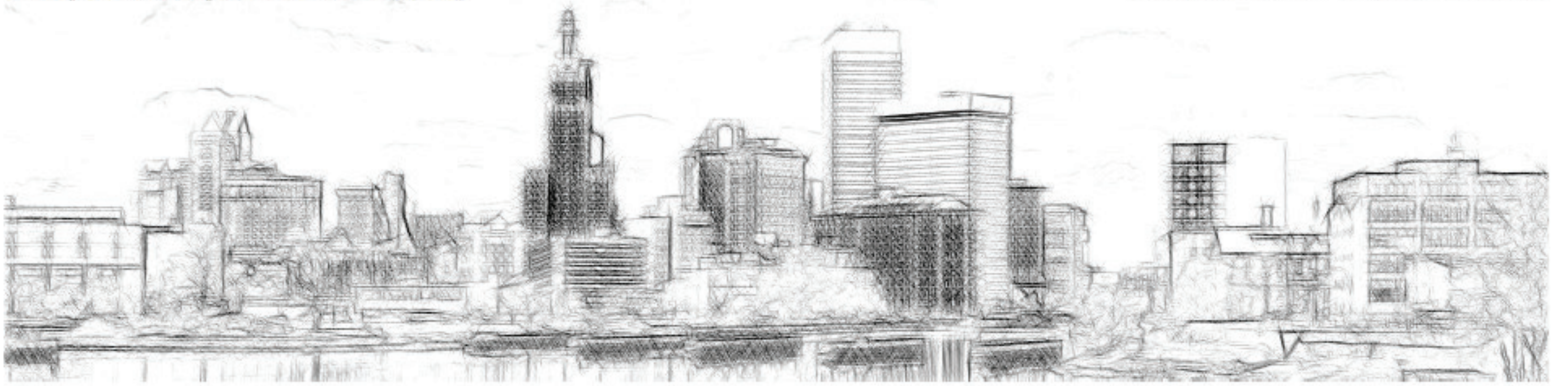
ONE HEALTH

PROVIDENCE AAVLD 2019



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AAVLD Strategic Plan

Updated August 7, 2019

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 One Health by engaging in development of animal health initiatives, policies, and dissemination of surveillance information.
2. Foster continuous improvement of diagnostic laboratories through accreditation and continuing education activities while encouraging discovery and innovation in veterinary laboratory diagnostic sciences.
3. Strengthen communication with members and promote their continued professional growth.

Our membership spans more than 32 countries worldwide.
Join us today and discover for yourself the benefits and
resources that AAVLD provides to its members.

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American Association of Veterinary Laboratory Diagnosticians

The American Association of Veterinary Laboratory Diagnosticians (AAVLD) is a not-for-profit professional organization.

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AAVLD Business Office
Phone 559-781-8900 Fax 559-781-8989
Website: www.aavld.org

PO Box 6396, Visalia, CA 93290
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Acknowledgments

Each year's meeting success depends on contributions from many individuals.

I want to recognize all attendees, who make the annual meeting possible. A special thank you to all who present their studies and findings, and to all exhibitors and sponsors for supporting the annual meeting. We would also like to give special recognition to our invited speakers for the AAVLD Plenary Session and the USAHA-AAVLD Keynote speaker.

Program Committee members deserve special thanks for reviewing and editing of the abstracts, and thanks are also due to moderators for hosting this year's interesting and engaging scientific sessions.

On behalf of entire AAVLD executive team, we are grateful for excellent administrative support provided by Reda Ozuna, flawless meeting coordination by Kaylin Taylor and program oversight offered by Dave Zeman in pulling together another successful annual meeting.

Sincerely,
Deepanker Tewari BVSc PhD DACVM

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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.

AAVLD AWARDS

2019 Trainee Travel Awardees

Molly Carpenter	Colorado State University
Juan Carlos Mora-Diaz	Iowa State University
Vanessa Oakes	Virginia-Maryland CVM
Sai Aravindh Sankara Narayanan	Oklahoma State University
Cristina Santana Maldonado	Iowa State University
Sreenidhi Srinivasan	Penn State University
Giovani Trevisan	Iowa State University
Yin Wang	Kansas State University
Jian Yuan	Iowa State University
Maodong Zhang	University of Saskatchewan
Grazieli Maboni Cosponsored by the Bacteriology Committee	University of Gerogia
Monika Samol Cosponsored by the Epidemiology Committee	University of California, Davis
Ava R. Jarvis Cosponsored by the Pathology Committee	Upstate Medical U. & Cornell U.
Viviana Gonzalez-Astudillo Cosponsored by the Pathology Committee	University of California, Davis

2019 Staff Travel Awardees

Renee Anderson	Cornell University
Brittany Chilson	Cornell University
Zachary Forbes	Cornell University
Suresh Kuchipudi	Pennsylvania State University
Nicole M. Andre Cosponsored by the Pathology Committee	Cornell University

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Dr. Francois Elvinger

The AAVLD Foundation with funds received from our members and committees have provided over $30,000 annually to support the advancement of our discipline and the development of the next generation of veterinary laboratory diagnosticians. Thank you to all donors!

The AAVLD Foundation Committee

AAVLD AWARDS

2018 AAVLD Organizational Awardees

E.P. Pope Award
Dr. Craig Carter

Distinguished Service Award
Dr. Kristy Pabilonia

Honorary Membership Award
Brad Mollet
Dr. Monique Eloit

Life Membership Award
Dr. Craig Carter

2018 AAVLD Committee Awards

BIOMIC Award for Excellence in Diagnostic Microbiology
Sponsored by Giles Scientific
Dr. Erdal Erol

Pioneers in Virology Award
Dr. Julia Ridpath

Richard L. Walker Best Classical Bacteriology Oral Presentation Award
Sponsored by Anaerobe Systems
April Aquino Estrada

J. Lindsay Oaks Best Molecular Oral or Poster Presentation Award
Sponsored by BioMerieux
Linto Anthony

Brenda Love Best Classical Bacteriology Poster Presentation Award
Sponsored by VMRD
Sreenidhi Srinivasan

2018 Joint AAVLD/ACVP Pathology Awards

AAVLD/ACVP Diagnostic Pathology Resident/Graduate Student Award

Yvonne Wikander

ACVP/AAVLD Diagnostic Pathology Resident/Graduate Student Award

Allison M. Watson

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Advances in Diagnostics and Impact on One Health

Saturday, October 26th, 2019 7:45am- 11:30 am

Exhibit Hall A

Moderators: Deepanker Tewari and Shuping Zhang

One Health: Challenges of Diagnostics for Zoonotic Infections

Capt. Jennifer McQuiston, DVM, MS, Deputy Director

Division of High Consequence Pathogens and Pathology/National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, GA

One Health is a collaborative, multisectoral, and transdisciplinary approach to public health that aims to optimize health outcomes for people, animals, plants, and their shared environment. CDC uses a One Health approach to prevent zoonotic disease outbreaks by working with physicians, veterinarians, ecologists, and many others to monitor and control public health threats. Using current diagnostics and surveillance platforms for rabies, hantaviruses, and orthopoxviruses as key examples, this talk will discuss the unique public health challenges—and opportunities—posed by zoonotic infections.

One Medicine, One Health: Dealing with Biological Risks and Mathematical Myths in Outbreaks of Highly Infectious Diseases

Gavin Macgregor-Skinner, BVSc, MSc, MPH, MRCVS, Director of Strategic Partnerships in Disaster Medicine

Department of Emergency Medicine, Beth Israel Deaconess Medical Center, Boston, MA, and Assistant Professor, Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA

Increasingly global threats including natural disaster, emerging pandemics, civil conflict, agricultural shocks are threatening the stability of countries and the health of millions, and can sometimes have an impact beyond country borders, as was seen during the 2014 West Africa Ebola outbreak and the global distribution of High Pathogenic Avian Influenza outbreaks. As the Ebola virus crossed borders, there was clear understanding that the outbreaks in West Africa were a potential threat to the world. This has been the commonly understood conceptualization for centuries. Yet, an equally important aspect to health security focuses on individual security and access to safe and effective health services, products, and technologies. Our work focuses on enhancing infection prevention and control activities; dealing with substandard drugs; addressing international migration, conflict and disaster; and building stronger health systems nationally and regionally. To be effective, key institutions such as Ministries of Health and Agriculture and National Disease Control bodies need to be functional with adequate capacity to carry out key functions such as coordination and communication in order to develop the infrastructure and capacity to protect the health of people, animals and societies worldwide and to ensure health system resilience

One Health and Omics age

Professor W. Ian Lipkin, MD, Director

Center for Infection and Immunity, Mailman School of Public Health, Columbia University, NY

The pace of pathogen discovery is dramatically increasing. This reflects not only factors enabling the appearance and globalization of new microbial infections, but also the improvements in methods for ascertainment. New molecular diagnostic platforms, investments in wildlife, domestic animal, and human microbial surveillance, and the advent of social media tools that mine the world wide web for clues to outbreaks of infectious disease are all proving invaluable in early recognition of threats to public health. Additionally, models of microbial pathogenesis are becoming more complex, providing insights into mechanisms by which microbes can contribute to chronic illnesses like cancer, peptic ulcer disease, and mental illness. I will review recent advances in methods for microbial surveillance and discovery, the strategies and pitfalls in linking discoveries to disease in both epigenetics and metabolomics, and methods for topological analysis having the potential to yield new insights into pathogenesis.

Zoonoses and One Health Diagnostics for regulatory animal diseases in Veterinary Labs

Suelee Robbe-Austerman, DVM, PhD, Director of Diagnostic Bacteriology and Pathology Laboratory

National Veterinary Services Laboratories, IA

Collaborations and partnerships with veterinary diagnostic laboratories, public health agencies, state animal health officials, researchers and international partners are critical for an effective regulatory response to a disease event. This talk will focus on the unique and important role veterinary diagnostic labs play in rapid diagnostics, notifications and recognition of new disease incursions, and the challenges we in veterinary labs face with new technologies, especially sequencing, that may have implications beyond their intended purpose.

Bacteriology 1

Saturday, October 26, 2019
Room 554

Moderators: Durda Slavic and John D. Loy

1:00 PM **Utility of different semisolid media for isolation of *Mycoplasma spp.* from canine lower respiratory specimens**
Joshua B. Daniels, Thomas Peppard *9*

1:15 PM **Whole Genome Sequencing investigation of a Carbapenem-Resistant *E. coli* Isolate from a Dog**
Stephen Cole, Shelley C. Rankin *10*

1:30 PM **Evidence for increasing and widespread antimicrobial resistance in *Salmonella* Dublin isolates associated with disease in cattle in California over the study period 1991-2018**
Heather Fritz, Richard Pereira, Kathy Toohey-Kurth, Kris A. Clothier *11*

1:45 PM **Joint FDA and USDA report on antimicrobial resistance pilot projects for 2018**
Beth Harris, Jennifer Rodriguez, Olgica Ceric, Greg Tyson, Renate Reimschuessel *12*

2:00 PM **Genomic analysis and virulent factors of *Klebsiella pneumoniae* isolated from mastitis cows in the United States * †**
Zhiyi Zheng, Nicholas John Backes, Phillips Gregory, Gorden Patrick, Xiaoqin Xia, Ying Zheng, Ganwu Li *13*

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Jennifer Malmberg, Terry Creekmore, Erika Peckham, Marguerite Johnson, Halcyon Killion, Madison Vance, Daniel Bradway, Noah C. Hull, Donal O'Toole, Kerry Sondgeroth *14*

2:30 PM **Isolation and characterization of invasive *Streptococcus pneumoniae* from an infant rhesus monkey (*Macaca mulatta*) died of fulminant meningitis**
Sung Guk Kim, Christine Summage-West, Lillie Sims, Leah Rowe, Kelly Davis *15*

2:45 PM **Whole-Genome Sequencing for predicting toxinotypes and antimicrobial resistance in *Clostridium perfringens***
Orhan Sahin, Lei Dai, Curt Thompson, Zuowei Wu, Nubia Macedo, Ganwu Li, Rodger Main *16*

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

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Utility of different semisolid media for isolation of *Mycoplasma spp.* from canine lower respiratory specimens

Joshua B. Daniels, Thomas Peppard

Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO

Mycoplasma spp. are thought to be components of the normal microbiota of the upper respiratory tract of dogs, however, their role as opportunists, potentially causing tracheitis, bronchitis, and bronchopneumonia is controversial, as Koch's postulates have only been satisfied for lower respiratory disease in dogs for *M. cynos*. Clinicians variably include requests for specific *Mycoplasma* culture upon submission of lower respiratory samples to our laboratory for microbiological testing, which is perhaps reflective of this controversy.

We have observed that *Mycoplasma spp.* may be isolated successfully without the use of specific *Mycoplasma* media, suggesting that it may be unnecessary to use specific *Mycoplasma* media to cultivate these organisms from canine respiratory samples. We analyzed *Mycoplasma spp.* (*n*=16) isolated from canine respiratory specimens that were isolated on either PPLO agar, Columbia blood agar (CBA), or both media to determine if recovery of the organisms on different media was associated with certain species of *Mycoplasma*. Isolates were subcultured in PPLO broth and speciated via DNA sequencing of the 16s-23s spacer region, and further subcultured to both types of media. 4/8 *M. canis*, 1/2 *M. edwardii*, 0/2 *M. cynos*, 1/3 *M. spumans*, and 0/1 *M. Maculosum* were originally isolated on CBA, and further subculture usually reflected the isolates' ability to grow on the media used for primary isolation. These results suggest that specific *Mycoplasma* media are indeed required to maximize recovery of *Mycoplasma spp.* from canine lower respiratory specimens, and it is notable that *M. cynos* were uncultivable on CBA.

Whole Genome Sequencing investigation of a Carbapenem-Resistant *E. coli* Isolate from a Dog

Stephen Cole, Shelley C. Rankin

UPenn, Philadelphia, PA

Carbapenem-resistant Enterobacteriaceae (CRE) are an important cause of nosocomial infections in humans but are rare in companion animals. CRE are resistant to most of the antimicrobials used to treat infections caused by Enterobacteriaceae. A carbapenem resistant strain of *E. coli* was isolated from a dog hospitalized at the University of Pennsylvania's School of Veterinary Medicine. CR-*E. coli* was isolated from an endotracheal wash specimen from an 11-year-old female-spayed Great Dane dog with megaesophagus and aspiration pneumonia. The MIC for imipenem was 4 ug/ml. Confirmatory tests included E-test and the modified carbapenem inactivation method (mCIM). Short-read sequencing on the Illumina MiSeq and analysis with ResFinder showed that the isolate possessed 19 known antibiotic resistance genes including an NDM-5 carbapenamase gene which has not been previously reported in isolates from animals in the USA. MLST analysis showed sequence type 167 (ST167). The predicted serotype was O89:H5. Virulence factor analysis detected *an iss* (increased serum survival) gene which is associated with the avian pathogenic *E. coli* (APEC) subpathotype. PlasmidFinder analysis identified an IncFII plasmid which has been associated with NDM-5 positive *E. coli*. Long read sequencing is pending to fully evaluate the complete plasmid structure. It is important to build infrastructure in veterinary medicine for detection and surveillance of CRE and to provide guidance for future studies. The development of protocols for the rapid identification, phenotypic confirmation and molecular characterization of CRE isolates from animals will be critical to containing the spread of this organism in the companion animal population in the United States.

Evidence for increasing and widespread antimicrobial resistance in *Salmonella* Dublin isolates associated with disease in cattle in California over the study period 1991-2018

Heather Fritz[1], Richard Pereira[2], Kathy Toohey-Kurth[1], Kris A. Clothier[1]

[1]CA Animal Health & Food Safety Lab, UC Davis, Sacramento, CA; [2]Population Health and reproduction, University of California, Davis, Davis, CA

Salmonella Dublin is a host-adapted *Salmonella* causing significant morbidity and mortality in calves and adult cattle. In addition to shedding in feces, milk and colostrum from acutely affected animals, development of a carrier state with intermittent shedding over months or years has been reported. Human infection from this serovar are often severe with septicemia and death as common sequelae, and antimicrobial therapy is critical to maximize successful outcomes in these patients. Current and historical antimicrobial resistance (AMR) pattern data is necessary to direct appropriate judicious antimicrobial use practices in animals. The purpose of this study was to evaluate *S.* Dublin isolated from cattle between 1991-2018 for trends in AMR and evaluate the predicted efficacy of veterinary drugs in the treatment of this pathogen. Microbroth dilution minimum inhibitory concentration (MIC) testing was performed on 188 isolates utilizing both the Sensititre BOPO6F and NARMS Gram Negative Plates to provide clinically relevant data for veterinarians as well as surveillance information for comparison with national databases. Using CLSI interpretive criteria for humans, AMR increased over time against amoxicillin-clavulanate (10% Resistant 1991-1999; 50% Resistant 2000-2009 and 65% Resistant 2010-2017), chloramphenicol (40% Resistant 1991-1999; 77% Resistant 2000-2009 and 84% Resistant 2010-2017), ceftriaxone (0% resistance 1991-1999; 43% resistance 2000-2009 and 65% Resistance 2010-2017) and trimethoprim-sulfamethoxazole (0% Resistance 1991-1999; 2% Resistance 2000-2009 and 5% Resistance 2010-2017). Additionally, decreasing susceptibility to Ciprofloxacin was measured (100% susceptible 1991-1999; 97% susceptible and 3% Intermediate 2000-2009 and 64% Susceptible and 35% Intermediate 2010-2017). AMR patterns against tetracycline decreased from 80% in 1990-1999 to 76% in 2000-2009 then increased to 83% in 2010-2017. Interestingly, resistance against ampicillin was relatively stable to slightly decreased over time (80%, 72% and 66%.) While bovine interpretations for *Salmonella sp.* are not available, extrapolation from other species yielded similar changes against veterinary drugs for the time periods 1991-1999, 2000-2009 and 2010-2017, with percent resistant increasing for ceftiofur (0%, 42% and 65%), danofloxacin (0%, 3% and 33%), and florfenicol (20%, 63% and 84%); and remaining consistently high for chlortetracycline (80%, 74% and 83%.) Although this data was obtained from a biased sample of diagnostic lab isolates for which animals likely had previous antimicrobial therapy, this information indicates that empirical antimicrobial therapy for *S.* Dublin is not likely to be effective. Alternative intervention strategies are needed to control this pathogen in bovine herds, particularly as antimicrobial drug use becomes more restricted, and to minimize risks of exposure in humans.

Joint FDA and USDA report on antimicrobial resistance pilot projects for 2018

Beth Harris[1], Jennifer Rodriguez[1], Olgica Ceric[2], Greg Tyson[2], Renate Reimschuessel[2]

[1]NAHLN, USDA APHIS NVSL, Ames, IA; [2]Center for Veterinary Medicine, FDA, Laurel, MD

Antibiotic resistant bacteria are a major concern for human and animal health. FDA and USDA are both charged with enhancing their efforts to identify emerging resistance in animal pathogens with the ultimate goal of increasing stewardship.

In 2018, FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) continued a pilot project with twenty "source" laboratories to conduct antimicrobial susceptibility testing (AST) of *S. pseudintermedius* and *E. coli* from dogs and *Salmonella sp*. from any host. These are collectively called "SES" isolates. For 6 months laboratories also tested 12 additional "other" bacteria per month, non-targeted isolates obtained from any host. FDA received AST data from a total of 2974 isolates (724 *S. pseudintermedius*, 716 E. coli, 576 Salmonella, and 958 "Other").

Concurrently, USDA APHIS' National Animal Health Laboratory Network (NAHLN) initiated the first year of the NAHLN AMR pilot project, covering the time period of January 1, 2018 to December 19, 2018. Nineteen laboratories provided AST data to the NAHLN from 3213 veterinary bacterial isolates. Four livestock species (cattle, swine, poultry and horses), and two companion animal species (dogs and cats) were covered. Bacterial isolates surveyed were *Escherichia coli (E. coli)* (1700 isolates across all animal species)*, Salmonella enterica* spp. (584 isolates across all species), *Mannheimia haemolytica* (380 isolates from cattle), and *Staphylococcus intermedius* group (548 isolates from dogs and cats).

This is a joint report of information from these two studies, highlighting the collaborations between FDA, USDA and all participating laboratories. The datasets do not overlap, the thirteen laboratories that provided data to both networks collected the first 4 SES isolates per month for Vet-LIRN, and subsequent isolates for NAHLN. Both networks followed Clinical and Laboratory Standards Institute (CLSI) AST testing methods.

In the NAHLN study, *E. coli* and *Salmonella* were most frequently associated with enteric infections in cattle, swine, and poultry, while urinary tract infections were the primary source of *E. coli* from dogs and cats. However, in horses, *E. coli* was correlated with reproductive tract infections. Similarly, the *E. coli* in dogs isolated by the Vet-LIRN laboratories came predominantly from urinary tract infections. As expected both networks found that the *S. intermedius* group isolates were most commonly associated with skin/wound infections in both dogs and cats. Similarly, certain Salmonella serotypes appear to be correlated with enteric infections and septicemia in cattle (serotypes Cerro, Dublin and Typhimurium), and enteric infections in swine (serotype 4,5,12:i:-).

Genomic analysis and virulent factors of *Klebsiella pneumoniae* isolated from mastitis cows in the United States * †

Zhiyi Zheng[1], Nicholas John Backes[1], Phillips Gregory[1], Gorden Patrick[4], Xiaoqin Xia[2,5], Ying Zheng[3], Ganwu Li[4]

[1]VMPM, Iowa State University, Ames, IA; [2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China; [3]VDL, Iowa State University, Ames, IA; [4]VDPAM, Iowa State University, Ames, IA; [5]University of Chinese Academy of Science, Beijing, China

Dairy Cattle Mastitis has long been one of the most common and costly diseases in the dairy industry worldwide due to its significant impact on milk production and animal welfare. Among all the mastitis bacterial pathogens, *Klebsiella pneumoniae* (Kp) was reported to cause the largest milk loss in dairy mastitis cases. As a consequence, additional studies are needed to characterize the bacterial pathogens that cause mastitis. To better understand the genomic epidemiology and pathogenesis of *Klebsiella pneumoniae* associated with bovine mastitis, we have sequenced the genomes of 93 *Klebsiella pneumoniae* strains isolated from cow mastitis outbreaks of 15 U.S. states. The phylogenetic analysis classified all 93 mastitis Kp into 2 major phylogroups, of which 85 were Kp-I and 11 were Kp-II. Among all genomes, there were more than 42 sequence types detected with a predominant sequence type 107. Capsular typing results showed some difference compared to Kp genomes from human infection. Only 2 mastitis Kp were identified with capsule type 1 and none with capsule type 2, which were both considered as hypervirulent capsule types in human Kp isolates. The O-antigen was typed based on related gene sequences and type O1 was found predominant. Antimicrobial resistant genes and susceptibility tests showed most of the mastitis Kp were resistant to ampicillin and few were resistant to kanamycin or chloramphenicol. In addition, several genes were identified that were unique to Kp associated with cow mastitis compared to human and meat Kp isolates. 97.85% (91/93) mastitis Kp strains possess two copies of the *lac* operon along with a ferric citrate operon, which might be important virulent determinants related to cattle mastitis. In contrast, most of Kp isolates from human infection cases contained only one *lac* operon and no ferric citrate operon. Further sequence analysis indicated these two operons exist on a single plasmid, along with some other putative virulent genes. To better understand the role of the virulence plasmid in Kp, we used a CRISPR-Cas9 system to cure the replicon from one of the Kp isolates. Ongoing studies to characterize this isolate should be valuable in improving our understanding of Kp virulence in bovine mastitis.

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

An outbreak of *Mycoplasma bovis* in free-ranging Wyoming pronghorn (*Antilocapra americana*)

Jennifer Malmberg[1,2], Terry Creekmore[3], Erika Peckham[3], Marguerite Johnson[2], Halcyon Killion[1], Madison Vance[1], Daniel Bradway[4], Noah C. Hull[5], Donal O'Toole[1,2], Kerry Sondgeroth[1,2]

[1]Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, WY; [2]Veterinary Sciences, University of Wyoming, Laramie, WY; [3]Wyoming Game and Fish Department, Laramie, WY; [4]Washington Animal Disease Diagnostic Laboratory, Pullman, WA; [5]Wyoming Public Health, Cheyenne, WY

Mycoplasma bovis (*M. bovis*) is one of the bacterial pathogens associated with bovine respiratory disease in cattle, however its role in wildlife pneumonia has not been consistently established. *M.bovis* associated pneumonia in captive North American bison, has recently increased the interest in this pathogen to bison producers. During February through April of 2019, approximately 60 pronghorn perished in northeastern Wyoming due to respiratory disease. Samples from nine of these mortalities were submitted to the Wyoming State Veterinary Diagnostic Laboratory. The consistent finding between cases was a severe, fibrinosuppurative pleuropneumonia, and detection of *M. bovis* by PCR and IHC. Isolates from five of these cases were grown in culture, for additional characterization by mass spectrometry and whole genome sequencing in comparison to other bovine isolates. Interestingly, genetic data revealed an indel in the alcohol dehydrogenase-1 gene (*adh*-1), a target of multilocus sequencing typing (MLST) of *M. bovis* in cattle and bison. Additionally, a retrospective study on lung samples from pronghorn with and without pneumonia from 2007-2018 (19 total) were evaluated by IHC and PCR; and *M. bovis* was not detected. Together, this suggests the emergence of *M.bovis* in pronghorn is a recent event, and should be considered as a differential in other free-ranging ungulates with pneumonia.

Isolation and characterization of invasive *Streptococcus pneumoniae* from an infant rhesus monkey (*Macaca mulatta*) died of fulminant meningitis

Sung Guk Kim[1], Christine Summage-West[1], Lillie Sims[1], Leah Rowe[1], Kelly Davis[2]

[1]Surveillance/Diagnostic Laboratory, NCTR/FDA, Jefferson, AR; [2]NCTR/FDA, Toxicologic Pathology Associates, Jefferson, AR

Heavily mucoid invasive *Streptococcus pneumoniae* was isolated from a 2-month-old male rhesus monkey (*Macaca mulatta*) that suddenly died with no clinical manifestations. Bacterial cultures of the brain, the lung, and the heart of the infant monkey were predominantly mucoid *S. pneumoniae*. The inflammation was observed in the meninges of all portions of the brain. The inflammation was also observed in the lung, particularly in subpleural parenchyma indicating cause of the inflammation via the hematogenous route rather than via the airways. No additional lesions in all other examined tissues were observed. *S. pneumoniae* isolates were determined to be serotype 3 with no significant antibiotic resistance profile by the *Streptococcus* Laboratory at CDC. Based on histopathological observations and bacterial culture results, streptococcal meningitis was diagnosed as the cause of the death of the infant monkey. These primate *S. pneumoniae* isolates were further characterized based on biochemical properties by Vitek2C and BioLog, fatty-acid compositions by MIDI, and proteomic profiles by Biotyper. There were no significant differences among those isolates, suggesting those isolates might be of the same origin. Whole genome sequences of these isolates determined using an Illumina MiSeq platform, analyzed, and compared. The genomes of the invasive serotype 3 isolate of *S. pneumoniae* were identical, consisting of a circular chromosome of 2,013,740 bp with G + C content of 39.8%. Of the 2208 CDSs, 1836 were assigned with functions, and 447 were assigned with pathways. The closest reference genomes are *S. pneumoniae* R6 (Genbank accession number AE007317) and *S. pneumonia* TGR4 (Genbank accession number AE005672) of human origin, with the Mash distances of 0.00113974 and 0.0117112, respectively. The presence of a serotype 3 *S. pneumoniae* isolate from rhesus monkeys in a lab animal facility is significantly important because of its greater virulence and higher mortality rate than other serotypes and could cause a serious occupational hazard especially to those people who work in close contact with non-human primates. Efficient monitoring programs and accurate diagnostic programs are required to early detect pneumococcal meningitis.

Whole-Genome Sequencing for predicting toxinotypes and antimicrobial resistance in *Clostridium perfringens*

Orhan Sahin[1], Lei Dai[2], Curt Thompson[1], Zuowei Wu[2], Nubia Macedo[1], Ganwu Li[1], Rodger Main[1]

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

Clostridium perfringens, a spore-forming anaerobic bacterium, is an important pathogen causing a range of diseases including gas gangrene, food poisoning, necrotic enteritis, enterocolitis and enterotoxemia in animals and humans. The organism produces a large array of harmful toxins, spectrum of which shows variation among strains and used for classification of the bacterium into five main toxinotypes (A-E) based on the production of four major toxins (alpha, beta, epsilon and iota). Different toxinotypes are usually associated with different pathological manifestations, and thus important to disease prevention and management, which includes vaccination, general hygiene and antibiotic therapy. Antimicrobial resistance (AMR) in *C. perfringens* is on the rise and poses a significant clinical threat for effective treatment. Whole-genome sequencing (WGS) has recently become an important tool in diagnostic microbiology, facilitating rapid and accurate characterization of bacterial pathogens. The purpose of the current study was to determine the toxinotypes and AMR profiles of *C. perfringens* isolates (n= 58) recovered from diseased animals using WGS. Traditional toxin typing was done via standard PCR; antimicrobial susceptibility testing (AST) was performed using broth microdilution to determine phenotypic resistance to 15 antibiotics based on CLSI breakpoints. WGS was performed on the Illumina MiSeq platform and raw reads were used for identification of virulence and AMR genes. The correlation between the PCR and WGS for toxin typing was 95% and WGS was able to further type an isolate with inconclusive PCR data. In regard to AMR correlation between AST and WGS, overall sensitivity (of WSG) was low (32%) though it was 100% for ampicillin and tetracyclines; however, overall specificity (of WGS) was high (96%) and it was 100% for the majority of antibiotics analyzed. These results show that WGS is a superior tool for identification of toxinotypes of *C. perfringens*, and has the potential for prediction of AMR in near future for this important animal and human pathogen.

Bacteriology 2

Sunday, October 27, 2019
Ballroom E

Moderators: Akhilesh Ramachandran and Sarmila Dasgupta

8:00 AM **Whole-Genome Sequencing to predict genotype and antimicrobial resistance in *Escherichia coli* isolated from pigs ◊**
Nubia Macedo, Rodger Main, Ganwu Li, Orhan Sahin 19

8:15 AM **Detection of bovine respiratory bacterial pathogens and determination of associated antimicrobial susceptibility profiles using clinical nanopore metagenomics**
Anatoliy Trokhymchuk, Musangu Ngeleka, Kazal Ghosh 20

8:30 AM **Tularemia (*Francisella tularensis*) recognition in cats - A public health message for veterinary clinicians and diagnosticians**
Marc D. Schwabenlander, Tory Whitten, Erik J. Olson 21

8:45 AM **Comparison of nested and quantitative PCR assays for detection of *Streptococcus equi* subspecies *equi* in clinical equine specimens**
Lance Wade Noll, Colin P. A. Stoy, Yin Wang, Elizabeth G. Porter, Xuming Liu, Jianfa Bai 22

9:00 AM **Evaluation of a high throughput nucleic acid extraction method for PCR-based detection of *Mycobacterium avium* ssp. *paratuberculosis* in bovine fecal samples**
Nagaraja Thirumalapura, Willard Feria, Eric Hue, Corey Zellers, Deepanker Tewari 23

9:15 AM **Break**

10:15 AM ***Theileria orientalis* Ikeda genotype is identified in cattle in Southwestern Virginia # + †**
Vanessa J. Oakes, Michael J. Yabsley, Diana Schwartz, Tanya LeRoith, Carolynn Bissett, Charles Broaddus, Jack L. Schlater, S Michelle Todd, Katie Boes, Meghan Brookhart, Kevin Lahmers 24

10:30 AM **Listerial meningoencephalitis and choroid plexitis in a saki monkey (*Simia pithecia*) +**
Abigail Finley, Alexandra Goe, Kristen Phair, Jason Struthers 25

10:45 AM **Increased pathogen diversity in the skin microbiome of bats affected by white nose syndrome # * †**
Ava R. Jarvis, Renee Anderson, Derek Rothenheber, Ben Golas, Amy Glaser, Laura B. Goodman, Elizabeth Buckles 26

11:00 AM ***Streptococcus pneumoniae*-associated septicemia in two cats**
Heidi Huffman Rose, Lifang Yan, JayKay Thornton 27

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

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Whole-Genome Sequencing to predict genotype and antimicrobial resistance in *Escherichia coli* isolated from pigs ◊

Nubia Macedo, Rodger Main, Ganwu Li, Orhan Sahin

VDPAM, Iowa State University, Ames, IA

Ninety *E. coli* strains isolated between 2016 and 2018 by the Iowa State University Veterinary Diagnostic Laboratory were characterized by genotyping PCR and WGS for detection of enterotoxins and adherence genes. The phenotype of antimicrobial resistance of a subset of 70 isolates was also tested by broth microdilution, and compared with their respective antimicrobial resistance genes detected by WGS. Preliminary results showed that 81 (90%) and 75 (83.3%) of isolates were classified as ETEC strains producing enterotoxins LT, STa and STb by both PCR and WGS, respectively. Kappa agreement between both tests were either very good (k=0.81-1) or good (k=0.61-0.80) for genes such as EAST, STA, STX2, F18 and K88, and moderate (k=0.41-0.6) for genes LT and STb. Kappa agreement was either fair (k=0.21-0.4) or poor (k≤0) when genes such as STX1, K99 and F41 were present in very low prevalence. Because the magnitude of kappa is affected by the prevalence of genes, if most of the genes are either present or absent, kappa agreement coefficient is reduced accordingly. Among the 70 strains characterized by antimicrobial susceptibility, all isolates were resistant to penicillin. Other common resistances were to oxytetracycline (98.6%), chlortetracycline (90%), ampicillin (84.3%), sulfadimethoxime (71.4%), spectinomycin (61%), enrofloxacin (61%), neomycin (50%) and gentamycin (41%). We detected relatively low levels of resistance to trimethoprim/sulphamethoxazole (31.4%), florfenicol (30%) and ceftiofur (23%). By WGS, 50 resistance genes were detected, which are involved in resistance to beta-lactams, aminoglycosides, chloramphenicol, quinolones, sulfonamides, and tetracyclines. When comparing WGS to antimicrobial resistance phenotypes, the resistances to ceftiofur, trimethoprim/sulphamethoxazole, florfenicol and sulfadimethoxime were highly correlated between genotypes and phenotypes. Because of the 100% presence of the *ampC* gene, the genotype of beta-lactam resistance was 100%, but for penicillin and ampicillin, their genotypic resistance was 100% and 84%, respectively. Resistance to ceftiofur was highly associated with presence of *bla-*$_{\text{CMY}}$ or *bla*$_{\text{CTX-M}}$ *genes*, with sensitivity and specificity of 94% and 100% respectively. Detection of genes involved in acquired quinolone resistance was very low, even though 61% of isolates were phenotypically resistant, probably due to mutations in the quinolone resistance-determining region. The majority of isolates carried genes conferring resistance to aminoglycosides and tetracyclines, even though a subset of isolates were still phenotypically susceptible to those antimicrobials, resulting in high sensitivities (90 and 80% respectively), but lower specificities (45 and <1%, respectively). In general, WGS accurately predicted *E. coli* genotypes and the majority of resistance phenotypes from *E. coli* strains, even though more studies are needed to better characterize some of the mechanisms of antimicrobial resistance.

◊ USAHA Paper

Detection of bovine respiratory bacterial pathogens and determination of associated antimicrobial susceptibility profiles using clinical nanopore metagenomics

Anatoliy Trokhymchuk, Musangu Ngeleka, Kazal Ghosh

Prairie Diagnostic Services, Saskatoon, SK, Canada

Bovine respiratory disease (BRD) is one of the most costly condition of beef cattle responsible for up to 65 to 80% morbidity and 45 to 75% mortality in some feedlots. Treatment and prevention of BRD lead to extensive use of antimicrobials in the cattle industry. Antimicrobial resistance (AMR) is an important public health threat worldwide due to the emergence of multidrug resistant bacteria. The World Health Organization promotes prudent use of antimicrobials and recommends that these drugs be prescribed for animals only when the etiological agent has been identified and the antimicrobial susceptibility test (AST) performed. However, the current laboratory diagnosis and AST for BRD treatment and metaphylaxis guidance is not a common practice due in part to long laboratory turnaround time (TAT; 3 to 5 days) from sample submission to availability of results. The purpose of this proof-of-concept study was to assess the rapid detection of BRD pathogens and their AMR profiles directly from clinical diagnostic samples using whole-metagenome sequencing (WMGS) with nanopore platform. Thirty samples including deep nasal swabs and lung tissues from feedlot calves with or without respiratory infection were cultured for the most common BRD pathogens *Mannheimia haemolytica* (Mh), *Pasteurella multocida* (Pm) and *Histophilus somni* (Hs). AST of isolates was determined by the broth microdilution method to the common antimicrobials used for BRD in the cattle industry: ampicillin, ceftiofur, danofloxacin, enrofloxacin, florfenicol, gamithromycin, penicillin, spectinomycin, tetracycline, tildipirosin, tilmicosin, trimetroprin-sulfamethoxasole and tulathromycin. Furthermore, these samples were tested for the presence of BRD pathogens and associated AMR gene profiles by WMGS. We recovered 33.3% of Mh and 10.0% of each of Pm and Hs from bacteriology cultures. In contrast, we detected more BRD pathogens by WMGS (Mh - 43.3%; Pm - 13.3% and Hs - 20.0%) as compared to culture. In addition, other pathogens such as Bibersteinia trehalosi (6.6%) and Mycoplasma bovis (23.3%) not isolated from culture were detected by WMGS. The AST phenotype of isolates was comparable to the genotypic profile obtained by WMGS. Our data indicate that WMGS can provide laboratory diagnostic information on BRD pathogen identification and AMR genotype similar or better to that obtained from routine culture and AST. This diagnostic information can be obtained faster using WMGS (under 6 hours) as compared to culture (3 to 5 days). Therefore, WMGS may be considered as a tool that improves diagnostic laboratory TAT and subsequently, may guide clinicians towards the informed choice of treatment options and promote antimicrobial stewardship.

Tularemia (*Francisella tularensis*) recognition in cats - A public health message for veterinary clinicians and diagnosticians

Marc D. Schwabenlander[1], Tory Whitten[2], Erik J. Olson[1]

[1]University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN; [2]Minnesota Department of Health, St. Paul, MN

Francisella tularensis is a gram-negative coccobacillus, which infects numerous animal species and is the causal agent of tularemia. Cats are the most likely domestic pet to transmit the bacteria to humans in Minnesota. The veterinary literature describes a clinical presentation in cats that may include sudden onset of fever; lethargy; generalized lymphadenopathy; splenomegaly and hepatomegaly; oral and/or lingual ulcers; icterus; respiratory distress; diarrhea; vomiting; seizures; and a history of being outdoors and/or predation on wildlife. Gross lesions may include markedly enlarged lymph nodes; hepatomegaly and/or splenomegaly; and multiple small, white to gray foci to large abscesses in the spleen, liver, lungs, lymph nodes, and/or heart.

In 2016-2018, the Minnesota Department of Health (MDH) and the University of Minnesota Veterinary Diagnostic Laboratory (MVDL) received an increased number of tularemia-positive cases in multiple animal species, including cats. Both agencies identified approximately twice as many positive cats in the last 3 years as compared to the previous 17 years. Concurrently, reports of human cases were also elevated. This retrospective project compared the clinical signs/histories and macroscopic necropsy lesions observed in tularemia-positive and tularemia-suspect cats at the MVDL from 2013-2018. The aim was to provide clinicians and diagnosticians a clarified picture of tularemia in cats and identify potential outliers, which could lead to misidentifying a suspect and possible pathogen exposure.

Clinical history was gathered from MDH follow-up data and necropsy submission forms. MVDL feline necropsy accessions were screened for key words (including: tularemia, *F. tularensis*, and francisellosis) indicating tularemia as a differential. The search was intended to identify tularemia cases and provide examples of tularemia look-alike cases expressing similar clinical and postmortem signs. Look-alike cases included neoplasia, feline infectious peritonitis, and bacterial septicemia. Eighteen tularemia-positive (Type A) cats originating from Minnesota were necropsied at MVDL. Clinical signs/histories most often involved lethargy (100%), fever (94%), anorexia (89%), and outdoor access (89%). Common macroscopic necropsy lesions involved enlarged lymph nodes (94%), splenitis (83%), and hepatitis (50%). Atypical cases included indoor-only history and involvement of only cervical lymph nodes.

Non-classical presentations of feline tularemia pose a greater risk of zoonosis for clinical and laboratory personnel due to the potential for decreased use of personal protection measures. Recognition and confirmation of tularemia-suspect cats is important in both clinical medicine and diagnostic pathology to reduce this risk. Throughout the study period, it was observed that both clinical and diagnostic recognition and communication of tularemia-suspect cats was initially variable and improved over time.

Comparison of nested and quantitative PCR assays for detection of *Streptococcus equi* subspecies *equi* in clinical equine specimens

Lance Wade Noll, Colin P. A. Stoy, Yin Wang, Elizabeth G. Porter, Xuming Liu, Jianfa Bai

Kansas State University, Manhattan, KS

Streptococcus equi subsp. *equi* is a Gram positive bacterial pathogen commonly associated with strangles in horses, a respiratory disease characterized by abscessation of submandibular and retropharyngeal lymph nodes which can lead to obstruction of the airway. Several real-time PCR assays have been developed for detection of *S. equi* from horses with most targeting conserved regions of the *S. equi* cell wall-associated M-protein (SeM), a major virulence factor and immunogen of *S. equi*. Our objective was to develop a nested PCR targeting SeM and an 18S rRNA internal control gene for detection of *S. equi* from horses with potential improvement in detection sensitivity compared to a real-time PCR (qPCR). Primers and probes from the Kansas State Veterinary Diagnostic Lab (KSVDL) *S. equi* clinical testing assay were utilized for all qPCR testing. Primers flanking the SeM qPCR target region were selected for an initial endpoint PCR step of the nested assay; PCR product from the endpoint reaction then served as template for the qPCR reaction step of the nested assay. Sample nucleic acid was also tested directly with qPCR to allow for assay comparison. Nucleic acid from clinical specimens (n=110) submitted to KSVDL were tested in parallel with each assay. All samples tested were positive, by each assay, for the 18S rRNA internal control target. The nested and qPCR assays identified 21.8% (24/110) and 13.6% (15/110) of samples positive for *S. equi*, respectively. None of the samples positive by qPCR were negative by nested PCR. The PCR products from all positive samples were submitted for DNA sequencing. Each of the 15 samples positive by both assays had a high nucleotide identity match (>96%) to the SeM gene. Among the 9 samples positive by nested PCR but negative by qPCR, 8 of 9 were sequence confirmed for SeM gene at greater than 97% nucleotide identity. Based on the nested PCR Ct (37.8) of the one sequence un-confirmed case, it's likely that the *S. equi* bacterial load in this sample was below the necessary concentration for successful sequencing. Nested PCR Cts of the 23 sequence confirmed samples ranged between 8.4 and 37.9, therefore limit of detection for the nested PCR was established at a Ct of 37. As demonstrated by sequencing confirmation, the nested PCR assay targeting the SeM gene is highly specific to *S. equi*. The increased sensitivity of the nested PCR, compared to the qPCR method, may reduce the number of false negative sample results in clinical testing.

