with direct life cycle that infects South American Camelids (SACs). In the United States, it was first reported in a llama from Oregon in 2014, but confirmed cases of the infection and disease are scant in the scientific literature. Here we report the occurrence of this trichostrongyloid in llamas native to California. Three 10- to 22-year-old llamas from a herd in California were euthanized and submitted to CAHFS for autopsy in July 2014. At postmortem examination, the carcasses were in good nutritional condition. Scattered throughout the liver capsule and parenchyma in llamas A-B-C, there were numerous, whitish/yellowish, 1-2 mm, round, hard, mineralized foci (calcareous granulomas), that were particularly widespread and severe in llama C. Additionally, in llama C, there were larger, up to 0.5 cm, less numerous, well-demarcated, irregular, soft, non-mineralized whitish areas, interspersed between the calcareous granulomas. Histologic examination of hematoxylin-eosin-stained sections of all livers was performed. Scattered throughout the parenchyma in all cases, more frequently in proximity to portal tracts, there were well-demarcated, discrete, mineralized granulomas surrounded by a mature fibrous capsule and infiltrated by few lymphocytes, plasma cells, and macrophages. Portal tracts were multifocally infiltrated by lymphocytes (portal hepatitis) and expanded by fibrosis. Additionally, randomly within the hepatic parenchyma of llamas B-C there were occasional transverse and tangential sections of migrating nematode larvae. These larvae were up to 50 micrometers wide, had a thin cuticle, platymyarian musculature, pseudocoelom and a large intestine composed of multinucleated cells. Around the larvae, there was dilation of the sinusoidal spaces, disruption of the hepatic cords, and moderate neutrophilic infiltration. In llama C, among the calcareous granulomas, there were a few lightly mineralized pyogranulomas/abscesses with a central area of liquefactive necrosis. One of these abscesses contained cross sections of degenerate nematode larvae. Frozen livers from llamas A-B-C were macerated and sieved for larval recovery and identification. Late third stage and fourth stage larvae were consistent with prior descriptions of *L. chavezi*; adult nematodes were not recovered. To the best of our knowledge this is the first time this parasitic disease is diagnosed in California. The epidemiology and geographic distribution of this nematode and the health impact for SACs in the US should be further studied. Ongoing work involves development of molecular diagnostic markers for larval and adult parasites.

Poster 34

The effect of zinc oxide nanoparticles on the antioxidant status, blood parameters and immune response in *Japanese quail* during starter period +

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This research was conducted to investigate the effects of zinc oxide nanoparticles (ZnO-NPs) on growth Performance, antioxidant indicator and some blood parameters from hatch to 21 days of broiler chickens (1-21d). 240 one-d-old male broilers (Ross-308) were randomly divided into four treatments including 60 birds with four replicates and 15 birds in each. The experimental diets were: T1) control: basal diet supplemented with 35.9 zinc (from zinc oxide), T2, T3 and, T4 basal diet supplemented with 30, 60 and 90 mg/kg ZnO-NPs, respectively. At the end of research, four birds from any treatment (one bird per replicate) as randomly selected and removed a sample blood from brachial vein. The blood sample was centrifuged (3000 ×g, 4°C with 15 min) and removed serum stored at -20 °C until further analysis. The results indicated that dietary ZnO-NPs had significantly increased ($P<0.05$) super oxide dismutase and glutathione peroxidase activity and total antioxidant capacity ($P<0.05$) level compared with control. The activity of LDH and level of MDA had significantly decreased ($P<0.05$) in the birds fed the diet inclusion of 80 mg of ZnO-NPs/kg basal diet in comparison to control and other treatments. The most concentration IgG, WBC count, total protein and globulin were observed ($P<0.05$) in the birds fed the diet inclusion of 80 mg of ZnO-NPs among experimental treatments. In conclusion, the results of the present study indicated that dietary zinc oxide nanoparticles improved the antioxidant status and immune indices. Also, the suitable level of ZnO-NPs was 80 mg per kg of basal diet.

+ AAVLD/ACVP Pathology Award Applicant

Poster 35

Quantification of aminoaciduria in dogs with jerky pet treat exposure

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Since 2012, Vet-LIRN, in collaboration with University of Pennsylvania, tested and confirmed Fanconi syndrome (FS) in 226 dogs with a history of jerky pet treat (JPT) ingestion. Follow up urine samples from FS positive dogs were periodically tested to characterize the severity and duration of glucosuria and aminoaciduria. Glucosuria usually resolved shortly after the owners stopped feeding JPT, and the animals received appropriate veterinary care. However, aminoaciduria often persisted many months after the glucosuria resolved. The Maltese and Shih Tzu were among the most common FS positive pure-bred and mixed breed dogs. FS positive Maltese and Shih Tzu urine samples were sent to the University of California, Davis for amino acid (AA) quantification, to evaluate changes during the recovery period. We compared the AA profiles from urine samples with normal, mild, moderate, and severe aminoaciduria. Approximately two thirds of the individual AA concentrations decreased over time. FS positive dogs with severe aminoaciduria had total AA concentrations (n=31 AA measured) over 35,000 nMol/mL. FS negative dogs with normal aminoaciduria had total AA concentrations below 10,000 nMol/mL. For two Shih Tzu dogs with a 10 week period between severe and mild aminoaciduria urine samples, the total AA concentration rate of decline was different; however, the rate of decline for two thirds of the individual amino acids was similar. Quantifying aminoaciduria will help FDA characterize the pattern of acquired FS in dogs eating JPT and gain insight into any possible breed predispositions.

Poster 36

Chronic canine parvovirus myocarditis in two puppies

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Two high-dollar, unrelated puppies were diagnosed with severe, chronic, myocarditis due to canine parvovirus (CPV) infection at an unusually late 8 and 9 weeks of age. An 8-week old, female, Boxer dog (case 1) and a 9-week old, female, Rhodesian ridgeback dog (case 2) died suddenly in their respective households without showing any previous clinical signs of disease. On necropsy, gross lesions in both cases were limited to the heart. Case 1 had multifocal to coalescing, bulging areas of severe myocardial pallor visible from the epicardial, endocardial and cut section surfaces that affected approximately 90% of the myocardium of the ventricular and atrial walls. Case 2 had locally extensive, non-raised areas of severe myocardial pallor visible from the cut section and endocardial surface that involved approximately 70% of the myocardium of the ventricular and atrial walls. Histologically, both dogs showed a severe, chronic, lymphoplasmacytic, histiocytic and neutrophilic, necrotizing myocarditis with multifocal early fibroplasia and occasional fibrosis. Viral inclusions were not observed in any of the numerous hematoxylin and eosin stained histological sections of heart examined in both puppies. Moderate to marked, diffuse, pulmonary congestion and edema and marked hepatic passive congestion compatible with heart failure were present in both cases. No enteritis was detected in either dog. In both cases, CPV antigen was detected in individually scattered cells in the heart by immunohistochemistry (IHC) and CPV PCR in formalin-fixed, paraffin-embedded sections of heart was positive. In case 2, the virus was typed as CPV type 2a. *Chlamydia* sp., *Neospora* sp., *Sarcocystis* sp. and *Toxoplasma gondii* IHCs were negative, and no bacteria were identified in select heart sections stained with Giemsa and Gram in both dogs. This is an unusual, late presentation of CPV infection, which usually affects neonatal puppies. In the absence of other lesions and given the extent of the cardiac involvement, sudden death was likely due to an acute decompensation of the severe chronic myocarditis in both cases. The puppies had arrived to California from two different states approximately 5-6 days prior to their death. Because of the chronicity of the cardiac lesions, infection with CPV was presumed to have occurred before they were purchased and brought into their new homes. In both instances, the owners used the evidence from the diagnostic investigation to get their money refunded.

Poster 37

Circovirus-like virus infection in a pig with myocarditis and cardiac arteritis of undetermined etiology

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Viruses in various families, including the *Circoviridae*, are responsible for diseases in swine throughout the world. Here we report cardiac lesions of undetermined etiology in a pig, associated with infection with a circovirus-like virus. Tissues from a 3-week-old pig with a history of anorexia, weight loss and swollen joints from a commercial operation were submitted to the University of Minnesota Veterinary Diagnostic Laboratory for diagnostic workup in May of 2015. The histologic examination of the tissues (trachea, esophagus, synovial capsule, heart, lung, thymus, thyroid and lymph node) revealed moderate fibrinous arthritis with necrotizing arteriolitis in the synovial capsule. *Haemophilus parasuis* joint infection was confirmed by real time PCR. Additionally, the histologic examination revealed multifocal moderate lymphoplasmacytic myocarditis with cardiac arteriolitis of undetermined etiology, necrotizing arteriolitis in the esophagus, and diffuse moderate lymphohistiocytic interstitial pneumonia. Because the etiology for the cardiac lesions was unresolved, a tissue homogenate was processed for viral metagenomics at the University of California. Circovirus-like virus, Porcine Astrovirus 4 and Rotavirus A sequences were detected. A DNA probe for the detection of the first virus by in situ hybridization (ISH) was developed and ISH was performed on formalin-fixed tissues. Circovirus-like virus was detected infrequently in the cytoplasm of myocardiocytes, a leiomyocyte of the tunica media of an inflamed arteriole, and in the cytoplasm of presumed inflammatory cells in the myocardium. Porcine Circovirus-2, Influenza A virus, PRRSV, Classical Swine Fever virus, Pestivirus, Foot-and-Mouth Disease virus, Porcine Parvovirus-1 and -2, West Nile virus, Encephalomyocarditis virus, *Mycoplasma hyosynoviae*, *Mycoplasma hyopneumoniae* and *Erysipelothrix rhusiopathiae* infections were ruled out by PCR, and *Toxoplasma gondii* infection was ruled out by immunohistochemistry. Bacterial aerobic cultures from lung, heart and joint were negative. Although the etiology of the myocarditis and arteriolitis in this case remained undetermined, the finding of a circovirus-like sequence in close proximity of the lesions raises a suspicion on whether this virus may have played a causative role. A 20 nm virus forming crystalline arrays has been found in cardiac endothelial cells in pigs with myocarditis of undetermined etiology in Australia. Broader studies to better understand the distribution and prevalence of this virus in the swine population and its eventual role in disease are warranted.