Evaluation of a high throughput nucleic acid extraction method for PCR-based detection of *Mycobacterium avium* ssp. *paratuberculosis* in bovine fecal samples

Nagaraja Thirumalapura, Willard Feria, Eric Hue, Corey Zellers, Deepanker Tewari

Pennsylvania Department of Agriculture, Pennsylvania Veterinary Laboratory, Harrisburg, PA

Johne's disease (paratuberculosis) is an economically important disease of cattle worldwide. The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and manifests as a chronic inflammation of the intestine in ruminants leading to loss of production, culling and mortality. Although, culture is considered the gold standard for the disease diagnosis, PCR is used in several diagnostic laboratories due to its rapid turnaround time, with sensitivity and specificity comparable to fecal culture. The objective of this study was to evaluate a high throughput nucleic acid extraction method for the detection of MAP in bovine fecal samples by PCR. We used the MagMAX™ CORE Nucleic Acid Purification Kit with Mechanical Lysis Module for extraction of DNA from bovine fecal samples. The MagMAX™ CORE Nucleic Acid Purification Kit uses a magnetic separation process for purification of nucleic acid and the Mechanical Lysis Module involves a bead beating step for efficient lysis of bacteria. Initially, the 2018 Johne's disease proficiency test individual and pooled panels, provided by the National Veterinary Services Laboratories (NVSL), were tested using the MagMAX CORE kit extraction protocol followed by PCR amplification using a MAP DNA test kit (Tetracore). Each individual panel consisted of 25 blinded samples and one positive control. The method correctly categorized all individual samples from non-shedding (6), high-shedding (9) and 80% (8 out of 10) of low and moderate-shedding animals. The pooled proficiency test samples consisted of 4 positive and one negative and all were correctly categorized. The new extraction method was further evaluated using 51 known Johne's positive and 6 known negative diagnostic fecal samples previously tested at the Pennsylvania Veterinary Laboratory. Of the 51 positive samples, 8 were culture positive and 43 were PCR positive. The previous DNA extraction method involved isolation of DNA on a BioRobot M48 workstation using the MagAttract DNA M48 Mini Kit (Qiagen). Use of the new extraction method resulted in categorization of all 57 diagnostic specimens correctly except one. The sensitivity and specificity of detection for the new method based on analysis of the diagnostic samples were 98.84% and 100%, respectively. In addition, 9 lab-prepared pooled fecal samples, each containing 4 known negative and 1 known Johne's positive fecal samples, were tested. Of the 9 pooled samples, 8 samples were correctly categorized. The data presented in this study demonstrate an efficient extraction of MAP DNA from bovine fecal samples using MagMAX CORE extraction protocol with mechanical lysis module for sensitive diagnosis of Johne's disease in cattle. The new extraction method is rapid and allows high throughput testing of samples.

Theileria orientalis Ikeda genotype is identified in cattle in Southwestern Virginia # + †

Vanessa J. Oakes[1], Michael J. Yabsley[2], Diana Schwartz[3], Tanya LeRoith[1], Carolynn Bissett[4], Charles Broaddus[4], Jack L. Schlater[5], S Michelle Todd[6], Katie Boes[1], Meghan Brookhart[7], Kevin Lahmers[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA; [2]University of Georgia, Athens, GA; [3]Kansas State University, Manhattan, KS; [4]Virginia Department of Agriculture and Consumer Services, Richmond, VA; [5]Animal and Plant Health Inspection Service, United States Department of Agriculture, Riverdale, MD; [6]Virginia Tech Animal Laboratory Services, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA; [7]Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA

Theileria orientalis Ikeda genotype/genotype 2 is a pathogenic, non-transforming hemoprotozoan. This genotype is increasingly implicated as the causative agent of bovine infectious anemia outside of the United States, and is a pathogen of significant economic importance in New Zealand and Australia. It can be mechanically transmitted by biting flies and contaminated needles, but is most frequently and effectively transmitted by *Haemaphysalis longicornis,* the Asian longhorned tick. In 2018, this tick was reported in numerous states throughout the eastern United States, including Virginia. Cattle within several herds in Virginia that were parasitized by *H. longicornis* developed clinical signs consistent with hemoprotozoal infection, including anemia, icterus, and general malaise. Blood collected from members of infected herds revealed the presence of intraerythrocytic piroplasms. ELISA and subsequent RT-PCR for *Anaplasma* spp. were negative. The morphology of the piroplasms was consistent with *Theileria* or *Babesia* species. Serology was negative for *Babesia* spp. There are non-pathogenic *T. orientalis* genotypes endemic to the United States, but the clinical signs in addition to the presence of a competent vector warranted further investigation. Conventional PCR was conducted on DNA extracted from whole blood. Sequences of partial major piroplasm surface protein (MPSP) and the small subunit ribosomal DNA (SSU) genes were similar to GenBank accessioned sequences of *T. orientalis* Ikeda genotype/genotype 2. Also, phylogenetic analysis of these sequences revealed clustering of clinical samples with Ikeda genotype/genotype 2. Additionally, clinical samples positive for *T. orientalis* Ikeda genotype/genotype 2 represented multiple, unrelated herds throughout Virginia, with no travel of individual cattle between herds. Some positive individuals were sampled repeatedly across several months' time, suggesting a chronic component to the infection. Although transmission studies for *H. longicornis* as a competent vector for *T. orientalis* Ikeda genotype/genotype 2 in the United States are pending, the increasingly described range of a tick known to be a competent vector of this hemoprotozoan in other areas of the world suggests that this disease is not limited to Virginia, and may represent an emerging disease within the United States. The clinical signs of animals in this study ranged in severity and included anemia, malaise, inappetence, and icterus, to apparently non-clinical carriers.

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

Listerial meningoencephalitis and choroid plexitis in a saki monkey (*Simia pithecia*) +

Abigail Finley[1], Alexandra Goe[1,2], Kristen Phair[2], Jason Struthers[1]

[1]Midwestern University, Glendale, AZ; [2]Phoenix Zoo, Phoenix, AZ

A 27-year-old, intact female saki monkey was submitted for postmortem exam when she was found deceased after an episode of vomiting and ptyalism. The day prior she was examined for acute lethargy and hyporexia, and was found to be thin, dehydrated, and sensitive on cranial abdominal palpation. Supportive treatments were administered. Postmortem exam detected gas distension of the alimentary tract, aortic arteriosclerosis, a focal, 0.5 cm, firm superficial nodule in the left hemisphere's frontal lobe (fibrosis attributed to previous vascular accident), and the cerebral ventricles contained opaque viscous material. Histopathology revealed proliferative glomerulonephritis with proteinuria and suppurative ventriculitis, choroid plexitis, periventricular encephalitis, and meningitis with intralesional Gram-positive coccobacilli and diploid rods. There was lytic suppurative hepatitis and mild suppurative enterocolitis with crypt necrosis. Bacteriology of the cranial cervical spinal cord and liver isolated numerous to moderate *Listeria monocytogenes*. Polymerase chain reaction and immunohistochemistry on brain tissue were positive for *L. monocytogenes*. This geriatric monkey suffered from fatal hematogenous listerial meningoencephalitis thought to have an intestinal origin. In nonhuman primates, the most common cause of bacterial meningoencephalitis is *Streptococcus pneumoniae*, and less commonly, *Klebsiella pneumoniae*, *Pasteurella multocida*, *Haemophilus influenzae*, and *Neisseria meningitidis*. *Listeria monocytogenes* infections in nonhuman primates are infrequent and mostly involve experimental infections of pregnant dams that cause reproductive failure without clinical signs. Although immunocompromised people are at an increased risk of septic listeriosis, hematogenous listeriosis has been reported in an immunocompetent macaque. This saki monkey likely ingested environmental listeria that caused disease potentially facilitated by preexisting co-morbidities.

+ AAVLD/ACVP Pathology Award Applicant

Increased pathogen diversity in the skin microbiome of bats affected by white nose syndrome # * †

Ava R. Jarvis[2,1]*, Renee Anderson*[2]*, Derek Rothenheber*[2]*, Ben Golas*[5]*, Amy Glaser*[3]*, Laura B. Goodman*[3]*,*
Elizabeth Buckles[4]

[1]Microbiology and Immunology, Upstate Medical University, Syracuse, NY; [2]Animal Health Diagnostic Center, Cornell University, Ithaca, NY; [3]Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY; [4]Veterinary Biomedical Sciences, Cornell University, Ithaca, NY; [5]Ecology, Colorado State University, Fort Collins, CO

Since its initial emergence in the US in 2006, white nose syndrome (WNS) has been confirmed in 33 states and has resulted in bat population declines exceeding 90% in some areas. The disease has been attributed to infection with *Pseudogymnoascus destructans* (*Pd*), a psychrophilic fungus. Despite the impact *Pd* has had on bat health, much remains unknown about the skin microbiome of *Pd*-infected and uninfected bats, and whether microbiome composition may impact development of WNS. In samples collected early in the outbreak, we examined the relationships between *Pd* detection by PCR and histological changes with fungal and bacterial profiles by metagenomics.

Samples (n=488) were collected from seven states (KY, MO, NY, OH, PA, VT, WV) in 2008 and 2009. A subset of bat wing tissues (n = 61) were examined histologically for signs of WNS and scored for the presence of fungus, bacteria, and/or inflammation. Scores were given as the average number of areas of a given category visible in a single microscope field viewed at 200x magnification. The scores ranged from 0.0 (no infiltration/inflammation observed) to 17.6 (most severe inflammation observed). Fungal scores for *Pd* positive slides were 2.8 without inflammation (F) and 0.6 with inflammation (Fi). Bacterial scores were 4.6 without inflammation (B) and 3.2 with inflammation (Bi). Negative slides had average scores of F=0.1, Fi=0.1, B=0.2, Bi=0.1. Overall a higher co-occurrence of inflammation was observed in positive samples in areas of bacterial infiltration than in areas of fungal infiltration.

Tissue and swabs from 423 of the bats were tested by PCR for presence or absence of *Pd*. A subset of *Pd* positive and *Pd* negative (n=48, each) swabs were selected for universal fungal and bacterial metagenomic profiling using Illumina paired end sequencing. Overall, assignments for higher taxonomic levels were: 29.64% Bacteria, 19.92% Fungi, 29.74% Metazoa, 1.39% other Eukaryota, and 19.28% returned no BLAST identification. The relative abundance of these taxa varied greatly between samples. 71% of samples had agreement between PCR and WGS results. Non-metric multidimensional scaling and Shannon diversity metrics were used to compare taxa diversity between PCR positive and PCR negative bats, and PCR positive bats were found to have increased skin microbiome diversity.

Our results indicate that infection with *Pd* can cause a shift in skin microbiome composition, leading to an abnormally high diversity of skin microbes. A number of potentially pathogenic bacterial species, including *Pseudomonas*, *Sphingobacterium*, and *Cryseobacterium* were found to be indicator species in WNS positive bats. Most slides examined that did contain bacteria contained Gram negative, rod-shaped bacterial cells, and most slides with high scores for inflammation were found to have inflammation associated with bacterial cells or with co-occurring bacterial and fungal clusters, but not with fungus alone.

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

***Streptococcus pneumoniae*-associated septicemia in two cats**

Heidi Huffman Rose, Lifang Yan, JayKay Thornton

MS Veterinary Research and Diagnostic Lab, Pearl, MS

Two cats from the same household, a 23-month-old neutered male Ragdoll and a 9-year-old neutered male Persian, presented to the referring veterinarian for sedation and grooming. Both cats recovered from sedation uneventfully, and according to the owners, appeared normal later that day. The following day, the Ragdoll was reportedly quiet, and two days post-sedation was presented to the RDVM. On physical examination, the cat was painful in the abdominal and lumbar regions and was treated with an injectable NSAID. The cat returned home and died at home the following day. The same day, the Persian presented to the RDVM moribund and in respiratory distress. Laboratory data demonstrated profound leukopenia, with severe neutropenia and lymphopenia; thrombocytopenia was also present. Serum chemistry identified markedly elevated ALP, as well as increased GGT and severe hypoglycemia. The cat died before treatment could be initiated. Both cats were submitted to the MVRDL for necropsy.

At necropsy, both cats had similar gross lesions. Both cats were in good body condition. In both cases, the lungs were somewhat rubbery and discolored pink to gray with petechiae (Ragdoll) to ecchymoses (Persian) and faint rib impressions on the pleural surfaces. Peripheral lymph nodes of the head and neck were mildly enlarged. In the Persian, the liver was pale tan and friable. Histologic lesions were also similar in both cases. Interstitial pneumonia was present in both, with foci of intra-alveolar hemorrhage in sections from the Persian. Throughout the lungs, as well as sections of all examined tissues, intravascular, Gram positive cocci were abundant. Additional significant lesions included splenitis, lymphadenitis, and hepatitis. *Streptococcus pneumoniae* was cultured from the lung in both cases.

Streptococcus pneumoniae infection in humans can result in meningitis, otitis, pneumonia, endocarditis, polyarthritis, and bacteremia; this bacterium is also part of the normal upper respiratory tract flora in humans. *Streptococcus pneumoniae* infection has been rarely reported in veterinary species. Case reports in cats include septic arthritis and septicemia in an adult cat and cellulitis, interstitial pneumonia, and septicemia in a kitten. In both cases, exposure to a human reservoir was postulated as the source of infection.

Epidemiology 1

Saturday, October 26, 2019
Room 555

Moderators: Orhan Sahin and Ashley E. Hill

Symbols at the end of titles indicate the following designations:

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Congenital abnormalities in neonatal or near-term aborted bovine and ovine clones

David J. Wilson, Thomas J. Baldwin, Chelsea W. Barker

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

Cloning is an important tool to improve livestock or create models for human disease. In most species clones have a low survival rate due to abortions or neonatal abnormalities. The objective of this record review study was to quantify congenital abnormalities seen in bovine and ovine clones. Cattle and sheep clones that died as neonates or near-term abortions (n = 29) and those born alive that died by 2 months of age (n = 6) were necropsied from 2011 to 2017 at the Utah Veterinary Diagnostic Laboratory. Data was harvested from the laboratory information management system. All clones were produced at Utah State University using somatic cell nuclear transfer (SCNT). The study population was non-transgenic bovine clones attempting to replicate superior bulls or cows (n=24) and attempted transgenic ovine clones (n=11) that did not acquire the targeted mutation. Standard necropsy procedures included collection and examination of heart, lung, trachea, brain, liver, kidney, thymus, thyroid, adrenal glands, spleen, pancreas, GI tract, cranium, skeletal muscle, joints, bone, spinal column, urinary bladder, ovaries and testes. Additional diagnostic procedures were conducted as indicated by necropsy findings. Many animals had multiple abnormalities, therefore the total number of abnormalities was greater than the number of clones. Within all 35 clones, the most common congenital abnormalities were: hydronephrosis (16, 46%), palatoschisis (cleft palate) (10, 29%), brachygnathism (8, 23%), hydroureter (5, 14%), fetal oversize (5, 14%), ventricular septal defect (4, 11%), and hepatic fibrosis (4, 11%). In the ovine attempted transgenic clones, the most common congenital abnormalities were: hydronephrosis (10, 91%), palatoschisis (8, 73%), and brachygnathism (6, 55%). Similar to within sheep, hydronephrosis (7, 29%) was one of the most common abnormalities in cattle. However, bovine clones had 9 abnormalities not found in sheep: patent foramen ovale (7, 29%), patent ductus arteriosus (7, 29%), arthrogryposis (4, 17%), tracheal hypoplasia (4, 17%), hydrocephalus (3, 13%), adrenal gland cortical hypoplasia (3, 13%), adrenal medullary dysplasia (2, 8%), renal cortical dysplasia/hypoplasia (2, 8%), and cerebral edema (2, 8%). Congenital defects associated with cloning affected multiple organ systems, often within the same animal. Investigation of the mechanisms for the most common abnormalities in ruminant clones is ongoing and vital to improvement of cloning efficiency

Which route of exposure is best for *Mycoplasma hyopneumoniae gilt acclimation?* * †

Ana Paula Poeta Silva[1], *Aric McDaniel*[1], *Bailey Lauren Arruda*[1], *Daniel C. L. Linhares*[1], *Luis Gabriel Gimenez-Lirola*[1], *Carmen Alonso*[1], *Rachel Derscheid*[1], *Locke Karricker*[1], *Paul Yeske*[2], *Jeff Zimmerman*[1], *Maria Clavijo*[1]

[1]Iowa State University, Ames, IA; [2]Swine Vet Center, St Peter, MN

Introduction

Mycoplasma hyopneumoniae (MHP) causes chronic respiratory infection and economic losses due to lower growth performance and higher treatment costs. Intentional exposure to MHP during gilt acclimation is intended to provide sufficient time for gilts to develop protective immunity and decrease MHP shedding before entering the breeding herd. Successful application of this approach reduces the number of positive piglets at weaning, leading to a decreased incidence of MHP in growing pigs. The question we addressed was, "*Which route of exposure is best for gilt acclimation?*"

Objective

Compare the efficacy of intratracheal, intranasal and aerosol MHP inoculation protocols of live lung homogenate, based on clinical signs, MHP shedding, antibody response, and lung lesions.

Materials and Methods

Six-week-old MHP- and PRRSV-negative gilts (n = 78) were randomized to one of four MHP exposure groups [aerosol (n = 24); intranasal (n = 24); intratracheal (n = 24); or no exposure (n = 6)] and followed through 49 days post exposure (DPE). The MHP inoculum consisted of lung homogenate (10^5 CFU/mL MHP 232). Weight was taken at -1, 28, and 49 DPE. Serum and tracheal samples for antibody and DNA testing were collected weekly through DPE 49. Pigs were humanely euthanized at 49 DPE. Lung was analyzed for gross lesions and histopathology (H&E and IHC). Weight gain, antibody responses (ELISA S/P), and shedding (qPCR) were analyzed using linear mixed regression, and area under curve. Differences among groups were evaluated by ANOVA.

Results

All routes of exposure resulted in infection. Intratracheal demonstrated earlier detection of DNA (7 DPE) and seroconversion (39 DPE); intranasal and aerosol showed similar time to DNA and antibody detection, i.e., ~14 DPE and ~49 DPE. MHP qPCR Cts were similar in exposed groups over DPE ($p>0.05$), but lower ELISA S/Ps were observed in intranasal and aerosol exposures. Aerosol group had the least impact on daily gain (0.64kg/day), performing similarly to the negative control group (0.73kg/day). At 49 DPE, gross and/or histopathologic lesions were observed in the lungs of 17/22, 19/24 and 19/24 gilts from the intratracheal, intranasal and aerosol groups, respectively.

Discussion

While intratracheal exposure resulted in the earliest detection of DNA and antibody, successful infection was achieved by all routes. Intranasal and aerosol routes might be better alternatives to the more labor-intensive and invasive intratracheal inoculation. Nonetheless, specific production circumstances may affect the choice of route. Practical and efficacious gilt exposure methods will improve MHP control and elimination programs.

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

Comparative genomics identifies methicillin-resistant *Staphylococcus pseudintermedius* ST71 as highly clonal across different geographical locations, irrespective of isolation sources

Narayan C. Paul[2,1], Lisa Page Jones[3], Margaret Alison Davis[3], Vincent Perreten[4], J Scott Weese[5], Joshua B. Daniels[6], Paul S. Morley[7], Rowland Cobbold[8], Devendra H. Shah[2,3]

[1]Texas A&M Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, TX; [2]Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [3]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA; [4]Institute of Veterinary Bacteriology, University of Berne, Berne, Switzerland; [5]Department of Pathobiology, University of Guelph, Guelph, ON, Canada; [6]Veterinary Diagnostic Laboratories, Colorado State University, Fort Collins, CO; [7]Department of Veterinary Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX; [8]School of Veterinary Science, University of Queensland, Gatton, QLD, Australia

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is frequently involved in surgical wound, pyoderma and skin infections in dogs. Recurrent infections and persistence of MRSP in hospital environments are very common, and in most cases antimicrobial choices are limited because MRSP is highly resistant to common antimicrobials. The objective of this study was to understand the population structure and comparative genomics of MRSP across different geographical locations. An international collection of 91 clinical and non-clinical MRSP isolates originating from the USA (n=69), Canada (n=10), Europe (n=4), Australia (n=3), Israel (n=4) and Granada (n=1) were selected for whole genome sequencing (WGS). Paired-end libraries were prepared and sequenced using a HiSeq Illumina following standard protocol. Whole genome sequences were aligned and mapped to a reference *Staphylococcus pseudintermedius* strain (CP002439). Bioinformatic analysis of sequence data determined that sequence type (ST) 71 (24%, 22/91) was the predominant one followed by ST45 (13%, 12/91) and ST181 (5%, 5/91). Interestingly, among the USA isolates, only one isolate (1%, 1/69) was identified as ST68, which was until recently considered as a predominant MRSP clone in North America. A phylogenetic tree based on single nucleotide polymorphism of all 91 MRSP core genomes revealed that individual STs cluster together, which correlates with the results observed using pulsed-field gel electrophoresis and multiple-locus variable number tandem repeat analysis. While ST71 is highly clonal regardless of source type (clinical v. non-clinical) and geographical location, the other STs including ST-45 and ST-181 are more diverse as shown by their forming different sub-clusters within individual ST clusters. ST-71, which is historically a European clone associated with high multidrug resistance, now also predominates in different geographical locations including the USA. WGS data will be further analyzed to develop hypotheses about the drivers of the success of this clone.

Retrospective survey of parasites identified in feces of 7,408 client-owned dogs at Oklahoma State University from 2007 through 2018

Yoko Nagamori[1], Mark Payton[2], Emily Looper[1], Hadley Apple[1], Katherine Herrick[1], Eileen Johnson[1]

[1]Veterinary Pathobiology, Oklahoma State University, Stillwater, OK; [2]Statistics, Oklahoma State University, Stillwater, OK

Our study objective was to determine the prevalence and trend of parasitic infection in client-owned dogs examined at Oklahoma State University over the past 12 years. All results of centrifugal flotation, saline direct smear, sedimentation, and Baermann examinations on fecal samples from client-owned dogs submitted to the Boren Veterinary Medical Hospital and Oklahoma Animal Disease Diagnostic Laboratory of Oklahoma State University from 2007 through 2018 were included. The impact of sex, age, seasonality, and years on the prevalence of infection were analyzed.

A total of 7,408 cases were included for this study. Majority of cases (79.5%; 5,893/7,408) did not include any parasites, eggs, oocysts, or cysts. Approximately 15.4% (1,144/7,408) of client-owned dogs were infected by at least one parasite, and 5.0% (371/7,408) of dogs were infected by multiple parasites. The most common parasite stage observed was *Ancylostoma* eggs (8.2%; 608/7,408), followed by *Cystoisospora* oocysts (5.0%; 372/7,408), *Giardia* cysts (4.1%; 301/7,408), *Trichuris vulpis* eggs (2.7%; 203/7,408), *Toxocara canis* eggs (2.5%; 187/7,408), *Dipylidium caninum* proglottids/egg packets (0.84%; 62/7,408), taeniid proglottids/eggs (0.47%; 35/7,408), *Sarcocystis* sporocysts (0.36%; 27/7,408), *Cryptosporidium* oocysts (0.30%; 22/7,408), *Strongyloides stercoralis* larvae (0.26%; 19/7,408), *Demodex* mites (0.22%; 16/7,408), *Alaria* eggs (0.19%; 14/7,408), *Toxascaris leonina* eggs (0.18%; 13/7,408), and capillarid eggs (0.16%; 12/7,408), small coccidian oocysts (0.16%; 12/7,408), *Uncinaria stenocephala* eggs (0.13%; 10/7,408), *Spirometra* eggs (0.11%; 8/7,408), *Physaloptera* eggs (0.09%; 7/7,408), *Heterobilharzia americana* eggs (0.08%; 6/7,408), *Nanophyetus salmincola* eggs (0.08%; 6/7,408), trichomonads (0.08%; 6/7,408), *Mesocestoides* proglottids/eggs (0.05%; 4/7,408), *Baylisascaris* eggs (0.01%; 1/7,408), *Macracanthorhynchus ingens* eggs (0.01%; 1/7,408), *Otodectes cynotis* mites (0.01%; 1/7,408), *Paragonimus kellicoti* eggs (0.01%; 1/7,408), *Rhipicephalus sanguineus* ticks (0.01%; 1/7,408), and *Sarcoptes scabei* mites (0.01%; 1/7,408).

There was no statistical significance between sexes ($p = 0.3231$); however, a significant trend was observed between ages, the younger the dogs, the higher the prevalence of parasitic infection ($p < 0.0001$). Statistical analyses also revealed the significant differences by months; the higher prevalence of infection occurred in early spring, early summer, and fall ($p = 0.0013$). Overall, the prevalence of infection decreased over years ($p < 0.0001$).

Retrospective survey of parasites identified in feces of 2,586 client-owned cats at Oklahoma State University from 2007 through 2018

Yoko Nagamori[1], Mark Payton[2], Emily Looper[1], Hadley Apple[1], Katherine Herrick[1], Eileen Johnson[1]

[1]Veterinary Pathobiology, Oklahoma State University, Stillwater, OK; [2]Statistics, Oklahoma State University, Stillwater, OK

Our study objective was to determine the prevalence and trend of parasitic infection in client-owned cats examined at Oklahoma State University over the past 12 years. All results of centrifugal flotation, saline direct smear, sedimentation, and Baermann examinations on fecal samples from client-owned cats submitted to the Boren Veterinary Medical Hospital and Oklahoma Animal Disease Diagnostic Laboratory of Oklahoma State University from 2007 through 2018 were included. The impact of sex, age, seasonality, and years on the prevalence of infection were analyzed.

A total of 2,586 cases were included for this study. Majority of cases (75.5%; 1,953/2,586) did not include any parasites, eggs, oocysts, or cysts. Approximately 18.8% (485/2,586) of client-owned cats were infected by at least one parasite, and 5.7% (148/2,586) of cats were infected by multiple parasites. The most common parasite stage observed was *Cystoisospora* oocysts (9.4%; 243/2,586), followed by *Toxocara cati* eggs (7.8%; 202/2,586), *Giardia* cysts (4.0%; 104/2,586), *Alaria* eggs (3.5%; 91/2,586), *Ancylostoma* eggs (1.2%; 32/2,586), taeniid proglottids/eggs (1.2%; 30/2,586), *Dipylidium caninum* proglottids/egg packets (1.1%; 29/2,586), *Demodex* mites (0.9%; 24/2,586), and *Eucoleus aerophilus* (0.7%; 18/2,586). Less commonly, *Physalopetra* eggs (0.19%; 5/2,586), *Toxascaris leonina* eggs (0.19%; 5/2,586), *Tritrichomonas blagburni* (0.15%; 4/2,586), *Cheyletiella* mites (0.15%; 4/2,586), *Ollulanus tricuspis* (0.12%; 3/2,586), *Platynosomum* eggs (0.12%; 3/2,586), *Aelurostrongylus abstrusus* larvae (0.08%; 2/2,323), *Sarcocystis* sporocysts (0.08%; 2/2,586), *Spirometra* eggs (0.08%; 2/2,586), *Mesocestoides* proglottids/eggs (0.08%; 2/2,586), *Trichuris felis* eggs (0.08%; 2/2,586), *Otodectes cynotis* mites (0.04%; 1/2,586), *Cryptosporidium* oocysts (0.04%; 1/2,586), and *Toxoplasma*-like small coccidian oocysts (0.04%; 1/2,586) were detected.

There was no statistical significance between sexes ($p = 0.3316$); however, a significant trend was observed between ages, the younger the cats, the higher the prevalence of parasitic infection ($p < 0.0001$). Statistical analyses also revealed the significant differences by months; the higher prevalence of infection occurred in summer through fall ($p = 0.0004$). Overall, the prevalence of infection increased over years ($p < 0.0001$).

An epizootic of Canine Distemper in Michigan wild carnivores: 2014-2018

Scott D. Fitzgerald[1], Amber R. Olson[1], Julie R. Melotti[2], Thomas M. Cooley[2], Daniel J. O'Brien[2], Roger K. Maes[1]

[1]Veterinary Diagnostic Laboratory, Michigan State university, Lansing, MI; [2]Wildlife Disease Laboratory, Michigan Department of Natural Resources, Lansing, MI

While Canine Distemper infections spill over from domestic dogs into wild carnivores commonly throughout North America, Michigan has recently experienced a marked rise in prevalence of the disease within wild carnivores from 2014 through 2018, and also appeared to exhibit geographic spread and dissemination into a variety of wild carnivore species. The outbreak was initially diagnosed in the Lower Peninsula of Michigan, but cases were subsequently identified statewide. Of the species examined, the greatest number of cases were recorded in raccoons. Striped Skunks and Gray Fox also exhibited relatively high prevalence of infection over the 5 years. Over time, less frequently submitted carnivore species were examined as suspects, including Red Fox, Coyotes, Wolves, Mink, and Short-tailed Weasels. A surprising finding was the considerably lower prevalence in Red Fox compared to Gray Fox. Both juvenile and adults were affected, with adults exhibiting higher prevalence, but this may be due in part to adult animals' larger size and their behavior allowing them to be more easily observed by people. We will outline the geographic distribution of the disease, discuss species prevalence, show examples of the typical histologic lesions in various tissues, discuss tissue selection for optimal surveillance, and illustrate the immunohistochemical staining which was utilized to confirm the diagnosis. In our experience, lungs and spleens are the optimal tissues for detection of Canine Distemper by immunohistochemical staining.

Catastrophic tibial fractures in racehorses # + †

Monika Aleksandra Samol[1], Susan Stover[2], Francisco Uzal[1], Rick Arthur[3]

[1]California Animal Health & Food Safety Laboratory- San Bernardino, University of California, Davis, Riverside, CA; [2]Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, CA; [3]Equine Medical Director, School of Veterinary Medicine, University of California, Davis, Davis, CA

The most prevalent causes of death in racehorses are musculoskeletal injuries, causing close to 80% of the deaths within the racehorse population in California and elsewhere. Fractures of the scapula, humerus, metacarpus, lumbar vertebrae, and pelvis have been characterized and associated with pre-existing lesions that predispose to the fatal injury. Knowledge of the main characteristics of each fracture and ability to recognize risk factors may help prevent catastrophic fractures. The purpose of this study was to characterize tibial fractures in racehorses. The California Horse Racing Board (CHRB) in association with the California Animal Health and Food Safety Laboratory System established a post mortem examination program in 1990, where every horse that dies spontaneously or is euthanized at a facility under the jurisdiction of the CHRB undergoes a post-mortem examination. The database of CAHFS, including 7,068 racehorse necropsy reports was searched for horses that died because of a tibial fracture between 1990 and 2018 and those reports were retrospectively reviewed. One hundred nineteen horses (108 Thoroughbreds; 10 Quarter Horses; 1 Standardbred) had a complete tibial fracture. The majority of fractures (66%) occurred during training, whereas racing-related and non-exercise fractures occurred in 17% and 14% of horses, respectively. For the remaining 3% of cases the information regarding circumstances of the injury was unavailable. Most fractures in Thoroughbreds occurred in 2- and 3- year old horses (39% and 33%, respectively), while 100% of the Quarter Horses for which age was available were 2 year-olds. Most horses (97%) had unilateral fractures that affected the left (50%) or the right (47%) hindlimb, the remaining 3% of horses fractured both tibiae. The most commonly recognized fracture site and configuration was diaphyseal (44%), and oblique (40%); 97% of the fractures were comminuted. Out of 65 cases examined for pre-existing callus formation, 60% of horses had raised periosteal callus bridging the line of catastrophic fracture. The most prevalent location for callus was the cortical bone in the proximal third of the diaphysis/proximal metaphysis, underneath the fibula (64%). The second most common area of callus formation was the distocaudal surface of the distal third of the diaphysis/distal metaphysis (26%). The collected data is highly suggestive that the stress fractures located at the proximolateral aspect of the tibia may be the most challenging to diagnose in the clinical setting. When undetected, these calluses may predispose to catastrophic fractures leading to euthanasia. Catastrophic fractures in racehorses are of high importance due to their great risk of injury for jockeys and, exercise riders, coupled with equine welfare and adverse effects on public perception.

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

PRRSV-specific antibody detection in fecal samples - a pilot study * †

Alexandra Henao-Diaz[1], Gang Wang[2], Ju Ji[3], Luis Gabriel Gimenez-Lirola[1], Jeff Zimmerman[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Chinese Academy of Agricultural Sciences, Harbin Veterinary Research Institute, Harbin, China; [3]Department of Statistics, Iowa State University, Ames, IA

Introduction

Routine surveillance is mandatory for the control and/or elimination of PRRSV, but breeding herds are rarely monitored because collecting diagnostic samples from adult animals is challenging. However, fecal samples (FS) are easily collected by a single person and do not require animal handling. The presence of antibody in feces (coproantibody) has been reported in human, sheep, mice, primates, and other species. In swine, coproantibody against ASF, CSF, HEV, and PEDV have been documented [1,2]. Therefore, the purpose of this pilot study was to evaluate PRRSV coproantibody and its potential use in surveillance.

Materials and methods

Pigs (n = 12) were vaccinated with a modified live virus vaccine (Ingelvac® PRRS MLV) and individually sampled from -5 to 42 days post vaccination (DPV). A total of 112 serum, 512 oral fluids (OF), and 513 FS were tested using commercial PRRSV IgG ELISAs (IDEXX OF Ab test, IDEXX PRRS X3 Ab Test, IDEXX Laboratories, Inc). Serum and OF samples were tested as directed by the ELISA manufacturer. Fecal samples were diluted 1:1 with ELISA kit diluent containing 1000ppm chitosan and then assayed on the PRRSV OF ELISA using a sample volume of 200 μL.

Results

Using a cutoff of $S/P \geq 0.4$, the first positive serum ELISA and OF-ELISA samples were detected on 8 DPV. Using a cutoff of $S/P \geq 0.1$, the first FS-ELISA-positive samples appeared on 10 DPV. A receiver operating characteristic (ROC) analysis was conducted under the assumption that samples collected prior to DPV 7 were true negatives and samples collected after DPV 11 were true positives. The analysis estimated the diagnostic sensitivity and specificity of both the serum and OF-ELISAs at (99%, 99%), whereas the FS-ELISA was (81%, 99%).

Conclusions

This pilot study demonstrated that detectable levels of PRRSV antibody are present in feces and that fecal antibody kinetics mirrors the antibody kinetics of serum and oral fluids. The FS-ELISA is sufficiently diagnostically specific and represents a practical approach to testing adult animals (sows or boars) in PRRSV surveillance programs. However, further research is necessary to improve the diagnostic sensitivity.

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

Epidemiology 2

Sunday, October 27, 2019
Ballroom C

Moderators: Laura B. Goodman and Robert E. Gibson

Symbols at the end of titles indicate the following designations:

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

Diagnostic performance comparison of two PRRSV oral fluid antibody ELISAs * †

Alexandra Henao-Diaz[1], Anne Harrenstein[3], Ju Ji[2], Luis Gabriel Gimenez-Lirola[1,3], Jeff Zimmerman[1], Dave Baum[1,3]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Statistics, Iowa State University, Ames, IA; [3]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Introduction

Antibody detection is useful in PRRSV surveillance and/or diagnostics because PRRSV-specific antibody reveals exposure to virus even when pigs are no longer viremic (RT-PCR negative) [1, 2]. PRRSV ELISAs were first developed for serum antibody detection and then adapted to oral fluid antibody detection using an overnight incubation procedure [3,4]. Recently, a commercial PRRSV oral fluid ELISA became available, i.e., an ELISA specifically purposed for detection of PRRSV antibody in oral fluids. The aim of this study was to compare the performance of two validated PRRSV oral fluid ELISAs: ELISA-1 a commercial PRRSV OF Ab ELISA (IDEXX Laboratories, Inc., Westbrook Maine) and ELISA-2 the overnight PRRSV oral fluid ELISA procedure based on the PRRSV X3 serum ELISA (IDEXX Laboratories, Inc.).

Materials and methods

Oral fluid samples of known status (Panel 1, n = 600) were collected from 12 pigs vaccinated using a commercial modified-live virus vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc.) and sampled from day post vaccination (DPV) -7 to 42. Oral fluid samples of unknown status (Panel 2, n = 600) consisted of oral fluid samples submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) for routine testing. All samples were tested by ELISA-1 and ELISA-2 in the ISU-VDL using standard operating procedures.

Results

Panel 1: At a cutoff of $S/P \geq 0.4$, receiver operating characteristic (ROC) analysis estimated the diagnostic sensitivity and diagnostic specificity of ELISA-1 as 98.8 (95% CI 97.2, 99.7) and 98.8 (95% CI 95.8, 99.9), respectively. At the same cutoff, ELISA-2 diagnostic sensitivity and specificity were 90.1 (95% CI 86.6, 92.9) and 99.40 (95% CI 96.7, 100). **Panel 2:** Among oral fluid samples of unknown status, 70/600 (11.7%) were positive on ELISA-1 and 53/600 (8.8%) on ELISA-2. This rate of positivity was significantly different between the two assays (Cochran test, $p < 0.05$).

Conclusions

The two PRRSV oral fluid antibody ELISAs evaluated in this study exhibited similar performance, albeit ELISA-1 provided higher diagnostic sensitivity and the advantage of same-day testing. However, for surveillance in contemporary production systems, diagnostic specificity is even more important than diagnostic sensitivity because producers quickly lose confidence when confronted with unexpectedly positive results. Therefore, future research is needed to further evaluate ELISA-1 diagnostic specificity and develop rapid confirmatory testing procedures.

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

Antibody response of different U.S. PEDV strains in pigs of different ages + * †

Loni L. Schumacher, Ashley Buerkley, Qi Chen, Hai Thanh Hoang, Maria Merodio, Phillip Gauger, Drew Magstadt, Ronaldo Magtoto, Luis Gabriel Gimenez-Lirola, Jianqiang Zhang

VDPAM, Iowa State University, Ames, IA

Currently, two main strains of porcine epidemic diarrhea virus (PEDV) i.e., high pathogenic U.S. non S-INDEL PEDV and moderate pathogenic U.S. S-INDEL PEDV, are circulating in U.S. swine and continue to impact pork production. While strain pathogenicity in different ages has been described, little is known about the differences in antibody response relative to virus strain and pig age. In this study, our objective was (1) to investigate antibody response differences for each virus strain in different pig ages and (2) identify which virus strain induces a stronger antibody response in each age group. Serum was collected from pigs experimentally inoculated with PEDV cell culture isolates USA/IN19338/2013 (non S-INDEL) and USA/IL20697/2014 (S-INDEL) across three pig age groups during 28 days post inoculation (DPI). PEDV naïve animals (n = 45) were divided into three age groups including 3-week-old or ‘weaned’ (n = 15), 8-week-old or ‘grower’ (n = 15), and 23-week-old or ‘finisher’ (n = 15). Pigs within each group were subsequently divided into three subgroups (5 pigs/group) and orogastrically inoculated with 105 TCID50 per pig of non S-INDEL or S-INDEL PEDV, or mock inoculated with culture media. Serum samples were collected at DPI 0, 2, 4, 7, 14, 21 and 28 and tested by PEDV fluorescent microsphere immunoassay (FMIA) based on the N-terminal portion (S1) of the non S-INDEL PEDV spike protein for IgG and by fluorescent focus neutralization assay for neutralizing antibody (NA) using non S-INDEL PEDV as the indicator virus. The first NA response was detected between 4-7 DPI with all pigs seropositive by 21 DPI until the end of the study regardless of age and PEDV strain used for inoculation. The NA response was significantly ($p < 0.0004$) lower in the S-INDEL group compared to the non S-INDEL group in ‘weaned’ (DPI 21-28) and ‘finisher’ pigs (DPI 21, $p <0.0212$) with no significant differences in ‘grower’ pigs throughout the study. For pigs inoculated with non S-INDEL PEDV, the first FMIA IgG antibody response was detected between 7-14 days regardless of pig age and lasted throughout the study. The magnitude of the FMIA IgG response in ‘weaned’ pigs was significantly higher ($p <0.001$) compared to ‘grower’ and ‘finisher pigs’ whereas there was no significant difference between the ‘grower’ and ‘finisher’ pigs. Interestingly, for pigs inoculated with S-INDEL PEDV, significant serum FMIA IgG response was only detected in ‘grower’ pigs with no significant response in the other two age groups. When the two PEDV strains were compared for antibody responses in each age group, the U.S. non S-INDEL PEDV appeared to stimulate a stronger serum FMIA IgG response compared to S-INDEL PEDV regardless of pig ages investigated in this study. Whether the differences in IgG response between S-INDEL vs. non S-INDEL PEDV-inoculated pigs are related to antigenic properties of the S1 protein used for FMIA testing is being investigated.

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

Diagnostic performance of the POCKIT™ for detection of African Swine Fever and Foot and Mouth Disease viruses using insulated isothermal PCR ◊

Jessie D. Trujillo[1], Russell Ransburgh[1], Anthony Craig[2], Mark Tsai[3], David Tsai[3], Thomas Wang[3], Livio Heath[4], Juergen Richt[1]

[1]DMP, Kansas State University, Manhattan, KS; [2]Vectors and Vector-borne Diseases Research Programme, Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa; [3]GeneReach, Boston, MA; [4]Transboundary Animal Diseases, Agricultural Research Council, Onderstepoort Veterinary Institute, Pretoria, South Africa

African Swine Fever virus (ASFV) and Foot and Mouth Disease virus (FMDV) are major concerns for the global agricultural industry. ASFV expansion out of Africa in the past decade and recurrent FMDV outbreaks within and outside of Africa demonstrate real risks due to epidemiological globalization. Surveillance and immediate response are critical to prevent, identify or control outbreaks. Our group has performed extensive evaluation of the analytical and clinical performance of the POCKIT™ for ASFV and FMDV. The POCKIT™ is a portable PCR machine for the point of need detection using lyophilized, insulated isothermal PCR reagents (iiPCR) and nucleic acids (NA) purified using a portable automated NA extraction platform, the Taco Mini (GeneReach). FMDV and ASFV qPCR detection assays (Callahan, 2002 and Zask, 2005) were adapted to the iiPCR with analytical sensitivity tested using the NAHLN controls and NAHLN proficiency panels, provided by USDA-FADDL. Analytical sensitivity determination was performed using serial dilutions of the NAHLN surrogate NA controls followed by testing 10x serial dilutions of NA purified from viral reference strains (n=10 for ASFV and n=7 for FMDV) using the Taco bead processor and the taco DNA/RNA extraction (GeneReach). This extraction platform was demonstrated to yield equivalent NA as the NAHLN protocol using respective extraction controls, reference standards and proficiency tests. Reference assay testing was performed side by side using NAHLN PCR/RT-PCR protocols using validated low cost PCR master mixes (Quanta qScript XLT 1-step RT-qPCR ToughMix or the PerfeCTa Fastmix II). Results demonstrate equivalent analytical sensitivity for the detection of ASFV and FMDV nucleic acids (10-500 copies per reaction) on the POCKIT™ when compared to testing on the reference assays on the CFX. Diagnostic sensitivity and specificity for ASFV and FMDV detection on the POCKIT™ was determined using 192 and 176 clinical samples, respectively. Clinical samples consisted of tissues or lymph node pools or vectors (ticks and lice) for ASFV and epithelium or swabs from vesicles, blood and probang samples for FMDV. Samples originated in the Republic of South Africa and were derived from experimental or natural infections of ruminants and swine, domestic or wild, with confirmed or suspected infections. The clinical sensitivity and specificity on the POCKIT™ was 100% /100% for ASFV and 98%/100% for FMDV, respectively. The exceptional diagnostic performance for detection of these two high priority infectious agents on the point of need POCKIT™ iiPCR platform suggest that when used properly, point of need PCR/RT-PCR could help mitigate livestock and economic losses or help control and eradicate such devastating diseases.

◊ USAHA Paper

Development and validation of an indirect ELISA for the serological diagnosis of African Swine Fever

Shawn Babiuk[1,2], Kaye Quizon[1,3], Joseph Wang[4], Dmytro Zhmendak[1], Alfonso Clavijo[1], Aruna Ambagala[1,5]

[1]National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, MB, Canada; [2]Immunology, University of Manitoba, Winnipeg, MB, Canada; [3]Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada; [4]BioStone Animal Health, Southlake, TX; [5]Animal Science, University of Manitoba, Winnipeg, MB, Canada

African swine fever (ASF) is an economically devastating viral disease of pigs caused by African swine fever virus (ASFV), a large enveloped DNA virus. The currently available commercial ASF ELISA kits are developed and licenced in Europe. Given the recent and rapid spread of ASF, there is a critical need for ASF diagnostic tests to be developed in North America. Three ASFV antigenic surface proteins, p30, p54 and p72, were expressed in recombinant baculovirus system and were evaluated in an indirect enzyme-linked immunosorbent assay (iELISA) for the detection of anti-ASFV antibodies using known anti-ASFV antibody negative serum and serum collected from pigs experimentally infected with different ASFV strains. A receiver operating characteristic curve (ROC) analysis of the data demonstrated that the p54 has the highest sensitivity (99.5%) and specificity (95.5%) of the three recombinant proteins, and was able to detect anti-ASFV antibodies in experimentally infected animals as early as 8 days post-infection (DPI). The p54 iELISA is a promising tool for the serological diagnosis of ASF. This novel iELISA is currently in the process of full validation in collaboration with a commercial partner.

Genotyping and Prevalence of Porcine Circovirus Type 3 (PCV3) and Type 2 (PCV2) in the Midwest of the USA during 2016-2018 # †

Yin Wang[1]*, Lance Noll*[1]*, Nanyan Lu*[1]*, Elizabeth G. Porter*[1]*, Colin P. A. Stoy*[1]*, Wanglong Zheng*[1,2]*, Xuming Liu*[1]*,*
Lalitha Peddireddi[1]*, Megan Niederwerder*[1]*, T.G. Nagaraja*[1]*, Jianfa Bai*[1]

[1]Kansas State University, Manhattan, KS; [2]Yangzhou University, Yangzhou, China

Similar to porcine circovirus type 2 (PCV2), PCV3 has becoming prevalent worldwide and associated with porcine dermatitis and nephropathy syndrome (PDNS)-like disease. A total of 2125 porcine samples from 910 cases were collected during 2016-2018, and tested for the presence of PCV3 and PCV2 by real-time PCR. The results showed that 28.4% samples from 41.2% cases were PCV3 positive while 16.4% samples from 16.7% cases were PCV2 positive. The coinfection rate was 5.4% at sample level and 8.4% at case level. Temporal analysis indicated that PCV3 positive case rate increased from 31.6% in 2016, 40.9% in 2017, to 55.6% in 2018. Although with lower prevalence, PCV2 positive case rate in 2018 (28.8%) doubled that in 2017 (14.4%). The coinfection case rate was also increased from 3.4% in 2016, 8.0% in 2017, to 16.1% in 2018. The high positive rate of PCV3 (56.9%) and PCV2 (33.8%) in oral fluids, PCV3 in fetus (57.1%) and PCV2 in tonsil (54.8%) implied potential viral transmission route and tissue tropism. In the 61 unique PCV3 full genome sequences, 12 strains are in a separate cluster, which characterized by five amino acid changes in the capsid protein: 24V, 27K, 56D, 98R and 168K, implied a different genotype and potentially with distinct immunogenicity. Other sequences are clustered into different clusters of published sequences indicating most of the Midwest US strains are similar to those collected from different regions and countries. The overall genetic diversity of PCV3 strains currently circulating in the globe is about 3%, a value that is much smaller than the about 10%-20% genetic diversity among different genotypes of PCV2 strains. In the 43 unique PCV2 full genome sequences, 31 belonged to PCV2d, 7 to PCV2a, and 5 to PCV2b. Thus, our study demonstrated that PCV2d was predominant genotype circulating in the Midwest of the USA during 2016-2018, and the PCV3 strains have certain level of genetic diversity, but it is smaller as compared to the level of PCV2 strains.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Whole Genome Sequencing of IAVs from Ohio pig farms

Melanie Prarat, Katherine Shiplett, Yan Zhang

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

Swine influenza is a highly contagious infectious disease of pigs, usually caused by type A influenza virus belonging to the family *Orthomyxoviridae*. Clinical signs include lethargy, fever, sneezing, coughing, and occasional abortion in pregnant sows. Economic losses due to influenza A virus (IAV-S) outbreaks are substantial. Two IAV hemagglutinin subtypes, H1 and H3, circulate in pigs globally in association with the neuraminidase N1 and N2 subtypes. Here, we report using next generation sequencing (NGS) technology for characterization of 148 IAV-S viruses from swine clinical samples submitted to our laboratory in 2018. All samples were initially tested using real-time RT-PCR. Positive samples were further characterized using NGS. Phylogenetic analysis indicated that three types of type A influenza viruses including H1N1, H3N2, and H1N2 circulate in Ohio pig farms. Human H1N1 influenza viruses were detected in multiple pig farms. Several commercial live-attenuated IAV vaccines also were sequenced.

Optimization of the sensitivity and specificity of the oral fluids PRRSV ELISA by modifying the cut-off s/p value

Albert Rovira, Devi P. Patnayak

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

Oral fluid (OF) samples are commonly used in swine populations to assess the exposure to porcine reproductive and respiratory syndrome virus (PRRSV). The presence of antibodies against PRRSV in OF can be tested by ELISA and some farms use this approach routinely. In some farms, this test is used to corroborate that the population is negative for PRRSV. In other farms, it is used to demonstrate that the population has been adequately exposed to the virus after vaccination or live virus inoculation. Unfortunately, false positives are not uncommon for this test and they result in delayed movement of animals, repeated testing and unnecessary stress to the producer. In this study, we evaluated the impact of changing the cut-off value of the test in its sensitivity and specificity. All the results obtained for the OF PRRSV IDEXX ELISA at the University of Minnesota Veterinary Diagnostic Laboratory during a one year period (4,521 results) were analyzed. To calculate specificity, results obtained from known negative herds from two large production companies were selected, based on a long history of negative results for PRRSV by serum PCR and ELISA in the farms of origin. There were 439 submissions with a total of 2,551 samples. The specificity of the test was estimated at 98.0% when using the cut-off of 0.4 recommended by the manufacturer of the test. When alternative cut-off values of 0.8 or 1.0 were used, the specificity was 99.6% and 99.7%, respectively. To calculate sensitivity, cases in which OF samples were also tested by PCR and had positive PCR results were selected (74 submissions with a total of 221 samples). The sensitivity of the test was estimated at 93.2%, 88.2% and 87.3% when using 0.4, 0.8 and 1.0 as the cut-off, respectively. As expected, an increase in the cut-off value resulted in an increase in specificity and a decrease in sensitivity. While these values apply to the performance of the test wen only one sample is available, most submissions consist of several samples. Therefore, we calculated the herd or case-level sensitivity and specificity, based on the diagnostic sensitivity and specificity estimates reported above, a sample size of 5 samples per case (average of the data in the study) and an estimated prevalence of 50%. The case/herd specificity estimates were 90.2%, 98.2% and 98.4% at cut-off of 0.4, 0.8 and 1.0, respectively. The case/herd sensitivity estimates were 96.6%, 94.9% and 94.9% at cut-off of 0.4, 0.8 and 1.0, respectively. Based on these calculations, a change in the cut-off s/p value from 0.4 to 1.0 is recommended.

Genetic diversity of type 1 porcine reproductive and respiratory syndrome virus in the United States from 2010 to 2018

Anping Wang[2,1], Huigang Shen[1], Ying Zheng[1], Qi Feng[2,1], Wannarat Yim-im[1], Jianqiang Zhang[1], Phillip Gauger[1], Ganwu Li[1]

[1]Iowa state university, Ames, IA; [2]Jiangsu Agri-animal Husbandry Vocational College, Taizhou, China

The first whole genome sequence of type 1 porcine reproductive and respiratory syndrome virus (PRRSV) was reported in the United States (U.S.) in 2004 and these isolates were usually called North American (NA) type 1 PRRSVs. Since then, there have been limited reports characterizing type 1 PRRSV in the U.S. especially in the past a few years. In order to explore the genetic characteristics and diversity of type 1 PRRSV circulating in the U.S. in recent years, 27 type 1 PRRSV PCR-positive samples collected from 7 states from 2010 to 2018 were included in the present study for whole genome sequencing by next-generation sequencing (NGS) technology followed by sequence analysis. Complete genome sequences were obtained for all of the 27 samples. The 27 type 1 PRRSVs had 88.4~91.3% nucleotide identity to the Lelystad PRRSV strain (the type 1 PRRSV reference strain initially identified in Europe) and 87.4~89.8% nucleotide identity to the previously reported NA type 1 PRRSV isolates. The individual proteins, such as Nsp2, Nsp12, GP3, GP4, and GP5, were identified to have some unique genetic characteristics. Only one of the 27 tested samples had the characteristic 17-aa deletion in the Nsp2, which was reported to serve as a genetic marker of NA type 1 PRRSV isolates. The remaining 26 samples contained different deletions in other regions of Nsp2 or a shorter deletion than the 17-aa deletion at the same site. Nine samples had a unique 1-aa insertion of leucine at aa position 378 of Nsp2. For the highly conserved Nsp12 gene, fourteen samples displayed a 3-aa C-terminal truncation as a result of premature stop codon. For GP3 and GP4 proteins, sixteen samples had a 21-aa or 18-aa truncation from the C terminus due to a premature stop codon, and 1-aa deletion was observed in one sample at the overlapping region of GP3 and GP4. GP5 protein showed the similar genetic variation as other reported NA type 1 PRRSV isolates with the exception for one 1 aa mutation at the highly conserved N-46 glycosylation site in one sample. The whole genome-based phylogenetic tree indicated that all the tested samples clustered within subtype 1 together with other available NA type 1 PRRSV isolates, supporting the assumption of the single origin of NA type 1 PRRSV. The recombinant analysis showed that no obvious recombination was detected. Collectively, although the number of type 1 PRRSV isolates included in this study is limited, the results provide up-to-date information about type 1 PRRSV circulating in the U.S. in the past 9-year period. The data clearly indicate that type 1 PRRSVs in the U.S. have undergone gradual variation and exhibited some previously undescribed genetic characteristics and diversity, which complicates the diagnosis and control of NA type 1 PRRSV. Therefore, comprehensive and deep characterization of more type 1 PRRSV circulating in the U.S. is warranted in future studies.

Prevalence of anti-PCV3 antibodies in swine diagnostic samples by a peptide-based ELISA assay †

Yin Wang[1], Wanglong Zheng[1,2], Xue Leng[1,3], Elizabeth G. Porter[1], Colin P. A. Stoy[1], Lance Wade Noll[1], Xuming Liu[1], Jianfa Bai[1]

[1]Kansas State University, Manhattan, KS; [2]Yangzhou University, Yangzhou, China; [3]Jilin Agricultural University, Changchun, China

In 2015, PCV3 was identified as the new porcine circovirus in the US, which could cause PCVAD-like clinical symptoms similar to that caused by PCV2. To study the serologic prevalence and to evaluate the animal immune response, we developed a PCV3 specific ELISA assay. Experimentally inoculated animals were used to generate PCV3 positive and negative standards for the assay development. Five peptides in the capsid (Cap) protein and one from the replicase (Rep) protein that were *in silico* predicted to have high immunogenicity were synthesized and evaluated with the standards. One peptide from Cap protein and one from Rep protein were recognized by the antibody from the positive standards, but not from the negative ones. The two peptides were then pooled as the coating antigen for the PCV3 antibody detection. The cutoffs were determined with the standards using the receiver operating characteristic (ROC) analysis. Validated with serial dilutions of PCV3 polyclonal antibody and clinical positive samples, the PCV3 ELISA had high sensitivity and specificity. The specificity was also verified by the negative results generated with a PCV2 ELISA assay for those PCV3 positive samples, and vice versa. The repeatability of the assay was assessed and confirmed by testing within plates, between plates and between runs. For diagnostic evaluation, 367 serum samples and 276 oral fluid samples collected in KSVDL were subjected to the prevalence investigation. The PCV3 positive rates were 46% (169/367) in serum samples and 35.1% (97/276) in oral fluid samples. In summary, we have established the peptide-based PCV3 specific ELISA assay for antibody detections that can be used to monitor serologic prevalence and animal immune response against the virus infection.

† Graduate Student Oral Presentation Award Applicant

Evaluation of Betacoronavirus fecal shedding among apparently healthy exhibition and racehorses

Hayley G. Hofmar-Glennon[3,4]*, Nicole M. Andre*[3]*, Alison Stout*[1]*, Gary Whittaker*[3,4]*, Laura B. Goodman*[1,2]*,*
Belinda S. Thompson[1,2]*, Manigandan Lejeune*[1,2]*, Wendy Hartman*[5]*, Erin L. Goodrich*[1,2]

[1]Population Medicine and Diagnostic Sciences, Cornell University, College of Veterinary Medicine, Ithaca, NY; [2]Animal Health Diagnostic Center, Cornell University, Ithaca, NY; [3]Department of Microbiology & Immunology, Cornell University, College of Veterinary Medicine, Ithaca, NY; [4]Master of Public Health Program, Cornell University, College of Veterinary Medicine, Ithaca, NY; [5]Hill Winds Veterinary Practice, Johnson City, NY

Equine coronavirus is a member of the Betacoronavirus (Beta-CoV) genus and has recently emerged as a pathogen of adult horses. Previously associated with gastrointestinal disease in foals, it is now known that Beta-CoV is also associated with outbreaks of anorexia, lethargy, and fever in adult horses. Less commonly it may result in gastrointestinal disease in adult horses. Very rarely it can cause necrotizing enteritis and can be associated with hyperammonemic encephalopathy. Diagnosis is typically performed via molecular detection of the virus in feces. Previous publications documenting outbreaks of Beta-CoV in adult horses have shown that a significant percentage of asymptomatic herd mates of clinically ill horses can test positive for Beta-CoV on fecal PCR. We hypothesized that apparently healthy adult exhibition and racehorses, in the absence of a Beta-CoV outbreak, would not shed Beta-CoV in their feces. In this study, fecal samples were collected a single time from apparently healthy horses at an exhibition in New York (NY) State and at two different NY State racetracks in late summer of 2018. The exhibition animals sampled included 100 horses and one female donkey. The horses consisted of a variety of both light and draft breeds, comprising 37 mares and 63 geldings. Ages of these animals ranged from 6 months to 40 years with a median of 8 years. The racehorse samples were collected from 42 Standardbred horses (15 mares, 18 geldings, and 9 stallions), ages ranging from 2 to 9 years with a median of 3 years. A single positive Beta-CoV fecal PCR was identified from an 8 year old gelding Percheron horse who had been present at the exhibition site for 3 days at the time of sample collection; all other samples tested negative. The findings from this study demonstrate that Beta-CoV is not typically shed in the feces of healthy adult horses. When fecal samples from clinically ill or apparently healthy horses do test positive on PCR for Beta-CoV, they should be considered diagnostically relevant.

Molecular Diagnostics and Bioinformatics 1

Saturday, October 26, 2019
Ballroom E

Moderators: Rebecca P. Wilkes and Yan Zhang

Symbols at the end of titles indicate the following designations:

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

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High-Throughput tick specimen processing for nucleic acid extraction §

Zachary C. Forbes, Renee Anderson, John S. Beeby, Amy Glaser, Laura B. Goodman

Animal Health Diagnostic Center, Cornell University, Ithaca, NY

Ticks are known vectors for numerous infectious diseases of concern to both animal and human health and are frequently on the front-line of newly emerging diseases. As the range of various tick species expands and overlaps in new ways with other organisms and environments, the demand for tick-borne disease testing may increase. Accredited veterinary diagnostic laboratories are ideal for this testing. Therefore, it is imperative that the ability to process ticks be ergonomically friendly, cost-effective, efficient, and scalable for high-throughput testing.

A conventional method for processing ticks for subsequent nucleic acid extraction is by hand grinding in a microcentrifuge tube with a pestle. This method has many drawbacks. The procedure is manual and introduces variability from technician grinding techniques. The procedure is done in open tubes and introduces the risk of cross-contamination. There can be loss of specimen during the procedure as well due to adhesion of the homogenate and larger pieces to the pestle that is removed from the tube, resulting in lower yield of nucleic acid. Finally, the procedure is labor-intensive, time-consuming, and introduces ergonomic issues to technical staff. To address these concerns, we have adapted a published small arthropod processing protocol using 4mm diameter hollow-core brass beads (BriteBeads™) into a high-throughput extraction procedure.

A 96-well plate format rack containing 1.1 mL round-bottom tubes with rubber caps was preloaded with one brite bead per tube, 400uL PBS, and a single, hydrated tick specimen in each tube. Ticks were disrupted for 5 minutes (2000 +/- 200 oscillations per minute) using a bead beater fitted with a 96-tube rack adapter. After disruption, the rack is centrifuged briefly in an ultracentrifuge to bring the tick-PBS homogenate down into the tube. The resulting PBS slurry has no visible fragments indicating effective homogenization. It can then be transferred to a 1.5mL microcentrifuge tube or other preferred sample container for subsequent nucleic acid extraction. In this way, up to 96-ticks can be rapidly homogenized at once. When verifying this new tick processing procedure, we used an exogenous internal control spiked into the lysis buffer to look for inhibition, and saw no inhibition of the nucleic acid. This technique is especially effective for ticks stored in ethanol for extended periods of time. Based on a selection of 16 ticks recently extracted, eluted total nucleic acid had a mean concentration of 10.6 ng/uL and mean purity (A260/A280) of 2.1. We have processed over 6,000 ticks from 8 species (4 genera) utilizing this procedure with consistent high quality results in terms of internal control consistency and pathogen prevalence compared to surveys conducted by other groups. This procedure is highly scalable and rapid, allowing for the potential of processing large numbers of ticks in a high-throughput workflow.

§ AAVLD Laboratory Staff Travel Awardee

Senecavirus A detection from piglets using processing fluid samples

Tracy Lene Otterson, Phillip Blomberg, Albert Rovira

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

In August 2018, six of the eleven sow farms in a swine production flow started experiencing diarrhea and increased pre-wean mortality in piglets during the first week of age. Tissue samples from each of the affected farms were submitted to the Molecular Diagnostic laboratory for viral and bacterial screening. Results for intestinal samples submitted showed strong positive Ct values for Senecavirus A (SVA) by real-time PCR from all six farms. Other potential causes of diarrhea were ruled out and Senecavirus A was diagnosed as the cause of diarrhea and increased pre-wean mortality. Each of the eleven farms in this swine production flow routinely submit processing fluids (PF) weekly for PRRSV testing. PF sample submissions have been more popular for viral screening, mainly PRRSV, due to ease of collection and pooled screening capabilities. However, the presence of Senecavirus A in PF had not been investigated. Therefore, the objective of this study was to evaluate PF as a diagnostic sample for Senecavirus A detection in sow farms. PF collected during the week of the outbreak from the same six farms that submitted intestinal samples for diarrhea diagnostics, as well as from five farms that were not experiencing increased pre-wean mortality from diarrhea were used. For the six affected farms, PF from the two preceding weeks were also tested to determine if the virus could be detected before the onset of clinical signs. Three PF samples were available for each farm and week, for a total of 69 samples. Intestinal samples were processed to create a tissue homogenate and PF were poured off and clarified by low speed centrifugation. Both sample types were extracted using the MagMax-96 Viral RNA Isolation kit (Thermo Fisher) on a 96-well magnetic particle processor. The samples were PCR'd using the EZ-SVA Real time RT-PCR assay kit (Tetracore) on the ABI 7500 Fast platform. From the six farms with increased pre-wean mortality, all 27 intestinal homogenates showed positive Ct values for SVA ranging from 20.07 to 34.07. Sixteen of 18 PF submitted from the same six farms during the same week were positive for SVA with Ct values ranging from 19.51 to 29.23. Two of the PF samples were negative. PF collected the previous week were positive from three of the affected farms and negative for the other three. Two weeks prior to tissue submission, all PF submitted from all six affected farms were negative. PF from the five farms that were not showing signs of increased pre-wean mortality from diarrhea were negative (14 samples) or suspect (one sample). These results indicate that PF would be an acceptable specimen type to use for the detection of SVA by real-time PCR in piglets experiencing increased pre-wean mortality and diarrhea. PF could also be a good sample to monitor the presence of Senecavirus A in sow herds and the effectiveness of control measures.

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Sequencing of *pilin* locus in *Moraxella bovis* reveals deletions and truncated proteins

Joshua Elston[1,2], *Jessica Peterson*[1,2]

[1]Diagnostics, Newport Laboratories, Worthington, MN; [2]Diagnostics, Boehringer Ingelheim Animal Health, Worthington, MN

Moraxella bovis is a cause of infectious bovine keratoconjuctivitis, the most common ocular disease in cattle worldwide(1). Presentation ranges from mild signs to corneal opacity, conjunctivitis, and ulceration, which lead to concerns for animal welfare and lost production costs(1). *Moraxella bovis* isolates are classified into seven serogroups, A through G, which are determined by the pilus antigen(2). Herein, we used high throughput sequencing (MiSeq) to generate the complete genome of 27 geographically diverse *Moraxella bovis* isolates and compared them to previously published serogroups from the NCBI sequence database. In total, 11 isolates failed to match a reference serogroup. Closer examination revealed 8 of 11 contained significant deletions in the coding region, ranging from 171 to 340 base pairs. The remaining 3 isolates possessed a full length coding sequence which failed to match to an existing serogroup reference. These 3 isolates share higher than 93.3% sequence identity in the coding region and lacked a common geographic origin. Further serology work is necessary to confirm the genetic variance confers an undiscovered serogroup.

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Repeatability of open reading frame 5 sequencing for Porcine Reproductive and Respiratory Syndrome Virus at different concentrations * †

Nicholas Streauslin[1], *Karen Harmon*[2], *Phillip Gauger*[2]

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

Open reading frame 5 (ORF5) is the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) gene for the major envelope protein. The ORF5 is an important epidemiological tool used by researchers and the swine industry to evaluate PRRSV phylogeny due to its high genetic variability. It is common practice for producers to monitor PRRSV strains circulating in their herds using ORF5 sequence data. The goal of this study was to determine the repeatability and sensitivity of PRRSV ORF5 sequencing.

A PRRSV 1-7-4 isolate was obtained from the lung of a pig with clinical respiratory disease submitted to the Iowa State University Veterinary Diagnostic Laboratory. Serial dilutions were created to examine the effect of viral concentration on the repeatability and sensitivity of the sequencing PCR reaction and generation of a high quality sequence. The targeted dilution groups were based on data corresponding to real-time PCR (qPCR) C_q values. The 8 groups targeted C_q ranges: 29-30 (set 1), 30-31 (set 2), 31-32 (set 3), 32-33 (set 4), 33-34 (set 5), 34-35 (set 6), 35-36 (set 7), 36-37 (set 8). The 8 dilution sets were extracted using the Thermo KingFisher 96 magnetic particle processor in conjunction with the MagMAX Pathogen RNA/DNA Kit. The extractions were conducted in triplicate. Five PCR reactions were set up from each of the triplicate extractions from the 8-dilution series for a total of 120 PCR reactions. The PCR products from these reactions were submitted to the Iowa State University DNA Facility for Sanger sequencing. Our data is based on all 15 reactions from each of the dilution series.

Our results showed that in sets 1-3 the repeatability of obtaining clean and useable sequencing data was 73.3% - 86.7%. The amount and quality of data that could be used to assemble DNA contigs dropped dramatically with later Cq values. In Set 4, only 53.3% of the data was of high enough quality to be able to be assembled. The sensitivity of series 5 – 8 decreased to 33.3%, 20.0%, 6.7%, and 6.7% respectively. We also examined if the viral concentration impacted the repeatability of the sequencing results. We compared the percent identity of each assembled contig in the dilution series to a contig of the undiluted virus. Our results show that there is little impact on the percent identity of the sequencing data based on the viral concentration. The lowest percent identity observed was 99.3%.

This investigation helps us further understand the sensitivity and repeatability of diagnostic PRRSV sequencing at C_q values commonly encountered in veterinary diagnostic laboratory testing. These data demonstrate that samples with low viral concentration may not result in a complete sequence. However, the sequencing data obtained from these samples with low viral concentration is repeatable, despite the lower sensitivity. This is important information for veterinarians when monitoring the health and viral history and phylogeny of their herds.

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

Escherichia coli virulence screening for veterinary diagnostics using whole genome sequencing data §

Patrick Mitchell[1], Brittany D. Chilson[1], Tong Ho[1], Esra I. Dogan[1], Renee Anderson[1], Reddy Bommineni[2], Shipra Dutta Mohan[2], Rebecca P. Wilkes[3], Hemant Kashinath Naikare[3], Cynthia Watson[3], Lanny Pace[4], Martha Pulido-Landinez[4], Amar Patil[5], Sarmila Dasgupta[5], Shannon Mann[5], Donna Kelly[6], Whitney LaRoche[1], Rebecca Franklin-Guild[1], Laura B. Goodman[1]

[1]Animal Health Diagnostic Center, Cornell University, Ithaca, NY; [2]Florida Department of Agriculture and Consumer Services, Kissimmee, FL; [3]University of Georgia, Athens, GA; [4]Mississippi State Veterinary Research & Diagnostic Laboratory, Pearl, MS; [5]New Jersey Animal Health and Diagnostic Lab, Trenton, NJ; [6]University of Pennsylvania, Kennett Square, PA

Escherichia coli pathotypes can vary in their host range and disease presentation, driven by the presence or absence of a wide array of virulence factors (VFs). As such, identifying the VFs carried by an *E. coli* isolate is key to understanding its pathogenic potential. Current PCR-based methods for VF detection are limited by the need to run multiple reactions per isolate and by the cost and effort needed to produce and validate a target gene specific assay. As whole genome sequencing (WGS) becomes standard practice in veterinary diagnostics, there is potential to replace these rigid, host-specific, stand-alone PCR assays with sequence-based analyses. Screening for virulence factors using WGS data allows for testing an unlimited number of targets with only a single wet lab process. It also allows one to go back and test for new targets should the need arise, with no additional bench work.

In order to use genomic data to screen for VFs, it is necessary to choose an appropriate database. Existing VF databases tend to include a large number of targets, producing unwieldy and difficult to interpret results, and sometimes lack key VFs of interest to the veterinary community. To address this gap, we developed a custom *E. coli* VF database oriented towards veterinary and zoonotic strains. This database includes the 11 targets covered by our current PCR assays (K99, F17A, F41, EAE, 987p, STa, STb, LT, Stx I, Stx II, and CNF1) as well as 10 others (K88, F18, F1845, CNF2, iutA, BFP, iroN, CS31A, ompT, and hlyF) and is searchable with assembled or unassembled WGS data using existing screening tools. In order to assess the quality of this WGS approach, we first screened unassembled sequencing reads from 58 *E.coli* isolates against our database using the short read-based typing program SRST2, then tested a subset of 18 of these isolates using our PCR assays. For targets covered by both assays, results were identical between the PCR and read-based WGS methods, with the exception of one low positive CNF1 hit that was not found in the genomic data. Shared hits included STa (1), STb (1), and CNF1 (12). Predictions also matched results for published colibacillosis isolates. We then assembled the genomes of these 18 samples using both SPAdes and SKESA and screened the assembled contigs against our database using ABRicate with a variety of coverage thresholds. Results of the assembly-based screening were similar to those from the read-based screening approaches, though they were sensitive to coverage threshold.

This screening methodology can be readily combined with serotype prediction, sequence typing, and antimicrobial resistance profiling in order to generate a comprehensive, host animal-tailored report for clients. The database will be made publically available. This study was funded and performed in collaboration with the Food and Drug Administration's Veterinary Laboratory Investigation and Response Network (FDA Vet-LIRN) under grant 1U18FD005144.

§ AAVLD Laboratory Staff Travel Awardee

Evaluation of targeted Next Generation Sequencing for detection of equine pathogens in clinical samples

Eman Anis[1,2], Marcia R.S. Ilha[3], Julie B. Engiles[2], Rebecca P. Wilkes[4,1]

[1]Infectious Diseases, University of Georgia, Tifton, GA; [2]Pathobiology, University of Pennsylvania, Kennett Square, PA; [3]TVDIL, University of Georgia, Tifton, GA; [4]Comparative Pathobiology, University of Purdue, West Lafayette, IN

Equine infectious disease outbreaks may have profound economic impact, resulting in millions lost in revenue due to horse loss, quarantine, and cancelled events. Early and accurate diagnosis is essential to limit the spread of infectious diseases. However, the laboratory diagnosis of infectious diseases, especially those that may be caused by numerous pathogens, is challenging to the clinician and diagnostic laboratory. Routinely, this requires submission of multiple samples to separate laboratory sections, even despite the development of multiplex PCR assays that can identify several pathogens in a single sample. Given the associated costs and time, there is a need to develop improved syndromic/comprehensive testing.

Next generation sequencing (NGS), which allows millions of DNA templates to be sequenced simultaneously in a single reaction, is an ideal technology for comprehensive testing. However, the wide implementation of NGS as a routine diagnostic tool is still facing many barriers, including high costs and the complexity of data analysis. Targeted NGS has been used to overcome these hurdles. Targeted NGS, using selective capture or amplification of specific genomic regions of interest prior to massive parallel sequencing, has proven to be a more cost-effective, simpler and efficient way to implement NGS technology for routing diagnostics.

In this study, 264 primers were designed to target the most common equine bacterial, viral, and parasitic pathogens (71 pathogens) and a bioinformatics tool was constructed for the detection of the targeted pathogens. Specificity of the primers was determined by testing known bacterial cultures, virus isolates and parasites. Relative detection limits of the targeted NGS method were evaluated with samples concurrently tested by real-time PCR. The method was then validated using equine clinical samples previously tested using other routine diagnostic techniques and percent agreement and Cohen’s kappa were calculated between routine methods and the targeted NGS assay. Targeted NGS identified multiple pathogens in the clinical samples in concordance with routine diagnostic techniques, and at a comparable price. Targeted NGS also identified additional pathogens missed by the routine techniques because the specific tests needed for these particular organisms were not performed. This study demonstrates that targeted NGS can be implemented in a cost-effective manner to greatly improve the diagnostic efficiency and sensitivity for veterinary microbiology samples.

Characterization of a novel human seasonal H3 influenza A virus spillover that is now endemic in US swine

Aditi Sharma[1], Michael Zeller[1], Tavis Anderson[2], Amy L. Vincent[2], Karen Harmon[1], Ganwu Li[1], Jianqiang Zhang[1], Phillip Gauger[1]

[1]VDPAM, Iowa State University, Ames, IA; [2]Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA

A reverse-zoonosis transmission of human-seasonal H3 subtype IAV from humans into swine was first detected in Oklahoma in March, 2017 and demonstrated sustained circulation within the U.S. swine population between 2017 to the present. To understand the origin of this virus and its evolution in the swine host, we genetically characterized the hemagglutinin (HA) and neuraminidase (NA) genes of 46 viruses, and whole genome sequenced 13 strains. A time-scaled Bayesian approach for the HA gene demonstrated that the time to the most recent common human H3 ancestor was August, 2016 to September, 2016. Spatial dissemination of genetically similar H3 HA across the central U.S. was observed with 1 detection in Ohio, 3 in Illinois, 5 in Iowa, 11 in Arkansas, 12 in Indiana and Oklahoma each and 2 from unknown states. Whole genome sequencing of 13 strains revealed that the N2 neuraminidase (NA) was of human-seasonal origin, with the matrix gene of H1N1pdm09 origin and all other internal genes of triple reassortant (TRIG) origin. Pairwise comparison between the first detected swine HA gene and the most similar human seasonal H3 revealed 99.9% similarity. Evaluation of glycosylation sites in HA gene revealed that after 2017, the HA protein lost a predicted glycosylation at position 133 (H3 numbering) within the antigenic site A and the 130-loop of the receptor binding site. Following spillover, early swine viruses had an antigenic motif of STHNYK (amino acid position 145, 155, 156, 158, 159 and 189) which was identical with the closest human seasonal virus. STHNYK was the primary motif post introduction whereas STHNYN was predominant after April, 2017. Changes in the antigenic motif and glycosylation patterns of the virus may be a consequence of antigenic drift and/or adaptation to the swine host. We report the second emergence of a sustained human seasonal H3 IAV spillover into swine in the 2010 decade (named “2010.2”). These data suggest human IAV continue to spillover into swine and become established in the population, contributing to the genetic and antigenic diversity of IAV circulating in swine. Continued monitoring for novel IAV will assist vaccine antigen selection and vaccine updates to improve the ability to prevent this costly disease.

Next Generation Sequencing(NGS)-Based Metagenomic Analysis: A promising diagnostic tool to identify pathogens in the blood of horses with febrile illness

Murugan Subbiah[1,2], Nagaraja Thirumalapura[1], Julia Livengood[1], Derek Veith[1], David Thompson[1],
Suresh Varma Kuchipudi[2], Bhushan Jayarao[2], Deepanker Tewari[1]

[1]PADLS, Pennsylvania Veterinary Laboratory, Harrisburg, PA; [2]Animal Diagnostic Laboratory, Pennsylvania State University, University Park, PA

The importance of Next Generation Sequencing (NGS)-based diagnostics is increasingly being recognized in human medicine and more recently in veterinary medicine. The major limitation of the existing molecular diagnostic tools such as PCR is that they are not readily designable to diagnosing novel pathogens and mixed infections. Here we demonstrated the usefulness of NGS-based metagenomic approach to identify the pathogens in the blood of horses with febrile illness collected (n=4) during 2017. Whole genomic DNA from blood samples was extracted and were enriched using NEBNext microbiome DNA enrichment and Genomiphi V2 DNA amplification kits. The enriched microbial DNA samples were subjected to NGS using Nextera DNA Flex library preparation kit and V2 chemistry sequencing kit in a MiSeq machine. On an average 234,896±35,620 reads per sample were analyzed for studying the composition of the microbial populations in the blood samples using Kraken metagenomic analysis platform. The analysis showed that 0.6±0.1% and 0.01% of reads belong to bacterial and viral genomes, respectively. Importantly, 10-20% of Alphaproteobacterial reads were identified as *Anaplasma phagocytophilum*. Notably, three samples that were identified as positive and one sample that was negative for *Anaplasma phagocytophilum* by NGS showed concordance with PCR testing. In addition, other bacterial and viral genomes such as Actinobacteria, Clostridium, and Herpesviridae were also detected in the blood samples. While the clinical significance of the other bacterial and viral genomes is not fully understood at this stage, it is clear that this approach was able to identify multiple pathogens in blood samples of horses with febrile illness. We are further analyzing additional samples to establish the correlation of these pathogens to febrile illness in horses. Our results provide a proof of concept that NGS-based metagenomic approach is a valuable tool which can facilitate veterinary diagnostic laboratories to not only accurately diagnose mixed infections but also to identify novel pathogens.

Molecular Diagnostics and Bioinformatics 2

Sunday, October 27, 2019
Room 555

Moderators: Hemant K. Naikare and Donna M. Mulrooney

8:00 AM **Evaluation of an easy and rapid Detection of Avian Leukosis Virus Subgroup J (ALV-J) by fully automated POCKIT™ Central PCR System**
Simon Chung, Choong Keat Fu, Wei-Fen Tsai, PingHan Chung, Chia-Chi Chang,
Yu-Chun Lin, Pin CHOU, Thomas Wang 63

8:15 AM **Whole Genomic Sequencing of BVDV-2 Isolates using Illumina iSeq 100 and MinION technologies**
Rebecca Mackey, Yi Candy Zhang, Michael Zhang, Lanny Pace, Lifang Yan 64

8:30 AM **Development of process controls for bacterial Whole Genome Sequencing §**
Renee Anderson, Patrick Mitchell, Denise L. Archer, Rebecca L. Tallmadge, Brittany D. Chilson,
Derek Rothenheber, Laura B. Goodman. 65

8:45 AM **Phylogenetic analysis of type 1 porcine reproductive and respiratory syndrome virus ORF5 sequences in the United States from 2003 to 2018 †**
Wannarat Yim-im, Tavis Anderson, Phillip Gauger, Karen Harmon, Jianqiang Zhang 66

9:00 AM **Application of a Nanopore MinION sequencing workflow in pathogen identification and detection of antimicrobial resistance gene analysis in a veterinary diagnostic setting # †**
Grazieli Maboni, Rodrigo de Paula Baptista, Joy Wireman, Anne Summers, Suan Sanchez 67

9:15 AM **Diagnosis of Bovine Tuberculosis using a defined skin test in Buffaloes in India # * †**
Sreenidhi Srinivasan, Maroudam Veerasami, Douwe Bakker,
Martin Vordermeier, Vivek Kapur 68

9:30 AM **Break**

10:15 AM **Genetic characterization of *Erysipelothrix rhusiopathiae* using Whole Genome Sequencing**
Jing Cui, Melanie Prarat, Katherine Shiplett, Dominika Jurkovic, Yan Zhang 69

10:30 AM **Quantitative real-time PCR detection of African Swine Fever virus on a field-based PCR platform, the Biomeme Franklin hand-held thermocycler ◊**
Jessie D. Trujillo, Russell Ransburgh, Anthony Craig, Livio Heath, Juergen Richt 70

10:45 AM **Development of a probe based real-time PCR for the accurate diagnosis of *Avibacterium paragallinaum* §**
Rhiannon Barry, Ruth Nissly, Jen Williams, Maurice Byukusenge, Traci Pierre, Tammy Matthews,
Eva Wallner-Pendleton, Patricia Dunn, Barnhart Denise, Sean Loughrey, Sherrill Davison Yeakel,
Donna Kelly, Deepanker Tewari, Bhushan Jayarao, Suresh V. Kuchipudi 71

11:00 AM **AgroDiag PorCoV : a multiplex immunoassay for the differential diagnosis of swine enteric coronaviruses**
Rémi Malbec, Kay Kimpson-Burkgren, Elisa Vandenkoornhuyse, Jose Carrillo-Ávila,
Christophe Audebert, Dave Baum, Luis Gabriel Gimenez-Lirola 72

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

Evaluation of an easy and rapid Detection of Avian Leukosis Virus Subgroup J (ALV-J) by fully automated POCKIT™ Central PCR System

Simon Chung[1], Choong Keat Fu[2], Wei-Fen Tsai[1], PingHan Chung[1], Chia-Chi Chang[1], Yu-Chun Lin[1], Pin CHOU[1], Thomas Wang[3]

[1]GeneReach Biotechnology Corp., Taichung, Taiwan; [2]Aviagen Inc., Huntsville, AL; [3]GeneReach USA, Lexington, MA

ALV-J is a prevalent retrovirus that causes lympho-proliferative disease or tumor and results in serious economic losses in poultry breeding industries.

The screening intensity is high especially in poultry breeding companies, SPF (specific pathogen free) chicken last but not least biological products such as poultry vaccines. Virus isolation coupled with antigen-capture ELISA is often referred to as the gold standard for ALV isolation and identification and ALV-J specific PCR is carried out for confirmation. However, the procedure is labor intensive and time consuming.

In order to facilitate the procedure utilized for ALV-J screening, POCKIT™ Central Nucleic Acid Analyzer (POCKIT™ Central, GeneReach) is a system benefit for timely detection of ALV-J with non-invasive sample types (serum and cloacal swab) in 85 minutes.

This evaluation and validation study were carried out in Asia region to compare with the available RT-qPCR system. An ALV-J RT-iiPCR reagent is commercially available to work with POCKIT™ Central system based on the detection of *env* gene, which is usually used for discrimination of the subgroups.

This study revealed the performance of the ALV-J RT-iiPCR/POCKIT™ Central system. It had analytical sensitivity 10-fold higher to reference RT-qPCR system and did not cross react with ALV-A, -B, -E, and –K, and endogenous avian viruses (EAVs). Side-by-side comparison with 70 retrospective avian samples showed moderate agreement between both systems. All discrepant samples are low-titer sample and showed positive with POCKIT™ Central system but negative with reference RT-qPCR system. Sanger sequencing were used to validate those samples and showed those samples are truly positive for ALV-J.

With analytical and clinical performance comparable to the reference RT-qPCR system, this user-friendly automated system can serve as a sample-in-answer-out, minimize human error risks to facilitate the screening procedure of ALV-J while providing easy qualitative results with cost effective reagents/consumables.

Whole Genomic Sequencing of BVDV-2 Isolates using Illumina iSeq 100 and MinION technologies

Rebecca Mackey[1], Yi Candy Zhang[1], Michael Zhang[2], Lanny Pace[1], Lifang Yan[1]

[1]CVM-MVRDL, Mississippi State University, Brandon, MS; [2]Biomedical Sciences, University of Missouri, Columbia, MO

Bovine viral diarrhea virus (BVDV) is a member of the *Pestivirus* genus and is separated into two genotypes, BVDV-1 and BVDV-2. BVDV-1 is associated with chronic mild or subclinical infections while BVDV-2 is frequently implicated in outbreaks of severe thrombocytopenia and acute fatal disease. Genetic and antigenic variations in field isolates can affect the performance of detection assays; therefore, whole genomic sequencing can be a useful tool in the evaluation of BVDV isolates.

The viruses used for complete genome sequencing were isolated from a beef breeder and a beef calf. Both animals presented severe clinical signs compatible with thrombocytopenia or hemorrhagic syndrome. The viruses were isolated on MDBK cells and the RNA extracted using the MagMax pathogen DNA/RNA extraction kit (Thermofisher). The two isolates were confirmed as BVDV-2 by real time RT-PCR and by sequencing of the E2 gene. RNA was reverse transcribed to DNA, and the library was prepared using Nextera DNA Flex. Whole genome sequencing of these two isolates was accomplished in the Illumina iSeq 100. Also, direct cDNA sequencing was done using the MinION sequencer for one isolate.

Illumina reads of isolate M10-5347 were assembled using spades, which generated one contig with a length of 12439 and 99.62% homology with published cytopathic BVDV-2 isolate 296c from 7 to 12336. Data from MinION was filtered using filtlong and assembled with minimap2. cDNA direct sequencing using MinION does not generate as much data as PCR-based sequencing from iSeq 100; however, there is one read for location 1 to 1018 and one read for location 11464 to 12629, sharing 93.2% and 91.24% homology with BVDV2 isolate 296c. The complete genome of M10-5347 was accomplished by combining reads from iSeq 100 and MinION direct cDNA sequencing. The accuracy of MinION sequencing is reduced compared to Illumina, but direct cDNA sequencing can avoid the bias of PCR amplification-based sequencing. The high levels of input mRNA required for direct cDNA sequencing, however, make comparable levels of accuracy difficult to achieve.