Poster 38

Enzyme-Linked Immunosorbent Assay (ELISA) Information Management System (EIMS): The Swiss army knife for managing the ELISA Value Stream Map (VSM)

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Veterinary diagnostic laboratories' (VDL's) fundamental system routinely generates customer value from these processes: client question(s) -> sample management -> diagnostic testing -> data creation -> information management -> client knowledge. Not long ago this system's sample inflow was readily managed in paper-based processes by a handful of talented professionals, technicians and clerks with, among other characteristics, photographic memories, encyclopedic storage and rapid recall. As the number of clients, client questions, samples, and test requests increased in volume and sophistication, it became abundantly clear that improved workflows and software solutions were needed. Driven by the de-novo development and implementation of a series of customizable software applications, the Iowa State University Veterinary Diagnostic Laboratory's Serology Section has digitalized many tasks that were once done manually. The ISU ELISA Information Management System (EIMS) is customizable software application that is used to electronically build ELISA plate maps; use test specific algorithms to interpret results; accept or reject plates based upon control values; links optical density values of controls to statistical process control charts; and automatically integrate results into the ISU VDL's Laboratory Information Management System (LIMS). Virtually all information transfer (including regulatory results) now occur paper-free. This presentation will show the serology section's historical value stream map (VSM), the current VSM with EIMS and a proposed future state VSM. This transformation is associated with capturing value for ISU VDL and its stakeholders.

Poster 39

Problem solving using SPC charts for PRRSX3 quality management at ISU VDL

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Introduction. Statistical process control (SPC) charts are the operational definition for 'on target with minimal variation ELISA results'. Herein we show how day-to-day SPC charts are used to identify and solve problems at the Iowa State University Veterinary Diagnostic Laboratory.

Materials and Methods. Low in-house positive control serum with an S/P value close to the positive-negative cutoff (0.400) is tested on every plate. SPC charts of the PRRSX3 ELISA negative control values and of the low house positive control S/P values were created from day-to-day testing. The charts were interpreted based on the type of variation displayed as: (1) normal variation characterized by data points within the 3-sigma limits or (2) special cause variation, as evidenced by any data point outside 3-sigma limits or 8 consecutive data points on either side of the central line. Dates of special-cause variation identified by SPC charts were compared to the diary of the PRRS ELISA lab where changes in operations are recorded. For example, changes in operations would include the introduction of a new PRRSX3 ELISA kit lot into the laboratory.

Results and Discussion. Two days' PRRSX3 ELISA runs results formed a cluster of special cause variation. The first incident was identified by the PRRSX3 ELISA technician through real-time SPC charts created within the ISU VDL ELISA Information Management Software (EIMS). While all plates were acceptable according to analysis of controls' OD values, the negative control values were out-of-control (high) and the low in-house positive S/P control values were out-of-control (low). In spite of passing the plate validity checks based on kit control sera, the decision was made to reject the run and call clients to inform them of a PRRS ELISA run failure. The same samples were tested the following morning with a similar outcome. Root-cause investigation revealed a mold-contaminated washer reservoir. After cleaning the washer reservoir, the kit negative control OD values and low in-house positive S/P results returned to "in-control". This example demonstrates how real-time SPC charts in the hands of skilled technicians are critical for day-to-day testing quality management.

Poster 40

ELISA quality: On target with minimal variation

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Introduction. A challenge in veterinary diagnostic laboratory testing processes is meeting clients' expectations of consistent and reliable results from assays rife with inherent variation. We demonstrate how SPC charts are used to achieve "On target with minimal variation" (Taguchi).

"A phenomenon will be said to be controlled when, through the use of past experience (read: "data"), we can predict, at least within limits, how the phenomenon will vary in the future," W.A. Shewhart (1931). There is nothing inherent to ELISA control sera OD values or their coefficient of variation (CV) that would assure technicians and clients of the consistency and reliability of the results; neither is an operational definition. Statistical Process Control (SPC) charts of positive and negative control OD values are the operational definitions for ELISA processes where their data are interpreted in the context of 3-sigma limits. The data within those limits are characterized as random or special-cause variation. If random lack patterns; the ELISA results will, with 99%-plus certainty, perform the same way in the future. Special-cause variation is data that contains patterns about the process average and relative to the upper and lower process limits. Sources of special cause variation must be discovered and removed in order for the process's variation to be characterized as random.

Materials and Methods. The two negative control values from PRRSX3 ELISA plate were used to create an average and range (R) chart for January 1, 2015 through May 23, 2016. The chart's limits were calculated globally and graphed as described by Wheeler and Chambers (1992). The test results' variation was interpreted as either random (predictable), or special-cause (unpredictable). Unpredictable being defined as 1 data point located outside the 3-sigma limits or 8 consecutive test results were on the same side of (Wheeler and Chambers, 1993).

Results and Discussion. The SPC chart of the two negative control values displayed unpredictability beginning on November 15, 2015. Unpredictability was associated with a change in kit lot, necessitating recalculation of the limits. The previous kit lot's negative control OD values' average, upper and lower limits were 0.047, 0.051 and 0.043, respectively; the next lot's, 0.074, 0.081 and 0.068, respectively. This knowledge is used, in part, to describe and manage ELISA process quality control.

Poster 41

Prevalence of *Coxiella burnetii* infection in livestock in Bangladesh * †

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Coxiella burnetii causes Q fever in humans and livestock. Coxiellosis occurs during late pregnancy leading to abortions and stillbirths in goats, infertility, mastitis and endometritis in cattle with associated financial losses to animal owners. The reservoir includes mammals, birds and arthropods, mainly ticks. Both public and animal health issues make Q fever a disease of interest for public policy makers and food industries. In the agro-based economy of Bangladesh livestock contribute to 1.73% of the total gross domestic product (GDP) and growth is 3.10% in 2014-2015. 75 % of rural people are directly or indirectly involved with livestock rearing. To control the spread of *C. burnetii* among animals to humans, the detection of shedders of *C. burnetii* and the knowledge of the seroprevalence of the infection are imperative. The objectives of this study were to determine the prevalence of *Coxiella burnetii* infection in domestic ruminants and to detect *Coxiella burnetii* DNA from ticks and serum samples. A total of 24 ticks, 91 goats and 81 cattle serum sample with the history of abortion and reproductive disorders were collected from the different areas in Bangladesh. The serum samples were tested by CHEKIT Q-Fever Antibody ELISA Test Kit and *Coxiella burnetii* DNA was detected by multiplex quantitative PCR in the Reference Laboratory for Q fever, Friedrich-Loeffler-Institut, Jena, Germany. The overall prevalence was 7.6% and 6.1% in goats and cattle, respectively. However, none of our seropositive samples and tick samples was positive in quantitative PCR.

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A comparison of two Enzyme-Linked Immunosorbent Assays (ELISAs) for determination of *Brucella ovis* seroprevalence in Wyoming domestic sheep * †

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Brucella ovis (*B. ovis*) is the primary causative agent of ovine brucellosis in the U.S. It is an infectious, sexually-transmitted bacterial disease that causes significant reproductive problems in domestic sheep worldwide. Infection is introduced into a flock after infected sheep are purchased, or following exposure to infected sheep on shared grazing allotments or open rangeland. If the disease is not properly controlled, major economic repercussions include decreased ram fertility, lowered conception rates in ewes, and increased premature and weak lambs with low birth weights. In addition to the direct negative economic effects of the lowered annual lamb crop, *B. ovis* infection can have negative genetic impacts when valuable rams are culled from the flock due to infection.

The primary study objective was to determine overall seroprevalence of *B. ovis* in Wyoming domestic sheep utilizing the indirect Enzyme-Linked Immunosorbent Assay (ELISA) approved by the National Veterinary Services Laboratory (NVSL). This diagnostic assay is the most widely used serological method to detect exposure to *B. ovis* in the U.S. We collected and tested approximately 2,500 sheep serum samples from multiple flocks in Wyoming and have found seroprevalence <1%. While the ELISA approved by NVSL comes with standardized antigens and controls, the assay requires each laboratory to antigen-coat their own plates, and requires reagents to be purchased from multiple companies to complete. The assay is also time-consuming and produces "indeterminate" results, which is an outcome that is difficult to interpret for producers, veterinarians, and diagnosticians. For these reasons, we pursued the use of an alternative, commercially available assay produced by a company named IDEXX.

This assay is a pre-manufactured ELISA kit that includes all the necessary equipment and reagents for completion. It has a comparable sensitivity (Se=91.7%) and specificity (Sp=95.2%) to the ELISA utilized in the U.S. and can be completed in half the time. We will compare the two ELISAs using the cross-sectional sample of sheep serum collected from various domestic flocks in Wyoming. If this alternative ELISA performs similarly or better than the current ELISA used in the U.S., we recommend diagnostic laboratories consider using the pre-manufactured ELISA kit produced by IDEXX to save on cost and time.

* Graduate Student Poster Presentation Award Applicant
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Ichthyophthirius multifiliis i-antigen stimulates *ex vivo* proliferation of channel catfish T cells: CD4 T cell immune responses and vaccine development *

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Aquaculture is the fastest growing food production industry and represents a viable source of animal protein for global human populations. In the United States it is estimated that sales of aquaculture products were 1.37 billion dollars in 2013. Despite the overall increase in aquaculture production, the channel catfish (*Ictalurus punctatus*) industry has experienced a recent decline in production due to multiple economic and biological factors. This industry faces challenges similar to other animal production industries such as maintaining healthy populations and preventing disease. One pathogen in particular, *Ichthyophthirius multifiliis* (Ich), a ciliated protozoan poses a significant threat to both freshwater aquaculture and the pet fish industry. Ich is an obligate, extracellular parasite that expresses a major surface protein referred to as the immobilization-antigen (i-antigen), which has been shown to provide immune protection in vaccination studies with channel catfish. Although the humoral immune response to i-antigen has been well characterized, less is known about the T cell-mediated immune response that is essential to elicit immunity. We hypothesized that CD4 T cells from immune fish would proliferate upon stimulation with i-antigen. Channel catfish were immunized by natural infection with Ich and treated with formalin to limit the infection. I-antigen was isolated from Ich theronts. Total spleen cells from naïve and immune fish were grown *ex vivo* for 72 hours in AL-3 media and co-stimulated with i-antigen and a sub-optimal dose concanavalin A (ConA). BrdU assays were used to detect proliferation of cells after i-antigen and ConA treatment. Real time PCR was used to measure CD4 T cell proliferation in comparison to the constitutively expressed housekeeping gene, β-actin. Our data suggest that a combination of i-antigen and a low dose of ConA stimulates proliferation of splenic CD4 T cells from immune fish. This research forms the basis for future work comparing T cell responses from naturally infected fish with those vaccinated with i-antigen.

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Indirect immunofluorescence assay for detection of antibodies to porcine delta corona virus

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Porcine delta coronavirus (PDCoV) has recently emerged as a novel enteric pathogen in swine in the United States (U.S.). The virus was first described in Hong Kong in the year 2012 and subsequently in the year 2013, it was isolated in the U.S. The sequence of U.S. virus strains has been found to be 99% identical to Chinese strains. At present there are no commercial tests available to detect presence of antibodies to PDCoV in swine population. The present study was used to validate an indirect immunofluorescence assay (IFA) for detecting antibodies against PDCoV. To evaluate the specificity of the test, 100 known-negative serum samples were obtained from a sow farm, a nursery and a finisher free of PDCoV. All these samples tested negative by IFA. To evaluate sensitivity, serum was collected from 30 sows that were infected with PDCoV through feedback administration at the sow farm. Samples were collected at approximately 2-week intervals post-feedback. More than 95% of sows had seroconverted by day 40 post-feedback. However, antibodies started declining thereafter and by day 55 only 33% of the sows remained positive. The IFA for PDCoV is a useful and accurate test to detect seroconversion at around 5 weeks post-exposure.

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Development of reagents & assays for Senecavirus A serodiagnosis

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Senecavirus A (SVA) is a non-enveloped, positive stranded, RNA virus belonging to the Picornaviridae family. Disease associated with SVA has spread to 16 states and is characterized by idiopathic vesicular lesions along the hoof, snout and oral mucosa. Currently there are minimal specific diagnostic reagents and serological tests for herd surveillance and confirmation of disease. To address these industry needs, we developed reagents for immunohistochemistry (IHC) and fluorescent antibody (FA) staining. These reagents were also applied towards a first generation of serological assays including an indirect ELISA, fluorescent microsphere immunoassay (FMIA) and a fluorescent focus neutralization (FFN) assay. Serological reagent development began with the cloning, expression and purification of specific SVA-P1 nucleoproteins including VP1, VP2 and VP3. Native expressed VP2 protein and re-folded VP1 and VP3 proteins were used to immunize mice and rabbits for monoclonal and polyclonal antibody production. For the VP1, VP2 & VP3 ELISA & FMIA development, microtiter plates were coated with 400 ng of each antigen while Luminex microspheres were coupled at a concentration of 15 ug per 3 x 10^6 microspheres. Both assays were optimized using a checkerboard titration to maximize signal-to-noise ratios using samples of known serostatus. Expected negative and positive serum samples were obtained from uninfected pigs (n=526) and naturally exposed field animals sampled 1 to 3 weeks post exposure (n=57). Initial FMIA testing showed that serum immunoreactivity of VP2 was 10-fold greater than the immunoreactivity of VP1 and VP3 and provided the rational for further development of a VP2 indirect ELISA and FMIA. Both tests were validated and ROC analysis showed calculated diagnostic sensitivities and specificities of 98.1, 89.7 & 95.2 and 89.8 respectively. Next, inter-rater (kappa) analysis was performed to show statistical measures of testing agreement between assays. The ELISA and FMIA assays were compared to a newly developed IFA and kappa values were calculated to be 0.923 and 0.925 respectively demonstrating significant testing agreement. ELISA and FMIA results show the detection of SVA antibodies as soon as one to two weeks post infection. Although the sensitivity of both assays are adequate, the number of false positive tests were concerning (n=57/526). Twenty-five false positive samples were chosen at random and subjected to FFN testing. Afterwards, all 25 samples were resolved as true negatives. Noteworthy, newly developed monoclonal antibodies against VP1 and VP2 have been employed in the construction of a blocking ELISA to increase specificity and to provide a confirmatory test where false positive analytes arise. These new diagnostic reagents and assays should aid in improved surveillance and control strategies for SVA.

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Histopathologic findings in a 3 month subchronic mouse microcystin LR study

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Water quality can adversely affect human and animal health. Microcystin LR (MCLR) is a common water-borne toxin, produced by harmful algal blooms which are increasing in frequency. Acute effects of MCLR are well known, however, there are meagre data on subchronic and chronic effects. We investigated the histopathology of subchronic exposure to MCLR using a mouse model. Male C57BL/6j mice (5 per group) were randomly distributed to control (0.9% normal saline); 40 ug/kg (low dose, LD); 200 ug/kg (medium dose, MD); and 1000 ug/kg (high dose, HD) bw groups for 4, 8, or 13 weeks by oral gavage. They were dosed daily Monday through Friday of each week. Body and major organ weights were obtained. Liver, kidney, brain, heart, lung, pancreas, and adrenal glands were collected, fixed in 10% neutral buffered formalin, routinely processed, paraffin embeded and stained with H&E. Data was statistically analyzed using a one-way ANOVA followed by Tukey's ad hoc test to compare treatment groups with the control. Body weights were significantly decreased in all groups ($p<0.05$). Other significant changes from control were observed for absolute liver and spleen weights in the HD group: a marked decrease at 4 weeks was followed by a return to control values by 13 weeks. Hepatic lesions were, in general, dose and time dependent, but were observed in some animals in all treatment groups. At 4 and 8 weeks, HD group animals had severe centrilobular hepatic lesions characterized by hepatocyte necrosis (both apoptotic and autophagic) and inflammation. Regenerative changes consisted of abnormal mitoses, multinucleation, karyomegaly and cytomegaly. Most of these changes had resolved by 13 weeks. Most MD and LD animals had mild lesions similar to 13 week HD animals except for a few outliers with changes similar to 8 week HD group. The spleen showed a dose and time dependent decrease in cellularity of both white and red pulp in all dose groups, ascribed to the poor condition of the animals. No significant changes were identified in brain, kidney, heart, lung, pancreas and adrenal. In conclusion, hepatotoxicity was the primary MCLR induced toxicity observed with the most severe changes observed at 4 weeks. Hepatic regeneration with apparent tolerance to further MCLR induced hepatotoxicity was observed after 4 weeks. Further studies are needed to determine the mechanism of this apparent tolerance.

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Mycotoxin and metal contaminants in peanut butter on the Ugandan market ◊

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Peanuts are a common human food in Uganda and are also used as animal feed ingredients globally. Peanut butter is a common food product worldwide. There are concerns about the safety of peanuts and peanut products in Uganda as well as globally. In particular, peanut contamination with mycotoxins, especially aflatoxins, is a longstanding issue. Peanuts from Uganda were responsible for hepatotoxicity in turkeys in 1950's in the UK. Trace and heavy metal contamination of peanut butter is also a food safety concern in Uganda, mainly because of the questionable quality of locally fabricated grinding machines. The objective of this study was to investigate mycotoxin and elemental concentrations in peanuts and peanut products purchased from various markets within Uganda and compare those with US products. Our hypothesis was that peanut products from Ugandan markets contain higher mycotoxin and elemental contaminants than those from the US products. A total of 38 peanut product samples were collected from 4 markets and 2 homesteads in Kampala, Uganda. The US peanut butter samples were purchased from local retail stores in Ames Iowa. Samples were analyzed for 13 mycotoxins and for 26 elements following routine Iowa State University Veterinary Diagnostic Laboratory procedures. Mycotoxins were analyzed by LC/MS/MS, while the elements were analyzed by both ICP/MS and ICP-OES. Inorganic results showed some differences in concentration of between market, homestead, and US samples. Most differences in concentration for the element were statistically significant, Ag, Al, Fe, Mn, Ni, S, and Zn concentrations were elevated in the Ugandan market samples. However, none of the metallic element contamination was at a concentration considered to be a food safety issue. Mycotoxin analysis, however, showed that 82% of peanut product samples from Ugandan markets contained aflatoxin residues, while 55% of the samples had concentrations above the 20ppb United States regulatory limit. These results indicate that aflatoxins are still a major public health and animal food safety issue.