Reads of M10-3432 were assembled using spades, which generated two contigs, one for location 1 to 5988 and one for location 6047 to 11986, which are 98.65% and 98.8% homologous with published noncytopathic BVDV2 isolate JV14. No MinION sequencing reads have been completed for isolate M10-3432 yet. Further sequencing will be performed to complete the whole genome.

Development of process controls for bacterial Whole Genome Sequencing §

Renee Anderson, Patrick Mitchell, Denise L. Archer, Rebecca L. Tallmadge, Brittany D. Chilson, Derek Rothenheber, Laura B. Goodman

Animal Health Diagnostic Center, Cornell University, Ithaca, NY

Bacterial whole genome sequencing (WGS) is a valuable tool for characterization of novel species, outbreak management, antimicrobial resistance (AMR) surveillance, virulence prediction, and investigation of microbial contamination in pet food. As WGS enters into clinical and regulatory applications, the documentation of a set of upstream and downstream standardized procedures is crucial. The AAVLD has established requirements for the implementation of a Quality Management System, which provides laboratories the basis for development of a standardized pipeline for WGS. Here, we describe process controls that were developed for including bacterial WGS on Illumina platforms under the auspices of AAVLD and ISO/IEC 17025 accreditation.

Selecting a starting point, such as a pure colony, allows the expansion of the WGS scope upstream and post sequencing. It is crucial to assign unique identifiers to each sample and/or sub-sample using a Laboratory Information Management System to allow for traceability. The process continues with extraction, library preparation, normalization, and pooling. This established stream allows for the acceptance of nucleic acid or libraries prepared externally with additional qualifiers, as quality input is essential. Metrics from four bench quality checks include DNA quality and concentration, individual library concentration, and pooled library quality and concentration. The Illumina sequence analysis viewer provides metrics after each sequencing run. These quality checks can be tracked, charted, and inspected for abnormalities. Any measurement falling outside of historical ranges may undergo further review prior to onsite data analysis or transfer of sequencing data to clients. Post-run management decisions include when and how to transfer sequencing data to clients.

Approaches to downstream bioinformatic analyses are dependent upon access to computing capacity and personnel expertise, but user-friendly tools are available to anybody able to use a web browser. Our current pipeline includes internal tracking of read and assembly quality control metrics including Phred scores, coverage depth, N50, and software versions. The report to the client focuses on applicable results with interpretations. For example, AMR gene detection with corresponding antibiotic classes for each gene found may be requested. *In silico* serotype prediction for *Salmonella enterica* and *Escherichia coli* is another common client need. Performing bioinformatic analyses with open-source tools that have been published in peer-reviewed journals facilitates the validation process, and disclaimers added to reports to indicate which analyses should be considered investigational pending validation.

This study was funded and performed in collaboration with the Food and Drug Administration's Veterinary Laboratory Investigation and Response Network (FDA Vet-LIRN) under grant 1U18FD005144.

§ AAVLD Laboratory Staff Travel Awardee

Phylogenetic analysis of type 1 porcine reproductive and respiratory syndrome virus ORF5 sequences in the United States from 2003 to 2018 †

Wannarat Yim-im[1]*, Tavis Anderson*[2]*, Phillip Gauger*[1]*, Karen Harmon*[1]*, Jianqiang Zhang*[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, USDA, Ames, IA

Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) can be divided into type 1 (PRRSV-1) and type 2 (PRRSV-2). In the U.S., PRRSV-2 dominates in swine populations (roughly 95-97%) with low PRRSV-1 circulation (3-5%) based on PCR testing. However, PRRSV-1 circulating in U.S. swine has not been well characterized. The objective of this study is to phylogenetically analyze PRRSV-1 ORF5 sequences determined in a U.S. veterinary diagnostic laboratory over fifteen years together with other global sequences.

Methods: 1,531 PRRSV-1 ORF5 sequences determined in the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) submitted from 15 states during 2003-2018, 2,497 PRRSV-1 ORF5 sequences available in GenBank from 31 countries, and 190 PRRSV-1 ORF5 reference sequences from previous studies were analyzed in this study. A total of 2,023 unique ORF5 sequences from GenBank were obtained after redundant ORF5 sequences were removed. ORF5 sequences from ISU VDL, GenBank, and reference sequences were aligned and a maximum likehood tree was inferred using FastTree with a general time-reversible model with gamma-distributed rate heterogeneity across sites. Subtypes and clades based on previous studies were identified.

Results: The results of phylogenetic analyses indicated that global PRRSV-1 ORF5 sequences were characterized into 3 subtypes including subtype 1 with 10 clades from various countries, subtype 2 from Russia, Lithuania, and Belarus, and subtype 3 from Belarus. All PRRSV-1 ORF5 sequences from ISU VDL were classified in the subtype 1 clade A together with sequences from Europe, Australia, and Asia with an intra-clade genetic distance of 10.1%. PRRSV-1 ORF5 sequences from ISU VDL overall had 82.5-100% nucleotide identity. PRRSV-1 ORF5 sequences in 2004 had 88.7-100% nucleotide identity and genetic variability increased especially during 2010-2018 to roughly 83.7-100%. Even though all PRRSV-1 ORF5 sequences from ISU VDL were grouped only in subtype 1 clade A, the phylogeny indicates that genetic diversity has increased. PRRSV-1 ORF5 sequences from ISU VDL had 83.1-100% nucleotide identity to other U.S. PRRSV-1 ORF5 sequences available in GenBank. Broad genetic diversity (73.7-100% nucleotide identity) was observed when PRRSV-1 ORF5 sequences from ISU VDL were compared to PRRSV-1 sequences from other countries available in GenBank.

Conclusion: This study revealed genetic diversity of PRRSV-1 ORF5 sequences circulating in the U.S. during 2003-2018. A gradual increase in genetic variability of PRRSV-1 sequences observed in this study warrants further monitoring and characterization of PRRSV-1 circulating in the U.S. in future studies.

† Graduate Student Oral Presentation Award Applicant

Application of a Nanopore MinION sequencing workflow in pathogen identification and detection of antimicrobial resistance gene analysis in a veterinary diagnostic setting # †

Grazieli Maboni[1], Rodrigo de Paula Baptista[2], Joy Wireman[2], Anne Summers[2], Suan Sanchez[1]

[1]Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA; [2]University of Georgia, Athens, GA

Current detection of antimicrobial resistance phenotypes is time consuming, delaying delivery of antimicrobial therapy. Oxford Nanopore MinION sequencing is now being adopted to predict antimicrobial resistance. The ability of bacteria to acquire resistance occurs mainly via plasmids. Plasmid DNA is separable from bulk chromosomal DNA, but total cellular DNA extractions may include plasmids. This study developed workflows for acquisition of total and plasmid DNA sequences using MinION to rapidly identify pathogens and antimicrobial resistance genes. We then compared total cellular DNA versus plasmid DNA extractions to assess which method predicts antimicrobial resistance in a reliable and rapid way that is easy to integrate into the workflow of a diagnostic laboratory.

Total cellular DNA and plasmid DNA were extracted from *Acinetobacter junii, Enterobacter cloacae, Escherichia coli Klebsiella pneumoniae* isolated from clinical samples. The DNA extracts were sequenced by MinION using an ultra-rapid barcoding library kit. Gene annotation was performed using Resfinder and the Comprehensive Antibiotic Resistance Database. In addition, we compared the gene annotation data with resistance phenotypes obtained from traditional phenotypic testing.

The total number of reads were sufficient to provide 73x to 290x sequencing coverage depth of all isolates for total DNA sequences, while for plasmids from all isolates the coverage depth was between 66x and 465x. Our analysis revealed that a higher number of resistance genes were obtained from the total cellular DNA than from plasmid sequences. Total DNA sequences from *A. junii* revealed two extra genes that were not present in the plasmids; for *E. cloacae*, three additional genes; *K. pneumoniae* 14 genes; while for *E. coli* 24 additional genes. Comparison with results of phenotypic susceptibility demonstrated that resistance genes detected from both total DNA and plasmids matched all corresponding categories of phenotypic resistance. For instance, resistance gene annotation from *E. coli* demonstrated the presence of *tetA* gene, with the prediction of resistance to doxycycline, *florR* to chloramphenicol, and *mexZ* to cephalexin; the same agreement was found for all isolates. Further work will compare the gene annotation from MinION with Illumina sequencing.

In summary, our sequencing workflows revealed that the total DNA extraction method yielded superior results than plasmid DNA extraction-only. It was also faster and less expensive. The turnaround time from the moment we received the clinical specimen until full identification of bacterial species and resistance genes was less than 2 days, which is less time than traditional phenotypic susceptibility testing. Our workflows can reliably predict antimicrobial resistance and may expedite animal treatment.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

Diagnosis of Bovine Tuberculosis using a defined skin test in Buffaloes in India # * †

Sreenidhi Srinivasan[1], *Maroudam Veerasami*[2], *Douwe Bakker*[1], *Martin Vordermeier*[3], *Vivek Kapur*[1]

[1]Penn State University, State College, PA; [2]Cisgen Biotech Discoveris, Chennai, India; [3]Animal and Plant Health Agency, Surrey, United Kingdom

Bovine tuberculosis (bTB) is a chronic inflammatory disease of zoonotic and economic significance caused by *Mycobacterium bovis*. While the disease is well controlled in most high income countries, it still remains endemic in India and many other low and middle-income countries. India has the largest cattle population in the world (~300 million), approximately one-third of which are buffaloes (*Bubalus bubalis*). In a recently conducted systematic review and meta-analysis of 29,037 buffaloes in India, we estimated the prevalence of bTB in this species to be 4.3% (95% CI: 2.7, 6.7), suggesting that efforts to control this disease in buffaloes are needed. Given the limitations associated with the tuberculin skin test, the primary ante-mortem diagnostic test for bTB, including the inability to differentiate infected and (BCG) vaccinated animals (DIVA), it is increasingly recognized that reliable, easy to produce, fit-for-purpose diagnostic assays with DIVA capability are an essential prerequisite for the implementation of future (vaccination based) control programs alongside conventional test and slaughter approaches. Here, we evaluated the diagnostic utility of a novel cocktail of chemically synthesized overlapping peptides representing *M. bovis* antigens ESAT-6, CFP-10 and Rv3615c for *in vitro* and *in vivo* bTB diagnosis in buffaloes in India. Our results showed that the peptide cocktail induced strong *in vitro* IFN-γ response in whole blood samples collected from naturally infected buffaloes ($n = 8$). Importantly, the peptide cocktail did not induce any measurable response in PBMCs isolated from uninfected buffaloes suggesting a high specificity of the cocktail. The peptide cocktail had a higher sensitivity than the current tuberculin standard in skin tests conducted in naturally infected buffaloes identified in field conditions in India, and it also induced significantly lower skin test induration reactions than PPD-B ($P < 0.0001$), without compromising the sensitivity of the test. Additionally, mapping the T-cell stimulating responses with an IFN-γ assay revealed individual peptides that contributed to the reactivity in naturally infected buffaloes ($n = 11$). Taken together, our studies provide proof-of-principle for the rational design of defined antigens for use in future generations of reliable and cost-effective bTB diagnostics in buffaloes, and provides a foundation for the development of BCG vaccination-based control programs in India and other regions where conventional test and cull strategies are neither feasible nor practicable.

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

Genetic characterization of *Erysipelothrix rhusiopathiae* using Whole Genome Sequencing

Jing Cui, Melanie Prarat, Katherine Shiplett, Dominika Jurkovic, Yan Zhang

Ohio Department of Agriculture, ADDL, Reynoldsburg, OH

Erysipelothrix rhusiopathiae is a Gram-positive, catalase-negative, rod-shaped, non-spore-forming, nonacid-fast, nonmotile bacterium. It is the causative pathogen of swine erysipelas with significant economic loss for the pig industry. *E. rhusiopathiae* also causes polyarthritis in lambs and calves, septicemia in turkeys and ducks, septicemia and urticaria in dolphins, and zoonotic cutaneous lesions in humans known as erysipeloid. Traditionally, strains of *E. rhusiopathiae* are classified into **22** serotypes. DNA-based methods including randomly amplified polymorphic DNA and ribotyping have also been developed for subtyping of the bacterium. Recently, a more sensitive method using PFGE for epidemiological studies of strains of the organism has been utilized. Due to the limited variation among the isolates and lack of resolution of traditional methods, sometimes it is difficult to differentiate strains of *E. rhusiopathiae*. In recent years, whole-genome sequencing (WGS) technology has become a gold standard with high solutions to what were once viewed as insurmountable challenges for genetic analysis of bacterial pathogens. However, the use of this method for subtyping of strains of the genus *Erysipelothrix* has not been reported. We describe herein the first analysis of *Erysipelothrix* strains by WGS. In addition to the subtyping, other information including virulence factors, antimicrobial resistance factors, and molecular fingerprint can be extracted from the WGS data.

Quantitative real-time PCR detection of African Swine Fever virus on a field-based PCR platform, the Biomeme Franklin hand-held thermocycler ◊

Jessie D. Trujillo[1], Russell Ransburgh[1], Anthony Craig[3], Livio Heath[2], Juergen Richt[1]

[1]DMP, Kansas State University, Manhattan, KS; [2]Transboundary Animal Diseases, Agricultural Research Council, Onderstepoort Veterinary Institute, Pretoria, South Africa; [3]Vector and Vector-borne Diseases Research Programme, Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, Pretoria, South Africa

African Swine Fever virus (ASFV) is a major concern for the global swine industry. The expansion of this virus from Africa into Asia and Eastern Europe and its established presence in populations of domestic and wild suids in new regions reinforces the notion that diligent surveillance coupled with rapid and extensive control programs are critical to identify and curtail outbreaks and prevent an endemic state. Our group has evaluated the feasibility of point of need detection of ASFV on a hand-held PCR platform, the Franklin three9 (Biomeme), which is capable of quantitative real-time PCR (qPCR) detection of pathogen nucleic acids. As a real-time PCR machine with nine PCR wells using three fluorophore detection channels, the internal battery-powered Franklin is controlled by a smartphone. Data sets including GPS location can be transmitted to a secure cloud via cellular phones or WiFi.

One limitation to performing real-time PCR in the field is the need to maintain polymerase enzyme function with ready-to-use room temperature stable PCR reagents. Lyophilized PCR master mix (LyoDNA, Biomeme), preloaded in reaction tubes, are available for use with the Franklin. Using DNA from tissue lysates (N=14) and ASFV reference strains (n=7), purified using a validated magnetic bead extraction protocol, we determined the sensitivity for ASFV detection using the USDA p72 qPCR assay (Zsak 2005) on the Franklin PCR machine using LyoDNA. A side by side performance comparison of the same PCR assay run on the CFX 96 (BioRad) using Fast mix II (QuantaBiosciences) was made. Tissue lysates were derived from clinical samples from pigs or arthropod vectors, confirmed or suspected of ASFV infection in the Republic of South Africa. Initial testing of the LyoDNA master mix on the field PCR machine resulted in equivalent sensitivity as the reference assay on the CFX 96 with equivalent or improved PCR Cq.

A syringe-based, silica DNA binding column extraction method with extraction materials preloaded in a closed cartridge (M1 Sample Prep, Biomeme) addresses a critical limitation to field PCR which is the ability to rapidly prepare PCR-quality nucleic acids without the need of special lab equipment or electricity. To test this system, we determined the clinical sensitivity (30 positive and 15 negative samples) of the end-to-end Biomeme platform (M1 Sample Prep, LyoDNA master mix, and the Franklin thermocycler). The diagnostic sensitivity/specificity of the deployable Biomeme platform for detection of ASFV was 100% when compared to the laboratory-based extraction and PCR protocols. Results warrant further investigation of the performance of the Biomeme deployable PCR platform for ASFV detection in the field which could prove to be a useful tool for ASFV preparedness and surveillance.

◊ USAHA Paper

Development of a probe based real-time PCR for the accurate diagnosis of *Avibacterium paragallinaum* §

Rhiannon Barry[1], Ruth Nissly[1], Jen Williams[1], Maurice Byukusenge[1], Traci Pierre[1], Tammy Matthews[1], Eva Wallner-Pendleton[1], Patricia Dunn[1], Barnhart Denise[2], Sean Loughrey[2], Sherrill Davison Yeakel[2], Donna Kelly[2], Deepanker Tewari[3], Bhushan Jayarao[1], Suresh V. Kuchipudi[1]

[1]Veterinary and Biomedical Science, Pennsylvania State University, University Park, PA; [2]Pennsylvania Animal Diagnostic Laboratory, New Bolton Center, University of Pennsylvania, Kenneth Square, PA; [3]PA Department of Agriculture, Pennsylvania Veterinary Laboratory, Harrisburg, PA

Infectious Coryza (IC) is an acute upper respiratory tract infection of chickens caused by the Gram-negative, non-motile bacterium *Avibacterium paragallinarum* (previously called *Haemophilus paragallinarum*). Three serovars of *A. paragallinarum*, namely A, B, and C, have been identified and can be distinguished by hemagglutination inhibition (HI) test using chicken erythrocytes fixed with glutaraldehyde. IC is one of the important infectious diseases that cause significant economic losses to poultry industry globally. Recently, outbreaks of IC have been reported in Pennsylvania in broilers, layer pullets and laying hens, causing significant respiratory disease and production losses. Tentative diagnosis of IC can be made based on the history, clinical signs, and characteristic gross lesions. However, isolation and identification of the organism is required for a definitive diagnosis. Major challenges with the bacteriological diagnosis of *A. paragallinarum* include that the organism is difficult to isolate, slow growing and can only be successfully isolated during the acute stage of infection and secondary bacterial infections are also common. Nucleic acid-based methods are highly useful for sensitive and rapid diagnosis of bacterial diseases. A gel-based PCR assay published in 1996 that targets a 511 bp region spanning two genes of *A. paragallinarum* namely pyrG gene and YihA family ribosome biogenesis GTP-binding Protein gene has been extensively used. There is very limited information on the genome sequences of *A. paragallinarum* isolates currently available in the public databases. We generated Whole Genome Sequences (WGS) of 18 *A.paragallinarum* isolated from recent outbreaks in Pennsylvania (January through April 2019). Based on the sequence analysis of PA isolates and several other sequences in GenBank, we determined that the DNA repair protein gene *recN* is the highly conserved gene across all the three serotypes of *A.paragallinarum*. It is well known that real-time PCR (rtPCR) assays employing amplicon-specific probes are highly sensitive and precise for the rapid and accurate detection of pathogens from clinical samples. Currently no probe based rtPCR assays are available for the diagnosis of *A.paragallinarum*. We developed a Taqman probe based rtPCR assay targeting a highly conserved 99bp region of the *recN* gene of *A.paragallinarum*. Efficiency of the rtPCR assay was 93.13% and we determined that the assay was highly specific by testing against a range of bacterial and viral pathogens known to cause respiratory disease in poultry. To our knowledge this is the first probe based rtPCR assay for the specific and rapid detection of *A.paragallinarum*.

§ AAVLD Laboratory Staff Travel Awardee

AgroDiag PorCoV : a multiplex immunoassay for the differential diagnosis of swine enteric coronaviruses

Rémi Malbec[1], Kay Kimpson-Burkgren[2], Elisa Vandenkoornhuyse[1], Jose Carrillo-Ávila[2], Christophe Audebert[1], Dave Baum[2], Luis Gabriel Gimenez-Lirola[2]

[1]GD Biotech, Lille, France; [2]ISU Veterinary Diagnostic Laboratory, Ames, IA

This study presents AgroDiag PorCoV, a parallel dot ELISA-like immunoassay for simultaneous serodetection and differentiation of the three porcine enteric coronaviruses currently circulating in North America swine herds, *i.e.* porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV) and porcine deltacoronavirus (PDCoV) in a single reaction, saving cost and time. The technology overall format and working principle are similar to those from the solid-phase standard indirect ELISA format. In this platform, multiple pathogen-specific 'probes' (antigens) can be printed in an array of spots at the bottom of 96-well microplate. Specifically, the amino terminal portion of the spike proteins (S1) of PEDV, TGEV and PDCoV were selected as printing antigens because of their high diagnostic sensitivity and specificity, and absence of serologic cross-reactivity due to the low amino acid sequence homology between them. Then, plates were blocked to avoid unspecific reactions during testing procedure. After the three typical incubation steps (sample, conjugate and substrate) the antibody positive reactions were easily visualized as blue dots of intensity directly correlated to the concentration of anti-PEDV, -TGEV, and -PDCoV specific antibodies in clinical serum samples. The diagnostic performance of this multiplex immunoassay was assessed on serum samples (n = 480) of known porcine coronavirus immune status collected from pigs experimentally inoculated with PEDV, PDCoV, TGEV Miller, and TGEV Purdue or culture medium (negative control), which infection was previously demonstrated by PCR (viral shedding) and ELISA (serocoversion). All serum samples were tested in duplicates on antigen spots printed in triplicates for assessment of both intra and inter- and intra-well reproducibility, respectively. This multiplex platform was capable of detecting specific serocoversion between 7-21 days post-inoculation (dpi) through the end of the study (dpi 42) in all infected pigs undergoing seroconversion. The seroconversion was pathogen-specific with 100% diagnostic and absence of serological cross reactivity between enteric coronaviruses. Altogether, the results of this study demonstrate that the AgroDiag PorCoV multiplex immunoassay is an efficient and reliable test for differential detection of porcine coronavirus-related enteric disease with an overall sensitivity and specificity comparable to other antibody-based tests currently available across U.S. veterinary diagnostic laboratories.

Molecular and ELISA assessment of risk of cyanotoxin poisoning by water supplies to swine facilities

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

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

Harmful algal blooms in freshwater are a significant ecological and toxicological concern, and toxic cyanobacteria are the culprit for bloom toxicity. *Microcystis* and *Anabaena* are two cyanobacterial genera that can produce cyanotoxins known as microcystin and anatoxin-a, respectively, which are two potent cyanotoxins detrimental to animal and human health. In this study, we developed rapid and accurate qPCR methods for detecting toxigenic *Microcystis* spp. and *Anabaena* spp. based on their microcystin synthetase C (*mcyC*) and anatoxin-a synthetase G (*anaG*) genes, respectively, and assess their diagnostic performance in comparison to toxin related assays.

The specificity of the methods was validated against 14 reference strains. The qPCR methods were employed to detect two toxigenic cyanobacteria in 105 water samples collected from five lakes and five nearby swine facilities served by the lakes in the US Midwest. Commercial ELISA kits for microcystin and anatoxin-a were used to test the water samples for the toxins. Pearson's *r* and *t*-test were done for linear regression and pairwise comparison, respectively.

Toxin biosynthesis genes (*mcyC* and *anaG*) were detected in 105 (100%) and 102 (97.14%) samples at 0.2 - 2484.4 copies/mL and 0.5 - 8490.0 copies/mL, respectively. Microcystin and anatoxin-a were found in 100 (95.24%) and 96 (91.43%) samples at 0 - 4.38 ppb and 0 - 0.64 ppb, respectively. While a discernible linear correlation was observed between the concentrations of *mcyC* and microcystin ($R^2 = 0.603$), such a relationship was not noticed between *anaG* and anatoxin-a ($R^2 = 0.224$). Furthermore, no significant difference in toxigenic cyanobacteria abundance and cyanotoxin level was found between lake waters and swine facility waters including10 chlorinated samples from one facility ($p > 0.05$).

These findings suggest the stable property of toxigenic cyanobacteria and cyanotoxins during transportation and holding. More importantly, failed decontamination of cyanobacteria and cyanotoxins by traditional chlorination urges more effective approaches for water purification in swine facilities to minimize cyanotoxin-related toxicosis.

AAVLD Trainee Travel Awardee

Development of a novel multiplex real-time PCR for the detection and differentiation of SADS-CoV, an emerging swine coronavirus

Rolf Rauh[1,2], Travis Clement[2], Jane Christopher-Hennings[2], Johnny Callahan[1], Shao-Lun Zhai[3], Diego G. Diel[2]

[1]Tetracore, Rockville, MD; [2]Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD; [3]Institute of Animal Health, Guangdong Academy of Agricultural Sciences, Guangzhou, China

A coronavirus variant known as Swine Acute Diarrhea Syndrome Coronavirus (SADS-CoV) has emerged in China where the first clinical signs in pigs were observed in late December 2016. In 2017, an outbreak in several Guangzhou farms was associated with fatal acute diarrhea in neonatal piglets. The introduction of this new swine enteric coronavirus in the US, could have significant economic consequences to the swine industry. Therefore, rapid detection methods are required for the early diagnosis of this pathogen. Here we report the development of a new real-time PCR assay for SADS-CoV that will be multiplexed with two other swine coronaviruses. The final configuration for the test in development will be PEDV / SADS-CoV / PDCoV / IC.

Currently there are only a few SADS sequences available on Genbank. These sequences were downloaded and imported into the Sequencher 5.4.5 Software. The software was used to assemble the sequences into a contig. The sequences were visually inspected for conserved region. For Corona Viruses mainly the Nucleocapsid (N) and RNA-dependent RNA polymerase (RdRp) gene regions seem to be highly conserved. For the new SADS rtRT-PCR assay, the N region was chosen. An In Vitro Transcript was designed, which included the target sequences for the SADS assay. The process to create the IVT began with designing a syntemp oligo. The oligo includes a T7 promotor which is used by the MEGAscript® T7 Kit to transcribe the In Vitro Transcript (IVT).

The IVT is then tested with the primer, probes and a chosen One-Step RT-PCR kit on the ABI 7500 Real-Time PCR Instrument. The results show the slope of -3.321280 and R2 value of 0.984144 and demonstrate that the chosen design of the rtRT-PCR SADS assay is efficient. The analytical specificity of the SADS-CoV PCR assay was evaluated against a panel of enteric swine corona viruses at SDSU to ensure that the test specifically amplifies SADS-CoV viruses and does not cross-react with closely related non-SADS swine coronaviruses. A commercial set of research reagents to detect swine coronaviruses has been on the market since 2013 from Tetracore. The PEDV-TGEV-SDCoV multiplex PCR has a three-year track record of consistent performance, although TGEV is very seldom seen in the US. Since the practical limit of multiplex real-time PCR is four fluorochromes; it is necessary to replace the TGEV assay with the SADS-CoV assay for a product that is a multiplex rRT-PCR consisting of PEDV, SADS-CoV, PDCoV and an internal positive control. The stand-alone SADS-CoV and multiplex PEDV-SADS-CoV-PDCoV-IC rRT-PCR was tested with collaborative partners, with a collection of swine coronaviruses including SADS-CoV in China.

It is important to the US Pork industry that there be well validated, commercially available tests that can be mass-produced under cGMP conditions and deployed to veterinary diagnostic laboratories for an outbreak contingency should this novel swine coronavirus make its way to the US.

Aggregating results and summarizing findings from multiple veterinary diagnostic laboratories in the US on a near real-time basis # + * †

Giovani Trevisan[1], Leticia Linhares[1], Bret Crim[1], Poonam Dubey[1], Kent J. Schwartz[1], Eric Burrough[1], Rodger Main[1], Paul Sundberg[5], Mary Thurn[4], Paulo T. F. Lages[4], Cesar A. Corzo[4], Jerry Torrison[4], Rob McGaughey[3], Jamie Henningson[3], Eric Herrman[3], Gregg Hanzlicek[3], Ram Raghavan[3], Douglas Marthaler[3], Shivali Gupta[2], Jon Greseth[2], Travis Clement[2], Jane Christopher-Hennings[2], Daniel C. L. Linhares[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]South Dakota State University, Brookings, SD; [3]Kansas State University, Manhattan, KS; [4]University of Minnesota, Saint Paul, MN; [5]Swine Health Information Center, Ames, IA

VDLs have independently, inconsistently and intermittently reported information regarding frequency of detection of swine pathogens and diseases, usually summarized in different formats including peer-reviewed publications, professional organization presentations and proceedings, lay-journal communications, and VDL summaries. There is no single resource in the US whereby the results of routine diagnostic testing from different VDLs are consistently gathered and reported in a standardized format. The purpose of this project was to develop a user-friendly informatics tool to summarize and report routine pathogen detection from samples submitted to participating VDLs over time.

Standardized submission data and results of tests performed on porcine samples were retrieved from 4 participating VDLs(ISU, UMN, SDSU, and KSU). Historical and anonymized data was retrieved from the VDL LIMS, shared in a CSV format, manipulated using SAS 9.4 scripts, and final results were uploaded in Power BI® for data visualization. The dataset was filtered by suppressing results from cases submitted for research and testing for animal/product export. Starting in 8/1/18 results from ISU and UMN were shared in near real-time using the Health Level Seven International (HL7) format. This first analysis utilized the results of PCR testing for porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus(PEDV), porcine deltacoronavirus (PDCoV), transmissible gastroenteritis virus (TGEV), and *M. hyopneumoniae* (MHP). This informatics tool is now known as the Swine Disease Reporting System-SDRS.

Until 5/1/19, data from 792,898 distinct cases were retrieved and analyzed. Clear cyclic seasonal trends for the frequency of detection of PRRSV, PEDV, PDCoV, and MHP were reported. Winter and spring months had the highest detection and summer or fall the lowest for PRRSV, PEDV, and PDCoV. MHP had the highest detection during fall and lowest during spring months. TGEV detection moved to near-zero after PEDV and PDCoV introduction in the US in 2013/2014. The data visualization dashboards and the analytical tools can be accessed online at https://fieldepi.research.cvm.iastate.edu/swine-disease-reporting-system/, with ability to apply dynamic filters allowing to display the information over time, age category, specimen, tissue presence, and site state. Monthly reports with the most relevant findings are published at SHIC webpage https://www.swinehealth.org/domestic-disease-surveillance-reports/ under the domestic disease surveillance reports.

A bio-informatics tool, SDRS, was developed to inform the US swine industry on key macro-epidemiological aspects of agent detection, with the capability to be updated in real-time, and is easily adaptable for other analytes relevant to stakeholders. Additionally, in this project the HL7 messaging system was used for a 9 months period, proving the concept that this messaging system can be effectively used among the VDL community.

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

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Pathology 1

Saturday, October 26, 2019
Room 557

Moderators: Grant N. Burcham and Maria Spinato

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

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Cytologic morphology of subcutaneous *Mycoleptodiscus indicus* infection in an immunocompetent cat +

Paula M. Krimer[1,2], Grazieli Maboni[1], Rodrigo de Paula Baptista[4,5], Ana Lorton[1], Christina Anderson[6], Suan Sanchez[1,3]

[1]Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA; [2]Pathology, University of Georgia, Athens, GA; [3]Infectious Diseases, University of Georgia, Athens, GA; [4]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA; [5]Institute of Bioinformatics, University of Georgia, Athens, GA; [6]Animal Hospital of East Cobb, Marietta, GA

Fungal infections in cats are relatively uncommon. Several fungal veterinary pathogens have distinct cytologic morphology that permits a presumptive diagnosis including blastomycosis, sporotrichosis, histoplasmosis, cryptococcosis, and coccidiomycosis. A fine needle aspirate from a subcutaneous front leg swelling in an 8-year-old immunocompetent cat had unique monotypic intracellular and extracellular fungal organism in a background of granulomatous to pyogranulomatous inflammation and multinucleated giant cells. Most organisms were non-pigmented septate fungi with irregular walls, short irregularly sized septa, and distinct non-staining wall. Round and larger shapes resembling yeast were also present, from which longer hyphae would often arise in a hub-and-spoke pattern. Intracytoplasmic organisms appeared ghost-like without internal features. Fungal culture, DNA sequencing, and phylogenetic analysis determined the organism to be *Mycoleptodiscus indicus*, a pigmented hyphomycete. Pigmentation was not apparent on cytology but was seen with *in vitro* culture. *M. indicus* is a plant pathogen rarely reported in animals, and often in immunocompromised hosts. Its cytologic features have not been published. It differs in appearance from other known distinct cytologic pathogens and other opportunistic fungi. As with other case reports of this opportunistic infection, antifungal treatment completely resolved the patient's lesion. Veterinary cytologists should familiarize themselves with the unique appearance and type of inflammation associated with this organism, especially as there is the potential for a positive clinical outcome with accurate identification.

+ AAVLD/ACVP Pathology Award Applicant

Chronic wasting disease in Mississippi white-tailed deer

Heidi Huffman Rose[1], Brittany S. Baughman[2], Alejandro Banda[1], William T. McKinley[3], Tina Hay Eichelberger[1], Rebecca Mackey[1]

[1]MS Veterinary Research and Diagnostic Lab, Pearl, MS; [2]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; [3]Mississippi Department of Wildlife, Fisheries, and Parks, Jackson, MS

In January 2018, an adult, male, free-ranging white-tailed deer in Issaquena county, Mississippi (MS) was observed over a three-day period to be ataxic and died. The deer was submitted by Mississippi Department of Wildlife, Fisheries and Parks (MDWFP) to MS Veterinary Research and Diagnostic Lab (MVRDL) for routine necropsy. The deer was in emaciated body condition. Fibrinosuppurative bronchopneumonia was identified grossly and histologically, and *Pasteurella multocida* was cultured from the lung. In sections of cerebral cortex and brainstem, the neuropil contained scattered, variably sized, clear vacuoles; focally, neurons were distorted by closely associated vacuoles. Per routine procedure, formalin-fixed obex and retropharyngeal lymph node were submitted to the National Veterinary Services Laboratories in Ames, Iowa, for CWD immunohistochemistry. Both tissues were IHC positive for CWD.

After this first confirmed positive case of CWD in Mississippi, MDWFP initiated the CWD Response Plan, which included identification of a management zone and testing of additional deer within this region. Extensive surveillance within the management zone between February and July 2018 did not yield additional positive cases. During the 2018-2019 hunting season, collection procedures were implemented for surveillance of hunter-harvested deer, and sampling by MDWFP continued.

Because of the increased requirement for CWD testing in Mississippi, MVRDL implemented CWD ELISA testing. NVSL protocols for testing are utilized, including specified equipment and supplies. Six laboratory technicians have been trained in the procedure, and two technicians completed NVSL proficiency testing. Routine testing at MVRDL began in October 2018. Between October 2018 and March 2019, 7,542 samples were tested. Most samples submitted by MDWFP were fresh retropharyngeal lymph node. A small number of formalin-fixed samples collected by MDWFP were submitted directly to NVSL for IHC (two positive cases were submitted in formalin). Per NVSL guidelines, all cases identified as initial reactors by ELISA were retested in duplicate, and samples that again reacted by ELISA were reported as “suspect positive” and forwarded to NVSL for confirmatory IHC. Sixteen CWD suspect positives by ELISA performed at MVRDL were confirmed by IHC at NVSL.

Since January 2018, a total of 19 CWD positive deer have been identified in Mississippi. Affected counties included Benton (7), Marshall (7), Issaquena (2), Panola (1), Pontotoc (1), and Tallahatchie (1).

Histopathological diagnosis of selected Equine and Canine viral diseases by *in situ* hybridization of Formalin-fixed paraffin-embedded tissues

Gerald E. Duhamel[1,2], Andrew D. Miller[1], Randall Renshaw[3], Edward J. Dubovi[3]

[1]Biomedical Sciences, Cornell University, Ithaca, NY; [2]Anatomic Pathology, Animal Health Diagnostic Center, Ithaca, NY; [3]Virology, Animal Health Diagnostic Center, Ithaca, NY

For the past several decades, traditional anatomic pathology diagnosis of infectious diseases has relied on hematoxylin and eosin-stained formalin-fixed paraffin-embedded (FFPE) tissue sections to identify lesions followed by confirmation with immunohistochemistry sometimes coupled with ancillary testing. The availability of curated public genomic sequence databases of veterinary infectious disease agents combined with recent technical advances in diagnostic *in situ* hybridization (ISH) methodology have provided a novel paradigm for the confirmation of infectious disease agents in archival and routine FFPE tissue specimens. This novel ISH methodology (RNAscope; Advanced Cell Diagnostics, Hayward, CA) relies on *in silico* design of small target-specific proprietary oligo ZZ pair probes that can hybridize to target regions ranging from 300 to 1,000 bases followed by conventional colorimetric detection. Here we describe the application of this ISH assay in detection of select equine and canine viral disease agents where immunoassay reagents are not currently available including : (i) bovine papilloma virus (BPV) 1 and 2 (E5-E6-E7 gene target) in horses with equine sarcoid and cattle with bovine fibropapilloma skin infection (Gaynor et al., Vet Pathol 53:567-573, 2016), (ii) equine gammaherpesvirus (EHV) 5 (gB gene target) in horses with equine multinodular pulmonary fibrosis and EHV2 (gB gene target) in horses with gastric ulcer (Pennington et al., Virus Research 242:30-36, 2017), (iii) canine alpha herpesvirus (CHV) 1 (thymidine kinase gene target) in dogs with meningoencephalitis (Jager et al., Vet Pathol 54:820–827, 2017), and (iv) canine vesivirus (CaVV; putative VPg, p30/peptidase C24, 2C endopeptidase cysteine protease family/non-structural polyprotein of peptidaseC37 and beginning of capsid leader gene targets) in dogs with systemic vasculopathy. The specificity of each ISH assay was validated by comparing ISH results with respective PCR assay positive and negative FFPE tissues (BPV1/2; EHV2/5; CHV1; CaVV), and when available, control un-infected and virus-infected cell cultures (EHV2/5 and CaVV). We found that these ISH assay provided a highly-reproducible and robust signal that localized virus-infected cells without non-specific background and reporting of diagnostic results overnight. Incorporating reference positive and negative control tissue sections on the same slide as the test sample allowed for quality assurance/quality control of individual test, while controlling costs. The combined experience of pathologists and technical assistance across different laboratories provides an alternative approach for correlation of histopathological findings with etiological diagnosis of either common, newly-emerging, or poorly understood equine and canine viral diseases.

Cervical pole necrosis of the equine allantochorion

Alan T. Loynachan

University of Kentucky, Lexington, KY

Cervical pole necrosis (CPN) is a poorly documented and infrequent cause of non-infectious abortion. The pathogenesis of this unique lesion is not definitively known, but it has been theorized to occur 1) secondary to placental detachment at the cervical star or 2) by ischemic insult related to hemodynamic abnormalities associated with long umbilical cords and vascular thrombosis. The UKVDL database was searched for cases of CPN to better characterize this distinctive entity.

Twenty-nine cases of CPN were diagnosed from 2013 to February of 2019. Thoroughbreds were overrepresented (27 cases). Clinical histories ranged from 1) no signs of impending abortion or no history, 15 cases; 2) placentitis, 7 cases; 3) vaginal discharge, 5 cases; 4) premature udder development, 4 cases; 5) placental separation, 2 cases; or 6) identification of a fluid filled fetal abdomen, 1 case. CPN resulted in abortion (21 cases), premature birth with subsequent neonatal death or euthanasia (3 cases), birth of a viable term foal (3 cases), and premature birth of a weak viable foal (1 case).

Gross necropsy findings consisted of well demarcated, paper thin regions of tan to green placental necrosis that were associated with the cervical pole and were located in close proximity to the cervical star. Necrotic regions were bordered by a red and raised border that was distinctly evident on the allantoic surface. In addition to the cervical pole lesion, single cases also exhibited similar lesions in the gravid and non-gravid allantochorionic horns, respectively. Necrotic regions averaged 245 cm^2 in size and ranged from 16-750 cm^2. Umbilical cord lengths averaged 98 cm (range 58-146 cm), and placental weights averaged 6.7 kg (range 3-12.7 kg). Fetuses were estimated to range from 210 days of gestation to term (average= 275), averaged 83 cm (range 52-109) from crown to rump, and weighed 25.9 kg (range 10-69.1 kg) on average.

Histopathology findings were similar in all cases and consisted of a locally extensive and transmural region of coagulative necrosis mixed with deposits of mineral. Associated stromal blood vessels variably contained mineral and fibrin thrombi. Necrotic regions were bordered by a moderate proliferation of fibrous to fibrovascular tissue mixed with small amounts of hemorrhage and scattered individual inflammatory cells. Microcotyledons in the adjacent chorion were frequently atrophied or necrotic, and there was mild allantoic epithelial hyperplasia in occasional cases. Bacteria were associated with the necrotic region in multiple cases.

Additional diagnoses in fetoplacental units with CPN included: fetal septicemia without significant inflammation (6 cases), bacterial placentitis (3 cases), various fetal malformations (3 cases), bacterial placentitis with fetal septicemia (1 case), fetal bacterial pneumonia (1 case), *in utero* meconium passage with aspiration (1 case), and premature placental separation (1 case).

Multiple cases of chondrodysplasia in beef calves in Iowa

Drew Magstadt, Scott Louis Radke, Vickie Cooper, Kent J. Schwartz

VDPAM, Iowa State University, Ames, IA

Over a 3-week period in the spring of 2019, four cow-calf beef farms in Iowa submitted calves with suspected dwarfism to the Iowa State University Veterinary Diagnostic Laboratory. An additional four farms reported cases of dwarfism around this same time via phone conversations. Most farms reported multiple affected calves and that did not share common genetic backgrounds. Dams from most affected farms were fed a diet consisting of a large percentage of corn silage for a significant portion of gestation, and nearly all farms reported affected calves from heifers while no affected calves were observed from mature cows.

Diagnostic investigation of these cases confirmed gross shortening of the long bones of the limbs, enlarged joints with widened epiphyses, and variable joint laxity. Superior brachygnathia was rarely observed. Histopathology of the physes from affected bones demonstrated mild to moderate widening of the physeal cartilage with moderate to severe abnormalities in cartilage maturation compared to age-matched controls. No lesions were observed in the evaluated soft tissues from these cases, and testing for BVDV was negative in all calves. Manganese content of liver from affected calves was consistently well below the normal reference range. Manganese deficiency associated with inadequate maternal nutrition was suspected as a causal factor involved in the development of these changes.

Published reports on congenital chondrodystrophy in beef calves have suggested various causes, but most investigations have implicated some deficiency in maternal nutrition.[1] Several reports have been associated with drought and poor quality nutrition during mid-to-late gestation. Others have reported diets with high amounts of corn silage or have implicated deficiency in manganese.[1] Experimentally, feeding a diet low in manganese resulted in an increase in calves affected with chondrodystrophy.[2] Potential genetic causes were considered but deemed unlikely due given the genetic background of affected calves and previous matings in these herds. *In utero* infection with a viral agent that is either unknown or undetectable at the time of birth remains a possibility. Questionnaires and verbal surveys of attending veterinarians and affected producers are in progress with an aim of finding commonalities in feed components, genetics, and other potential risk factors. The results of these surveys will be included in the presentation.

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Comparison of urine cytokines from Dogs with urinary carcinoma and UTI

Stephen Cole

UPenn, Philadelphia, PA

Urinary tract carcinoma (UTC) accounts for approximately 2% of all reported malignancies in dogs. The clinical signs of UTC are clinically indistinguishable to that of urinary tract infection (UTI) including stranguria, dysuria and hematuria. Previous studies have shown distinct urinary cytokine profiles of equivalent diseases in people that may be of diagnostic value. The aim of this preliminary study was to identify urine cytokine markers that differed between dogs with UTC and UTI. Urine from 37 dogs (9 dogs with UTC, 14 with UTI, 4 with both and 10 free of either) was analyzed using a customized canine cytokine panel (Millipore Sigma) on the Luminex 100 for Ifn-g, IL-6, IL-8, KC-like, Il-10 and MCP-1. Samples were diluted (1:8) to limit matrix effect on the assay and results standardized as a urine cytokine to urine creatinine ratio. A Wilcoxon-rank sum test was used to evaluate if a difference between cytokine levels that existed within the tested clinical populations. Patients diagnosed with UTC had significantly different levels of IL-10 (p=0.0078) and MCP-1 (p=0.0036) compared to patients without carcinoma. Patients diagnosed with UTI had significantly different levels of IL-8 (p=0.039) and KC-like (p=0.0343) compared to those without. This study showed that the urine of canine patients with UC and UTI may have distinct profiles that vary by disease. This may be of diagnostic utility for UTC, particularly if these differences exist early in disease when intervention may be more successful. Future directions include validation in a diagnostic setting including establishing normal levels in healthy animals and monitoring levels during progression of disease and therapy.