Key words: mycotoxin, aflatoxin, heavy metal, peanut

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Acute lead arsenate poisoning in beef cattle in Uruguay

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Arsenic (As) is a ubiquitous toxic element that is concentrated in the environment as a result of industrial activities. Before the 1960s As was used extensively in insecticides, herbicides, fungicides, paint and preservatives. Lead arsenate (LA) is an inorganic insecticide that was used extensively in agriculture. Although acute As poisoning is infrequent in domestic animals, it still happens when animals have access to areas where the element persists. Here we describe an outbreak of LA poisoning in cattle which occurred in September 2015 at a beef operation in Uruguay. A herd of 70, 1.5- to 2-year-old, Aberdeen Angus, Hereford and crossbred steers/heifers were grazing on a pasture composed of fescue (*Festuca arundinacea*), lotus (*Lotus corniculatus*) and white clover (*Trifolium repens*). An abandoned building lodging waste materials, including an unidentified bucket containing a white-gray powder, was present in the paddock and was accessed by the cattle. Over a period of 21 days, 14 cattle (20%) became sick, 10 (14%) of which died. Affected animals had diarrhea, melena, mild ataxia and/or difficulty standing. The clinical course of fatal cases was 12-18 hrs. Three cattle were autopsied. Multiple tissues were collected and fixed in 10% neutral buffered formalin for histology. Pathological findings included severe extensive acute hemorrhagic abomasitis with submucosal edema, necrohemorrhagic erosive/ulcerative rumenitis and omasitis with multifocal epithelial/mucosal sloughing, and diffuse renal pallor with acute tubular necrosis (nephrosis). Formalin-fixed samples of liver from all cases were submitted to the California Animal Health & Food Safety Laboratory for quantification of As, lead (Pb), mercury (Hg), and selenium (Se). As concentrations were 20, 31 and 24 ppm; Pb concentrations were 8.3, 25 and 9.4 ppm; and Se concentrations were 1.2, 0.84 and 0.86 ppm (all expressed in dry weight basis), respectively. Hg was not detected in any case. A sample of the powder found in the bucket in the abandoned building was submitted to the "Laboratorio Tecnologico de Uruguay" for determination of its chemical composition, and proved to be LA. The diagnostic investigation allowed for confirmation of As poisoning and Pb exposure, with LA as the source for the elements. On several occasions the farmer had seen cattle entering the abandoned building where the insecticide had been stored. Retrospectively, it was found out that the farm used to be an orange orchard a few decades before this outbreak occurred. The current property owner had purchased the farm in 2012, and was unaware of the presence of this insecticide in the building. Acute As poisoning is now uncommon, although, as this case illustrates, exposure to old and improperly stored chemicals can result in livestock exposures and possible mortalities. Timely diagnosis of such exposures is important to prevent contamination of food intended for human consumption.

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Thyroid parafollicular (C) cell hyperplasia and carcinoma in a sheep with enzootic calcinosis due to *Nierembergia rivularis* (synonymous *N. repens*) poisoning in Uruguay

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In ruminants, proliferative lesions of thyroid C cells usually occur in response to hypercalcemia caused by ingestion of calcinogenic plants, calcium-rich diets, or paraneoplastic syndrome, as a compensatory mechanism to increase the secretion of the hormone calcitonin that reduces plasma calcium. The histological distinction between hyperplasia, adenoma and carcinoma is often difficult. C-cell hyperplasia may be focal or diffuse and represents a physiological response, although it is considered a pre-neoplastic lesion as usually precedes the progression to neoplasia, which is favored by chronic hypercalcemia. Adenomas are single or multiple uni- or bi-lateral nodules, they are smaller than carcinomas and are separated from the thyroid parenchyma by a thin fibrous capsule. The adjacent thyroid is usually compressed, but not invaded, by the neoplastic cells. In C cell carcinomas there is excessive enlargement of one or both thyroid lobes, neoplastic cells are more pleomorphic and there is evidence of invasion. Several calcinogenic plants (*Solanum malacoxylon*, *Cestrum diurnum*, *Trisetum flavescens*, *Nierembergia* spp.) are responsible for hypercalcemia and enzootic calcinosis (EC). EC produced by *N. veitchii* and *N. rivularis* in Uruguay, and *N. veitchii* in Brasil, have been described in sheep. While C-cell hyperplasia is documented for *N. vietchii*, to our knowledge proliferative lesions of C cells have not been described for *N. rivularis*. The aim of this work is to describe lesions in a sheep with EC due to *N. rivularis* poisoning, focusing on thyroid lesions. The case was diagnosed in February 2016 in Uruguay, in a flock of 110 sheep grazing native grassland invaded with *N. rivularis*. Eleven animals (10%) showed progressive weight loss, weakness and/or decubitus, with a course of 2-3 months. Eight of the affected animals died. A 4-year-old sheep was autopsied and tissues were processed for histology and immunohistochemistry. Major arteries including the thoracic and abdominal aorta, carotid, coronary and renal arteries, had diffusely rigid walls with loss of elasticity, and were thickened and expanded by irregular, variably-sized, roughly-surfaced, firm to hard, white, crepitant (mineralized) intramural plaques and nodules that narrowed their lumens. Similar deposits expanded the bicuspid and aortic semilunar valves. Multiple, coalescing, irregular, tan/white, homogeneous nodules, up to 18 mm, with indistinct borders expanded the thyroid glands bilaterally. These correlated histologically with severe diffuse and nodular C cell hyperplasia with co-existing foci of carcinoma with intrathyroidal infiltration. Hyperplastic and neoplastic cells were immunoreactive for calcitonin. The etiology of the C cell hyperplasia and carcinoma in this case is unknown, but chronic hypercalcemia induced by *N. rivularis* poisoning may have been played a predisposing factor, as has been suggested in other conditions associated with chronic hypercalcemia in sheep.

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Evaluation of a rapid antigen kit for the detection of porcine epidemic diarrhea virus

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A brief comparison study was conducted to evaluate and compare VDRG PEDV Ag Rapid kit (Median Diagnostics) for the detection of porcine epidemic diarrhea virus (PEDV) in fecal samples from pigs. The comparison was done with RT-PCR test, which is used for routine diagnosis of PEDV at the Minnesota Veterinary Diagnostic Laboratory. A total of 36 samples were tested by both RT-PCR and VDRG Rapid kit. Of these, 25 and 12 samples were positive by RT-PCR and rapid kit, respectively. Most of the samples detected positive by rapid kit had a Ct value of less than 19. Further, serial 10-fold dilutions of PEDV were prepared and tested by RT-PCR and rapid kit. The rapid kit detected virus up to a dilution of 10^{-3} (original virus titer = $10^{6}TCID_{50}$/mL).

To determine specificity, the kit was also tested for other viruses causing diarrhea in pigs e.g., transmissible gastroenteritis virus, porcine delta corona virus and rotavirus. None of these viruses were detected by the rapid kit. We conclude that, although the antigen kit is specific, its sensitivity is low in comparison to RT-PCR.

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The Wisconsin electron microscopy diagnostic proficiency program

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Negative stain imaging of specimens for virus identification is on the decline in some electron microscopy (EM) diagnostic laboratories. Our laboratory went from over 2000 negatively stained [1] samples to less than 100 per year. This dramatic decrease in samples can have a deleterious effect on keeping one's eye keen for spotting unknown pathogens that do come in for transmission electron microscopy (TEM) examination. In 2007, the Centers for Disease Control (CDC) and Duke University partnered in a program to improve the use of TEM laboratories in bioterrorism preparedness. Hosts Cynthia Goldsmith from the CDC and Sara Miller from the Department of Pathology at Duke sought to fill a gap in expertise. It was apparent that a number of laboratories did not have access to a variety of viruses or adequate diagnostic samples to improve identification skills. Virologists at WVDL prepare and ship inactivated viruses to the participating laboratories. Each participant then has the opportunity to prepare and evaluate their own grid as well as make the diagnosis. This was the start of the "Wisconsin EM Diagnostic Proficiency Program" and is currently the only one in the United States. The laboratories that have participated show improved expertise in their abilities to prepare and identify unknown viruses grown in tissue culture [Figures 1, 2]. PCR has become the standard in many diagnostic tests; however it can miss unsuspected pathogens if the correct primer is not chosen or the sequence is not available. If one simply suspects a viral pathogen, based on symptomatology and runs a PCR test for that pathogen, the "guess" may be incorrect, and the test result would be negative; yet, a virus could, nevertheless, be present. Additionally, some viruses do not grow or are fastidious in culture, making large-scale production of reagents difficult. Thus, commercial diagnostic primers and antibody reagents do not exist for all viral pathogens. In the case of electron microscopy, if a virus is present in sufficient numbers, and if the microscopist is experienced in viral morphology, the pathogen can be recognized and identified. This examination of "whatever is there" has been termed the "Open View" [2] by Hans Gelderblom, one of the giants in the field of virus diagnosis and whose program we have emulated here. In summary, one should make use of all the tools in their diagnostic arsenal, including EM, when it comes to detecting unknown viruses in samples. We encourage all diagnostic laboratories with EM facilities to participate in this proficiency program.

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Avian influenza virus inactivation by environmental factors and disinfectants: Premises treatment during the 2014-2015 H5Nx outbreak in the United States ◊

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Avian influenza virus can contaminate natural and agricultural environments due to fecal and oronasal shedding by infected animals. It can then persist, (for up to months in cool water), which is believed to contribute to its transmission and maintenance in wild waterfowl. Such persistence is facilitated by the presence of organic materials, particularly those high in protein, and contradicts the view that enveloped viruses are 'sensitive' to inactivants. Laboratory and field studies of influenza virus' sensitivity to environmental factors such as heat, desiccation, pH and radiation help with assessing the risk of contaminated materials and environments to uninfected birds or other animals. The materials likely contaminated are varied (e.g., water, cloth, wood, concrete, feces, litter, eggs and carcasses). Materials influence the virus' persistence and availability to inactivant or to the host, and so can challenge the external validity of controlled studies. Manipulation and monitoring of environmental factors offers promise for ensuring the reduction or inactivation of influenza contamination without use of chemical disinfectants. During the 2014-2015 highly pathogenic avian influenza outbreak in the United States, dry cleaning and subsequent heating of the affected facility (100-120 F for 7 days) as well as extended fallow periods were used as alternatives to traditional methods (wet disinfectants or fumigation) of cleaning and disinfection (C&D). Post-C&D environmental samples were tested by PCR to detect viral RNA and by virus isolation to detect viable virus. Tests were performed by the National Veterinary Services Laboratories and by National Animal Health Laboratory Network laboratories. The Emergency Management Response System was used to collect, store, and analyze outbreak information. It was recently queried to analyze treatments of premises vs. test results, and analysis as of June 10, 2016 is noted here. Of the 232 infected and dangerous contact premises in 15 states undergoing C&D, the numbers of premises receiving each treatment were: 189 wet disinfectant; 26 heat; 15 extended fallow period; and 2 a combination of methods. Viral RNA was detected in 145 post-C&D samples from 8 different premises (of >5400 total samples for the 232 premises), in multiple treatment groups. Samples from which RNA was detected included egg belt, feeders, waterers, footwear and manure belts/pits. If viable virus was recovered after C&D, additional treatments were performed. Further studies are needed to evaluate sampling approaches, identify environmental samples to target by production system, and to determine whether such samples might be useful as flock monitoring tools in addition to pre-clean and post-C&D assessment. The wide variation in production systems (layout, equipment, and materials) and in mitigation strategies will necessitate a review of testing, sampling and strategies to determine what improvements would be valuable.

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Complete genome constellation of group A rotavirus from deer identifies common evolution with bovine rotaviruses

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Rotaviruses are major enteric pathogens of humans and livestock animals, especially in young calves and piglets. Clinical presentations range from asymptomatic infection to acute diarrhea, which may lead to death due to severe dehydration or other complications. Rotavirus A has been identified and isolated from several artiodactyls including deer species, reindeer (*Rangifer tarandus*), waterdeer (*Hydropotes inermis*), and muntjac deer (*Muntiacus reevesi*), however, there aren't any whole genome sequences of deer rotavirus A in GenBank. Newport Laboratories received lung, heart, kidney, spleen, and intestine samples from a farm where several newborn fawns appeared extremely weak and died within few days of birth. Samples were positive for rotavirus A (Ct=22.81) and negative for rotavirus B and C. Rotavirus A was isolated from the sample and confirmed by quantitative reverse-transcription polymerase chain reaction (RT-PCR). Deer Rotavirus A (14-02218-2) genome was sequenced using Ion-Torrent personal genome machine. 14-02218-2 possessed genome constellation G8-P[1]-I2-R2-C2-M2-A3-N2-T6-E2-H3 and was named RVA/Deer-wt/USA/14-02218-2/2014/G8P[1] according to the guidelines for the uniformity of rotaviruses proposed by the Rotavirus Classification Working Group (RCWG). Genome constellation of strain 14-02218-2 is identical to bovine and bovine-like strains, commonly found in rotavirus A strains from order *Artiodactyla* which includes pigs, peccaries, hippopotamus, camel, deer, giraffe, pronghorn, antelopes, sheep, goats and cattle. Significant genomic similarity of deer rotavirus strain 14-02218-2 with bovine and bovine-like rotavirus strains suggest that the deer G8P[1], 14-02218-2 rotavirus strain, originated from interspecies transmissions from a common pool of bovine rotaviruses. The G8 type is also found in a number of other members of the ruminant family *Bovidae,* such as buffalo, sheep, goats and antelopes, and in guanacos, members of the family *Camelidae.* Intermingled distribution of deer and bovine herds over the same geographical location might be one of the reasons for the isolation of bovine-like G8P[1] from deer. The present study was the first to sequence and analyze the whole genome of deer rotavirus strain 14-02218-2 with the G8P[1] genotype from newborn fawn's clinical sample. The current study provides insights into the complete genetic makeup of a deer rotavirus strain and its genetic relatedness to rotavirus A from other host species. Furthermore, the results support the increasing evidence of interspecies transmission and reassortment events.

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Genotype constellation analysis of bovine and porcine rotavirus A isolates

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Group A Rotaviruses are major enteric pathogens of humans and livestock animals, particularly in young calves and piglets, and can be zoonotic. Clinical presentations of Rotavirus A can range from asymptomatic infection to severe diarrhea, which can be fatal if left untreated. To detect the association and variability among bovine and porcine Rotavirus A, we sequenced Rotavirus A isolated from clinical samples submitted to Newport Laboratories during 2013-2016. Whole genomes of 47 field isolates (25 bovine and 22 porcine) were sequenced using Ion Torrent Personal Genome Machine sequencing platform (Life Technologies, Grand Island, NY). Sequence reads were assembled into contigs using SeqMan NGen (DNAstar, Madison, WI) and subsequently genotyped using the RotaC v2.0 automated genotyping tool. The results show a clear distinction in predominant genotypes between bovine and porcine isolates, as well as variation within species. G6P[5] (80%) followed by G6P[11] (12%) were the predominant G/P genotypes among bovine isolates.. G9P[7] (36%) followed by G9P[23] (22%) and G5P[7] (22%) were the predominant G/P genotypes among porcine isolates. P5 (80%), followed by P11 (16%), and P13 (4%) were the predominant P types among bovine isolates. P7 (63%), followed by P23 (23%), and P5 (14%) were the predominant P types among porcine isolates. G6 (92%), followed by G9 (4%) and G10 (4%) were the predominant G type among bovine isolates. G9 (59%) followed by G5 (23%), G6 (14%), and G4 (5%) were the predominant G type among porcine isolates. The non-G/P genotype of bovine and porcine were I2/5-R1/2-C1/2-M1/2-A3/8/13-N1/2-T1/6-E1/2-H1/3 and I2/5-R1/2-C1/2-M1/2-A1/8/13-N1/2-T1/6/7-E1/2-H1/3 respectively. Except for the A type (NSP1) the non-G/P genotype remained identical for bovine and porcine isolates. The comparison of these genotypes can provide important perspective into the understanding of Rotavirus A pathogenesis, and immune response. Alongside sequence comparison, genotyping could provide additional insight into selecting isolates for vaccine production.

Poster 55

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

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VDPAM, ISU-VDL, Ames, IA

Senecavirus A (SVA) is a single-stranded, positive-sense RNA virus belonging to the genus *Senecavirus* in the family *Picornaviridae.* This virus has been recently detected in commercial swine operations in several swine producing states throughout the United States, causing sporadic vesicular lesions and increased neonatal mortality. It is critical to discriminate SVA from other infectious and economically devastating vesicular diseases, including foot-and-mouth disease, vesicular stomatitis, swine vesicular disease, and vesicular exanthema of swine. Etiological diagnosis of SVA has been based on specific qRT-PCR, virus isolation, immunohistochemistry and *in situ* hybridization. The VP1 protein has proven to be highly immunogenic in SVA and other members of the *Picornaviridae* family. However, there is no available information regarding the immunodominant regions of SVA-VP1. The objective of this study is to identified the immunodominant regions of SVA-VP1 using an ELISA epitope mapping approach.

Eighteen overlapping peptides shifted by 5 amino acids, spanning the complete SVA-VP1 amino acid sequence, were synthesized and conjugated to Keyhole limpet hemocyanin (KLH) at N-terminus and hydroxyl at C-terminus. Peptides were coated on high binding microtitration plates at 1 μg/ml in PBS (pH 7.4), and incubated 16 h at 4°C. After incubation, plates were washed 5 times, and blocked with a solution of 1% BSA, 2h at 25°C. Plates were then dried 4 h at 37°C and stored at 4°C.

Two anti-SVA whole virus polyclonal antibodies (SVA-Pab), experimentally generated were serially diluted from 1:50 to 1:6400. Sera from clinically affected sows, tested positive by SVA-VP1 ELISA, were diluted at 1:50. Pre-injection mouse serum and serum from naïve pigs were evaluated to establish a baseline for SVA-Pab and clinical samples respectively. Experimental and clinical samples were incubated 1 h at 37°C. After a washing step, SVA-Pabs were incubated 1 h at 37°C with goat anti-mouse IgG (Fc) and clinical samples with goat anti-swine IgG (Fc), both conjugated with horseradish peroxidase (HRP). Reactions were measured as optical density (OD) at 450 nm.

All 18 peptides generated from the VP1 protein were reactive against both mouse SVA-whole virus Pab and clinical sow serum. No difference in S/P ratio was observed amongst eighteen epitopes evaluated against both SVA-Pab. However, amongst 18 peptides evaluated good discriminatory S/P ratio values were observed with clinical sow serum on six peptides. Specifically, 4 independent linear epitopes and 2 linear overlapping peptides showed significantly higher S/P ratio compared with the rest of the epitopes, when evaluated with clinical sow sera. These results indicate that immune response generated by SVA-VP1 in pigs can be defined by a set of lineal epitopes. Further investigation warranted to determine potential neutralizing activity induced by this set of linear epitopes.

* Graduate Student Poster Presentation Award Applicant
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American Association of
Veterinary Laboratory Diagnosticians

Exhibit Directory

Greensboro, NC
October 13-20, 2016

Advanced Technology Corp.

Booth 301

79 North Franklin Turnpike, Suite 103
Ramsey, NJ 07446
www.vetstar.com
Contact: Joseph Bove
(201) 934-7127
jbove@vetstar.com

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

Anaerobe Systems

Booth 205

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

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

Biolog, Inc.

Booth 314

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Hayward, CA 94545
www.biolog.com
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smontgomery@biolog.com

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BioMed Diagnostics

Booth 312

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rvinayak@biomeddiagnostics.com

BioMed Diagnostics is an innovative manufacturer of microbiology diagnostic devices that save money, time, improve workflow and reduce sample exposure and contamination. Veterinarians, medical professionals, and researchers worldwide use these devices to accurately identify bacteria, parasites, fungi, and more.

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To learn about these and other products please visit: www.biomeddiagnostics.com and speak with us at our booth at AAVLD.

bioMérieux

Booth 200-202

595 Anglum Road
Hazelwood, MO 63042
www.biomerieux.com
Contact: Karen Mullen
(314) 731-8884
karen.mullen@biomerieux.com

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

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

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

Bioplastics/Cyclertest, Inc.

Booth 213

2933 South Miami Blvd., Bldg. 102, Suite 121
Durham, NC 27703
www.bpcti.com
Contact: Liane West
(919) 806-8811
lianew@bpcti.com

BIOplastics/CYCLERtest Inc. is uniquely positioned to assist with two key variables in PCR/qPCR, thermal cyclers and plastic vessels. Our DRIFTCON and MTAS dynamic multi-channel temperature calibration systems are calibrated to ISO 17025 standards and meet accreditation requirements for thermal cycler temperature calibration. The systems measure heat rate, cool rate, overshoots and undershoots, as well as accuracy and uniformity throughout the block, providing easy to understand reports. BIOplastics superior state-of-the-art disposables combine new and innovative user-friendly designs, optimum materials, low evaporation rates, low binding, high signal-to-noise ratios, and uniform wall thickness. These elements provide excellent performance and ease of handling to work in both standard and real time cyclers, so you can be assured of the most consistent and reproducible results possible for your assays.