Characterization of an RNA ISH probe for the diagnosis of feline infectious peritonitis virus and comparison with the current gold standard §

Nicole M. Andre[1], Arjun Sweet[1], Gary R. Whittaker[1], Andrew D. Miller[2]

[1]Department of Microbiology & Immunology, Cornell University College of Veterinary Medicine, Ithaca, NY;
[2]Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY

Feline infectious peritonitis virus (FIPV) is an important cause of morbidity and mortality in both domestic and wild cats. There are two serotypes of FIPV; type 1 viruses infect 85 - 95 % of the feline population and type 2 viruses infects 5 - 15 % of the feline population. The "gold standard" of diagnosis is the demonstration of FIPV antigen in formalin fixed tissue via immunohistochemistry (IHC); however, data suggests that there are variations in the sensitivity of the FIPV IHC antibody between different laboratories. In situ hybridization (ISH) is a potentially more sensitive method for identifying RNA within tissue and has been increasing used in infectious disease diagnostics. In this study, a comparison of RNA ISH staining for FIPV was performed with probes designed against either type 1 or type 2 viruses. The sensitivity of the two RNA ISH probes was compared to the monoclonal antibody FIP 3-70. IHC using FIP 3-70 and ISH using type 1 and type 2 probes was performed on 30 Formalin fixed paraffin embedded (FFPE) tissues from FIPV infected cats and their staining of similar lesions was described and characterized, and a semi quantitative score determined where 0 – (no staining or <1 dot/ 10 cells), 1 – (1 - 3 dots/cell), 2 – (4 - 9 dots/cell and none or very few dot clusters) 3 – (10 – 15 dots/cell and/or <10% dots in clusters), 4 – (>15 dots/cell and/or >10% dots in clusters). The type 2 probe was found to be the most sensitive compared to either IHC or the type 1 probe. Based on the data presented herein, ISH has increased sensitivity for the identification of FIPV in feline tissues compared to IHC.

§ AAVLD Laboratory Staff Travel Awardee

Pathological findings associated with Eastern equine encephalitis virus and West Nile virus infections in wild ruffed grouse (*Bonasa umbellus*) from Wisconsin in 2018-2019

Elizabeth J. Elsmo[1], Phil Bochsler[1], Lorelei Lynne Clarke[1], Kathleen Deering[1], Ailam L. Lim[1], Jaimie Miller[1], Nancy Businga[2], Lindsey Long[2]

[1]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI; [2]Wildlife Health, Wisconsin Department of Natural Resources, Madison, WI

During the summer and fall of 2018 and spring and winter of 2019, a total of 32 wild ruffed grouse (*Bonasa umbellus*) from Wisconsin were tested for Eastern equine encephalitis (EEEV) virus and West Nile virus (WNV) by real-time PCR. One or both arboviruses were detected in tissues from 28% (8/32) of ruffed grouse. Histopathological findings of lymphoplasmacytic meningoencephalitis, myocarditis, and interstitial nephritis were only identified in four infected birds, and only during the summer and fall of 2018. Three of the four birds with histological evidence of arboviral infection tested positive for EEEV only, one tested positive for both viruses. A historical serosurvey found that 50% of ruffed grouse in Wisconsin had detectable antibodies to EEEV, indicating that this viral infection can be successfully cleared by infected ruffed grouse. However, these cases are the first evidence that EEEV infections can cause disease and potentially mortality in wild ruffed grouse.

Pathology 2

Sunday, October 27, 2019
Room 556

Moderators: Kelli Almes and Jonathan P. Samuelson

Symbols at the end of titles indicate the following designations:

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

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Canid predators kill bites and the injuries to their animal victims

Nanny Wenzlow

Pathology and Microbiology, Université de Montréal, St Hyacinthe, QC, Canada

Multiple deceased dogs and cats were found in different geographic locations. Suspicious presentation of the bodies let law enforcement initiate a death investigation.

The cat victims were found either as half bodies or with large abdominal openings and most internal organs absent. The bodies, as well as the surrounding recovery site, lacked obvious bloodstains and led investigators conclude that: a) the site of the deadly incident had been elsewhere, b) the perpetrator was likely a human, and c) the use of large tools had caused the sustained injuries. The dog victims were found without obvious external wounds and bloodstains, in either suspicious locations or after a canine police unite had raided a property with multiple other dogs.

All animal victims were submitted for forensic necropsy. The skin wounds of the cat bodies were free of bloodstains, and were lacerated with irregular, mildly hemorrhagic edges. The adjacent fur was arranged in wet streaks that had partially dried. Foreign fur was adherent to the hind claws of one cat. Dog and cat bodies showed in most cases the typical deep tissue crushing wounds in the ventral neck area with or without associated skin perforations. These lesions are typical for kill bites by canid predators.

Asphyxiation due to these kill bites was the mechanism of death in most cases. Coyote DNA was isolated from the skin wounds of two cases. Human interference was excluded.

Virulent Newcastle disease in Southern California, 2018-2019

Francisco A. Uzal, Francisco R. Carvallo, Janet Moore, Akinyi Nyaoke, Beate Crossley

UCDavis, San Bernadino, CA

Virulent Newcastle disease (vND) is a contagious and almost always fatal viral disease affecting the respiratory, nervous and digestive systems of poultry and other birds, caused by highly virulent strains of avian paramyxovirus type 1. Clinical signs include sudden death, increased mortality in the flock, depression, respiratory difficulty, nasal discharge, diarrhea, neurological disturbances, and/or swelling around the eyes and neck. A death rate of almost 100 percent can occur in unvaccinated poultry flocks, but vND can also infect and cause death in vaccinated poultry. A diagnosis of vND in California was confirmed on May 17 2018. Since then, and as of May 20, 2019 the United States Department of Agriculture (USDA) has confirmed 440 premises in California as infected with vND, including 139 in San Bernardino County, 256 in Riverside County, 44 in Los Angeles County, 1 in Ventura County, and 1 in Alameda County. Most of these cases occurred in backyard exhibition birds, but since December 2018, several commercial flocks in California were found to infected via routine PCR surveillance. USDA also confirmed 1 infected premises in Utah County, Utah and 1 infected premises in Coconino County, Arizona. During the current outbreak, all field cases are being tested by CAHFS with real-time PCR on oropharyngeal and/or cloacal swabs, according to the National Animal Health Laboratory Network protocols, to establish a presumptive diagnosis. This diagnosis is later confirmed by the USDA's Animal and Plant Health Inspection Service. Necropsies are performed in some cases, such as when urgent preliminary results are required. Gross lesions are considered compatible or not compatible with vND. Gross lesions identified so far in backyard birds submitted for necropsy, which later tested positive for vND by PCR, included i) conjunctival, tracheal, proventricular, intestinal and cloacal hemorrhages, ii) fibrino-necrotizing stomatitis, pharyngitis, esophagitis and laryngotracheitis, and/or iii) cecal tonsil necrosis. Occasionally, no gross lesions were observed in a few cases with positive PCR results. Lesions observed in commercial chickens were much more subtle or completely absent. Gross pathology remains a critical tool for early recognition of vND as demonstrated in the current outbreak.

Diagnostic histopathological features of small intestinal lymphatic hypoplasia in Dogs with protein-losing enteropathy

Gerald E. Duhamel[1,4], *Adam J. Miller*[2], *Tristan Weinkle*[3]

[1]Biomedical Sciences, Cornell University, Ithaca, NY; [2]Cornell University Hospital for Animals, Cornell University, Ithaca, NY; [3]South Carolina Veterinary Specialists & Emergency Care, Columbia, SC; [4]Anatomic Pathology, Animal Health Diagnostic Center, Ithaca, NY

Intestinal lymphatic hypoplasia (ILH) is a rare cause of protein-losing enteropathy (PLE) in human infants. We recently reported the clinicopathological findings in 3 dogs with ILH and PLE. Here we report on the clinical and intestinal histopathological findings in 3 additional dogs with ILH and PLE. In our original report, the onset of PLE was early in an 18-month-old Great Pyrenees, while the other 2 dogs, a Pug and a Tibetan terrier had late onset at 4- and 12-years of age, respectively. The onset of PLE in our new cases was 19-months in an Australian Shepherd and a Labrador Retriever, while the third dog was a 2-year-old mixed breed. In all cases, ILH was confirmed by immunohistochemical (IHC) staining with prox-1 antibody, a lymphatic endothelial nuclear transcription factor. Because small intestinal subepithelial myofibroblasts play critical regulatory functions in intestinal villous morphogenesis, remodeling, epithelial cell development, polarity and function, the small intestinal lamina propria myofibroblastic system of all dogs with ILH was assessed by IHC staining with anti-alpha-smooth muscle actin antibody. Consistent with our previous report, the small intestine of all dogs with ILH has prominent mucosal edema with dilated and tortuous villous capillaries, club-shaped villi outlined by low cuboidal epithelium in the upper 1/3, scattered villous tip extrusion zone epithelial inversion, and mild mixed inflammatory cell infiltrate. Inconsistent findings in dogs with ILH also included villous epithelial cell cytoplasmic microvacuolar changes in 3 dogs and increased numbers of intraepithelial lymphocytes in 1 dog. In all dogs, the villous smooth muscles had relatively normal bundles at the base that progressively became disorganized into thin wispy strands that formed tangles at the tip of villi around variably sized, often large clear spaces within the upper 2/3 of intestinal villi. When compared with control dogs, dogs with ILH had more prominent subepithelial myofibroblasts that arborized from smooth muscle bundles. Although dogs with ILH have obvious mucosal lymphedema and a lack of lacteals by prox-1 IHC staining, additional changes within the lamina propria myofibroblastic system add to the diagnostic confidence of ILH. While ILH is an uncommon diagnosis, it should be considered as a differential particularly in young dogs presented with PLE.

Small ruminant abortion cases at the Ontario Animal Health Laboratory: Shifting patterns, novel and re-emerging pathogens

Maria Spinato, Margaret Stalker, Murray Hazlett, Josepha DeLay, Jan Shapiro, Andrew Brooks, Emily Brouwer, Amanda Mansz, Hein Snyman, Hugh Cai, Durda Slavic

Animal Health Laboratory, University of Guelph, Guelph, ON, Canada

The Animal Health Laboratory at the University of Guelph has implemented a standardized testing protocol for small ruminant abortion submissions, based upon the results of a previously reported study. This protocol includes postmortem, histopathology, routine bacterial culture, specialized culture for *Campylobacter* spp., and quantitative RT-PCR for *Coxiella burnetii* and *Chlamydia abortus.* IHC or PCR for *Toxoplasma gondii* is conducted if warranted by histologic lesions.

Diagnostic patterns at the AHL have shifted over the past 5 years, likely related to factors such as the development of new vaccines, improved treatment protocols and management practices. Several novel and re-emerging pathogens have also been identified over the past 5 years. During the last week of December 2015 and the first 2 weeks of January 2016, the AHL received several submissions of deformed stillborn and aborted ovine fetuses for postmortem examination. Gross examination of these fetuses revealed flexural limb deformities, kyphoscoliosis, torticollis, and hypoplastic musculature. Most lambs had varying degrees of brain malformation ranging from cerebellar hypoplasia to hydranencephaly. Positive fetal and maternal antibody titres for Cache Valley virus ranged from 1:64 to 1:512, confirming CVV as the cause of fetal abnormalities in this outbreak.

More recently, the AHL diagnosed an unusual case of ovine abortion due to *Neospora caninum*, confirmed by IHC. The affected lambs had more extensive foci of non-suppurative myositis and meningoencephalitis containing intralesional protozoal cysts than typically observed in cases of *Toxoplasma* abortion (IHC and PCR negative). Another flock experienced multiple mid- and late-term abortions, and samples were submitted for diagnostic evaluation. Extensive suppurative placentitis, mild vasculitis and thrombosis were noted in placentas. Bacterial cultures and PCR tests were negative for commonly-diagnosed pathogens. Reports in the literature of sporadic cases of ovine abortion due to *Flexispira rappini* in New Zealand and the United States instigated PCR testing of placenta using genus-specific *Helicobacter* primers. The obtained PCR product was sequenced and sequence results (100% coverage and 99.4% identity) revealed the presence of *Helicobacter trogontum*, previously known as *Helicobacter* (*Flexispira*) *rappini*.

Despite the increased diagnostic success fostered by molecular techniques that can now identify a broad range of infectious etiologies, the proportion of idiopathic abortion cases in small ruminants at our laboratory remains relatively high at 30-35%. However, nutritional and management factors also have a significant influence on the rates of abortion in these species.

Semilunar valve malformations in two Quarterhorses

Mayane Faccin[2], Beth Graham-Williams[3], Esther Crouch[1], W. Shane Sills[1], Kathleen Mullen[3], Kathleen Kelly[1]

[1]Biomedical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY; [2]Animal Health Diagnostic Center, Ithaca, NY; [3]Littleton Equine Hospital, Littleton, CO

Bicuspid aortic valve (BAV) is a common congenital heart defect in humans; comparatively, malformations of the semilunar valve leaflets are relatively rare in domestic animals with most reports in Syrian hamsters and murine models and few reports in dogs and pigs. Bicuspid pulmonic valve is considerably less frequent compared to human BAV. The clinical features and cardiac pathology of two Quarterhorses with semilunar valve malformations were examined: a 5-year-old mare with BAV and a 2-year-old mare with bicuspid pulmonic valve. The horse with BAV was euthanized due to weight loss and severe aortic insufficiency, grade VI/VI continuous murmur with the point of maximum intensity over the left heart base, and palpable thrill. Necropsy revealed BAV with a raphe between the left and right coronary sinuses. In humans, BAV are classified according to one main category (Type 0-2) based on the presence and position of raphe(s) and two subcategories based on spatial position relative to coronary vessels (L, left coronary sinus; R, right coronary sinus; N, non-coronary sinus) and valvular function (I, insufficiency; S, stenosis). According to this system, the horse had BAV Type 1, L-R, I, a frequent malformation in humans. Secondary cardiac findings included severe dilation of the aortic arch, left ventricle, and left atrium. The horse with bicuspid pulmonic valve was euthanized due to cervical vertebral stenosis and ataxia; incidentally, at necropsy, the pulmonic valve leaflets were non-equal with elongated irregular right and left leaflets and a rudimentary dysplastic intermediate leaflet having a free margin diffusely tethered to the pulmonary artery intima. There was also dilation of the main pulmonary artery. These equine cases resemble the clinical features in humans with semilunar valve malformations: patients with BAV develop aortic stenosis, insufficiency, or combined stenosis and insufficiency which necessitate surgical valve replacement and possibly aortic repair. In contrast, bicuspid pulmonic valve is often incidental and is an infrequent incidental autopsy finding. The incidence of equine semilunar valve malformations is unknown but possibly under-reported.

Cause of death and pathology in search-and-rescue Dogs deployed to Sept. 11, 2001 terrorist attack sites

Scott D. Fitzgerald[1]*, Cynthia M. Otto*[2]*, Kathleen M. Kelsey*[2]*, Elizabeth Hare*[2]*, John Philip Buchweitz*[1]

[1]Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI; [2]Penn Vet Working Dog Center, University of Pennsylvania, Philadelphia, PA

A fifteen year surveillance study was conducted on the Search-and-Rescue dogs utilized at the Sept. 11, 2001, terrorist attack sites which used both deployed and non-deployed control dogs. These dogs were evaluated for cause of death, organ system pathology, neoplasms, toxicologic screening, and longevity. Cause of death was not significantly impacted by deployment status. Most frequent causes of death include degenerative conditions (35%), neoplasms (32%), and undetermined (21%); while less common causes of death included inflammatory disease (8%), and traumatic (4%). The organ systems most frequently affected were neurologic (19%), cardiovascular (16%), and urogenital (14%); while lesser affected systems included gastrointestinal (11%), musculoskeletal (8%), respiratory (5%), lymphoid (4%) and hematopoietic (2%). There was no increased incidence of neoplasia in deployed dogs (30.5%) compared to non-deployed dogs (32.7%). Neoplasms detected included cardiovascular (9), primarily hemangiosarcomas; lymphatic (6), primarily lymphosarcomas; and respiratory (1), a pulmonary carcinoma. No significant toxicologic agents were found in any deployed dogs based on screens for PCBs, organic compounds by GC/MS screen, or heavy metals by ICP. Finally, longevity of the deployed dogs was virtually the same as for control dogs. The conclusion being that in spite of deployment and exposure to many different potential toxins, dogs' lifespans were not significantly affected by their experience.

One of the most interesting conclusions from this study was that Search-and-Rescue dogs are able to tolerate exposure to a dangerous environment filled with many airborne dusts, gases and toxins with little or no long term effects, in spite of having no protective respirators. In contrast, many human first responders with respirator protection developed a variety of chronic coughs and decreased pulmonary capacity. Interestingly, there was a significant amount of inhaled carbon (anthracosis) and refractile foreign particulate matter within many of the deployed dogs' lungs, in spite of an absence of significant pulmonary inflammation or neoplasms. These are valuable findings which should allow the continued use of Search-and-Rescue dogs at both natural and man-made disaster sites in the future.

Eastern Equine Encephalitis Virus: recent avian cases diagnosed in New England + †

Emily Reinhardt, Neha Mishra

Pathobiology, CVMDL/ University of Connecticut, Storrs, CT

Eastern equine encephalitis virus (EEEV), a regional and seasonal Arboviral disease, causes severe neurological disease in equids and humans. While previously considered a sporadically occurring disease in New England, over the past decade, patterns of seasonal occurrence and an expanding geographical distribution have been described, bringing renewed attention to this zoonosis. Recent work on the sylvatic enzootic lifecycle of this virus suggests that the transmission between ornithophilic mosquito vectors (*Culiseta melanura*) and their passerine hosts plays a key role in viral amplification and maintenance. *Culiseta melanura* preferentially feeds on avian hosts with a potential seasonally diverse host specificity, which may dictate the recent changes in prevalence and distribution of EEEV. Due to their importance in viral transmission and the presence of silent carriers, avian species have been proposed as a sentinel for equine and human cases, in which the virus carries a comparatively high mortality rate. Aberrant hosts are effected when bridging vectors, non-ornithophilic mosquitoes (*Aedes vexans, Coquillettidia perturbans, & Ochlerotatus canadensis*, etc), transmit the virus from the amplifying avian host to other avian or mammalian species. Reported aberrant hosts include horses, swine, cattle, new-world camelids, dogs, pheasants, ostriches, emus, turkeys, and whooping cranes. In the summer of 2019, the Connecticut Veterinary Medical Diagnostic Laboratory received two avian cases; one wild Turkey (*Meleagris gallopavo*) from Massachusetts and another a Bald Eagle (*Haliaeetus leucocephalus*) from Connecticut which were diagnosed positive for EEEV. In this presentation we describe EEEV as was seen in aberrant avian host species across two New England states. In particular, we will discuss the recent developments in the life cycle and transmission of this virus, the prevalence and surveillance in New England, and the pathology, diagnosis, and differential diagnosis of this virus.

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

Pulmonary hypertension and copper deficiency in four yearling Angus bulls

John Ragsdale, Norbert Takacs, Peter G. Moisan, Tim Hanosh

New Mexico Veterinary Diagnostic Services, Albuquerque, NM

Four yearling Angus bulls were submitted for postmortem examination. The bulls were from a herd that has a repeated history of outbreaks of respiratory disease in all age groups with the owners suspecting vaccine failure. One of the four bulls had brisket edema. All four bulls were poor doing with chronic weight loss. The bulls had a frothy nasal discharge and a cough. The bulls also “walked like their chest hurt” with pleuritis suspected. At necropsy, all four bulls had similar findings. The bulls were thin with body condition scores of four out of nine. They had mild to moderate ascites. One bull had subcutaneous edema in the brisket. There were numerous fibrous adhesions of the visceral pleura of the lungs to the parietal pleura of the thoracic wall. The lungs were heavy, wet and edematous. There was moderate to severe hydropericardium. The hearts were enlarged and round with a flaccid myocardium. All four chambers of the hearts were dilated. The myocardium of the right ventricle was thickened and had a similar thickness to the myocardium of the left ventricle. The livers were congested with an accentuated reticular pattern. The kidneys and intestine were congested. The microscopic lesions were similar in the four bulls. The myocardium was edematous with multiple foci of myocardial degeneration, myofiber loss and fibrosis, and perivascular to multifocal infiltrates of lymphocytes and macrophages. In the lungs, there was pulmonary edema as well as marked medial and adventitial hypertrophy of pulmonary arteries and arterioles by smooth muscle and fibrous tissue, respectively. The alveolar septa were thickened by infiltrates of small numbers of lymphocytes and macrophages. There was passive congestion of the liver. The hepatic copper concentration was low to critically low in all four bulls. The diagnoses of bovine pulmonary hypertension (high altitude disease) and myocardial degeneration due to copper deficiency were made.

Bovine pulmonary hypertension occurs most commonly in cattle living at an elevation above five thousand feet in Wyoming, Colorado, Wyoming and New Mexico. That being said, there is an increasing incidence of the disease in heavy feedlot cattle at two thousand to four thousand feet of elevation in the Great Plains. Pulmonary hypoxia at high altitudes results in pulmonary arterial vasoconstriction and hypertrophy reducing the lumen diameter of pulmonary arterioles. This causes pulmonary hypertension eventually resulting in right-sided heart failure. Although it is not common, prolonged copper deficiency can result in myocardial necrosis in multiple species and can result in sudden death in cattle (falling disease). Within the myocardium, there are often foci of acute degeneration mixed with foci of myocardial scarring such as in this case. In these bulls, the comorbid conditions of pulmonary hypertension and myocardial necrosis probably complicated one another and combined to result in both right and left-sided heart failure.

Suspected fatal adverse reactions to a commercial *Mannheimia haemolytica* Toxoid vaccine in young holstein heifers on two New York dairy farms

Gerald E. Duhamel[1,2], Belinda S. Thompson[3]

[1]Biomedical Sciences, Cornell University, Ithaca, NY; [2]Anatomic Pathology, Animal Health Diagnostic Center, Ithaca, NY; [3]Animal Health Diagnostic Center, Ithaca, NY

Preventative vaccination is a critical component of biosecurity programs on cattle farms that can assist with prevention and control of infectious diseases, improve productivity, decrease economic losses and minimize antibiotic usage. Although, administration of biological preparations has potential risks, adverse reactions are generally rare, and can range from variable degree of injection site reaction to systemic signs including hive, collapse, abortion, and rarely death. Here we describe the clinical and histopathological features of two separate suspected fatal systemic adverse reactions in 4, 2- to 6-month-old Holstein heifers from New York dairy farms. In each instances, heifers died within less than 4 hours following subcutaneous administration of a commercial *Mannheimia haemolytica* toxoid preparation (Presponse SQ, Boehringer Ingelheim Vetmedica, Inc., Duluth, GA). Farm 1 was a 1,200-cow dairy with approximately 600 young stocks. Shortly after vaccination of a group of 6-month-old heifers, 2 heifers displayed increased respiratory rate and distress, followed by approximately 10 additional heifers. All heifers received epinephrine, and while 9 recovered, 1 heifer died. Farm 2 was a 100-cow dairy with approximately 80 young stocks. Two hours after vaccination of a group of 2-month-old heifers, 2 out of 7 died, while a third heifer died 2 hours later with foaming at the nose and signs of respiratory distress. The remaining 4 heifers in this group were administered epinephrine and recovered. Complete postmortem field autopsies were performed by referring veterinarians and fresh and fixed tissue specimens were submitted for laboratory investigation. At autopsy, all heifers were in good body condition, and the only significant finding was diffuse reddening and expansion of the lungs that oozed abundant red fluid on cut section. Histologically, the lungs of all four heifers had moderate-to-severe, acute interstitial pulmonary hemorrhages. All four heifers also showed evidence of a systemic inflammatory response attributable to Gram negative bacterial endotoxin characterized by diffuse pulmonary alveolar capillary and hepatic sinusoidal neutrophilia (4 out of 4), together with frequent splenic red pulp neutrophilic infiltrate in one heifer and marginal zone neutrophilia in another. Aerobic and *Mycoplasma* bacteriological culture of fresh lung samples from each heifer and molecular diagnostics for common respiratory viral pathogens of cattle were unrewarding. The American Veterinary Medical Association recommends reporting adverse events to administration of animal vaccines to the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS). However, potential underlying disease process should be ruled out when cattle die shortly after administration of biological preparations by performing complete postmortem examination followed by histopathological and laboratory assessment of tissue specimens.

Use of diagnostic codes to summarize respiratory and enteric diseases detected in tissues submitted to the ISU-VDL from 2010-2018 + * †

Giovani Trevisan, Kent J. Schwartz, Leticia Linhares, Bret Crim, Poonam Dubey, Eric Burrough, Rodger Main, Daniel C. L. Linhares

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

Introduction: Tissues from diseased swine are routinely sent to VDLS for diagnostic testing. Pathogen detection is based on tests such as PCR, isolation, antigen detection or antibody presence. Confirming particular pathogen(s) as the cause of the disease process at hand requires diagnostic investigation of information and findings, which include clinical presentation, submission and history details, test results, and compatible macroscopic and/or microscopic lesions by a capable diagnostician. At the ISU-VDL, diagnosticians assign diagnostic codes-(DXcode) to each case which is recorded in the LIMS in the form of specific disease name(s), offending agent(s), and/or predominant lesion(s). The purpose of this project was to use DXcode to develop a user-friendly informatics tool to allow summarizing and reporting the key pathogens associated with swine respiratory-(RES) or enteric-(ENT) diseases found in tissues received at ISU-VDL between 2010 and May of 2019.

M&M: Anonymized historical data from cases including DXcode were retrieved from the ISU-VDL LIMS for the period between 2010 to 2019, manipulated using SAS® scripts, and uploaded to Power BI® for data visualization. The first analysis utilized cases having enteric and/or respiratory tissue only. Final results were organized by year and season, age category, site state, diagnostic, and agent.

Results: Available information related to disease detection from 86,092 distinct cases were retrieved and analyzed. For respiratory cases, fall and winter months had the highest and summer the lowest detection rate. The 2 majors RES diseases were IAV and PRRS, and the combination of both diseases was the most frequent in the category where cases had multiple pathogens reported. IAV and *M. hyopneumoniae* had the highest detection rate during fall and PRRSV during winter months. The most frequent causes of ENT disease were rotaviruses, and *E. coli*, individually or combined. Rotaviral enteritis was more frequently detected in winter and spring whereas *E. coli* infection was more common during summer and fall. PED had the highest number of diagnoses in 2013/2014, lowering thereafter. These data can be visualized online at https://fieldepi.research.cvm.iastate.edu/swine-disease-reporting-system/ under RES and ENT disease detection dashboards.

Conclusions: The bio-informatics tool developed as part of this study has a near real-time capability to inform the US swine industry on aspects of ENT and RES disease detection at the ISU-VDL. The tool can be expanded to inform disease detection of other systems (e.g. musculoskeletal, systemic, reproductive, nervous, integumentary, and urogenital), species, and can also incorporate other relevant diagnostic data points. The use of aggregated DXcode, which are assigned by diagnosticians, is a novel way to inform disease detection over time allowing to better understand the macro-epidemiological aspects of disease affecting animal populations versus pathogen detection alone.

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

Sudden death caused by fetlock failure in a Thoroughbred racehorse + †

Monika Aleksandra Samol[4], Susan Stover[2], Francisco A. Uzal[1], Rick Arthur[3]

[1]California Animal Health & Food Safety Laboratory- San Bernardino, University of California, Davis, Riverside, CA; [2]Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, CA; [3]School of Veterinary Medicine, University of California, Davis, Davis, CA; [4]California Animal Health & Food Safety Laboratory- San Bernardino, University of California, Davis, San Bernardino, CA

The most prevalent causes of death in racehorses are musculoskeletal injuries, causing app. 80% of deaths within the racing industry in California and elsewhere. The vast majority of these injuries (about 90%) have pre-existing lesions that predispose to fatal injury.

A 4-year-old Thoroughbred colt suffered from an acute suspensory apparatus failure, including biaxial proximal sesamoid bone fracture of the right front fetlock, causing loss of support of the fetlock joint and consequent fall with cervical and sacral vertebral fractures.

Evidence of pre-existing pathology was observed in association with the complete transverse fracture of the medial proximal sesamoid bone. A focal discoloration within a region of sclerotic bone was found to be osteopenic on microcomputed tomography examination. Most likely, focal osteopenia acted as a stress riser that caused initiation of complete bone fracture. A similar osteopenic lesion was present in the intact medial proximal sesamoid bone of the left forelimb.

The morphological features of the vertebral fractures are compatible with acute injury. Probably, these acute fractures occurred subsequent to the fall during the horse's collision with the racetrack. The presence of bilateral osteopenic lesions is consistent with repetitive overuse injury, with the most severe lesion leading to bone fracture and fetlock failure.

Approximately 30% to 50% percent of California Thoroughbred racehorse deaths have been associated with proximal sesamoid bones injuries. Therefore, the understanding of the chronic pathogenesis and character of these lesions is paramount to try to reduce the number of catastrophic injuries in the racehorse population.

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† Graduate Student Oral Presentation Award Applicant

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Serology

Saturday, October 26, 2019
Ballroom C

Moderators: Pamela J. Ferro and Devi P. Patnayak

1:00 PM **A new indirect ELISA to improve accuracy and consistency of *Mycoplasma bovis* diagnostics**
Josh Blase, Alix Carpentier, Kevin Martin, Loic Comtet, Philippe Pourquier 103

1:15 PM **Trouble-shooting and improving assay performance at the benchtop level**
Jamica Fonseca, Suzy Block, Sheila Heinen, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola, Dave Baum 104

1:30 PM **Performant competitive and indirect ELISAs for the diagnosis of African Swine Fever on domestic swine and wild boar**
Josh Blase, Loic Comtet, Mickael Roche, Fabien Donnet, Philippe Pourquier 105

1:45 PM **Heat-inactivation of cell culture fetal bovine serum and diagnostic test serum samples – is it necessary?**
Jillian Fishburn, Jeremiah T. Saliki 106

2:00 PM **A bead-based assay for the detection of risk of infection and protective immunity against EHV-1 infection in horses and other equids**
Cassandra M. Guarino, Alicia Rollins, Bettina Wagner 107

2:15 PM **Applying the Deming Plan-Do-Study-Act (P-D-S-A) cycle of continuous improvement to reduce variation in the IDEXX *Mycoplasma hyopneumoniae* ELISA**
Qiaoling Gong, Mingxi Guo, Sheila Norris, Suzy Block, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola, Dave Baum 108

2:30 PM **Development of an *Bartonella henselae* specific ELISA**
Andreas Latz, Markus Jost, Volkhard Kempf 109

2:45 PM **Use of chimeric Eastern Equine Encephalitis virus in an IgM antibody capture enzyme-linked immunosorbent assay for diagnosis of EEE Infection in Horses**
Karthik Kumar K. Shanmuganatham, Randy G. Atchison, Sue M. Klein, Ashley R. Nagel, Joshua H. Thompson, Albert Van Geelen, Sabrina L. Swenson 110

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A new indirect ELISA to improve accuracy and consistency of *Mycoplasma bovis* diagnostics

Josh Blase, Alix Carpentier, Kevin Martin, Loic Comtet, Philippe Pourquier

IDvet, Grabels, France

Bovine diseases due to *Mycoplasma bovis* cause considerable economic losses in cattle production. While it is principally responsible for mastitis in dairy farms, it also causes Enzootic Infectious Broncho Pneumoniae (EIBP) in calves in feedlots, commonly complicated by opportunistic infections with other pathogens. Prevention is the most effective way to stop disease spread, and effective disease diagnosis plays an important role in controlling the disease. The **ID Screen® Mycoplasma bovis Indirect** ELISA kit is an efficient tool which can be used to detect antibodies against *Mycoplasma bovis* in bovine serum or plasma samples and also in milk samples (individual or pooled samples and bulk milks).

The kit specificity was evaluated with 100 sera from 6-8 weeks-old calves that were considered negative because there was no mycoplasma growth detected from nasal swabs by culture and PCR, and the herds had no history of *M. bovis* infection. The measured specificity was 100% (CI95%: 96.3% – 100.0%). The sensitivity was evaluated by testing 23 samples from 8-11 week old calves with clinical signs and positive by the Western Blot ; the sensitivity relative to Western Blot as a gold standard, was 95.7% (CI95%=87.3%-100.0%). To study the correlation between serum and milk, 324 individual paired milk/serum samples (collected from four different herds, France) were tested using the ELISA serum and milk protocols. The result correlation was 94.7% (CI95% = [91.7 - 96.7]) and the kappa 0.858 (CI95% = [0.793; 0.924]), indicating a good correlation in terms of disease status.

In 2018, the European COVETLAB (COllaborating VETerinary LABoratories) group organized a European ring trial comparing different diagnostic methods (Western Blot, and different commercial ELISAs). In this ring trial, the ID Screen® ELISA showed excellent sensitivity and specificity - 95,6% [IC95% 91,6-99,0] and 99,3% [IC95% 98,3-99,8], respectively. Results were presented in a congress (Medvet Pathogens congress, october 8 -11 2018, Prato, Italy.) and are under review for publication.

These results demonstrate the ability of the ID Screen® ELISA to efficiently detect antibodies against *M. bovis* in individual serum and milk samples. This ELISA may be used to test animals moved to *Mycoplasma bovis*-free areas, for disease monitoring or for epidemiological studies.

Trouble-shooting and improving assay performance at the benchtop level

Jamica Fonseca, Suzy Block, Sheila Heinen, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola, Dave Baum

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

Simply meeting the specifications of kit manufacturers' control values cannot be the definition of assay quality. Technicians must pay attention to the variation of each of the assays for which they are responsible and distinguish normal from special cause variation. This requires definitions of normal and special cause variation. The definition of normal variation is that which happens without pattern within three-sigma limits about the average assay output. Special cause variation is that which produces patterns relative to the average assay output and it three-sigma limits. Four data patterns of special cause variation are: any 1 data point outside three-sigma limits, 2 of 3 consecutive data points outside two-sigma limits and on the same side of the average, four of 5 consecutive data points outside one-sigma limits and on the same side of the average and 8 consecutive data points on the same side of the average. This is particularly critical when new technicians are on-boarded and benchtop space is restricted. These two changes can be sources of special cause variation. Such was the case of a multi-plate *Actinobacillus pleuropneumoniae* ELISA assigned to a new team member, who recognized changes in test output and sought out the Section's quality team for help. Process behavior charts identified special-cause variation of the assay's negative kit control OD range and the lab's house negative S/P moving range chart values. The average range (R-bar) and upper control limit (UCL) for the kit negative control were 0.012 and .033, respectively, for 45 consecutive assays prior to 26Feb19. This assay's plates are washed by hand. There being no counter space available in the lab, the technician held the plates in their hand while washing whereas the previous technician had counter space to set the plates on a counter for washing. The technician promptly corrected this practice and performed the assay in another area where the plates were placed on a counter for washing. This change produced immediate reduction in variation: the R-bar and UCL became .005 and .017. While satisfactory, these results were not similar to the previous technician. An audit of procedures revealed four washing steps performed rather than five per kit instructions. With five washes, wash variation declined further with the R-bar and UCL of 0.0036 and 0.012, similar to those of previous technician's work. This work demonstrates the importance of cultivating technicians' awareness of assay performance characteristics and ongoing control value analysis.

Performant competitive and indirect ELISAs for the diagnosis of African Swine Fever on domestic swine and wild boar

Josh Blase, Loic Comtet, Mickael Roche, Fabien Donnet, Philippe Pourquier

IDvet, Grabels, France

Introduction

African Swine Fever Virus (ASFV) control programs require reliable diagnostic tests. IDvet offers an indirect ELISAs and a competitive ELISA for the detection of antibodies. This work presents validation data obtained on these ELISA.

Materials and methods

ELISAs The ID Screen® ASF Indirect ELISA kit includes plates coated with three recombinant ASFV antigens (P32, P62, and P72). The ID Screen® African Swine Fever Competition ELISA allows the detection of P32 antibodies. The test was performed as per manufacturer's instructions. Panels of known positive and negative samples, as describe below, were tested.

Results

Indirect ELISA Specificity: - 763 disease-free sera from domestic pigs were tested, wild boars, and Iberian pigs, measured specificity was 99.61% (CI 95%: 98.96% - 99.90%). - 90 negative sera tested by both the serum and filter paper protocols were correctly identified by both protocols.

Sensitivity: - 3 sera from vaccinated and challenged pigs gave positive results. - 8 reference sera from the ASF EURL were correctly identified as positive. - 3 positive sera, titrated and tested by both the serum and filter paper protocols, the analytical sensitivity was similar regardless of the sample type tested. - All spiked meat juice samples were correctly identified as positive.

Competitive ELISA Specificity: It was evaluated through the analysis of 280 disease-free sera from domestic and Iberian pigs. Measured specificity was 100.0% (CI 95%: 98.7% - 100.0%).

Sensitivity: 8 positive reference sera from the ASF European Reference Laboratory (EURL-ASF, Madrid, Spain) were correctly identified. Seroconversion was detected between 6 and 13 dpi. The test correctly identified genotypes tested, including genotype II. The test was also evaluated by the EURL. Results indicate a specificity of 99.4% (n=177) and a sensitivity of 95.8% (n=213). Perfect agreement (k=0,95) with the immunoperoxidase test was obtained.

Discussion:

The ID Screen® African Swine Fever Indirect ELISA is the only test presenting no false positive on wild boars. The use of filter papers makes sampling easier, especially for wild boars. It shows excellent specificity and sensitivity, correctly detected reference sera from the EURL for ASF (INIA-CISA, Madrid, Spain). This test is a reliable in both domestic pigs and wild boars. It has been validated by the ASF EURL.

Conclusion:

ID ELISAs offer a very good performance, with the highest analytic sensitivity on domestic pigs. Both kits are currently massively used in Europe for the control of the current outbreak.

Heat-inactivation of cell culture fetal bovine serum and diagnostic test serum samples – is it necessary?

Jillian Fishburn, Jeremiah T. Saliki

Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA

Heat inactivation of fetal bovine serum (FBS) and test serum samples has generally been used as an unquestioned basic protocol transmitted from one generation of scientists to the next. Although a few recent studies have shown that heat inactivating FBS may not be helpful to cell cultures, the practice is still common in veterinary diagnostic laboratories. We conducted a study to determine if heat inactivating FBS and diagnostic serum samples is necessary or useful.

To study the effect of heat inactivated (HI) FBS on cell growth, one portion of a commercial FBS lot was heat inactivated by immersing in a water bath at 56°C for 30 minutes, while the other portion was not heat inactivated (NHI). The two portions were used to prepare cell culture medium at 9%. Six routinely used cell lines (A-72, CRFK, MDBK, MDCK, RK-13 and VDS) were passaged weekly for 10 weeks in the 2 media. At each passage, cells were counted, viability recorded, and the data used to create growth curves were established for the 6 cell lines.

To study the effect of heat inactivating diagnostic serum samples on virus neutralizing (VN) antibody titers, 11 VN tests were performed on 4 of the cell lines (MDBK – 5 tests; MDCK – 1 test; VDS – 3 tests; RK-13 – 2 tests) using HI and NHI serum samples and cells grown in media supplemented with HI and NHI FBS. Additionally, HI and NHI serum samples were tested for leptospiral antibody titers using the microscopic agglutination test (MAT).

Growth of the six cell lines was identical when cells were grown in media with HI or NHI FBS, as indicated by virtually overlapping growth curves. Our results differ with those from a study by Hyclone, which showed that heat inactivation actually diminished the growth of MDBK cells. The bottom line is that heat inactivation of FBS had no additional beneficial effect on the growth of any of the cell lines included in this study.

When VN tests were performed using HI or NHI serum samples, HI sera generally yielded lower titers when tested using cells grown in media with HI FBS than with NHI FBS. Using serum as the first identifier in each pair of test conditions (example: HI/NHI = HI serum tested on cells grown in media with NHI FBS), antibody titers decreased in the order: HI/NHI > NHI/HI > NHI/NHI > HI/HI.

The *Leptospira* antibody MAT was performed using six serovars: *L. canicola*, *L. bratislava*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, and *L. pomona*. Using the Wilcoxon signed-rank test for comparison of titers obtained with HI and NHI sera on each serovar, there was no significant difference ($p \geq 0.25$) between HI and NHI titers for any of the six serovars.

Taken together, our results suggest that: 1) heat-inactivation of FBS for cell cultures is not necessary, at least for the majority of cell lines routinely used in veterinary diagnostic laboratories; 2) heat-inactivation of diagnostic serum samples appears to be useful for VN tests, but neither helps nor hurts the *Leptospira* antibody MAT.

A bead-based assay for the detection of risk of infection and protective immunity against EHV-1 infection in horses and other equids

Cassandra M. Guarino, Alicia Rollins, Bettina Wagner

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

Equine Herpes Virus type-1 (EHV-1) is known to cause respiratory disease, abortion, and also has the potential to cause neurologic disease, commonly known as equine herpesvirus myeloencephalopathy (EHM), in horses and other equids. In an effort to prevent outbreaks at shows, races, and events, stringent requirements for EHV vaccination have been implemented by some organizations, while others have no vaccination requirements for EHV. Horses that are vaccinated typically get one or two booster doses per year. In some cases, this frequent vaccination can lead to an increase in adverse reactions in horses, including pain and swelling at the injection site or swollen legs, which impact the horse's performance. Currently, the standard tool for measuring antibody responses to EHV-1 vaccination or infection is a serum neutralization test, however this test takes days to perform and does not provide a cut-off that can be used to support evidence of protection. Here, we present an assay that quantifies specific serum antibody biomarkers and correlates these values with protective responses in horses challenged with the neuropathogenic Ab4 strain of EHV-1. This assay can have a same-day turn-around time and will be a valuable asset to the equine community. The assay can be used to monitor vaccine responses and help guide vaccination decisions in an effort to obtain protective antibody values in susceptible horses. Further, use of this assay can help to avoid over-vaccination in horses that show side-effects to vaccination. Finally, this assay has the potential to allow for the modification of vaccine recommendations, replacing vaccine requirements in protected horses in places where regulators recognize the use of this new technology.

Applying the Deming Plan-Do-Study-Act (P-D-S-A) cycle of continuous improvement to reduce variation in the IDEXX *Mycoplasma hyopneumoniae* ELISA

Qiaoling Gong, Mingxi Guo, Sheila Norris, Suzy Block, Jeff Zimmerman, Luis Gabriel Gimenez-Lirola, Dave Baum

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

Swine production systems conduct *Mycoplasma hyopneumoniae* (Mhp) surveillance in order to establish the Mhp status of sow farms, growing pigs, and replacement breeding stock. The expansion of testing from diagnostics to routine surveillance results in both in an increased demand for testing and more “11th - hour” submissions. The former puts pressure on kit manufacturers to produce more kits and on testing laboratories to perform more tests. The latter drives just-in-time delivery of Mhp-negative pigs. Thus, unexpected positive results reported from a population of animals scheduled for shipment in the next day or two brings the scheduled delivery to a halt. Then, the farm must cancel previously-scheduled transport vehicles and personnel in order to resolve the unexpected result. Resolution often occurs by retesting and resubmitting serum samples for antibody testing along with tracheal swabs of several animals to detect Mhp by PCR. ISU VDL applied The Deming Cycle (Plan-Do-Study-Act, PDSA) to its daily Mhp antibody testing in order to assure assay quality and repeatability for its Mhp serology clients. First, a process behavior chart described the output of the assay and defined the process’ average S/P value and the 3-sigma limits of the house negative control and its ranges: -0.458, -0.290, -0.062; 0.190, 0.028 and 0.000, respectively. Then, a fishbone (cause-and-effect) diagram helped the team narrow its focus to inputs manageable by the Serology Section. The immediately-discovered concerns were pipette tips that fell off and fluctuations in ambient temperature. First, new pipettes which hold tips with a clip were ordered, calibrated, and put into the testing process. This process change reduced the average S/P, narrowed its limits, and reduced its variation. These changes were sustained for approximately 6 weeks. Incubation of ELISA plates on tray warmers was the next process change, with further reductions in variation. Thus, continued improvement projects will improve the quality of ELISA test results as defined by reduction in variation. The next step for improvement, identified by the fishbone diagram, will be to study the process of ELISA plate washing. These improvements enable the ISU VDL to add an additional quality check for its ELISA’s: when process variation exceeds the process limits, those plates will be rejected and samples rerun.

Development of an *Bartonella henselae* specific ELISA

Andreas Latz[1], Markus Jost[2], Volkhard Kempf[2,3]

[1]Research and Development, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany; [2]Institute for Medical Microbiology and Infection Control, University Hospital, Goethe-University, Frankfurt am Main, Germany; [3]German National Consiliary Laboratory for Bartonella, Frankfurt am Main, Germany

Bartonella henselae causes cat scratch disease (CSD), an often self-limiting lymphadenitis in immunocompetent patients, and several other clinical entities. While cats are the natural reservoir for *B. henselae,* the pathogen is transmitted by cats, cat fleas and eventually by other arthropods. The clinical symptoms underlying CSD might be similar to those being suspicious for malignant tumors. Thus, an easy and reliable test for *B. henselae* infections is highly desirable.

Microscopic immunofluorescence assays (IFA) are widely used for serodiagnostics of B. henselae-infections, but are laborious, time consuming and interpretation is subjective. An easy and reliable method for the serological diagnosis of B. henselae infections is needed to overcome the shortcomings of the current IFA. The aim of this study is to design an ELISA for detection of *B. henselae* to improve the shortcomings of the currently used immunofluorescent test (IFT), e.g., objective and reproducible results and less hands-on time.

Test development is based on different *B. henselae* strains and quality assured patient sera [(a)sera positively tested for anti *B. henselae* antibodies via IFT, (b) patients/animals with typical symptoms, (c) sera of patients/animals with PCR-based infection diagnosis]. Antigens were separated by ion exchange chromatography and fractions examined in lineblots. Potential fractions were further tested and optimized for ELISA.

Patients/animals with *B. henselae* infections show different patterns of antibody expression in western blots. Thus, there is obviously no universally usable antigen for diagnosis detectable. However, our tests show that there are certain protein fractions from *B. henselae* which react reliably and results from lineblots were successfully transferred to an ELISA-format with sufficient sensitivity.

By the use of well-characterized human sera from the strictly quality-controlled serum library of the German National Consiliary Laboratory for Bartonella infections, sensitivity of this ELISA was 100% for molecular proven infections and 76% for clinically suspected infections at a specificity of 93%, respectively.

Conclusions: This ELISA is therefore a reliable high-throughput method allowing serodiagnostics of B. henselae infections.

We show a strategy for antigen testing and selection from *B. henselae* protein preparations for ELISA-based serology. Further processing of antigens is under investigation so that in future an ELISA for *B. henselae* is possible.

Funding: This study is financed by the state Hesse within the LOEWE III project.

Reference: Jost et al., 2018 Journal of Clinical Microbiology Nov 2018, 56 (12) e01329-18; DOI: 10.1128/JCM.01329-18

Use of chimeric Eastern Equine Encephalitis virus in an IgM antibody capture enzyme-linked immunosorbent assay for diagnosis of EEE Infection in Horses

Karthik Kumar K. Shanmuganatham, Randy G. Atchison, Sue M. Klein, Ashley R. Nagel, Joshua H. Thompson, Albert Van Geelen, Sabrina L. Swenson

DVL, NVSL-VS-APHIS-USDA, Ames, IA

Eastern equine encephalomyelitis virus (EEEV) is an arbovirus in the family Togaviridae, genus Alphavirus that causes severe often fatal neurologic disease in horses, human beings, and other vertebrates. EEEV has been classified into North American EEEV and Madariaga virus (formerly South American EEEV). Wild type EEE viral antigen currently used in diagnostic ELISAs is inactivated and produced in either sucrose-acetone extracted suckling mouse brain or in cell culture with serological reactivity preserved. Wild type EEEV should be worked with in biosafety level 3 (BSL-3) containment facilities and is classified as a select agent in the U.S. Surrogate viruses or chimeras with equivalent antigenic makeup to wild type virus that confer comparable sensitivity and specificity to the wild type viruses have been developed to alleviate the need to be approved by the select agent program for serologic assays. Here we assessed and evaluated the potential of two chimeras in replacing the wild type EEE currently being used in the diagnostic IgM antibody capture ELISA (MAC ELISA). The two recombinant Sindbis virus (SINV)/EEEV have the nonstructural protein genes and cis-acting RNA genome elements from benign wild-type SINV and the structural protein genes are from either North American EEEV strain or from a South American strain. The chimeras were propagated in two different cell lines (mammalian and mosquito), chemically inactivated, and tested against a panel of horse sera from North and South America which were previously identified as EEEV positive. The specificity of the EEEV chimera based assay was assessed by testing them against Venezuelan Equine Encephalomyelitis (VEE) and Western Equine Encephalomyelitis (WEE) positive sera. The chimera grew to relatively high titers (10^6 to 10^7) in the insect cell line and to a modest titer (10^4 to 10^6) in the Vero cell, requiring concentration before the Vero propagated antigen could be used in the MAC ELISA. Both antigen preparations when tested in the MAC ELISA had similar performance characteristics, with the chimera from the insect cell line performing better for samples with lower IgM titers. Neither chimera showed any kind of cross reactivity with VEE or WEE positive sera. These results suggest that the chimera are comparable to the wild type EEE antigen and may be viable alternatives in replacing the WT EEEV in the MAC ELISA.

Toxicology

Sunday, October 27, 2019
Room 557

Moderators: Catherine Barr and Karyn Bischoff

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

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Quantitation of ionophores in animal feed samples

Gwendolyne Alarcio, Elizabeth R. Tor, Linda S. Aston, Robert H. Poppenga

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

Ionophores are feed additives that are widely used in animal agriculture to improve feed efficiency in beef and control coccidiosis in poultry. Monensin, lasalocid, narasin, and salinomycin are some of the commercially available ionophores. They are available in different forms and concentrations from the low ppm levels in finished feed products to percent levels in premixes, slow-release bolus products, and concentrates intended for mixing into feeds. Because of the availability of various formulations, accidental overdose, misuse, and mixing errors can occur and result in toxicity in off-target species.

Simultaneous screening, confirmation and quantitation of ionophores in animal feeds has been performed by the CAHFS-Toxicology laboratory using LCMS technique with a detection limit in the ppb levels (<100ppb). Although the method is very sensitive, the quantitation at high ppm levels (>500ppm) can be difficult due to limited linear range and electrospray signal suppression due to matrix interferences. Currently there are no commercially available isotope-labeled internal standards for ionophores.

As an alternative to quantification by LCMS, an AOAC method utilizing post-column derivatization of monensin with acidified vanillin and UV detection has been validated in-house to quantitate monensin levels in medicated animal feeds with a 100ppm detection limit, <10% intra- and inter-assay precision, and accurate results within 2x standard deviation of the expected levels. Although the technique is not as sensitive, it provides a wide linear range (10ug/mL to 2000ug/mL) and eliminates many of the limitations associated with ESI quantitation. Comparison of results from LCMS and post column results will be presented.

GC/MS confirmation of Atrazine exposure in a cow

Abigail Props[1], Hilary Richards[1], Stephen B. Hooser[1,2], Christina Wilson[1,2], Grant N. Burcham[3,2]

[1]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN; [2]Comparative Pathobiology, Purdue University, West Lafayette, IN; [3]Heeke Animal Disease Diagnostic Laboratory, Purdue University, Dubois, IN

Atrazine is a triazine herbicide commonly used in crops such as corn, sugarcane and sorghum. Because it is one of the most widely used herbicides in the U.S., concerns regarding animal and human exposure to atrazine persist. Although uncommon, toxicity has occurred in animals, particularly cattle, who can have access to formulated atrazine and ingest lethal quantities. Clinical signs reported in cases of toxic ingestion can include increased body temperature and respiratory rate, ataxia, stiffness, hypersalivation and death. In this case report, 10 cows of 40 died within 48 hours of having access to a barn in which a variety of pesticides and herbicides were being stored. Some number of remaining cows exhibited hypersalivation, muscle tremors and agitation. Post-mortem examination of one cow revealed non-specific moderate to severe pulmonary congestion. Samples of liver and rumen contents, each from a different cow, were submitted to the Toxicology Section at the Indiana Animal Disease Diagnostic Laboratory for GC/MS analysis.

Five grams of liver and 25 grams of rumen contents were homogenized in acetonitrile and NaCl (10:1, v/w). The acetonitrile extracts were applied to ENVI-Carb™-II/PSA solid-phase extraction cartridges, the eluate evaporated to dryness under N_2 and reconstituted with 0.5 mL acetonitrile:toluene (3:1) for GC/MS analysis. The GC instrument method included an injector port temperature of 220 C, a column flow of 1.0 mL/minute and a column temperature program initially set at 90 C (held 0.10 minutes) with a 7.1 C/min ramp to 300 C. One microliter of atrazine standard, quality controls and samples were injected onto a VF-5ms column (30m x 0.32mm, ID; DF = 0.25). EI-MS scans from 40 to 650 m/z were used to monitor ions.

The liver and rumen content samples were confirmed positive for atrazine. GC/MS analysis of atrazine and liver fortified with atrazine (positive control liver) revealed a retention time of approximately 13.07 minutes for the standard with NIST library matches > 97%. Major ions detected for confirmation included 200 m/z, 215 m/z, 173 m/z, and 58 m/z. The rumen content sample contained a higher amount of atrazine when compared to the liver and the NIST library matches to atrazine were > 97% for both diagnostic samples.

This GC/MS method can isolate and confirm the presence of atrazine in liver and rumen contents. It is suspected that atrazine toxicity is likely the cause of death and clinical signs observed in this herd.

Determining approximate minimum toxic dose of diphacinone in horses, and corresponding serum, blood, and liver diphacinone concentrations †

Megan C. Romano[1], Kyle A. Francis[1], Jennifer G. Janes[1], Amanda A. Adams[1], Robert H. Poppenga[2], Michael Filigenzi[2], Cynthia Gaskill[1]

[1]Veterinary Science, University of Kentucky, Lexington, KY; [2]California Animal Health and Food Safety Laboratory Systems, University of California at Davis, Davis, CA

Introduction:

Anticoagulant rodenticide (ACR) toxicosis in nontarget species remains a concern, despite regulations limiting use of long acting ACRs. Differentiating ACR toxicosis from incidental exposure is sometimes difficult, as clinically significant serum, blood, and liver concentrations have not been established in horses. Diphacinone is currently the most widely used ACR available to the public.

Goals:

Estimate minimum toxic dose (MTD) of diphacinone in horses. Measure postmortem liver diphacinone concentrations at various doses. Characterize liver diphacinone distribution Compare blood and serum diphacinone concentrations

Methods:

Three healthy university-owned mares were used in this study. Baseline physical examinations (PEs) were performed. Complete blood count (CBC), serum chemistry panel, serum and blood diphacinone concentration, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were obtained. Diphacinone mixed with sweet feed was administered once to each horse as follows:

Horse 1: 0.4 mg technical grade diphacinone/kg body weight (mg/kg)

Horse 2: 0.2 mg/kg diphacinone liquid bait (Liqua-Tox® II, Bell Laboratories)

Horse 3: 0.09 mg/kg diphacinone liquid as above

PE, serum and blood diphacinone concentration, PT, and aPTT were repeated at 24, 48, and 65 h (Horse 1), or 24, 48, 72, and 96 h (Horses 2 and 3). CBCs were repeated at 65 h (Horse 1) or 96 h (Horses 2 and 3). PT-International Normalized Ratio (PT-INR) and aPTT Patient:Control (P:C) ratio were calculated (Horses 2 and 3). PT-INR is the ratio of patient PT to assay mean PT, raised to the International Sensitivity Index value of the PT reagent. Horses were euthanized, postmortem examinations performed, and liver diphacinone concentrations measured.

Results:

All PEs and CBCs were normal throughout. PT, aPTT, and serum chemistry panels were normal at baseline and at 24 h. Post-dosing coagulation changes are as follows: Horse 1 (0.4 mg/kg) 48 h: PT and aPTT prolonged (>1.5 x baseline). 65 h: PT and aPTT prolonged. Horse 2 (0.2 mg/kg) 48 h: PT normal, aPTT prolonged (P:C ratio >1.5). 72 h: PT prolonged (PT-INR > 1.5), aPTT prolonged 96 h: PT and aPTT normal. Horse 3 (0.09 mg/kg) PT and aPTT normal throughout. No evidence of coagulopathy or hemorrhage was present on postmortem examinations. Diphacinone distribution was uniform in all livers; mean concentrations (wet weight) were 2230 ppb, 1790 ppb, and 1600 ppb in Horses 1, 2, and 3 respectively. Serum diphacinone concentrations ranged from 170 ppb (Horse 1, 24 h) to 3.7 ppb (Horse 3, 96 h). Blood concentrations were comparable except at 24 hours.

Discussion:

This study suggests an approximate MTD after single exposure to diphacinone in horses of 0.2 mg/kg; corresponding hepatic diphacinone concentration was 1790 ppb at 96 h post dosing. Serum and blood are both suitable for diphacinone analysis. Additional work is underway to further characterize MTD and liver clearance of diphacinone in horses.

† Graduate Student Oral Presentation Award Applicant

Inter-laboratory evaluation of a high performance liquid chromatography-fluorescence method for the quantitative measurement of aflatoxins B_1 and M_1 in animal urine

Xiangwei Du[1], Dwayne Edward Schrunk[1], Paula Martin Imerman[1], John Tahara[2], Andriy Tkachenko[3], Jake Guag[3], Renate Reimschuessel[3], Wilson Kiiza Rumbeiha[1]

[1]VDL, Iowa State University, Ames, IA; [2]UC Davis, Davis, CA; [3]FDA, Silverspring, MD

Diagnosis of exposure to aflatoxins in live animals has been hampered by lack of blood or urine-based tests directed at detection of the aflatoxins or their metabolites. We recently published our work on intra-lab validation of a urine assay for detection of aflatoxin B_1 (AFB_1) and aflatoxin M_1 (AFM_1). In this abstract, we present results of the inter-laboratory blinded method test (BMT) evaluation.

First, we completed a single laboratory blinded method test (BMT) evaluation by the originating laboratory. For this, the study consisted of canine urine spiked at low (1.1 ppb for AFB_1 and 1.0 ppb for AFM_1), medium (4.6 ppb for AFB_1 and 4.5 ppb AFM_1), and high (10.0 ppb for AFB_1 and 12.0 AFM_1) levels. At these three levels, over 6 replicates were used. For AFB_1, the accuracy ranged from 89 to 94%, while the intra-laboratory reproducibility ranged from 6 to 10%. For AFM_1, the accuracy ranged from 90 to 93%, while intra-laboratory reproducibility ranged from 3 to 10%. The accuracy was excellent for both aflatoxins and was within AOAC and FDA guidelines (40-120%).

Second, this inter-laboratory BMT evaluation was performed in two laboratories. Canine urine was spiked at low (1.1 ppb for AFB_1 and 1.0 ppb for AFM_1), medium (4.7 ppb for AFB_1 and 4.6 ppb AFM_1), and high (11.0 ppb for AFB_1 and 13.0 AFM_1) levels. At all three levels, over five replicates were used. Results indicated that the inter-laboratory accuracy was 86-92% for AFB_1 and 89-94% for AFM_1. Inter-laboratory reproducibility was 10-20% for AFB_1 and 10-20% for AFM_1. This indicates satisfactory results for the three levels for both AFB_1 and AFM_1 between the two laboratories. The correlation coefficients for standard curves for each aflatoxin were >0.99 in each laboratory. This study demonstrates a robust urine-based antemortem method for the diagnosis of aflatoxicosis in animals, which can be used by the Vet-LIRN network laboratory members. This work was funded by FDA grant number 1U18FD005006-05.

Key words: aflatoxicosis; aflatoxin B_1; aflatoxin M_1; urine; inter-laboratory evaluation; accuracy; repeatability

Biphasal extractive pentafluorobenzylation of monofluoroacetate

John Philip Buchweitz[1,2], Andreas F. Lehner[2]

[1]Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI; [2]Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI

Sodium monofluoroacetate, also known as compound 1080, has limited and restricted use as a vertebrate pesticide to control feral animals. The mechanism of action of compound 1080 is the inhibition of the citric acid cycle via its conversion to fluorocitrate which binds tightly to the enzyme aconitase, thus halting the cycle. A rash of malicious poisoning events involving the use of compound 1080 in the southwest United States prompted the Michigan State University Veterinary Diagnostic Laboratory to develop an analytical method for its detection that applied liquid-liquid extraction of bait and stomach contents with chemically stable reagents to provide a method sensitive to 10 ppm. Accordingly, we utilized phase transfer catalysis with tetrabutylammonium hydrogen sulfate in the aqueous phase and base-catalyzed derivatization with 2,3,4,5,6-pentafluorobenzyl bromide in a biphasal system. The dichloromethane soluble derivative was detectable by gas chromatography tandem-quadrupole mass spectrometry (GC-MS/MS) using multiple reaction monitoring (MRM) for qualification and quantitation.

Residual analysis of ivermectin in White-tail deer in Texas for safe human consumption

Juan Carlos Castro

Toxicology, Texas A&M University Veterinary Medical Diagnostic Laboratory, College Station, TX

Bovine babesiosis, most commonly known as Texas Cattle Fever, is a disease that was introduced in North America in the early 1600s, by cattle imported from Spanish colonies of the West Indies and Mexico. This disease is caused by the organism *Piroplasma bigemina* (later reclassified as *Babesia bigemina)*, which are blood born parasites that cause piroplasmosis (Babesiosis), often a deadly disease of cattle, horses and other various animals. This parasite was found in 1890 to be carried by ticks. The application of arsenic dip treatments resulted in the eradication of both species of cattle ticks [*Boophilus (Margaropus) annulatus* and *B. microplus*] except for a narrow area between the Mexico-Texas border, due to the high infestation of ticks south of the border. The ticks responsible for this disease are believed to have breached the nearly 500 mile permanent quarantine zone and place the cattle population of Texas at risk of a new epidemic. One of the current approaches to prevent the spread of tick infestation in this geographical region is the treatment of whole kernel deer corn with an ivermectin containing product, such as Merial, Inc. and Ivomax®. However, consumption of ivermectin treated corn by white tail deer (WTD) may lead to unsafe tissue residues. Therefore, a quick and reliable method was developed to screen WTD tissues for ivermectin. Here, we present the approaches used by TVMDL to detect ivermectin in adipose tissue and feces, including our findings, limits of detection, quantitation and margin of errors.

Liver and muscle tissue Pentobarbital concentrations following euthanasia

Robert H. Poppenga, Michael Filigenzi, Robyn Noonan

California Animal Health and Food Safety Laboratory System, Davis, CA

It is well recognized that improper disposal of animals euthanized by pentobarbital presents intoxication risks to scavengers such as turkey vultures and bald eagles that have access to the carcasses. There are also documented cases where privately owned exotic carnivores have been given meat from euthanized animals resulting in death. In addition sources of rendered animal by-products such as protein (e.g., meat meal) and tallow have included euthanized animals. These by-products have been used in pet foods that have caused pet morbidity and mortality and pet food recalls due to the presence of pentobarbital. We were interested in determining concentrations of pentobarbital in liver and muscle from live animals requiring euthanasia prior to postmortem examination. Liver and leg muscle samples were collected from 13 cows, 2 horses, and 1 dog euthanized according to product label directions within a short period of time after death. Samples were tested for pentobarbital by liquid chromatography – mass spectrometry using negative ion mode.

The concentrations of pentobarbital detected in liver samples ranged from 13 ppm to 230 ppm (75.8 +/- 18.5 ppm) and 0.48 to 69 ppm in muscle samples (16.0 +/4.5 ppm). The FDA Center for Veterinary Medicine (CVM) has determined a daily dose of pentobarbital of 50 micrograms or less per day would have no biological effect in dogs. An oral LD_{50} for dogs is reported to be 85 mg/kg body weight. This can be compared to 2002 data from the FDA-CVM indicating that no more than 4 microgram/kg/day would be ingested by a dog eating pet food containing the highest concentration of pentobarbital detected in tested pet foods of 32 ppb. A much higher concentration of pentobarbital, attributed to the inclusion of horse meat, was found in a more recent case of dog food contamination (approximately 80 ppm). At the highest concentration of 69 ppm (69 mg/kg meat) detected in the muscle samples that we tested, and using the oral LD_{50} of 85 mg/kg as a lethal dose, 10 kg and 25 kg dogs would have to consume approximately 12 kg and 31 kg of meat, respectively, to reach an LD_{50}. Similar LD_{50}s have been reported for avian species. A typical turkey vulture weighing 2 kg would need to ingest 2.5 kg of meat and 0.74 kg of liver to reach an LD_{50} threshold. Stricter control of sources of animals for rendering can eliminate exposure of pets to pentobarbital. Proper disposal of individual euthanized animals requires on-going education of veterinarians and animal owners in order to eliminate risk to wildlife.

Detection of Black Walnut wood in equine bedding by PCR

Stephen B. Hooser[1,2]*, Keith Woeste*[3]*, Rebecca P. Wilkes*[1,2]*, Hilary Richards*[1]*, Angela Chan*[1]*, Christina Wilson*[1,2]

[1]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN; [2]Comparative Pathobiology, Purdue University, West Lafayette, IN; [3]Forestry and Natural Resources, HTIRC, Purdue University, West Lafayette, IN

American black walnut (*Juglans nigra*) is a common hardwood tree. Exposure to black walnut wood in bedding can cause severe laminitis and ventral edema in horses. Currently, when bedding from suspected cases of black walnut toxicity is submitted for diagnostic testing, the sample is visually and microscopically inspected for the presence of black walnut wood. Unfortunately, if the bedding is composed of sawdust, or if the bedding is heavily soiled, it is extremely difficult to determine if black walnut is present. Individuals who have the necessary training and experience in wood anatomy are few, difficult to find, and expensive to hire. The goal of this study is to develop a polymerase chain reaction (PCR)-based test to identify black walnut wood in bedding used for horses.

Pure samples of wood shavings or sawdust from black walnut and other trees, such as pine, spruce, cherry, ash, etc., were obtained from the Purdue University Wood Products Laboratory. The samples were individually ground using a freezer mill and extracted using a modified Qiagen DNEASY Plant DNA isolation kit, or were extracted using a MagMAX Core Nucleic Acid Purification Kit. PCR primers were designed to amplify three sequences specific to black walnut and often used as DNA barcodes, i.e., ITS1 – ITS4, the matK gene, and the trnT-trnF intergenic spacer. Amplified DNA products were verified to have come from black walnut wood by reaction with restriction enzymes and evaluation of the resulting DNA fragments, or by direct sequencing of the amplicons. Preliminary results revealed that ITS1- ITS4 was the most reliable template, and that primer nesting (nested PCR) was able to produce visible amplicons in positive controls containing low amounts of black walnut DNA. Assay sensitivity was determined by performing PCR analysis on decreasing concentrations of DNA from pure samples of black walnut. Assay specificity was determined by performing PCR on pure samples of black walnut or other individual woods, or by mixing samples of varying concentrations of black walnut with combinations of those other woods.

These studies indicate that PCR can be used as a specific and sensitive assay to positively identify black walnut wood in bedding used for horses.

This work was supported by the State of Indiana, and Purdue University College of Veterinary Medicine research account funded by the Total Wagers Tax.

Illicit drugs of abuse screen in biological samples by LCMS

Gwendolyne Alarcio, Elizabeth R. Tor, Linda S. Aston, Robert H. Poppenga

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

Exposure of animals to illicit drugs can be accidental, intentional, or malicious. In suspected cases of exposure, diagnosis will be dependent on accurate history and the presentation of symptoms. However, accurate information provided to veterinarians is commonly skewed because of the illegal nature of these abused drugs and symptoms of exposure may be subtle and varied. In addition, many illicit drugs are mixed with other harmful substances making diagnosis and treatment more difficult. Therefore, it is important to have a screening method that can detect and identify the drugs and metabolites that are potentially present in a sample. Over-the-counter (OTC) human urine drug testing kits are available and can screen for many illicit drugs, but without confirmation, these kits have the potential for false positives due to cross reactivity with other compounds. Furthermore, they may not be able to detect glucuronidated metabolites.

An LCMS method has been developed to simultaneously screen and confirm 28 illicit drugs in bait and biological samples utilizing Thermo VelosPro ion trap and HRMS technology (Thermo Exactive). Chromatographic separation is achieved using the Synergi PolarRP HPLC column (Phenomenex Corp.) with mobile phases containing (A) 10mM ammonium formate in 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Biological fluids and tissue samples undergo enzyme hydrolysis with beta-glucuronidase prior to extraction. Tissue, bait, and stomach contents samples are extracted with methylene chloride at a basic pH. Biological fluids are extracted using the mixed mode, Hyersep VerifyCX SPE columns (Thermo Scientific).

Applying LCMS techniques have provided less limitations compared to previously applied GCMS techniques. With simple extraction procedures and the ease of adding new compounds, this method is a benefit to the diagnosis and confirmation of drug exposure in animals. Diagnostic cases to which this method was applied will be discussed.

Congenital skeletal abnormalities in a Michigan dairy herd associated with manganese deficiency and comparison to classical cases of rickets

Dodd Sledge, Gordon Ehrensing, John Philip Buchweitz, Matti Kiupel

Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI

Approximately 12-15 calves with varying degrees and types of skeletal malformation were born on a 1,300 cow dairy over a 5-month period. The most common abnormality was severe sickle hock, but few animals also presented with abnormal gait, valgus malformation of the hindlimbs, small stature, and bulldog appearance. Necropsies were performed on three calves including a clinically unaffected calf sacrificed prior to ingestion of colostrum, a 1-week-old calf with limb abnormalities, and a 1-week-old calf with swollen joints. In all animals, the ribs had increased pliability and were easily broken. In addition to the clinically abnormal stance, the affected calf had midshaft fractures of the first seven ribs of the right side, which were surrounded by variably dense fibrous callouses. Growth plates of long bones of all animals were grossly unremarkable. Histologically in the affected animals, there was overall a decreased thickness and prominence of cortical bone and medullary trabeculae, and the physeal zone of hypertrophy was markedly shortened or absent. Cartilage cores within primary and secondary spongiosa were often thick and retained deep into the metaphysis of long bones. In regionally extensive areas of the diaphysis of multiple bones including ribs and long bones, there was evidence of chronic fractures characterized by regions of fragmentation and loss of osseous medullary trabeculae and replacement by dense fibrosis and islands of woven bone. Mineral analyses of the livers from each animal detected manganese levels ranging from 3.19-4.95 ug/g dry weight (reference interval 5.50-15.00 ug/g). Manganese is essential for activation of glycosyltransferases that function in the generation of sulfated glycosaminoglycans of cartilage. While dietary imbalances or congenital anomalies affecting calcium and phosphorus balance associated with rickets in growing animals can cause similar limb deformities and predispose animals to fractures as was seen in this herd, there are clear differences between the classic presentations of rickets and manganese deficiency-associated skeletal abnormalities. Nutritional forms of rickets are progressive with age and not typically associated with skeletal abnormalities at birth. Congenital forms of rickets can result in physical abnormalities at birth; however, the skeletal abnormalities of manganese deficiency are generally described to be more varied. Most importantly, manganese deficiency produces a thinning of physeal cartilage associated with a narrowed zone of hypertrophy such as was seen in these animals, in contrast to rickets where there is expansion of the physeal cartilage and retainment of tongues of cartilage into the metaphysis. The cause of manganese deficiency in this herd is unclear; however, the dams of these calves had been given calcium chelators raising the concern that manganese may have been inadvertently bound and not bioavailable.

Determination factors associated with the mercury-selenium ratio in cat food and tuna * † ◊

Qin Yuan, Lakshman Balaji, Matthew Freer, Jennifer Moiseff, Karyn Havas, Karyn Bischoff

Cornell University, Ithaca, NY

Mercury (Hg) is a toxin that can have deleterious effects on humans and other animals. It arises from natural and anthropogenic sources; as an element, it does not degrade in the environment. Mercury bioaccumulates and biomagnifies in the aquatic food chain, thus people and other fish consumers are likely to have higher Hg concentrations, leading to the risk of neurotoxic and teratogenic effects. Numerous studies have shown the protective effects of selenium (Se) against Hg, as well as the usefulness of the Se: Hg molar ratio as the indicator of selenium's protective capacity. With more study, the Se: Hg molar ratio is a potential guide for risk assessments in fish consumers. However, there is little research on the Se: Hg ration in fish based wet cat food and tuna. In this study, we collected 42 samples (cans with unique lot numbers) of different brands of cat food and 30 samples (cans with unique lot numbers) of tuna from local grocery stores, and determined the dry weight, Se, and Hg concentrations. We are interested in whether the Se: Hg molar ratio is consistent in these specific food sources, and if there are implications on the effects of mercury on consumers. Linear regression will be performed to evaluate if the Se: Hg molar ratio is impacted by commercial brand, dry weight, measured Se and Hg concentrations, and, for cat food, type of fish involved. If necessary mixed effects will be included.

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

Development and validation of a method for quantification of Vitamin D in pet food

Vamsi Thiriveedhi[1,2], Michael Filigenzi[1], Robert H. Poppenga[1]

[1]California Animal Health and Food Safety Laboratory, University of California at Davis, Davis, CA; [2]Graduate Program in Forensic Science, University of California at Davis, Davis, CA

Vitamin D is an essential nutrient for dogs, important in regulating calcium and phosphorus. It is also highly toxic to dogs when ingested at levels above those required for proper nutrition. In late 2018, the FDA announced a recall of a number of different brands of dog food due to potentially hazardous concentrations of vitamin D. Few methods have been published for analysis of vitamin D in pet food, so there became an obvious need for such a method.

AOAC Method 2016.05[1] is a validated method for the analysis of vitamin D in infant formula. This method involves high temperature saponification of the sample, extraction of lipid-soluble components into isooctane, and derivatization of vitamin D with 4-phenyl-1,2,4-triazoline-3,5-dione. Extracts are analyzed using LC-MS/MS on a triple-stage quadrupole mass spectrometer. A stable isotope-labeled analog of vitamin D is spiked into samples prior to extraction to provide accurate and precise quantification. This presentation will discuss the extension of AOAC 2016.05 into the dog food matrix and results of the method validation process.

(1) Gill & Indyk. Journal of AOAC International, 101(1), 2018

Virology
Saturday, October 26, 2019
Room 556

Moderators: Suresh V. Kuchipudi and Ailam L. Lim

Symbols at the end of titles indicate the following designations:

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

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Detection of *Deer atadenovirus A* in vaginal samples from mule deer (*Odocoileus hemionus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) and potential transmission to offspring

Kayla Kauffman[1], Todd Cornish[1], Brant Andrew Schumaker[1], Kevin Monteith[2], Mathhew Hayes[2], Tayler Lasharr[2], Katey Huggler[2], Myrna M. Miller[1]

[1]Veterinary Sciences, Wyoming State Veterinary laboratory, Laramie, WY; [2]University of Wyoming, Laramie, WY

Young animals are the most susceptible to Adenovirus Hemorrhagic Disease (AHD) and the impact of AHD on recruitment is poorly understood due to the elusive nature of neonatal ungulates. Mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus nelsoni*) typically spend the first days or weeks of life hiding, isolated from potential conspecific reservoirs besides their dam. Dams that are persistently infected and intermittently shedding *Deer atadenovirus A* (previously *Odocoileus hemionus* deer adenovirus 1, OdAdV-1) could expose their neonates to the virus during that time. Recruitment studies on two mule deer and one elk herd in Western Wyoming provided us the opportunity to research perinatal transmission of OdAdV-1. Prior to this study, all three herds had experienced AHD related mortalities, and in 2015, 20% of neonatal mortalities in one mule deer herd were positive for OdAdV-1. We hypothesized that dams were transmitting the virus to their offspring at or near the time of parturition. We collected vaginal samples from dams prior to parturition when vaginal implant transmitters (VIT) were placed (n=136), and from VITs collected after parturition (n=114). Neonate samples were collected by swabbing the oral and anal cavities when tracking collars were applied (n=153), and necropsy samples were collected from recovered mortalities (n=74). The year of this study had a relatively low number of AHD related mortalities (n=7). At VIT insertion, OdAdV-1 was detected in one elk and 4 mule deer, and was also detected from VITs collected from 8 mule deer after parturition. OdAdv-1 was detected in 7/135 mule deer neonatal swabs, and from 3/14 mule deer does and 4/51 mule deer juveniles at necropsy. Correlations between dams and juveniles positive for OdAdV-1, and between antemortem and post mortem samples could not be established in this study. The low incidence of AHD mortality in the year of sample collection, intermittent nature of shedding from persistent carriers, and poor sample quality were limitations of the study. OdAdV-1 has rarely been detected in healthy individuals, though persistent infections are suspected. Our findings demonstrate that OdAdV-1 can be detected in vaginal samples from healthy dams prior to and at parturition, and may serve as a source of infection to their offspring.

Generation and characterization of heterohybridoma clones stably producing porcine immunoglobulin monoclonal antibodies to Foot-and-Mouth Disease Virus Serotype O1 Manisa

Chungwon Joseph Chung[1]*, Erica Martel*[2]*, Jacob Rutherford*[2]*, Jose Barrera*[3]*, Michael Puckette*[1]*, William Hurtle*[1]*, John Neilan*[1]

[1]DHS Science, PIADC, Greenport, NY; [2]PIADC Research Participation Program, Oak Ridge Institute for Science and Education, Greenport, NY; [3]PIADC, Leidos, Greenport, NY

Using an improved protocol, heterohybridoma clones producing porcine immunoglobulins specific to Foot-and-Mouth disease virus (FMDV) were generated following chemical fusion of murine myeloma (Sp2/0) cells with splenocytes obtained from a BEI-inactivated FMDV (O1 Manisa) immunized pig. FMDV-specific subclones (n=31) were further characterized *in vitro*. Of the 239 subclones tested, all stably produced (>20 passages) porcine immunoglobulins based on immunohistochemical staining (IHC) and/or indirect immunofluorescent antibody (IFA) assays using FMDV O1Manisa-infected LFPK-αVβ6 cells. FMDV-specificity was determined by western blot using whole FMDV and individual recombinant subunit proteins (VP1, VP2 VP3). Among the 59 subclones evaluated, six, two and one reacted to FMDV VP1, VP2 and VP3, respectively. Six VP1-specific heterohybridoma monoclonal antibodies recognized FMDV O1 Manisa as well as FMDV A24 Cruzeiro, but not FMDV Asia-1 Shamir or FMDV C3 Resende, suggesting limited cross-reactivity against other FMDV serotypes. Heterohybridoma subclone clonality was determined by reverse-transcription PCR-based typing (n=13) and sequencing (n=4) of porcine immunoglobulin heavy and/or light chain genes. All four sequenced clones had unique variable region sequences not reported in GenBank, and all 13 PCR-typed clones had a lambda light chain. This is the first known report on the generation and characterization of genetically stable heterohybridoma producing FMDV-specific porcine monoclonal antibodies.

First isolation and in vivo characterization of porcine circovirus 3 # †

Juan Carlos Mora-Diaz, Pablo E. Pineyro, Huigang Shen, Ganwu Li, Kent J. Schwartz, Bailey Lauren Arruda, Luis Gabriel Gimenez-Lirola

VDPAM, Iowa State University, Ames, IA

Porcine circovirus 3 (PCV3) has recently been identified as a putative pathogen in the U.S. swine herd, with a subset of infections resulting in stillbirths and mummies and multisystemic inflammation in perinatal and growing pigs. This study describes the isolation and characterization of PCV3 from three diagnostic case submissions to the Iowa State University Veterinary Diagnostic Laboratory from three different sites reporting weak-born piglets, abnormal piglets, or elevated stillbirths and mummified fetuses. Lung, heart, cerebrum, and kidney collected from two cases that consisted of perinatal pigs with multisystemic inflammation characterized by lymphocytic myocarditis and/or lymphocytic perivasculitis and gliosis in the cerebrum were used for virus isolation in pig kidney epithelial (PK-15) cells. In addition, tissues from a third case of stillborn or mummified fetuses positive for PCV3 and negative for other abortogenic pathogens were also used for virus isolation. PCV3 was isolated from each of the three cases. Virus production in cell culture was confirmed by qPCR, IFA, and RNA *in situ* hybridization. Eight full-length genome sequences of different passages of the three PCV3 isolates were determined using metagenomics sequencing: PCV3/USA/MO/ISU27734/2018, PCV3/USA/NC/ISU58312/2018, and PCV3/USA/IA/ISU44806/2018. Isolate ISU27734 was serially propagated in cell culture for 9 passages. All the genomes contained 2,000 nucleotides with two ORFs encoding the *Cap* and *Rep* proteins. Phylogenetic analysis based on both the complete genome and ORF2 sequences suggested that the serially propagated isolate ISU27734 had identical sequences at different passages and belong to genotype PCV3a-1, while ISU58312 and ISU44806 belong to genotypes PCV3a-2 and PCV3b, respectively. Following isolation, eight 6-week-old cesarean-derived, colostrum-deprived (CDCD) pigs were inoculated with 2 ml (1 ml intranasal and 1 ml intramuscular) of PCV3 ISU27734 (passage 6). Viremia was first demonstrated at 14 days post-inoculation (DPI) and was present in all pigs by 28 DPI. Neither clinical signs nor pyrexia were observed in any animal. This is the first description of PCV3 isolation from perinatal piglets and fetal tissue, and the first experimental inoculation of a PCV3 isolate in CDCD pigs.

AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

A novel astrovirus from a pig with non-suppurative polioencephalitis, Australia

Mark Hawes[1], Honglei Chen[2], Som Walker[2], Gemma Harvey[2], Brenda Van der Heide[2], David T. Williams[2], Debbie Eagles[2], Jianning Wang[2]

[1]Agriculture Victoria Research, Department of Jobs, Precincts and Regions, Bundoora, VIC, Australia; [2]CSIRO Australian Animal Health Laboratory, Geelong, VIC, Australia

Astroviruses are small non-enveloped, single-stranded viruses, with 6.2–7.8 kb RNA genome of positive polarity. The family *Astroviridae* currently consists of two genera: the genus *Mamastrovirus* and the genus *Avastrovirus*. Astroviruses can infect a wide range of mammalian and avian species, and are generally associated gastroenteritis. Astrovirus infections with central nervous system (CNS) involvement have been reported recently in mink, human, bovine, ovine, and swine hosts. Porcine astrovirus (PAstV) is found worldwide with variable prevalence among pig populations. So far five genotypes of porcine astrovirus (PAstV1–PAstV5) have been identified. PAstV infection has been detected in pigs with enteric, respiratory, as well as neurological diseases. The clinical importance and impact of PAstV are increasing.

In 2015, a neurological disease situation occurred in a pig farm with 50 6-week-old piglets, in Western Victoria, Australia. One piglet from two separate litters over a period of three months were affected. The clinical symptoms of affected piglets included lethargy, hind limb paresis, ataxia, and progressing to recumbence over 24 hours. Samples (blood, brain, and spinal cord) from one euthanized piglet were collected for disease investigation. Histopathological examination demonstrated lesions of severe sub-acute multifocal non-suppurative polioencephalitis in CNS (spinal cord and brain), suggesting possible viral infection. Real-time and conventional PCR assays for several suspected diseases, including Aujeszky disease, classic swine fever, malignant catarrhal fever, Hendra virus, porcine teschovirus, porcine sapelovirus, and flavivirus, were conducted with the samples. All tests were negative. Virus isolation was conducted using porcine kidney cells and Vero cells and no virus was isolated after three consecutive passages. Next Generation Sequencing (NGS) was subsequently carried out with the pooled spinal cord and brain. By using De novo metagenomic analysis, an astrovirus-like virus with full length of genome (5985 bp) was detected. Sequence analysis demonstrated that this virus is distinct from all known members of astroviruses. Phylogenetic analysis clustered this virus into bastrovirus group, a recently-identified lineage in astrovirus family. A quantitative RT-PCR (qRT-PCR) was then developed based on the sequences detected by NGS, and applied to the tissue samples from the affected piglet. High viral load was detected by qRT-PCR in spinal cord and brain. The findings indicate that the virus could be the causative agent of the disease in the piglet affected. Further studies are warranted to determine the pathogenicity and epidemiology of this virus.

What was old is new again: viral culture and nanopore sequencing for identification and characterization of novel viral pathogens

Kevin Lahmers[1], James Evermann[2], James B. Stanton[3], S Michelle Todd[1], Alexander Bradshaw[1], Caitlin Mason[1], Tanya LeRoith[1], Jessica Shiflett[2], Lorraine Tanaka[2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Maryland College of Veterinary Medicine, Blacksburg, VA; [2]WADDL, Washington State University, Pullman, WA; [3]Department of Pathology, University of Georgia, Athens, GA

Culture has been a mainstay of viral diagnostics in veterinary medicine. However, its application in routine diagnostic investigation has been supplanted by molecular techniques. This has been due to multiple factors, such as cost, required facilities, needed expertise, turnaround time, and lack of sensitivity when analyzing samples for non-culturable, or unexpected novel pathogens. Recent advances in deep sequencing may reinvigorate the value of viral culture in investigations of new and changing diseases. One of the challenges of deep sequencing for pathogen identification is enriching for the pathogen over the host and accompanying flora nucleic acid. In this study, we evaluated the ability of nanopore-based sequencing paired with viral culture to identify and characterize two viral isolates that could not be identified with traditional methods, as well as a third that was not fully characterized. Results to be discussed include characterization of a novel adenovirus in goats with respiratory and ocular disease, a bovine herpesvirus-4 like virus isolated from a bison respiratory disease outbreak, and epizootic hemorrhagic disease in bighorn sheep with respiratory disease. In addition to identifying the pathogen, correlation of the amount of genetic information allows for an evaluation of the virulence factors and phylogenetic differences with previously characterized viruses of veterinary importance.