Bio-Rad

Booth 201

2000 Alfred Nobel Drive,
Hercules, CA 94547
www.foodscience.bio-rad.com
Contact: Sonya Sano
(510) 741-4486
sonya_sano@bio-rad.com

Bio-Rad Laboratories is a worldwide leader in developing and manufacturing rapid methods for food and water safety testing and veterinary diagnostics. Bio-Rad's TSE kit is used throughout the world for consistent and accurate diagnosis of various spongiform encephalopathies such as Scrapie in sheep and goats, CWD in deer and elk, and BSE in cattle. The Bio-Rad kit is the only NAHLN approved ELISA for CWD testing of obex and lymph nodes samples for wild and captive mule deer, white –tailed deer and elk. Bio-Rad has a full range of chromogenic media for detection of pathogens such as *Listeria* and *Salmonella*. In addition, Bio-Rad produces a full range of antisera for serological identification of *Salmonella* using the Kauffman-White scheme. Visit the Bio-Rad booth for more information and to see how Bio-Rad methods can help improve the efficiency of your lab.

Biovet

Booth 100

9025 Penn Avenue South
Minneapolis, MN 55431
www.biovet-inc.com
Contact: Sheila Braun
(877) 824-6838
biovetusa@biovet-inc.com

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

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Booth 207

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Billerica, MA 01821
www.bruker.com
Contact: Nancy Salt
(978) 663-3660 x1492
nancy.salt@bruker.com

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Carolina Liquid Chemistries Corp.
Booth 307

575 N. Patterson Ave., Suite 430
Winston-Salem, NC 27101
www. carolinachemistries.com
Contact: Jennifer Hardy
(877) 722-8910
jhardy@carolinachemistries.com

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Centaur, Inc.
Booth 109

1351 Old 56 Hwy West, Bldg. F
Olathe, KS 66061
www.centauranimalhealth.com
Contact: Howard Jones, DVM
(800) 236-6180
sales@centauranimalhealth.com

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

Computer Aid, Inc.
Booth 204

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Harrisburg, PA 17111
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erin_pelletier@compaid.com

Computer Aid, Inc. is a global information technology application management and outsourcing firm focused on helping clients gain a competitive advantage through the effective use of IT. CAI was selected by the Commonwealth of Pennsylvania and the **National Agribusiness Technology Center** to develop and maintain the original herds software and after, all of NATC's AgraGuard products. As a $600 million IT company with more than 30 branch offices worldwide, we are entrepreneurial in focus, specializing in technical and management disciplines associated with business and government IT services and consulting. CAI is the original technical architect for all products in the AgraGuard suite. We work in various agencies including departments of agriculture, veterinary diagnostic laboratories, departments of health and bureaus of plant industry. This work has generated several awards, including the American Council for Technology 2006 Award and multiple honors from NASCIO.

CosmosID
Booth 215

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Rockville, MD
www.cosmosid.com
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Jon.ryan@cosmosid.com

CosmosID is a genomic big data company offering a cloud-based software that focuses on rapid identification of microorganisms for infectious disease diagnostics, antibiotic stewardship, outbreak investigation, and microbiome analysis for health and wellness. Our software platform offers unrivaled sensitivity and specificity in microbial identification and characterization. From a single universal test, we provide precise identification of bacteria, viruses, fungi and parasites at strain level with individual relative abundance and comprehensively characterize their antibiotic resistance genes and virulence factors. Our company was founded by Dr. Rita Colwell, a highly distinguished microbiologist who also served as a director for the United States National Science Foundation.

Eagle Biosciences, Inc.

Booth 102

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Nashua, NH 03063
www.EagleBio.com
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dkeefe@EagleBio.com

Eagle Biosciences has quickly become a leading provider of ELISA and Molecular Biology assay kits. With the new CKD staging guidelines for dogs from the International Renal Interest Society (IRIS) suggesting that SDMA concentrations in blood plasma or serum may be a more sensitive biomarker of renal function than creatinine, Eagle Biosciences is proud to introduce the SDMA ELISA assay from DLD Diagnostika in Germany which has been validated for multiple species.

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Booth 206

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Contact: Keith Ernst
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Booth 105

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www.elsevier.com
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GeneReach Biotechnology Corporation

Booth 107

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Central Taiwan Science Park
Taichung, 407 Taiwan ROC
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886.4.2463.9869
sales@genereach.com

GeneReach Biotechnology is a worldwide biotechnology company dedicated to bringing the innovation to global health management. By developing, manufacturing and marketing products for applied nucleic acid detection technology, we offer disease detection platforms, including equipment and reagents, to multiple industries such as aquaculture, agriculture, livestock, companion animals, and in vitro diagnostic industries. We developed and manufacture the world's one and only OIE-certified PCR diagnostic system, IQ2000. Our quality system is in compliance with ISO9001 and ISO13485.

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

IDEXX

Booths 306-308

One Idexx Drive
Westbrook, ME 04092
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Contact: Erin Ware
(207) 556-8313 or (207) 210-8288
erin-ware@idexx.com

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

Liferiver Bio-Tech (United States) Corp.

Booth 310

9855 Towne Centre Drive
San Diego, CA 92121
www.liferiverbiotech.com
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Louis_chen@liferiverbiotech.com

Liferiver develops and manufactures real-time PCR/RT-PCR kits/reagents and instruments for detection of infectious diseases in animals and human. We currently provide over 100 sets of reagents for examination of infectious pathogens in various animals including birds, cats, dogs, swine, cattle, goats, shrimps, etc. The products are user-friendly designed with convenient one-step assay, high specificity, sensitivity, and accuracy. With over 11 years of experience in this field, Liferiver is your supporter and partner in fighting against contagious diseases in animals.

Longhorn Vaccines and Diagnostics LLC

Booth 315

2 Bethesda Metro Center, Suite 910
Bethesda, MD 20814
wwwlhnvd.com
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chris@lhnvd.com

Privately-held Longhorn Vaccines and Diagnostics LLC is focused on vaccine and diagnostic product development harnessing the capabilities of molecular biology to develop and commercialize products.

Longhorn's PrimeStore® Molecular Transport Medium facilitates and simplifies sample collection and non-hazardous cold chain-free transportation (including long distance international shipping without dry ice) by effectively killing viral and bacterial pathogens and preserving and stabilizing naked RNA and DNA at ambient or elevated temperatures for extended periods.

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MACHEREY-NAGEL Inc.

Booth 203

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Bethlehem, PA 18020
www.mn-net.com
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(888) 321-6224
sales-us@mn-net.com

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National Institute for Animal Agriculture

Booth 217

13570 Meadowgrass Drive, Suite 201
Colorado Springs, CO 80921
www.animalagriculture.org
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katie.ambrose@animalagriculture.org

The mission of the National Institute for Animal Agriculture is to provide a forum for building consensus and advancing solutions for animal agriculture and to provide continuing education and communication linkages to animal agriculture professionals.

NIAA's purpose is to provide a resource for individuals, organizations, and the entire animal agriculture industry to obtain information, education and solutions for animal agriculture. NIAA accomplishes this by coordinating and promoting industry conferences, symposiums, meetings, etc. and materials that assist animal agriculture professionals in addressing current and emerging issues.

The organization is dedicated to programs that: work towards the eradication of diseases that pose a risk to the health of animals, wildlife and humans; promote a safe and wholesome food supply for our nation and abroad; and promote best practices in environmental stewardship, animal health and well-being.

PRI Bio

Booth 305

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Dupo, IL 62239
www.pri-bio.com
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jlaarman@progressive-recovery.com

PRI Bio is a global leader in Alkaline Hydrolysis Technologies (Tissue Digesters) and Effluent Decontamination Systems servicing Veterinary Research and Diagnostic Labs; Zootonic Research Facilities; Centers for Disease Control; and Pharmaceutical R&D and Manufacturing operations. For more than 30 years, PRI has advanced the science in hazardous liquid and solid waste treatment, specializing in the design and manufacture of sterilization and digester equipment. PRI's thermal products are currently used in 26 countries worldwide, with over 3,000 units installed.

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Explore us at www.pri-bio.com.

QIAGEN, Inc.

Booth 209-211

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Germantown, MD 20874
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QIAGEN is the leading global provider of Sample to Insight solutions that enable customers to gain valuable molecular insights from samples containing the building blocks of life. Our sample technologies isolate and process DNA, RNA and proteins from blood, tissue and other materials. Assay technologies make these biomolecules visible and ready for analysis. Bioinformatics software and knowledge bases interpret data to report relevant, actionable insights. Automation solutions tie these together in seamless and cost-effective workflows.

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Qualtrax

Booth 309

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www.qualtrax.com
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tdriver@qualtrax.com

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Contact: Christian Meinunger
(800) 364-2149
sales@quantabio.com

Quanta Biosciences is now Quantabio!

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Tetracore, Inc.

Booth 303

9901 Belward Campus Drive, #300
Rockville, MD 20850
www.tetracore.com
Contact: Olivia Mihalik
(240) 268-5400
omihalik@tetracore.com

Tetracore is a leading biotechnology company providing innovative diagnostic assays and reagents for infectious diseases. The Tetracore VetAlert™ product line features real-time Polymerase Chain Reaction (PCR) test kits and reagents for rapid and sensitive detection of animal pathogens. USDA licensed test kits are available for Johne's disease, in addition to specific detection reagents for PRRSV, Influenza, PED/TGE/PDCoV, SVA, ASFV , CSF, FMDV, and other targets. Our EZ Series qPCR assays have been shown to have superior coverage and performance in numerous studies. Our EZ Series includes EZ-PRRSV MPX 4.0, EZ-PED/TGE/PDCoV MPX 1.1, EZ-Universal Flu A 2.0 and EZ-SVA.

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Thermo Fisher Scientific

Booth 219-223

2130 Woodward Street
Austin, TX 78744
www.thermofisher.com/animalhealth
Contact: Elizabeth Lohse
(512) 721-3610
elizabeth.lohse@thermofisher.com

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

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VacciXcell

Booth 311

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mail@vaccixcell.com

VacciXcell is the bioprocessing division of Esco Healthcare, particularly specializing in adherent bioprocessing. VacciXcell provides design and manufacture of single-use, multiple-use, and hybrid bioreactor solutions for the vaccine and cell therapy industries. VacciXcell envisions a world of vaccine-self-sufficiency and easy access to advanced therapeutics and high quality biologics for all nations. VacciXcell is Esco's social entrepreneurship, in line with the Group's vision to provide enabling technologies to make human lives safer and healthier.

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VMRD, Inc.

Booth 210

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Pullman, WA 99163
www.vmrd.com
Contact: Edward Felt
(800) 222-8673
vmrd@vmrd.com

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

Zoetis

Booth 208

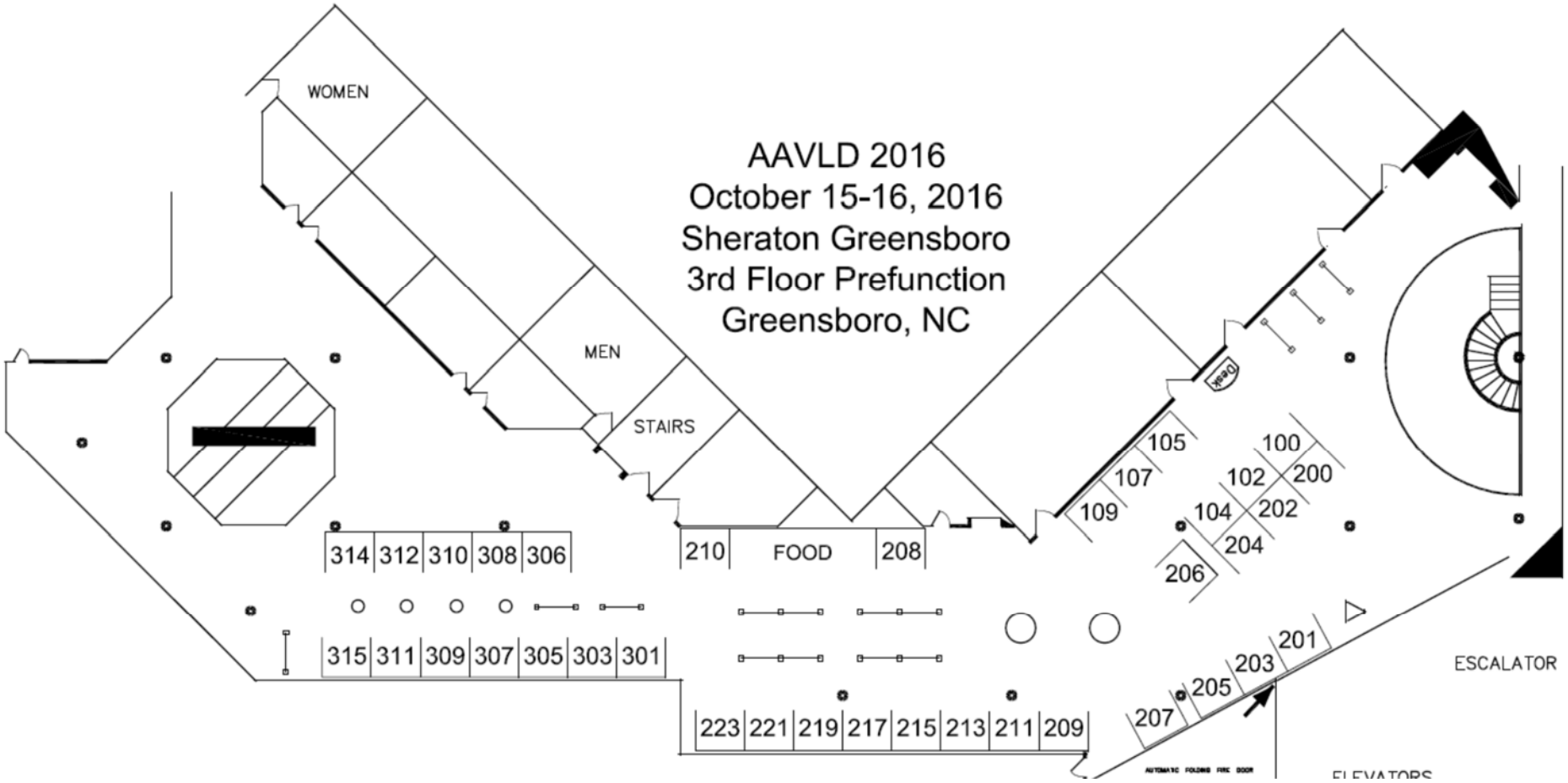
100 Campus Drive
Florham Park, NJ 07932
www.zoetis.com
Contact: Chris Demiris
(973) 443-3139
chris.demiris@zoetis.com

Zoetis (zô-EH-tis) is the leading animal health company, dedicated to supporting its customers and their businesses. Building on more than 60 years of experience in animal health, Zoetis discovers, develops, manufactures and markets veterinary vaccines and medicines, complemented by diagnostic products and genetic tests and supported by a range of services. Zoetis serves veterinarians, livestock producers and people who raise and care for farm and companion animals with sales of its products in more than 100 countries. In 2015, the company generated annual revenue of $4.8 billion with approximately 9,000 employees. For more information, visit www.zoetis.com.

Upcoming AAVLD/USAHA meetings:

San Diego, California
October 12-18, 2017

Kansas City, Missouri
October 18-24, 2018



Visit Exhibitors:

October 15 Saturday 9:00 am—6:00 pm

October 16 Sunday 9:00 am—1 :00 pm

Observe poster sessions throughout the exhibit area.

Authors will be at the posters

Saturday, Oct 15 3:00-4:00 pm

2016 AAVLD Exhibitors

Booth #	Exhibitor
301	Advanced Technology Corp. VADDS
205	Anaerobe Systems
314	Biolog, Inc.
312	BioMed Diagnostics, Inc.
200 202	bioMérieux
213	Bioplastics/Cyclertest, Inc
201	Bio-Rad
100	Biovet Inc.
207	Bruker Daltonics
307	Carolina Liquid Chemistries Corp.
109	Centaur, Inc.
204	Computer Aid, Inc.
215	Cosmos iD
102	Eagle Biosciences, Inc.
206	ECL2/Q-Pulse
105	Elsevier
107	GeneReach Biotechnology Corporation
306 308	IDEXX Laboratories
310	Liferiver Bio-Tech(US) Corp
315	Longhorn Vaccines and Diagnostics LLC
203	Macherey-Nagel, Inc.
217	Nat'l Institute for Animal Agriculture
305	PRI Bio (Progressive Recovery, Inc.)
209 211	QIAGEN, Inc.
309	Qualtrax
104	Quantabio
303	Tetracore, Inc.
219 221 223	Thermo Fisher Scientific
311	VacciXcell
210	VMRD, Inc.
208	Zoetis

Sponsor Presentations

Saturday, Oct 15

6:00 pm - 6:30 pm Imperial F

Biovet

Biovet innovates again with Multiplex serology, ELISA diagnostic kits and a brand new qPCR product line

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6:30 pm - 7:00 pm Imperial F

Qiagen

Explore new frontiers with Qiagen's sample to insight solutions for animal health

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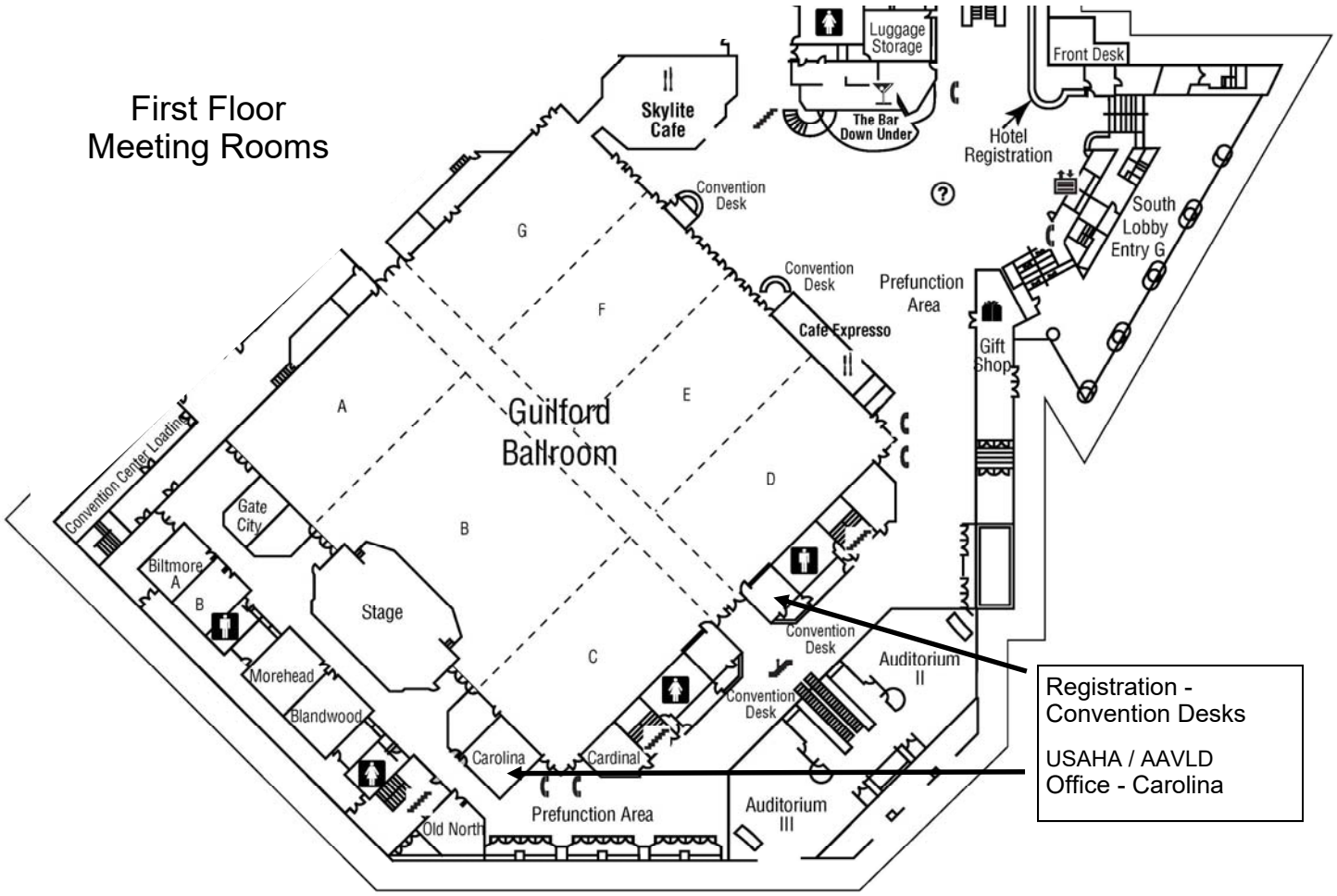
6:30 pm - 7:00 pm Imperial B