Application and comparison of iSeq100 and MinION sequencing in identifying Avian Infectious Bronchitis Virus

Lifang Yan[1], Rebecca Mackey[1,2], Yi Candy Zhang[1], Alejandro Banda[2], Scott Baughman[2], Lanny Pace[1]

[1]Mississippi Veterinary Research & Diagnostic Lab, Mississippi State University, Pearl, MS; [2]Poultry Research & Diagnostic Laboratory, Mississippi State University, Pearl, MS

Avian infectious bronchitis virus (IBV) is an enveloped, positive sense, single-stranded RNA virus with an approximate size of 27.6 kilobases (kb). IBV causes respiratory and nephrogenic symptoms in broilers and reduces fertility and hatchability in layer birds. Different genotypes circulate in commercial and backyard poultry, and new genotypes are continually emerging in the field due to mutation and recombination. These alterations present challenges in designing a general primer set to genotype IBV isolates. Therefore, multiple sets of primers for IBV are currently used in our laboratory to partially amplify the S1 gene of IBV. However, due to their capability to mutate, it is not possible to amplify and sequence some isolates. Therefore, application of whole genome sequencing reduces turn-around time, increases the capability to identify new isolates, and differentiates wild type and vaccine strains.

IBV isolates used in this study were isolated using 9-11 day old embryonated chicken eggs, and RNA was extracted using Pathogen RNA/DNA kit (Thermofisher Scientific). RNA was reverse-transcribed to cDNA, amplified by PCR, and sequenced on iSeq 100. For MinION sequencing, a Poly A tail was added to the RNA, and the RNA was reverse transcribed to cDNA, which was then directly sequenced. Sequencing reads from iSeq100 were checked by FastQC and assembled by minia with optimal kmer to assemble the longest contig. Reads from MinION were filtered by filtlong and trimmed and assembled by minimap2.

Four IBV isolates were sequenced on iSeq 100, and different assembly methods were compared. Minia (https://github.com/GATB/minia) generated the best result with an optimal k-mer selected for each isolate. Assembly results indicated that one isolate with the complete genome size of 27.6kb was generated, one isolate with 19k, and two isolates with 24k. All four isolates share over 97.5% similarity with IBV serotype Arkansas DPI (Gen Bank Accession EU418976.1). One isolate was sequenced on MinION9.4, and the full genome was assembled, which shares a similarity of 97.64% with published strain CK/CH/LJL/130908(Gen Bank Accession KP868573.1) and shares 96.61% similarity with serotype Arkansas DPI. Within this study, both iSeq 100 and MinION generate comparable results. Therefore, whole genome sequencing can be used to identify isolates that are unable to be amplified by conventional PCR.

Development and validation of a new real-time PCR for African Swine Fever

Elodie Pautet, Sandrine Moine, Richard Conrad

Thermo Fisher Scientific, Austin, TX

Introduction:

African Swine Fever Virus (ASFV) is a notifiable, highly contagious disease of domestic pigs and wild boar that can cause enormous economic losses. The disease is widely endemic in many parts of Africa, in parts of Southern Europe and increasingly becoming a serious threat in Eastern Europe. As there is still no vaccine or treatment available, monitoring and controlling the disease is of outmost importance. We developed a new ASFV rtPCR assay and validated the assay against the French standard (U 47-600-2). These studies were performed in Lissieu, France.

Material and Methods:

For the development of a reliable, sensitive and specific rtPCR system more than 450 different ASFV sequences representing the p72 protein encoding region were aligned. The assay composes a duplex rtPCR including an internal control to ensure reliable DNA extraction. For extraction of viral DNA from field samples the MagMax Pathogen RNA/DNA Kit and the MagVet Universal Isolation Kit were validated.

In order to demonstrate the sensitivity and specificity of the new VetMAX™ ASFV Detection kit we performed internal and field studies that included animal infection experiments (INIA, Valdeolmos, Spain; CVI, Netherlands; Germany). In total about 1600 negative samples from ASFV free regions (Germany/ Spain) and 33 non-ASFV pathogens were tested to demonstrate specificity of the assay.

For validation of the sensitivity about 100 ASFV positive samples from Africa and Europe were tested. The assay efficiency and Limit of detection (LD_{PCR}) was determined by serial dilution of an artificial plasmid carrying a specific ASF sequence (pASF).

Results:

Test results on ASFV positive samples showed 100% sensitivity in all tested sample materials (blood, serum and tissue samples). The LD_{PCR} was determined to be 16 genome copies per PCR reaction (95 % confidence interval). The experimental LD_{PCR} was 5E+03 copies per ml in serum and 1E+04 copies per ml in blood. Test results demonstrated 100% specificity with 1600 negative samples and sample infected with non-ASFV pathogens, and 100% specificity with all positive samples tested.

Summary:

In conclusion, the VetMAX™ ASFV Detection kit meets all validation criteria of PCR characteristics and complete method required by the French standard (U 47-600-2). The kit helps enable control of the spread of the disease and monitors circulating virus outbreaks.

Regulatory requirements vary by country; products may not be available in your geographic area.

Detection and genetic characterization of porcine sapovirus from pigs with diarrhea in the U.S.

Huigang Shen[1], Leyi Wang[2], Ying Zheng[1], Anping Wang[1], Phillip Gauger[1], Jianqiang Zhang[1], Eric Burrough[1], Thomas Petznick[3], Ganwu Li[1]

[1]Iowa State University, Ames, IA; [2]University of Illinois, Urbana, IL; [3]Arkcare, Fairbury, NE

Sapovirus is a single-stranded positive-sense non-enveloped RNA virus belonging to the genus *Sapovirus* in the family *Caliciviridae*. Although sapovirus can cause enteric disease in pigs, it is often in mixed infection with other pathogens. Here we report the detection of a single porcine sapovirus infection in young pigs with diarrhea. There has been an ongoing problem with diarrhea in the lactation phase for more than 2 years in the farm. The pigs exhibited a pasty to occasional gravy-like diarrhea starting around 10 days of age. The diarrhea is self-limiting, but the pigs lost 1-2 lbs of expected weaning weight. Fecal and intestinal tissue samples were submitted to the Veterinary Diagnostic Lab at Iowa State University. Histopathological examination of five piglets revealed that 5/5 small intestines had mild to moderate villus atrophy, vascular congestion, and lymphocytic infiltration in the small intestine suggestive of an enteric viral infection. No porcine epidemic diarrhea virus (PEDV), porcine deltacoronavirus, transmissible gastroenteritis virus (TGEV), or rotavirus was detected from small intestines using real-time PCR. There was no significant bacteria growth in small intestines either. Next-generation sequencing (NGS) was directly applied to the fecal and intestinal tissue samples and a sapovirus was detected. A near complete genome of porcine sapovirus was assembled. The phylogenetic analysis revealed that this sapovirus was clustered within the sapovirus genogroup III. Further analysis of amino acid sequence showed that the sapovirus has a 2-aa deletion in VP2 protein as compared to the reference strain JJ259. A follow-up investigation was performed to examine the intestinal tissue samples, and additional 100 fecal swab samples from both clinical (50 samples) and non-clinical (50 samples) pigs using sapovirus real-time PCR. The results showed that all (5/5) the small intestines were positive for sapovirus PCR, with Cts of 17.2 to 21.6. For the fecal swab samples, the sapovirus PCR positive rate was 50/50 for clinical pigs and 32/50 for non-clinical pigs, with significantly ($p < 0.01$) lower Cts in clinical pigs (15.9 ± 0.59) than that in non-clinical pigs (35.8 ± 0.71), indicating significantly higher porcine sapovirus loads in the clinical pigs than the non-clinical pigs. Taken together, these results suggest that porcine sapovirus may be the sole aetiological agent of diarrhea in these pigs and future studies are required to better understand the pathogenicity and control of clinical disease associated with sapovirus infection.

POSTER SESSION

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Posters

Saturday, October 26 — Sunday, October 27

Ballroom A

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 109

Low Level exposure to Hydrogen Sulfide increases the pathogenicity of Influenza A Virus in swine # *

Cristina Marie Santana Maldonado[1], Phillip Gauger[1], Drew Magstadt[1], Amber Vegter[1], Denusha Shrestha[2], Chandrashekhar Charavaryamath[2], Wilson Kiiza Rumbeiha[1]

[1]VDPAM, Iowa State University, Ames, IA; [2]BMS, Iowa State University, Ames, IA

Hydrogen sulfide (H_2S) is a gas that affects the respiratory, cardiovascular and central nervous systems. H_2S present in animal production units and is an environmental stressor for both caretakers and pigs. Influenza A virus (IAV) is a zoonotic disease of public health significance. Both H_2S and IAV affect the respiratory system. IAV infection is a disease of economic importance as it lowers productivity in pigs. Environmental stressors can potentially increase disease transmission within intensive livestock operations. However, the effects of low-level H_2S exposure on the pathogenicity of IAV in pigs have not been investigated.

We hypothesized that repeated exposure to low concentrations of H_2S increases the pathogenicity of IAV. To test this hypothesis, thirty, 3-week old crossbred pigs were exposed to H_2S via inhalation at concentrations ranging from 0 to 50 ppm for 6 hours daily for 12 days; five controls were exposed to breathing air (BA) and were challenged with a placebo. Pigs were exposed either to BA or H_2S for 7 days before intratracheal and intranasal challenge with approximately $3x10^5$ $TCID_{50}$ H_3N_2 IAV(C) or given placebo (NC) on day 0. The H3N2 challenge IAV is a human-like virus that spilled-over from people into pigs in 2010 and has become the most common H3 clade circulating in swine The experimental design was as follows: BA/NC; BA/C; 0.5ppm/C; 5ppm/C; 50ppm/NC; and 50ppm/C. Pigs were monitored for changes in body weight, temperature and clinical signs. Following euthanasia, bronchoalveolar lavage fluids (BALF) were collected and the lungs processed for histopathology, among other endpoints.

Results showed that 50 ppm H_2S decreased growth rate compared to other groups. H_2S exposed and IAV challenged pigs exhibited more severe respiratory clinical signs compared to those in BA/C group. 0.5ppm/C pigs exhibited the most severe respiratory signs. Pigs in 50ppm/C group had the most severe pneumonia both grossly and histologically. H_2S, at any concentration in this study, demonstrated a trend of increased severity of lung lesions. Additionally, IAV titers in the nasal swabs and BALF were significantly elevated in some H_2S/C groups compared to BA/C, suggesting that H_2S exposure increased IAV [GPC[1] [SCM[2] viral load and nasal shedding at certain time points. Cytokine concentrations in the BALF were lowest in 50ppm/C suggesting a dose-dependent H_2S/IAV suppression of these cytokines. Interestingly, reactive nitrogen species, biomarkers of oxidative stress, were significantly increased in the 50ppm/C group compared to BA/NC. Overall, these results suggest that low-level H_2S increased the severity of IAV infection in pigs and that there is a complex interaction between IAV and H_2S in lung injury. Thus low level H2S commonly present in intensive swine confinement houses may negatively affect pig health by exacerbating respiratory tract infections.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant

Poster 110

A novel online metagenome-based pathogen detection tool # *

Sai Aravindh Sankara Narayanan[1,2]*, Andres Espindola*[1]*, Jerry Malayer*[1]*, Kitty Cardwell*[1]*, Akhilesh Ramachandran*[1,2]

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

Nucleic acid sequencing-based techniques are becoming commonplace in infectious disease diagnostics. Analysis of metagenome sequences can theoretically accomplish simultaneous identification of multiple pathogen types in a single procedure. One of the biggest hurdles for such an approach is the complexity involved in analyzing and interpreting enormous amounts of data. We report a new user-friendly online bioinformatics platform[BK1] that utilizes a set of curated pathogen-specific *in-silico* nucleic acid probes for easy detection of pathogen signatures in metagenomic data. The unique *in-silico* probes were designed using phylogenetic suffix tree based strategies by comparing the genome of the pathogen of interest to genomes of taxonomic neighbors. A modified NCBI-BLAST protocol was used for further curation of probes to ensure high pathogen specificity. Metagenome sequencing of clinical samples submitted to Oklahoma Animal Disease Diagnostic Laboratory was performed using the MinION (Oxford Nanopore Technologies) and iSeq100 (Illumina Inc.) sequencers. Successful detection of bovine respiratory pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, *Bovine Herpesvirus-1* and intestinal pathogens such as *Salmonella* sp. was achieved in lung and fecal specimens. Remote access to the platform was tested and rapid data upload was successfully achieved from international locations.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant

Poster 111

Identification and genomic analysis of inter-serotype reassortant epizootic hemorrhagic disease viruses in the United States

Leyi Wang[1], Saraswathi Lanka[1], Debbie L. Cassout[1], Nohra Mateus-Pinilla[2], Ganwu Li[5], William C. Wilson[6], Dongwan Yoo[3], Paul Shelton[4], Richard Fredrickson[1]

[1]Veterinary Clinical Medicine, University of Illinois, Urbana, IL; [2]Illinois Natural History Survey-Prairie Research Institute, University of Illinois, Champaign, IL; [3]Department of Pathobiology, University of Illinois, Urbana, IL; [4]Division of Wildlife Resources, Illinois Department of Natural Resources, Springfield, IL; [5]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [6]Agricultural Research Service, United States Department of Agriculture, Manhattan, KS

Epizootic hemorrhagic disease (EHD) is an insect-transmitted infectious disease which affects wild and domestic ruminants. In 1955, an outbreak with EHD was first described in New Jersey and has since spread throughout most of the USA. EHD infections occur in deer populations each year, typically from summer to late autumn, with varying degrees of severity. The etiological agent is EHD virus (EHDV), an orbivirus in the Reoviridae family whose genome consists of double stranded segmented RNA. Due to its viral genome, EHDV maintains viral fitness during infections by utilizing point mutations and reassortment strategies. In 2018, EHDV serotype 2 was predominantly detected in deer in Illinois at the ISU-VDL. Of 96 submissions, virus was detected in 16 with 14 identified as EHDV-2. Whole genome sequencing was conducted for two 2018 EHDV2 isolates (IL41747 and IL42218) and the sequence analyses indicated that IL42218 was a reassortant between different serotypes whereas IL41747 was a genetically stable strain. Our findings indicate the presence of multiple strains which may contribute to the occurrence of annual outbreaks in the USA.

Poster 112

Creation and validation of a novel real-time PCR for Cervid Adenovirus

Jennifer McKenna, Myrna M. Miller, Marce Vasquez

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

Cervid adenovirus hemorrhagic disease has been known to impact wildlife populations since 1993, when an outbreak in California resulted in over 1,000 mule deer deaths. The disease has caused epidemics in the western United States, Alaska, Iowa, and Ontario, Canada, and has affected mule deer, white-tailed deer, moose, and elk populations. In the wild, adenovirus is found primarily in deceased animals, as animals that recover from mild or subclinical disease are not easily identified or screened. Fawns seem to be the primary age group affected, and morbidity and mortality in this grown may significantly affect herd populations. While the Wyoming State Veterinary Laboratory has seen cases of adenovirus since the late 1990s, it is likely there have been others that were not diagnosed correctly due to less than ideal methods. Previously, virus isolation and fluorescent antibody staining with bovine reagents were the standard diagnostic tools. We have since developed, optimized, and validated a real-time PCR assay for diagnosis of adenovirus in cervids, as well as in pronghorns, and receive samples for testing from several agencies throughout the Rocky Mountain region, as well as from Washington and Alaska. This assay targets the RH2 gene, and does not detect bovine, ovine, or canine adenoviruses. Since implementing this assay, we have detected adenovirus in elk, moose, mule deer, white-tailed deer, and possibly pronghorns.

Poster 113

Virulent Newcastle disease in backyard exhibition chickens in Utah

Jane Kelly[2], David Frame[1], Arnaud J. VanWettere[1], Barry Pittman[2], Thomas J. Baldwin[3]

[1]Animal Dairy and Veterinary Sciences, Utah State University, Springville, UT; [2]Utah Department of Agriculture and food, Salt Lake city, UT; [3]Utah Veterinary Diagnostic Laboratory, Logan, UT

Three chickens (one hen and two cocks) were submitted to the Utah Veterinary Diagnostic Laboratory, Spanish Fork for necropsy on January 15th, 2019. They were from a small domestic flock of exhibition poultry (250 birds) in Utah County. The flock had been experiencing high mortality. Fifty to 70 birds had died. Clinical signs seen included nasal discharge and depression and death occurred in 2-3 days or sooner. Gross lesions included mucosal hemorrhages in the esophagus, proventriculus, intestine and cecal tonsils and serosal hemorrhages on the ventriculus and other organs. The history and gross lesions were highly suggestive of virulent Newcastle disease (vND). Antigen capture immunoassay test (FluDETECT™- Zoetis) for type A influenza virus was performed on cloacal swabs and was negative. Avian paramyxovirus serotype 1 (APMV-1) matrix gene and fusion gene were detected in tracheal swabs and kidney from all 3 birds by qPCR indicating that the virus was a velogenic strain of Newcastle disease. Samples (trachea, lung, and kidneys) were submitted to NVSL which confirmed the qPCR results and the diagnosis. Partial sequencing of the fusion gene performed at NVSL was consistent with a virulent Newcastle disease strain and the segment sequence is >99% similar to the virus strain causing the outbreak in California.

This is the first diagnosed case of vND in Utah. It was believed to be connected to the current outbreak in California because 3 of the birds at the premises were recently moved to Utah from Los Angeles county in California. The premise was quarantined and all chickens depopulated. There have been no further known outbreaks in Utah since January 2019.

Poster 114

Evaluation of pre-enrichment and pooling for direct detection of *Mycoplasma bovis* in milk samples

Gloria Gioia[1], Laura B. Goodman[2], Brittany D. Chilson[2], Renee Anderson[2], Carlos Santisteban[1], Paolo Moroni[1,3]

[1]Department of Population Medicine and Diagnostic Sciences - Quality Milk Production Service, Cornell University, Ithaca, NY; [2]Department of Population Medicine and Diagnostic Sciences - Molecular Diagnostics, Cornell University, Ithaca, NY; [3]Medicina Veterinaria, Universita' degli Studi di Milano, Milan, Italy

Mycoplasma infections constitute a significant concern for dairy and beef cattle industries, since they are associated with bovine respiratory disease, arthritis, keratoconjuctivitis, otitis and mastitis. Among all *Mycoplasma* species, *M. bovis* is recognized as the most important agent with higher prevalence, contagiousness, severe pathogenicity and antimicrobial resistance. *M. bovis* infections require rapid and accurate diagnosis to prevent and control disease outbreaks, however bacterial culture takes 5-7 days for growth and requires additional speciation testing. A number of molecular assays are now available for direct detection in milk, however the cost of this testing may be unaffordable for farmers. The goal of this study was to assess the sensitivity and specificity of pre-enrichment and pooling, in order to reduce costs.

Milk was obtained from routine diagnostic submissions either for screening or disease diagnosis. Detection by culture was conducted as per National Mastitis Council standards (milk streaking on modified Hayflick agar plates incubated at 37°C and 5% CO2). Pre-enrichment of milk samples in broth was assessed at 8 different spike levels (into negative milk) first with 3 different inoculum ratios (milk vs broth) and then with different incubation conditions including presence or absence of CO2 and time points up to 2 days. Pooling both before and after enrichment was tested with 5, 8, or 10 individual samples per pool. With the optimized conditions, milk submissions with different bacterial loads were assessed either with or without any enrichment. Two different automated extraction methods were also compared on spiked milk. Real-time PCR was carried out on ABI 7500-FAST system. The enrichment protocol established was in Mycoplasma Hardy broth for 30-32 hours with 5% CO2. The optimal inoculum ratio was 1:5 in the selective broth above mentioned. The optimal number of individual samples for pooling was 5. Pooling prior to enrichment had the best performance on milk with low mycoplasma counts. The optimized scheme was compared to direct detection without pre-enrichment of milk samples using both bacterial culture and Real-Time PCR. Pre-enrichment, prior to culture or DNA extraction, enhanced detection rates in terms of colony count and Ct values. Direct *M. bovis* detection without enrichment resulted in LoD of 10^2 cfu/ml for culture and 10^1 cfu/ml for qPCR, with a pre-enrichment step these values improve of 1-2 logs. However, this increased sensitivity has been mainly observed on fresh spiked milk samples in which the bacteria were mostly still viable. For 128 field samples tested after different storage conditions, the pre-enrichment advantages were less pronounced, possibly due to the dilution of non-viable bacteria in the original samples resulting in no further growth. Overall, results of *M. bovis* detection by real-time PCR were more consistent than culture in animals with low or intermittent shedding.

Poster 115

Recurrent gastrointestinal foreign bodies with disseminating gastrointestinal mycetoma in a dog

Shih-Hsuan Hsiao, Natalie M. Kirk

Veterinary Diagnostic Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL

An 11-year-old, castrated male, Golden Retriever dog was presented for necropsy after passed away naturally at home. Approximately 1 year prior, the dog had a gastrointestinal foreign body (a corn cob), which was removed via laparotomy. In the week prior, he was vomiting, febrile, lethargic and was treated with IV fluids and injectable maropitant (Cerenia). During postmortem examination, a large, poorly delineated, firm, mass-like structure was identified on the body of the stomach, which extended across the greater curvature into the greater omentum. A smaller, yet similar, mass-like structure was also noted on the pylorus of the stomach, extending along the lesser curvature into the lesser omentum. Duodenojejunal flexure was firmly adhered to the large stomach mass. Two segments of proximal jejunum were also adhered to the mass by fibrous stalks. Between the two stalks, a loop of the proximal jejunum was entrapped by a falciform ligament extending from the right medial liver lobe to the ventral abdomen. Adjacent loops of distal jejunum and ileum were also firmly adhered to one another. Additionally, a linear foreign body, composed of twisted bundles of fibrous material (a rope), was found in the descending duodenum, causing partial outflow obstruction of the pylorus. Histologically, both the stomach masses consisted countless, interspersed colonies of filamentous bacteria embedded in pools of lightly eosinophilic, amorphous material, forming large, grain- or granule-like structures. These structures were surrounded by rims of neutrophils and macrophages with abundant, fibrous stroma. Parts of the gastric submucosa and muscularis were effaced by the masses while the mucosa was mostly unaffected. Similar lesions were also found in segments of the duodenum and jejunum, particularly at the adhesion sites. The lesion was most consistent with a mycetoma, which is often caused by a member of order Actinomycetales, such as Actinomyces and Nocardia. An aerobic culture confirmed the diagnosis by recovering Nocardia sp. (most close to Nocardia arthritidis) from the stomach masses. The infection was probably opportunistic from repeat injuries by the foreign bodies.

Poster 116

Jamestown Canyon virus Isolated from horse swabs in South Dakota

Melinda Jenkins-Moore[1], Katherine Mozingo[1], Rachel M. Tell[1], Mary Lea Killian[2], Leo Koster[1], Kerrie Franzen[2], Alicia Janas-Martindale[1], Todd Tedrow[3]

[1]BPA, USDA/NVSL, Ames, IA; [2]Avian, USDA/NVSL, Ames, IA; [3]South Dakota Animal Industry Board, Pierre, SD

Jamestown Canyon virus (JCV) is in the genus *Orthobunyavirus* and is a member of the California serogroup. This zoonotic pathogen is transmitted by mosquitos and possibly by tabanid flies. White tailed deer are thought to be the primary reservoir in nature. In humans, symptoms range from runny nose, cough, fever, headache, and nausea to encephalitis. While considered a potential cause of equine encephalitis, there is little published data about clinical signs in JCV-infected horses. JCV has been found in Canada and at least 12 states in the U.S., including Minnesota, Wisconsin, Montana, and Wyoming. On July 14, 2018, a horse owner in northwest South Dakota noticed his horse was lethargic, off feed, and drooling. Two days later the owner noticed erosions in the oral cavity and contacted his veterinarian. The next day, the horse was deceased with effusion in its limbs and erosions at the muco-cutaneous junctions. Two swabs of oral lesions were taken and submitted to the National Veterinary Services Laboratories (NVSL) for vesicular stomatitis virus (VSV) foreign animal disease (FAD) testing. Initial rRT-PCR testing and a 24-hour immunofluorescent (FA) stain on the inoculated cell cultures were negative for VSV. At 7 days post inoculation, cytopathic effects were observed in the cells and the sample was repassed. Additional VSV FA staining of the isolate was negative. The isolate was then tested by PCR for influenza A, equine rhinitis A and B, and equine herpesvirus. All PCRs were negative. Particles resembling the size and structure of orthomyxovirus, bunyavirus, or bornavirus were observed with negative staining electron microscopy. The isolate was submitted for whole genome sequencing using the Ion S5 System. Results indicated 97-98% homology to all three segments of a 2004 JCV isolate (3324-04) from Connecticut. The virus was reisolated to confirm the results. This is the second report of Jamestown Canyon virus isolation from oral lesions in a horse.

Poster 117

A commercially available competitive ELISA for the detection of EDHV antibodies

Josh Blase, Mickael Roche, Muriel Malzac, Loic Comtet, Philippe Pourquier

IDvet, Grabels, France

Epizootic hemorrhagic disease (EHD) of deer is an arthropod-borne Orbivirus that causes infection in wild and domestic ruminants, in which EHDV may induce clinical and pathologic signs similar to those of bluetongue (BT), but can also be asymptomatic. Laboratory diagnosis is most often based on the detection of anti-EHDV antibodies in serum. This study presents validation data obtained for the **ID Screen® EHDV Competition ELISA**.

The **ID Screen® EHDV Competition** ELISA allows for the detection of anti-VP7 antibodies in serum or plasma. It is based on a VP7 recombinant protein as coated antigen and an anti-VP7 monoclonal antibody, HRP labelled, as conjugate. Results are obtained in 1h30.

*Test specificity was evaluated by testing s*erum samples from cattle (n=383), domestic deer (n=172), sheep (186) and goats (124) ; sample origin : France and Europe, collected before 2004 BTV outbreaks and tested negative for BTV using the ID Screen® Blue Tongue Competition ELISA kit. They are considered free of antibodies to Obriviruses. All samples were found negative with the ID Screen® EHDV ELISA, giving a measured specificity of 100.0% (CI95% : bovine [99,0–100%] ; domestic deer : [97.8-100%] ; sheep [98.0–100%] ; goat [97.0-100]).

Sensitivity was evaluated by testing 42 samples from deer, experimentally-infected with EHDV US serotypes 1 or 2. All samples were found positive with the **ID Screen® ELISA,** including samples classified as weak positive by the AGID technique.

Inclusivity was tested with a reference panel composed of 5 samples from serotypes 1, 2, 7, 8 (The Pirbright Institute – TPI-, UK), and samples from serotype 6 (ANSES, Maisons-Alfort, France). All samples were found positive.

Different seroconversion kinetics from experimental infections were tested; seroconversion was detected between 4 and 15 dpi.

Test exclusivity was assessed by testing samples from a reference panel raised again 21 different BTV serotypes (TPI, UK). While all samples were positive for BTV antibodies, 19 samples were found negative by the EDHV ELISA; and only one was doubtful.

Bovine (n=153) and ovine (n=67) field samples containing anti-BTV antibodies from natural infection and/or vaccination (origin: France and Europe) were tested. These samples had been found positive with the ID Screen® Blue Tongue Competition ELISA. 217 out of 220 samples were found negative, giving a exclusivity of 98.6% ($CI_{95\%}$: [96,1 – 99.5%]. These results demonstrate the excellent specificity of the ELISA, even in the presence of high levels of anti-BTV antibodies. However cross-reactivity cannot be excluded, especially with samples with high VP7 BTV titers.

The **ID Screen® EDHV Competition ELISA** is a robust, easy-to-use, highly sensitive and specific ELISA for the detection of EHDV antbodies, showing similar or better performance compared to AGID.

Poster 118

Molecular mycological detection and phylogenetic analysis of *Mycoleptodiscus indicus* infection in a cat * † ◊

Grazieli Maboni[2], Paula Krimer[2], Rodrigo de Paula Baptista[1], Ana Lorton[2], Suan Sanchez[2]

[1]University of Georgia, Athens, GA; [2]Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA

Mycoleptodiscus indicus is a dematiaceous hyphomycete fungus found on plant leaves. It has been reported rarely as a cause of human or animal disease, possibly because it is difficult to culture and identify from clinical specimens. Infections are presumably acquired by traumatic implantation of the fungus, with plants implied as potential sources of infection. *M. indicus* is typically found in immunocompromised human hosts causing subcutaneous lesions, cellulitis and sometimes myositis. Here we report a previously undescribed presentation of *M. indicus* causing a subcutaneous infection in an immunocompetent cat with successful antifungal treatment. An 8-year-old non-immunosuppressed cat from Georgia, USA, presented with a left front leg swelling without lameness. A fine-needle aspirate was collected from the swelling area and submitted for fungal culture. A mold with circular shape, white fleecy surface and a light beige reverse was isolated. After five days of incubation, colonies were brown with a diffusive brown-yellow pigment. Microscopically, the hyphae appeared septate and branched. Application of a pan-fungal PCR followed by Sanger sequencing of the PCR product revealed 99% homology to sequences from *M. indicus.* Phylogenetic analysis was performed to determine the possible epidemiological and ancestral correlation to other known cases of *M. indicus.* Our results indicate that the strain infecting the cat was closely related to *M. indicus* strains that infected a dog and humans in North America. In addition, these sequences were closely related to sequences from fungal isolates obtained from infected plants. This may support the hypothesis that this fungus, which is well adapted in plants, may have initially evolved from plants to adapt and infect different mammals. This also supports previous reports suggesting that plants were the potential source of *M. indicus* infection in humans. In summary, our findings highlight the potential of *M. indicus* to infect immunocompetent animals and suggest that the veterinary medical community should be aware of its unusual clinical and microbiological presentation.

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

Poster 119

Characterization of multidrug resistant *Histophilus somni* associated with Bovine Respiratory Disease

Caitlyn Deal, John Dustin Loy

University of Nebraska-Lincoln, Lincoln, NE

Bovine Respiratory Disease Complex (BRDC) is a multifactorial respiratory disease of feedlot cattle, causing great economic loss in North America. *Histophilus somni*, a bacterial pathogen associated with BRDC, has shown an increase in the number of isolates with elevated minimum inhibitory concentrations (MIC) to approved antimicrobial therapies. To combat the increase in resistance and reduce economic loss and to facilitate judicious treatment, rapid identification of isolates with enhanced likelihood for resistance is of critical importance. This study utilized a real time hydrolysis probe polymerase chain reaction (rtPCR) assay to detect macrolide and tetracycline resistance genes and to determine if they are present in *Histophilus somni* isolated from BRDC cases. Isolates were classified as susceptible or resistant based on MIC using approved breakpoints for tulathromycin and oxytetracycline. Susceptible (n=23) and resistant (n=22) *H. somni* isolates were tested for the presence of *erm(42), mrs(E), mph(E)* associated with macrolide resistance and the integrative conjugative element associated tetracycline repressor, *ICEtetR*, associated with tetracycline resistance. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) and Fourier-transform Infrared Spectroscopy (FTIR) were used to generate mass spectrum profiles (MSP) or infrared spectroscopic signatures (IR), Phylogenetic trees were generated using hierarchical cluster analysis (HCA) to visualize relatedness between susceptible and resistant populations on MSPs (Bruker Biotyper) and IR (Bruker Biotyper IR). rtPCR was successful in identifying the presence of resistance determinants in *H. somni*, with presence or absence of determinants in agreement with 80% (36/45) of MIC-determined susceptibilities to tulathromycin and 82% (37/45) to oxytetracycline. Agreement between resistant phenotype and genotypes were good with a kappa statistic of K=0.69 for both resistance determinants. MALDI-TOF HCA analysis of MSPs identified 4 major clusters consisting of susceptible and resistant isolates. Two clusters consisted of only susceptible isolates. One cluster had predominantly resistant isolates (14/17 isolates) and the remaining cluster was heterogenous (6 resistant/13 isolates). FTIR analysis identified two major clusters by HCA analysis, one of which consisted of only resistant isolates. In conclusion, tetracycline and macrolide resistance in *H.somni* appears to be associated with genetic resistance determinants, and that these phenotypes associate with identifiable clusters by MALDI-TOF MS and FTIR. Since these methods are rapid, cost effective and robust, these clustering strategies and MSP and IR signatures enable future test development to allow comparative analysis of strains that may be more likely to have resistant phenotypes in diagnostic workflows.

Poster 120

ONE HEALTH: Veterinary faculty perceptions in international public health

Ronaldo Magtoto

VDPAM, Iowa State University, Ames, IA

One Health: Veterinary Faculty Perceptions in International Public Health

Ronaldo L. Magtoto and PrecyDizon-Magtoto

Introduction

Animal health practitioners affiliated with land-grant universities in the United States have been providing technical assistance in the areas of disease prevention, control and management in developing countries.

The purpose of this study was to identify the perceptions and motivational factors affecting faculty veterinarians who were born from different countries regarding their involvement in the One Health as applied in international setting.

Materials and methods

A survey instrument with a Scoring Likert scales was used in this study. Both descriptive and inferential analyses were used. The descriptive procedure included frequencies, percentages, means and standard deviations. The software program Statistical Package for Social Sciences (SPSS) was used in the computer analysis of the data.

Results and discussion

Five highly significant differences were found on the sources of original motivation by country of birth. The U.S.-born respondents had a mean score of 4.07 on the item *because it is interesting and important work*. The non-U.S. born respondents had a mean of 2.57 on this item which was significantly lower than the mean score of the U.S.-born respondents. The U.S.-born respondents gave higher mean score of 4.05 on the item *a desire to provide humanitarian service to people in developing countries*. This mean score was significantly higher than the mean score of 2.50 given by the non-U.S.-born respondents. U.S.-born respondents were more *curious to see other parts of the world.* This was shown on the mean scores given to this item of 4.09 and 3.0 by the U.S.-born respondents and non-U.S. born respondents, respectively. Non-U.S.-born respondents had less *interest in knowing the problems of developing countries*. This was revealed by the mean score of 2.80 they had given compared to the mean score of 4.02 given by the U.S.-born respondents. The interest in the problems of developing countries is a better motivation for U.S.-born respondents than for non-U.S.-born respondents. U.S.-born respondents did not view their participation in international development activities as a *means to add to their income.* The non-US-born respondents looked at additional income as a motivation in participating in international development activities.

The results provided useful information to help maximize the contribution of faculty veterinarians can make to themselves, to their university, and to developing countries. It is also useful in policy formulation and strategic planning.

This study suggested tapping faculty veterinarians regardless of their country of birth to participate in international One Health efforts. A periodic assessment on the level of interest by way of a survey or other forms of inquiry can prove to be useful in strategic planning at the college level.

Poster 121

A universal approach to animal health sample preparation using the MagMAX CORE nucleic acid purification kit for laboratory use including veterinary and environmental uses

Quoc Hoang, Lillian Manley, Ivan Leyva Baca, Richard Conrad

Agribusiness R&D, Thermo Fisher Scientific, Austin, TX

In many animal health testing labs, the kit used for nucleic acid purification differs not only according to sample types and pathogens tested, but also to downstream applications. For an example, a lab may use different sample preparation kits for viral nucleic acid purification and bacterial nucleic acid isolation for detection by real-time RT-PCR, and an entirely different kit for genomic DNA extraction for sequencing applications. Using a single sample preparation kit to unify these applications would significantly reduce the confusion and result in fewer errors. Additionally, using a single product on a single sample extraction run with different sample types and different pathogens allows for improved efficiency and cost savings. The new MagMAX™ CORE Nucleic Acid Purification Kit was designed in order to meet these testing needs.

Here, we will show single extractions used for various applications. Extractions from ear notch tissues are tested for the presence of Bovine Viral Diarrhea Virus (BVDV), and the same extractions used for genomic DNA studies including fragment analysis by electrophoresis and next generation sequencing (NGS). Additional data will show a single run using the MagMAX CORE kit to detect a wide variety of pathogens from a wide variety of sample types. The data show that the MagMAX CORE Nucleic Acid Purification Kit matches or exceeds performance of currently-available magnetic bead-based isolation kits for both real-time PCR and sequencing applications, and demonstrates that the isolated RNA/DNA was of high quality, sufficient to be applied to a wide variety of molecular analysis techniques.

Poster 122

Animal poisoning in the Republic of Korea: 45 years of epidemiological data from the Animal and Plant Quarantine Agency

hyunkyoung lee, Soon-Seek Yoon, KyungHyun Lee, Eunjin choi, ByungJae So

Animal and Plant Quarantine Agency, Gimmcheon, Korea (the Republic of)

Animal poisoning has occurred in the Republic of Korea. However, the lack of reports about animal poisoning in the Republic of Korea makes it difficult for clinicians and diagnosticians to obtain information on poisoning cases. In this study, we gathered information about animal poisoning in the Republic of Korea. Animal and Plant Quarantine Agency (APQA) record database was used to examine the trends in animal poisoning. Data collected from January 1, 1974, to December 31, 2018 were retrieved and analyzed. A total of 358 animal poisoning cases were diagnosed in APQA from 1974 to 2018. Pesticides (237/358, 66.2%) and botulinum toxin (82/358, 22.9%) were responsible for most of the recorded cases. Several cases of salt, nitrate, lead and urea poisonings, a few cases of carbon monoxide, grayanotoxin, alcohol, acetic acid, arsenic acid, chloride and cyanide poisonings, were recorded. Animal poisonings occurred year round across the country, frequently in January and in Gyeonggi province. Birds (195/358, 54.5%), particularly wild birds, were more commonly reported as victims of poisoning than mammals. Cattle (109/358, 30.4%) were the species most frequently reported in poisoning of mammals. The majority of cases in birds and cattle resulted from exposure to pesticides (181/195, 92.8%) and botulinum toxin (67/109, 61.5%), respectively. Pesticides (27/29, 93.1%) constituted the primary group of toxicants in poisoning of companion animals. Pesticides are extensively used for the control of crop pests in agriculture and forestry. Pesticide poisoning of birds is presumed to be the result of exposures to pesticides used in crops or misuse of those in agricultural land. Botulinum toxin was reported as a major toxicant in cattle due to explosive botulism in northern Gyeonggi province from 2011 to 2012. Pesticide poisoning in companion animals was mostly caused by intentional exposure. Although the data presented in this study is a little, it will be helpful to understand the general trends of animal poisoning in the Republic of Korea. Knowledge of the common features of animal poisoning cases is crucial to any endeavor to reduce morbidity and mortality and it is therefore essential that collaborated and integrated efforts are made between countries to facilitate the flow and sharing of information to minimize the risks of accidental and deliberate poisoning.

Poster 123

Histopathological characterization and tissue distribution of pathogen in canine granulocytic anaplasmosis in the Republic of Korea

hyunkyoung lee, Seunghee Lee, Jung-Won Park, Soon-Seek Yoon, Hyun-Ji Seo, Jinhyeong Noh, Yun Sang Cho, ByungJae So

Animal and Plant Quarantine Agency, Gimmcheon, Korea (the Republic of)

Anaplasma phagocytophilum was detected in the blood of a shelter dog by PCR in a prevalence study on canine tick-borne diseases. The dog showed anorexia and lethargy when the blood was collected. One month later, the dog was dead and submitted for autopsy. On gross examination, pale gingiva, redness in lung and white spots in liver were also observed. Histopathologically, lymphoid depletion and a starry sky appearance in the spleen and lymph nodes, pyogranulomatous pneumonia and hepatitis and crypt necrosis in intestine were observed.
A. phagocytophilum was detected in all of the organs by PCR. PCR and phylogenetic analyses targeting the 16S rRNA, groEL, and msp2 genes of *A. phagocytophilum* revealed that the *A. phagocytophilum* identified in this study subgrouped into alanine and USA groups according to the groEL and msp2 gene sequences, respectively. Based on the authors' knowledge, this is first report showing histopathological characterization and tissue distribution in canine granulocytic anaplasmosis. The results of this study are meaningful, although the dog was also infected with canine parvovirus, canine calicivirus and *Streptococcus canis.*

Poster 124

Iba1 Immunohistochemical Evaluation of a Case of Transmissible Venereal Tumor: Further Support Against a Histiocytic Immunophenotype

Caitlyn Callaghan[1]*, Kristin Varner*[1]*, Karla Nova*[2]*, Kenneth Kim*[1]

[1]SDx Histopathology, San Diego, CA; [2]B Street Veterinary Hospital, San Diego, CA

Canine Transmissible Venereal Tumor (TVT) has classically been described to have a histiocytic immunophenotype. However, a recent study demonstrated that 100% of twenty-two TVT cases did not express lysozyme or CD18. In the present case, a 10-month-old female miniature schnauzer presented with vaginal masses. Histopathology demonstrated sheets of neoplastic round cells with features diagnostic for TVT. We performed immunohistochemistry for Iba1, a histiocytic marker. Neoplastic cells did not express Iba1 whereas single Iba1-expressing cells, interpreted as infiltrating macrophages, were scattered throughout the neoplasm. The findings in this case lend further support to refute the classically accepted immunophenotype of TVT, and begin to raise the question of whether histiocytic markers can be used as criteria to exclude a diagnosis of TVT.

Poster 125

New Real-time PCR Assays for Detection of Equine Herpesvirus 1 and Equine Herpesvirus 4 and Genotyping of Equine Herpesvirus 1

Zhenyu Shen, Michael Zhang, Shuping Zhang

University of Missouri, Columbia, MO

Equine herpesvirus 1 (EHV-1) and EHV-4 are common etiologic agents of equine viral rhinopneumonitis and abortion. A point mutation (A2254 to G2254) in the DNA polymerase gene of EHV-1 is linked to equine neurologic disease. EHV-1 shares approximately 85% nucleotide sequence similarity with EHV-4, and 86% to 96% homology with EHV-9 in several open reading frames. Although natural EHV-9 infection mainly occurs in wild equids, it has the potential to spread to domestic horses. The high sequence similarity between these viruses complicates laboratory diagnosis of EHV infections. The objectives of the present study were to develop a duplex real-time PCR (qPCR) for the specific identification and differentiation of EHV-1 and EHV-4 and a qPCR for genotyping of neurologic form EHV-1.

Analysis of the 38 EHV-1 and 1 EHV-9 complete genome sequences deposited in the GenBank revealed an EHV-1-specific region between the ORF 62 and 63. Alignment of 38 EHV-1 and 36 EHV-4 glycoprotein B sequences indicated a 5'-end region specific to EHV-4. Primers and probes for EHV-1 and EHV-4 were designed to target the aforementioned regions. EHV-1 and EHV-4 probes were labeled with FAM and CY5, respectively, to allow simultaneously detecting the two viruses. For the genotyping qPCR, a locked nucleic acid (LNA) modified probe was used to enhance the differentiation of G2254 from A2254. DNA fragments containing the amplification regions (gBlocks) were used as templates in qPCR development and positive controls in assay validation. To determine qPCR performance and analytical sensitivity, standard curves were constructed using 10-fold serially diluted gBlocks. Assay specificity was assessed using DNA isolated from EHV1 and EHV4 as well as other common equine pathogens. Validation of the qPCRs was conducted using EHV-spiked negative samples (equine nasal swabs, lung and other tissues) and 30 diagnostic cases.