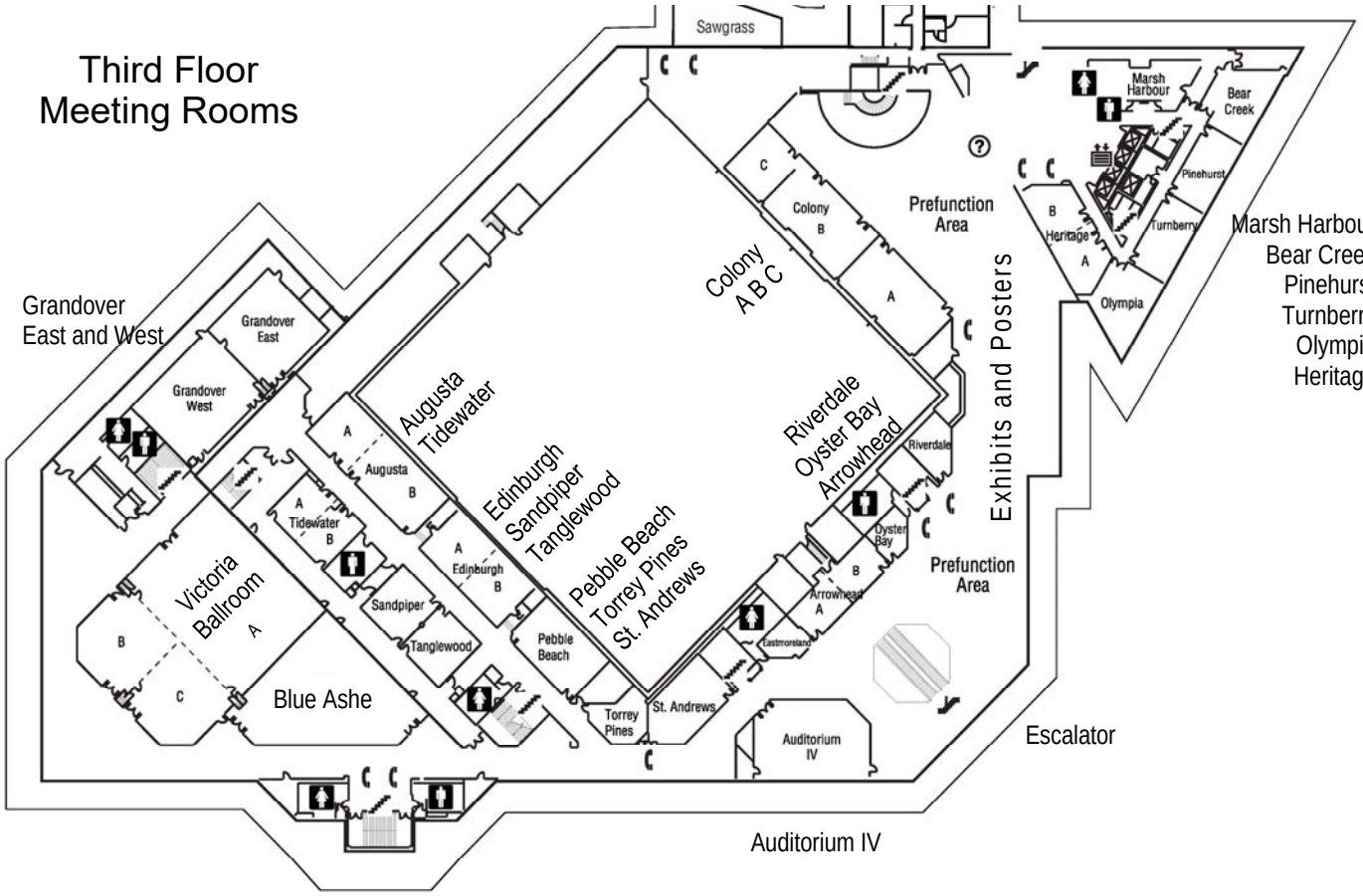
Thermo Fisher

Meeting today’s diagnostic needs – innovating for tomorrow’s challenges

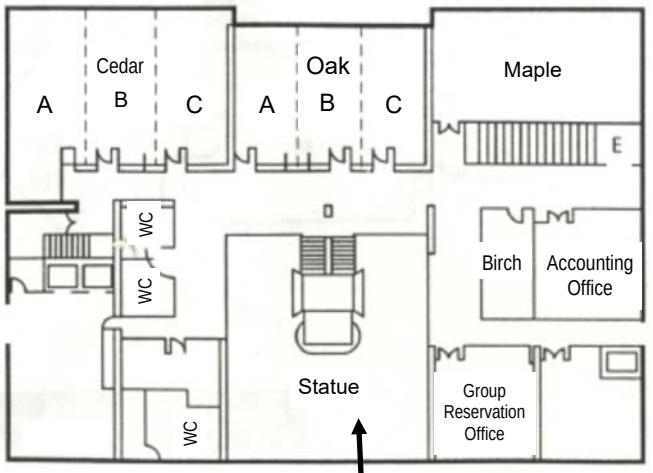
First Floor Meeting Rooms



Third Floor Meeting Rooms



SECOND FLOOR

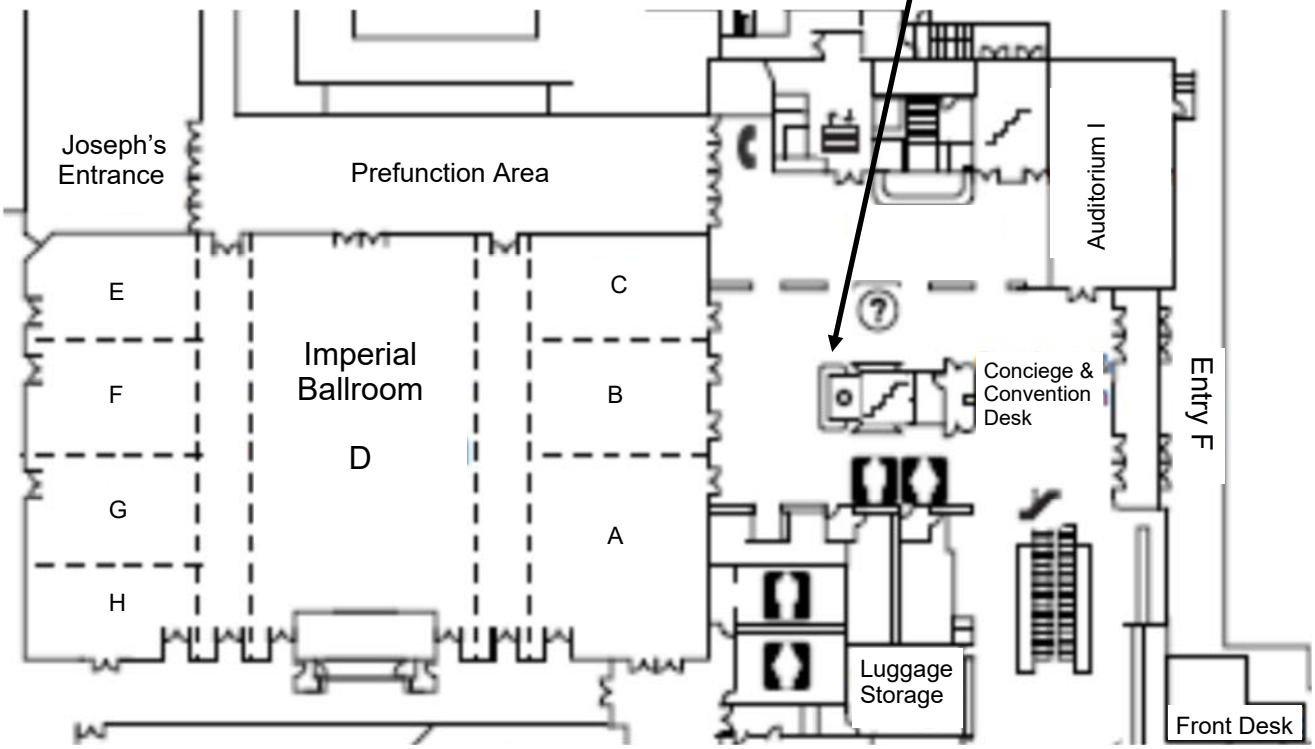


IMPERIAL BALLROOM

Committee meetings
AAVLD Plenary Session-Saturday
Scientific Sessions
Joint Plenary Session-Monday

Take stairs by statue to these rooms on the second floor; or use a nearby elevator

FIRST FLOOR



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