The standard curve analysis showed an excellent linearity (R squared values are 0.9942, 0.9986, and 0.9945 for EHV1, EHV4, and EHV1 neurologic form, respectively). The analytical sensitivity of the duplex qPCR for both EHV-1 and EHV-4 was 1 and 10 copies per reaction for pure gBlocks and gBlock-spiked samples, respectively. The detection limit of genotyping qPCR was 10 copies per reaction for both pure gBlock and gBlock-spiked samples. The duplex qPCR was specific without cross reacting to *Actinobacillus equi*, *Klebsiella pneumonia, Rhodococcus equi, Streptococcus equi ss equi,* and *Streptococcus equi ss zooepidemicus*. Of the 30 diagnostic cases, 3 were positive for EHV-1 and 2 were positive for EHV-4, which were consistent with the results obtained using previously validated conventional PCRs. Two of the three EHV-1 positive samples were identified as neurologic form, which were confirmed by DNA sequencing. In summary, the assays developed in the present study are sensitive, specific, and suitable to detect EHV-1, EHV-4, and neurologic form EHV-1.

Poster 126

Effect of mismatch between extraction kit and viral nucleic acid type on PCR testing

Jin-Ho Kim, Baoqing Guo, Hanjun Kim, Kyoung-Jin Yoon

Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Extraction of enriched nucleic acid materials from clinical specimens is the first and important step for PCR-based detection of viruses or bacteria in the samples. There are numerous commercial products serving this purpose. In general, laboratory personnel selects an extraction kit based on the type of nucleic acid (i.e., DNA or RNA) of the intended target. Yet, the impact of mismatched use has not been evaluated. The following study was conducted to address this.

Commercially available RNA and DNA extraction kits manufactured by the same company were selected for the study using a 2x2 factorial design. The performance of these kits was evaluated on a set of serially diluted cell-culture derived swine poxvirus (SPV) and porcine epidemic diarrhea virus (PEDV) as DNA and RNA targets, respectively. Nucleic acids from both virus isolates were made using both DNA and RNA extraction kits concurrently and tested by both conventional and real-time PCR-based assays for amplification. PCR products were then analyzed by a capillary gel-electrophoresis instrument for imaging while real-time PCR (qPCR) results were determined based on the established cut-off Ct values.

While PEDV RNA was detectable by RT-PCR and RT-qPCR only when the RNA extraction kit was used, the viral RNA was also detected by RT-qPCR in an extract from the lowest dilution of the virus which was made by the DNA extraction kit. In contrast, SPV DNA was extracted equally by both DNA and RNA extraction kits as it could be detected by PCR. The RT-PCR did not negatively affect the detection of SPV DNA extracted by either of the extraction kits when compared to the result of the PCR on SPV DNA extracted by the DNA kit. Furthermore, the efficacy of DNA extraction by the RNA kit was similar, if not the same, to that of the DNA kit since no discernable difference in the detection of SPV DNA extracted from serially diluted virus materials was observed between the two kits.

The study results suggest that the impact of mismatch between viral nucleic acid type and extraction kit varies and the use of an RNA extraction kit may be required for RNA viruses. In some instances, use of an RNA extraction kit for both DNA and RNA targets can be economically beneficial for PCR assays, particularly a multiplex PCR assay devised to detect both DNA and RNA viruses at the same time, even though total nucleic acid extraction kits which can extract both DNA and RNA from samples are commercially available.

Poster 127

Marked panuveitis in a case of uveal schwannoma of a Blue-eyed dog

Kristin Varner[2], Caitlyn Callaghan[2], Megan Climans[3], Gillian C. Shaw[3], Kenneth Kim[2], Marcella Ashton[1]

[1]Animal Eye Vet, Inc, Murrieta, CA; [2]SDx Histopathology Inc., Carlsbad, CA; [3]Comparative Ocular Pathology Laboratory of Wisconsin, University of Wisconsin-Madison, Madison, WI

Uveal schwannoma is a spindle cell neoplasm that is typically seen in the iris of blue-eyed dogs. These neoplasms have been reported to variably express GFAP, and concurrent mild lymphoplasmacytic uveitis has been shown. In the present case a four-year-old, spayed female, blue-eyed husky/pit bull presented for change in eye color and buphthalmia OS. Clinically, iridal hyperpigmentation, lens subluxation, and refractory glaucoma were seen. Uveitis was also detected and refractory to topical glucocorticoids. Enucleation was performed, and histopathology demonstrated a neoplasm composed of spindle cells with numerous accompanying infiltrating lymphocytes and plasma cells in the iris. The inflammation also extended into the peripheral choroid. The neoplastic cells were immunoreactive for GFAP, but inflamed areas showed little GFAP expression. Approximately 60% of infiltrating lymphocytes expressed CD20 whereas the remainder expressed CD3. No additional underlying cause for the panuveitis was seen histopathologically. Further, glaucomatous changes and ectropion uvea of the pigmented iris epithelium explained the additional clinical signs. This case demonstrates that uveal schwannomas can be markedly inflamed. These neoplastic cells may be mistaken for fibroplasia and should be included in the differential for blue-eyed dogs with chronic uveitis and spindle cells in the iris.

Poster 128

Unique antimicrobial susceptibility patterns in animal-associated *Acinetobacter* spp. reveal high rate of multidrug resistance * †

Grazieli Maboni[1], Mauricio Seguel[2], Ana Lorton[1], Suan Sanchez[1]

[1]Athens Veterinary Diagnostic Laboratory, University of Georgia, Athens, GA; [2]Odum School of Ecology, University of Georgia, Athens, GA

Acinetobacter spp. are important nosocomial human pathogens due to their remarkable ability to acquire antimicrobial resistance. Knowledge about the clinical relevance and antimicrobial resistance profiles of *Acinetobacter* species of animal origin is limited. We used statistical modelling analysis to investigate a collection of *Acinetobacter* spp. isolates obtained from a veterinary diagnostic laboratory with regard to their occurrence in different animal species, clinical relevance, antimicrobial susceptibility profiles and multidrug resistance rates.

Seven *Acinetobacter* species were identified, including *A. baumannii*, *A. lwoffii*, *A. jonhsonii*, *A. radioresistens*, *A. haemolyticus, A. ursingii* and *A. junii*. The most common species was *A. baumannii* (83/226), and along with *A. haemolyticus* they were more likely to be isolated from blood samples, suggesting their involvement in systemic infections. *A. baumannii* also exhibited high rates of multidrug resistance (72%), and isolates from dogs were four times more likely to be multidrug resistant than other animal species, suggesting a possible role for domestic animals as reservoirs in the human environment. A comprehensive analysis of the susceptibility data identified resistance patterns that appeared to be associated with individual species of *Acinetobacter*. Further examination of the distribution of minimum inhibitory concentration (MIC) values clearly confirmed this observation, revealing that each *Acinetobacter* species presented a unique antimicrobial susceptibility profile. These results emphasize the importance of bacterial speciation and the need to determine specific breakpoints for antimicrobial susceptibility testing of *Acinetobacter* spp. of animal origin. Contrary to the intrinsic resistance predictions of the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing, our *A. baumannii* isolates were not resistant to certain antimicrobials that have been identified in human strains as intrinsically resistant. Therefore, we propose that veterinary diagnostic laboratories should cautiously review resistance guidelines when reporting antimicrobial susceptibility testing of *A. baumannii* isolated from animals.

In summary, this study provides novel information on MIC distributions and different antimicrobial resistant profiles of *Acinetobacter* species of animal origin, while highlighting the role of animals in the dynamics of multidrug resistance in bacteria. This study is a valuable source of information for further establishment of MICs breakpoints and antimicrobial susceptibility testing guidelines for animal-associated *Acinetobacter* species.

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

Poster 129

Development of two multiplex Real-Time qPCR (RT-qPCR) assays for detecting main pathogens associated with bovine respiratory disease complex (BRDC)

Christian Savard, Andre Broes

Biovet, Saint-Hyacinthe, QC, Canada

Bovine respiratory disease complex (BRDC) is one of the most economically significant diseases of cattle. Various pathogens, including both viruses and bacteria, are involved in the development of BRDC. Main viral pathogens commonly involved in BRDC include Bovine herpesvirus type 1 (BHV1), Bovine parainfluenza virus type 3 (BPIV-3), Bovine respiratory syncytial virus (BRSV), Bovine coronavirus (BCoV) and Bovine viral diarrhoea virus (BVDV). On the other hand, the most common bacterial pathogens include *Mycoplasma bovis (M. bovis)*, *Mannheimia haemolytica (M. haemolytica)*, *Pasteurella multocida (P. multocida)*, and *Histophilus somni (H. somni).*

Given the multiple agents associated with BRDC, the most appropriate method to detect them is Real-Time qPCR (RT-qPCR). Indeed RT-qPCR is faster, more sensitive and specific than traditional virologic and bacteriological methods. Moreover, its multiplexing capacity allows simultaneous detection of several agents at the same time allowing to reduce the cost of testing. We have developed eight singleplex and two 4-plex TaqMan RT- qPCR for detecting the main viruses (BHV, BRSV, BPIV-3, and BCoV) and bacteria (*M. bovis*, *M. haemolytica*, *P. multocida,* and *H. somni*) involved in the development of BRDC. Each individual singleplex reaction was optimized separately and subsequently multiplexed. Assay sensitivity and specificity were assessed by testing clinical samples simultaneously with corresponding commercial singleplex RT-qPCR kits (EXOone qPCR, Exopol, Zaragoza, Spain) as reference assays.

We tested 125 nasopharyngeal samples for the viruses. The 4-plex viral assay detected 92,9 % (13/14), 100% (30/30), 92,3% (12/13) and 100% (29/29) of the BHV1, BRSV, BPIV-3, and BCoV positive samples, respectively. On the other hand, 99,1 % (110/111), 92,5% (88/95), 100% (112/112) and 84,4% (81/96) of the samples which tested negative for BHV1, BRSV, BPIV-3 and BCoV, respectively were also negative in the multiplex assay.

Moreover, we tested 57 nasopharyngeal samples for the bacteria. The bacterial 4-plex assay detected 100 % (17/17), 96% (24/25), 96,7% (29/30) and 100% (13/13) of the *M. bovis*, *M. haemolytica*, *P. multocida* and *H. somni* positive samples, respectively. On the other hand, 97,5 % (39/40), 100% (32/32), 92,6% (25/27) and 95,5% (42/44) of samples which tested negative for *M. bovis*, *M. haemolytica*, *P. multocida* and *H. somni*, respectively were also negative in the multiplex assay.

Our preliminary results suggest that the two 4-plex RT-qPCR assays are sensitive and specific tools to detect the main viruses and bacteria associated with BRDC. Moreover they allow to significantly reduce the cost of testing for these agents. The discordant results with the reference singleplex assays concerned samples with low viral or bacterial loads. Additional results will be presented at the meeting.

Poster 130

Atypical porcine pestivirus (APPV) prevalence in the US swine herds

Yuan Feng, Fangfeng Yuan, Jianfa Bai, Lalitha Peddireddi

Department of Diagnostic Medicine&Pthobiology; Kansas State Veterinary Diagnostic Laboratory,
Kansas State University, Manhattan, KS

Atypical Porcine Pestivirus (APPV), a highly divergent emerging pestivirus, has a wide geographical distribution including Americas, Asia and Europe. APPV is known to cause neurological signs and type A II congenital tremors in newborn piglets. The main objective of this study is to investigate APPV prevalence in US swine herds utilizing a newly developed quantitative real-time RT-PCR (qRT-PCR) assay at Kansas State veterinary molecular diagnostic laboratory. The qRT-PCR assay was developed using all available sequences from GenBank as well as newly generated APPV sequences from diagnostic samples. After the test was fully validated, a retrospective study was conducted by screening of 1785 clinical samples submitted to KSVDL over a period of ~ 3 years (2016-2018). Data from this study revealed ~19% prevalence of APPV in the US swine herds. Of all the specimens tested, oral fluids are shown to have high viral loads, suggesting their use as reliable diagnostic specimen for APPV detection followed by serum, nasal swab and tissue. Among all the APPV positives, 82 samples are also tested positive for one or more swine pathogens such as PEDV, PRRSV, PCV2 or SIV suggesting APPV coinfections with other common swine pathogens. The virus has been detected in samples from pigs of all age groups indicating the possibility of persistent infection by APPV in infected pigs. Our qRT-PCR assay detected APPV in clinical samples submitted from Canada, which is the first report of APPV in this region. Partial NS5b sequences (9451nt-9837nt of the polyprotein gene) obtained form 45 APPV positive clinical samples revealed considerable sequence diversity, ranging from 85.8% to 100% nucleotide identity. In addition, two full-length sequences were obtained in this study demonstrated significant sequence diversity (~83% NS5B sequence identity) compared to the first APPV strain from US reported in 2014.

In summary, the qRT-PCR assay developed in this study identified ~19% APPV prevalence in in the US swine herds. Sequence diversity among APPV strains within the US provide a basis for our understanding of genetic diversity and molecular epidemiology of APPV in the field.

Poster 131

Pathology of cryptosporidiosis in raccoons (*Procyon lotor*) # + * †

Viviana Gonzalez-Astudillo, Mauricio A. Navarro-Badilla, Matthew F. Sheley, Francisco Uzal

California Animal Health and Food Safety Laboratory, University of California, San Bernardino, CA

Cryptosporidiosis is a protozoal disease of public health importance, caused by *Cryptosporidium* spp., a group of apicomplexan parasites that cause intestinal disease known to infect ~150 mammal species, including humans. *Cryptosporidium* spp. appear to have a strong host-adaptation and thus wildlife infections do not appear to contribute to human disease. Nevertheless, recent evidence suggests that genotypes known to be wildlife-adapted have caused human disease. In addition, some genotypes can be detected in numerous host species, especially when there is a common infection source (e.g. contaminated water). Studies describing the pathology and diagnosis of *Cryptosporidium* spp. infection in synantropic wildlife, such as the raccoon, are lacking. We therefore characterized the pathology of cryptosporidiosis in two juvenile raccoons with history of progressive illness (# 1), and upper respiratory signs, hyporexia and weight loss (# 2), respectively. Grossly, both animals had pneumonia, and animal # 1 had also evidence of diarrhea. Microscopically, both animals had small intestinal villus atrophy and crypt necrosis, as well as eosinophilic infiltration with intralesional *Cryptosporidium* spp., and pneumonia. In addition, animal # 2 had and encephalitis. Intra-nuclear and intracytoplasmic inclusion bodies were observed in pulmonary bronchiolar epithelium and macrophages of both animals and in neurons of animal #2; these tissues were positive for canine distemper virus (CDV) via immunohistochemistry (IHC). IHC for canine parvovirus, was negative in the intestine of both raccoons. *Cryptosporidium* sp. was detected by PCR targeting the 18S rRNA gene on mucosal scrapes of the small intestine of animal #1. The sequenced amplicon clustered with the *Cryptosporidium* skunk genotype. A retrospective search of the CAHFS database (1990-2019) produced eight raccoon cryptosporidiosis cases out of 318 raccoon submissions during that period. Seven of these cases were co-infected with canine distemper virus (CDV). To our knowledge this is the first description of the pathology of cryptosporidiosis in raccoons, including co-infection with CDV. The cause of crypt necrosis is still under investigation.

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

Poster 132

Prevalence and genotyping of Bovine viral diarrhea virus in cattle across Wisconsin

Ailam L. Lim, Kate Palmer, Donald C. Sockett

WVDL, UW-Madison, Madison, WI

Bovine viral diarrhea virus causes significant disease and losses for the dairy and beef cattle industries. Diagnostic screening for cattle persistently infected with Bovine viral diarrhea virus (BVDV) has been routinely utilized across Wisconsin. Prevalence of BVDV in Wisconsin is estimated using diagnostic data generated from the years 2009–2018 at Wisconsin Veterinary Diagnostic Laboratory. The BVDV persistent infection screening tests were done using ear notches, nasal swabs, whole blood, or serum samples. Potential persistent or acute infection cases were predicted based off the real time polymerase chain reaction results, which correlate to viral load in samples tested. Of 867,033 animals tested, 1.06% were tested positive for BVDV, and only 0.21% were potentially persistently infected. Representative samples from persistently infected animals were genotyped to understand the genetic diversity of the BVDV isolates in Wisconsin.

Poster 133

Confirmation of *Avibacterium Paragallinarum* by Next Generation Sequencing

Yi Candy Zhang, Rebecca Mackey, Martha Pulido-Landinez, JayKay Thornton, Lifang Yan

MVRDL/PRDL, Mississippi State University, Pearl, MS

Avibacterium paragallinarum (A. paragallinarum), a gram-negative bacterium, causes infectious coryza, a severe upper respiratory tract disease in chickens. All chickens are susceptible, although most natural outbreaks occur in pullets and layers, causing a significant economic impact. *A. paragallinarum* can be isolated from sinus exudate on chocolate agar, but phenotypic identification is challenging. Last year, three *A. paragallinarum* isolates were isolated and confirmed by partial 16S rDNA by MVRDL. However, these results were questioned by the client, whose sequencing results indicated that all three isolates were *Ornithobacterium rhinotracheale*. Whole genome sequencing was used for further confirmation.

A. paragallinarum were isolated from sinus exudate on chocolate agar incubated at 35°C for 48 hours. DNA was extracted with DNeasy blood & tissue kits (Qiagen). The library was prepared by using Nextera DNA flex library prep kit (Illumina). Two of three isolates (P18-4486 and P18-4633) were sequenced by using iSeq 100 (Illumina). Species identification was performed on Kmer-Finder (https://cge.cbs.dtu.dk/services/KmerFinder/), which compares number of co-occuring k-mers between the query sequences and the database template. Kmer-Finder can only be used to identify known pathogens. Three major parameters can be used to generate results. Score is the number of k-mers that map to the template or species within the database, query coverage indicates the percentage of k-mers from query samples to template, and template coverage reveals the percentage of k-mers from the template genome that is covered by k-mers from query samples. ResFinder3.1 (https://cge.cbs.dtu.dk/services/ResFinder/) was used to determine the resistance gene by comparing the query sequence with the database.

KmerFinder results of P18-4486 showed a score of 761319, 55.18% query_coverage and 93.77% template coverage compared to template NZ_CP034110.1 A. paragallinarum strain FARPER-174 chromosome. Additionally, it showed a score of 149357, 10.83% query_coverage and 27.16% template coverage compared to template NC_CP006828.1 Ornithobacterium rhinotracheale ORT-UMN88. Kmer-Finder recommends to use the best match. Comparing score, query coverage, and template coverage, the P18-4486 isolate is *A. paragallinarum*. ResFinder indicates that P18-4486 has two resistance genes, one is is Macrolide-lincosamide-streptogramin B(MLS) resistance gene erm(F), which is from position 1 to 699 and shares 99.86% identity with erm(F) accession no. M62487. The other resistance gene is tetracycline resistance gene tet(Q), which is from location 4475 to 6400 and shares 99.38% identity with tet(Q) accession no. L33696. KmerFinder-3.1 results for P18-4633 found one perfect match with NZ_CP034110.1 A. paragallinarum strain FARPER-174 chromosome with score 2185779, query coverage 91.26% and template coverage 93.88%. There was no resistance gene found from ResFinder.

Poster 134

Validation of MagMAX CORE nucleic acid purification of Porcine Reproductive and Respiratory Syndrome Virus

Tracy Lene Otterson, Phillip Blomberg, Elisabeth Edgerton, Julie Pilot, Lindsey Raymond, Elizabeth Rogers, Jennifer Voison, Michele Leiferman, Stephanie Rossow, Jerry Torrison

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

The University of Minnesota Veterinary Molecular Diagnostic Lab is a high-throughput testing facility that is responsible for isolating nucleic acid for the detection of various viruses and bacteria in clinical samples. This validation was conducted to compare the MagMAX-96 Viral RNA Isolation Kit with the MagMax CORE Nucleic Acid Purification Kit for porcine specimen types associated with PRRSV infection. The MagMax CORE reagents are more stable at room temperature and require fewer plastic consumables. An analytical sensitivity was first performed on selected sample matrices by spiking PRRSV viral isolates into serum, oral fluids, and tissue homogenates on three different occasions. Side by side comparisons were then run on clinical samples with known PRRSV status and clinical samples of unknown status to determine diagnostic sensitivity and specificity values. Each set of isolates and the diagnostic samples were extracted by the MagMAX-96 Viral RNA Isolation Kit and by the MagMax CORE. Sanger sequencing was also performed on clinical samples to determine downstream sequencing effects on CORE extracted nucleic acid. The results from the analytical sensitivity showed an increased sensitivity by one dilution factor for serum down to 0.018 $TCID_{50}$/mL for Type 2 PRRSV and 0.0001 $TCID_{50}$/mL for Type 1 PRRSV and oral fluids down to 0.18 $TCID_{50}$/mL for Type 2 PRRSV and 0.001 $TCID_{50}$/mL for Type 1 PRRSV. Analytical sensitivity for tissue homogenate sample types showed an increased sensitivity for the detection of Type 1 PRRSV down to 0.001 $TCID_{50}$/mL, and showed a comparable sensitivity down to 0.018 $TCID_{50}$/mL for Type 2 PRRSV. Two-hundred and fourteen tissue homogenate samples were extracted on both platforms and Ct values were compared and found to have a diagnostic sensitivity of 100% and a diagnostic specificity of 94.6%. Two-hundred and sixteen serum samples were compared and found to have a diagnostic sensitivity of 100% and a diagnostic specificity of 98.8%. Three- hundred and seventy seven oral fluids were compared and found to have a diagnostic sensitivity of 96.3% and a diagnostic specificity of 98.1%. When Sanger sequencing was performed on CORE extracted RNA, a decreased sensitivity represented by relative band brightness was observed when compared to RNA extracted using the MagMAX-96 Viral RNA Isolation Kit. These results confirm that the MagMax CORE extraction platform produces end point results equivalent to the MagMAX-96 Viral RNA Isolation Kit for the isolation of PRRSV from multiple sample types. Future work will need to be done to optimize the extraction to increase success in Sanger sequencing amplification from CORE extracted RNA.

Poster 135

Antiviral activity of synthesized IFN-λ3 and PEGylated IFN-λ3 against Bovine viral diarrhea virus *in vitro* # *

Molly J. Carpenter[1], John Arguello[2], Matthew Caldemeyer[3], Kirsten Reed[2], John W. Wallen[3], Christie Mayo[2]

[1]MIP, Colorado State University, Fort Collins, CO; [2]Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO; [3]Exalt Therapeutics, LLC, San Diego, CA

Bovine viral diarrhea virus (BVDV) is a complex and multi-faceted enteric disease that is embodied by a diverse range of clinical and sub-clinical symptoms and is estimated to cost the US cattle industry between $1.5 and $2.5B annually. BVDV affects beef and dairy operations by causing reproductive disorders (abortion, delayed calving, reduced fertility) and by reducing productivity by increased culling, morbidity, and mortality. Additionally, BVDV elicits immunosuppression in infected cattle and may increase pathogen virulence that further impacts vaccine efficacy. Interferons (IFN) are important cytokines in the initial defense against viral infections that inhibit virus replication in virus-infected cells and protect uninfected cells. A relatively new family of IFNs, IFN-l type three (IFN-λ3), has been identified in several animal species including cattle. IFN-λ3 is particularly active on, and specific for, epithelial cells of the gut and respiratory tract, which are the primary initial target cells for BVDV infection. Previous studies have demonstrated therapeutic potential of IFN-λ3 against viral diseases such as influenza and foot and mouth disease. To extend the pharmacokinetic (PK) profile of therapeutic proteins, a PEGlyation strategy can be applied in which the protein is conjugated to a poly-ethylene glycol (PEG) molecule of about 30K molecular weight. Colorado State University in partnership with Exalt Therapeutics sought to determine the biological activity of bovine IFN-λ3 (boIFN-λ3) and PEGylated boIFN-λ3 against BVDV *in vitro*. DNA synthesis of the known boIFN-λ3 sequence was performed by GeneScript. BoIFN -λ3 was cloned into standard bacterial expression vectors. Recombinant protein was produced, isolated and purified. Additional IFN-λ3 was conjugated to a PEG molecule to create a PEGylated form of boIFN-λ3. Four-fold dilutions of boIFN-λ3 and PEGylated boIFN-λ3 recombinant proteins were tested for their ability to inhibit virus replication in plaque reduction assays and cell viability assays using the bovine epithelial cell line MDBK. Results demonstrated significant plaque reductions in both boIFN-λ3 and PEGylated boIFN-λ3 in a dose dependent manner. The antiviral effects of boIFN- λ3 may have potential therapeutic applications in ameliorating the negative effects of BVDV in cattle.

AAVLD Trainee Travel Awardee
* Graduate Student Poster Presentation Award Applicant

Poster 136

Small ruminant necropsy diagnoses in Wisconsin 2009-2018

Lorelei Lynne Clarke, Elizabeth J. Elsmo, Doug Lyman, Kathleen Deering, Jaimie Miller, Phil Bochsler

Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI

In recent years, the Wisconsin Veterinary Diagnostic Laboratory (WVDL) has experienced an increase in the number of small ruminant necropsy submissions. A search of the literature did not find a recent survey of common diagnoses in small ruminant deaths in the Midwestern US. A retrospective search of archived necropsy reports from the WVDL from 2009 to 2018 found 369 sheep submissions and 639 goat submissions. Information recorded from each case including the type of submission (necropsy or fresh and fixed tissues), signalment (sex, age, breed), city of origin, date of submission, pathologic diagnosis(es) (as reported by the primary pathologist of each case), whether autolysis was noted in the necropsy report (Y/N), and specific etiology(ies) when found. Some animals had more than 1 diagnosis or more than 1 pathologic etiology involved. Between 2009 and 2018, there was a 152% increase in small ruminant necropsy submissions. Abortions and stillbirths were the most frequent age group submitted (30% of all cases) and also had the highest percentage of undiagnosed cases (41% of sheep, 45% of goats). Neonatal (1 day-2 weeks) goats were 4.2 times more likely to be diagnosed with enteritis than sheep of a similar age. Juvenile goats (2 weeks-3 months) were 2.0 times more likely to be diagnosed with pneumonia or enteritis than sheep of a similar age. Young adult (3 months-1 year) sheep were 10.0 times more likely to be diagnosed with pathologic parasitism (gastrointestinal, pulmonary, hepatic, or neural) than goats of a similar age. Adult goats (1-5 years) were 5.5 times more likely to be diagnosed with urinary tract disease than sheep of a similar age. The most commonly diagnosed etiologic agent of pneumonia was *Mannheimia haemolytica* in goats and *Pasteurella multocida* in sheep. The overall percentage of undiagnosed cases was 23% in sheep and 21% in goats. Autolysis was noted in the necropsy report in 23% of all cases and in 29% of undiagnosed sheep and goat cases (difference was not significant). Overall, this study indicates that sheep and goats are presenting in increasing numbers to our diagnostic lab, and while diagnoses are primarily of well-known entities, there are significant differences between the two species that can guide diagnostic workup. This study can also be used to guide the development of diagnostic tests and resource allocation at diagnostic laboratories that regularly see small ruminant submissions.

Poster 137

Prevalence of *Salmonella* serovars in submitted clinical samples of food and companion animals to Texas A&M Veterinary Medical Diagnostic Laboratory(TVMDL): Year 2014 to 2018 ◊

Owais Ahmed Khan[1], Jessica D. Monday[2], Russell H. Raleigh[3], Sonia W. Lingsweiler[4], Amy K. Swinford[5]

[1]Bacteriology and Serology, Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX; [2]Diagnostic Services, Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX; [3]Bacteriology and Serology, Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX; [4]Bacteriology, Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX; [5]Bacteriology, Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX

Salmonellosis is an economically important disease of food and companion animals of different age groups mainly of younger age. It has various clinical manifestations viz; fever, diarrhea, dehydration, anorexia, respiratory illness, septicemia, abortion and decreased production. *Salmonella enterica* is important zoonotic agents and the foodborne Salmonellosis is attributed to consumption of uncooked, contaminated meat and dairy products as well as in contact with Salmonella shedding animals.

The aim of present study was to determine the prevalence percentage of *Salmonella* isolates at TVMDL from submitted clinical samples of food and companion animals during years 2014 to 2018. Furthermore, the available serovar results from Salmonella NVSL serotyping were analyzed to examine the serovar diversity among studied species. Data were retrospectively collected from USALIMS on all accessions submitted into two TVMDL lab locations (Amarillo and College Station) for *Salmonella* isolation during years 2014 to 2018. These samples received from 15 states with the majority from Texas.

A total of 1946 (20.73%) *Salmonella* isolates were recovered from 9384 clinical samples. These samples were comprised of feces, fecal swabs, enteric tissues, and swabs from various organs. Of these samples, cattle 32.45% (1212/4946); horses 21.34% (503/2357); donkey and mule 19.35% (13/65); goat 3.6% (9/244); sheep 7.94% (10/126); Pig 23.20% (103/443); dog 8.25% (76/921) and cat 7.12% (19/267) were positive for *Salmonella* isolates. Out of 1212 cattle *Salmonella* isolates 1073 isolates were serotyped and 71 serovars were isolated, the top five serovars were Dublin (12.58%), Montevideo (12.40%), Heidelberg (10.72%), Typhimurium (8.38%) and Newport (7.82%). In horses out of 503 isolates 405 were serotyped, 51 serovars were isolated. The top five serovars were Newport (18.02%), Typhimurium (13.58%), Muenster (10.62%), Anatum (8.64%) and *Salmonella* ser. 4, [5], 12:i-. Similarly in pigs out of 103 *Salmonella* isolates, 86 were serotyped and 21 serovars were isolated, the top five serovars were *Salmonella* ser. 4, [5], 12:I (44.19%), Derby and Infantis (9.30%), *Salmonella* ser. 4, 12:i:- and Montevideo (4.65%). In dogs out of 76 *Salmonella* isolates 73 were serotyped and 29 serovars were isolated, the top five serovars were Newport (23.29%), Anatum and Rubislaw (8.22%), Heidelberg and Infantis (6.85%). In cats out of 19 isolates 18 were serotyped, 12 serovars were isolated, the top four serotypes were Newport and Typhimurium (16.66%), Enteritidis and Typhimurium var 5 (11.11%). In the rest of the species few serovars (<9) were isolated.

The above study will provide an overview of the prevalence of various *Salmonella* serovars in the studied species and diversity of serovars among *Salmonella enterica* species. The presence of serovars Newport and Typhimurium in cattle, horses, dogs and cats is noteworthy as these are of public health concern due to emergence of multidrug resistant (MDR) bacteria

◊ USAHA Paper

Poster 138

Phenotypic characterization, antibiogram and risk factors of Salmonella isolates from Chicken, Stool, Farm and Market's eggs in Jimma Town, Ethiopia * ◊

Diriba Taddese Legesse

Veterinary Medicine, Jimma University, Jimma Town, Ethiopia

Salmonella is the most important causes of foodborne illness globally. Foods of animal origin, especially poultry and poultry products, including eggs have been consistently implicated in sporadic cases and outbreaks of Salmonellosis. The objectives of this study was to carryout phenotypic characterization, antimicrobial susceptibility pattern and risk factors of *salmonella* isolates from chicken, stool, farm and market's egg in Jimma town. A cross-sectional study was conducted from January 2018 to September 2018 in Jimma town, on egg samples collected from farm and market, and on cloacae swab of laying chicken and stool of egg collectors from poultry farm. Samples were, processed, cultured and *salmonella* isolates were identified by OmniLog test. Phenotypically identified *salmonella* isolates were tested for antimicrobial susceptibility. Over all; 13(2.98%) of *salmonella enterica* species were phenotipically characterized out of 436 sample from farms egg content (n=83), farms eggshell (n=83), cloacae (n=83), stools (n=21) market eggshell (n=83) and market egg contents (n=83) at a rate of 2.4%, 0%, 2.4%, 9.52% 4.8 % and 3.6% respectively. From antimicrobial susceptibility tested isolates, 9(69%) displayed multidrug resistance. All the isolates showed susceptibility to Gentamicin, Kanamycin and Streptomycin. Lack of separating cracked eggs, not washing hand, eggs stay longer unsold, and mixing excreta with feed were associated risk factors for *salmonella* presence (p-value<0.05) at farm's and market's egg. The presence of drug resistant *salmonella enterica* within egg/and chicken can pose serious health problem. Good hygienic practices are important to reduce the risk of *salmonella enterica* contamination.

Key word: - *Antibiogram, Egg, Chicken, market, Salmonell*

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster 139

Lameness, medication, surgery, and exercise as risk factors for catastrophic musculoskeletal injury in racehorses

Peta Lee Hitchens[2], Ashley E. Hill[1], Susan Stover[3]

[1]California Animal Health and Food Safety Laboratory, University of California, Davis, CA; [2]University of Melbourne, Werribee, VIC, Australia; [3]Anatomy, Physiology, and Cell Biology, University of California, Davis, CA

Background: The rate of catastrophic musculoskeletal injuries (CMI) in racehorses is high in the United States compared to other countries. Few modifiable risk factors related to lameness, medication, and surgery history have been identified.

Objective: To detect management factors that increase risk of catastrophic musculoskeletal injury (CMI)

Methods: Cases were identified using racehorse necropsy data; matched controls were selected from horses that had participated in the same race or in the same training session in which the CMI occurred. Veterinarians of case and three matched control horses were invited to complete an online veterinary medical history survey. Associations between CMI and lameness, medication, surgery, and exercise history were evaluated using multivariable logistic regression.

Results: 146 TB and 17 QH surveys were completed. TB cases were more likely to show signs of lameness within the three months prior to death compared to controls. A high proportion of both cases and controls were administered medications. Unraced TB case horses were more likely to have been administered systemic medications compared to those that previously raced. TB cases were more likely to have raced with greater intensity during their career, but had eased off in the month preceding CMI. Indicators of lameness were associated with CMI.

Conclusion: The study provides information that can be used to aid in identification of horses at high risk for catastrophic injury, and management factors that can be modified to reduce the risk for all horses.

Poster 140

Equine cytokine and chemokine multiplex assays

Cassandra M. Guarino, Alicia Rollins, Heather Freer, Diane Kilts, Bettina Wagner

Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY

Development of antibody pairs for equine cytokines, chemokines, and other equine biomarkers through the Wagner Laboratory at Cornell University has allowed for the development of a set of assays that can detect and quantify these protein biomarkers in a variety of fluids from horses. This technology can improve the diagnostic assessment of clinical samples, allowing for better understanding of disease state, pathology, immune activation or suppression, and overall health status of a horse. Currently, the NYS Veterinary Diagnostic Laboratory (NYSVDL) offers a Cytokine 5-plex assay for quantification of IL-4, IL-10, IL-17, IFN-gamma, and IFN-alpha. This assay is frequently used for research purposes and shows promise for monitoring immune response to viral infection. In addition, the NYSVDL offers an assay to quantify sCD14, a soluble biomarker that has been used for the evaluation of different clinical conditions including septicemia in neonates, recurrent airway obstruction (RAO), gastrointestinal diseases, horses with clinical signs of endotoxemia, horses with EHV-1 infections or other respiratory infections, and allergic horses. Results indicate that serum sCD14 values can distinguish samples from horses with many of these conditions from those of healthy horses. Validation of antibody pairs for additional chemokine and cytokine analysis is currently underway to further improve the repertoire of biomarkers available for overall health and disease staging of horses.

Poster 141

Rapid sequence-based diagnostic characterization of African Swine Fever virus using Oxford Nanopore Minion sequence sensing device along with a companion analysis software tool ◊

Vivian K. O'Donnell, Frederic R. Grau, Gregory A. Mayr, Tracy L. Sturgill Samayoa, Kimberly A. Dodd, Roger William Barrette

Diagnostic Services Section, Plum Island Animal Disease Center/USDA/APHIS/FADDL, Greenport, NY

African swine fever virus (ASFV) is the causative agent of a severe and highly contagious viral disease of swine, that can have devastating economic consequences to the swine industry due to the high mortality rate and impact on international trade. There is no effective vaccine to control African swine fever (ASF), and therefore, efficient disease control is dependent on early detection and diagnosis of ASFV. The large size of the ASFV genome (~180 kB) has historically hindered efforts to rapidly obtain full-genome sequence.

Here we investigate the utility of the Oxford Nanopore MinION sequence sensing device to act as a rapid diagnostic tool when coupled with our novel companion software script; African Swine Fever Fast Analysis Sequencing Tool (ASF-FAST). This tool enables the user to evaluate the output data in real-time and generate genome assemblies. Viral genome sequences were generated from various ASFV infected samples, including virus grown in cell culture and blood samples obtained from experimentally infected pigs. Removal of methylated DNA increased the number of ASFV-specific reads in relation to total reads. When samples were enriched by removal of host methylated DNA, sequencing reads specific to ASFV were improved due to a modest shift in the ratio of virus to host nucleic acid. The ASF-FAST detected viral genomic sequence generated by the MinION within 6 minutes after the start of sequencing and provided sufficient sequence for complete genome resolution within 20 minutes. In our hands, generation of sequencing libraries ready to load into the primed flow cell, required less than 2 hours and 15 minutes from nucleic acid extraction and enrichment. These ASF targeted sequencing libraries supported rapid the generation of ASFV genomic data that leverages the near real-time data processing and data generation capability of this platform.

We show that the nanopore technology, when coupled with the ASF-FAST, is a promising diagnostic tool to support rapid disease surveillance and monitoring. The MinION is accessible to most laboratories at a reduced cost and does not require sophisticated infrastructure in the field. This work demonstrates the potential to expand sequencing capabilities to low-resource settings to support sequence-based diagnostics for detection and surveillance of ASFV.

◊ USAHA Paper

Poster 142

Fast and convenient nucleic acid extraction method for mastitis diagnostics

Quoc Hoang, Anne Quijada, Emeline Ripoche, Denisse Meza, Robert Sterling Tebbs

Thermo Fisher, Austin, TX

Identifying pathogens in mastitic milk traditionally requires culturing, which is labor-intensive and time-consuming. The advent of molecular testing provides a convenient alternative. The Animal Health group at Applied Biosystems™ Thermo Fisher Scientific offers an easy-to-use and fast nucleic acid extraction method for mastitis diagnostic.

MagMAX™ CORE Nucleic Acid Purification Kit is a universal magnetic bead-based separation system designed for rapid purification of high-quality DNA and RNA for downstream molecular analysis. The new MagMAX™ CORE Mastitis and Panbacteria Module is offered here in combination with the universal kit to aid in the processing of all bacteria, especially those important for the detection of mastitis in cattle and from fresh, frozen, or preserved milk samples. The protocol offers benefits such as minimal hands-on time and easy-to-use (no centrifugation and no aspiration steps), fast (less than 1 hour for 96 samples) and convenient with both automated (using the KingFisher™ Purification System) and manual purification methods.

Nucleic acid from milk samples coming from North-America and Europe were extracted using this new extraction method. The extracted DNA were tested for a total of 19 different species.

MagMAX CORE extraction method was validated on a broad range of mastitic milk field samples containing each of the target organisms, including gram-positive and gram-negative bacteria. It provides equivalent or better DNA recovery compared to the leading competitor with minimal extraction and reagent preparation time, to get faster diagnostic results.

Nucleic acid extraction is an important part of molecular testing and the MagMAX CORE Kit provides quality RNA and DNA for accurate results in order to ensure the efficacy of surveillance and control programs.

Regulatory requirements vary by country; products may not be available in your geographic area.

Poster 143

Mastitis diagnosis utilizing a turnkey solution from Thermo Fisher Scientific's Animal Health

Quoc Hoang, Robert Sterling Tebbs, Anne Quijada, Emeline Ripoche, Denisse Meza, Richard Conrad

Thermo Fisher, Austin, TX

INTRODUCTION

Identifying pathogens in mastitic milk traditionally required culturing, which is labor-intensive and time-consuming. The advent of qPCR testing provides a faster and much more convenient alternative. The Animal Health group at Applied Biosystems™ (AB) Thermo Fisher Scientific developed a complete mastitis solution.

MATERIALS AND METHODS

The Mastitis solution includes a new sample prepartio nmodule to be use with the MagMAX™ CORE Nucleic Acid Purification Kit for nucleic acid isolation. The MagMAX CORE kit offers benefits such as ambient-temperature shipping and storage, inclusion of all reagents in one box, and verified automated processing on the Thermo Fisher™ King Fisher™magnetic particle processor. The extracted DNA can then be tested with three different 4-plexed VetMAX™ MastiType PCR kits, on either the 7500 or QuantStudio 5 (QS5) series of AB Real-Time PCR Systems. Data analysis is performed with the VeriVet™ cloud-based software with a user-friendly graphical interface.

RESULTS

Detection of mastitic pathogens using the MastiType complete mastitic detection workflow were validated using a broad range of mastitic milk field samples. The field samples contained all target organisms detected by the VetMAX™ MastiType PCR kits (Micro4, Myco8 and Multi kits), and analysed by VeriVet software. This entire workflow takes about 3 hours and provides equivalent results on the 7500 and QS5 Real-Time PCR Systems.

CONCLUSION

Detection of mastitic pathogen infections needs diagnostic tools that are easy to handle and can provide rapid and accurate results in order to ensure the efficacy of surveillance and control programs. The Thermo Fisher Scientific mastitis solution is designed to meet these expectations.

Regulatory requirements vary by country; products may not be available in your geographic area.

Poster 144

Influence of technician on PRRSV OF ELISA test results * †

Alexandra Henao-Diaz[1], Anne Harrenstein[2], Ju Ji[3], Luis Gabriel Gimenez-Lirola[1,2], Jeff Zimmerman[1], Dave Baum[1,2]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA; [3]Department of Statistics, Iowa State University, Ames, IA

Introduction

Commercial PRRSV oral fluid (OF) ELISAs are considered to provide provide good diagnostic performance [1] and testing swine OF specimens for PRRSV antibody is a convenient way to evaluate the PRRSV status of pigs [2]. Like most serum ELISAs, PRRSV OF ELISA reactions are read as optical density (OD), then ODs are converted to sample-to-positive (S/P) ratios using a formula that uses plate negative and positive control ODs to standardize reactions across plates. The results (positive or negative) are then determined by comparing sample S/Ps to the kit cut-off (≥ 0.40). The objective of this study was to evaluate the variation in PRRSV OF ELISA test results when the same set of samples was tested by two trained technicians on the same equipment.

Materials and methods

Oral fluid samples ($n = 600$) from pigs of known PRRSV status were tested on the IDEXX PRRS OF Ab Test (IDEXX Laboratories, Inc.) by two trained technicians (T1 and T2). Quantitative (S/P) and qualitative (negative/positive) test results were compared by non-parametrical analyses.

Results

T1 produced higher S/P values than T2 (median S/P 1.993 vs 1.906; Wilcoxon, $p < 0.0001$) (Figure 1). In terms of qualitative results, the proportion of positive results was similar between T1 and T2 393/600 (65.5%) vs. 390/600 (65%); Cochran, $p = 0.18$) (Figure 2). Receiver operating characteristic (ROC) analysis detected no difference between T1 or T2 results in terms of diagnostic performance (ROC-AUC Pairwise comparison, $p = 0.41$). However, 2/168 (1.2%) negative samples were misclassified as positive by both technicians; 2/372 (0.54%) and 4/372 (1.07%) positive samples were negative by T1 and T2, respectively. (Figure 3).

Investigation into the cause of the quantitative differences in results revealed that T1 loaded the positive and negative plate controls immediately after the samples had been allocated to the plate, whereas T2 loaded the positive and negative plate controls before loading the samples (as recommended by the manufacturer). This difference in control incubation time affected control ODs and, therefore, all sample S/P calculations.

Conclusions and discussion

During any testing process, a variety of factors can affect results, e.g., sample handling, incubation time, temperature, and others. Veterinary diagnostic laboratories (VDLs) implement a variety of standard operating procedures (SOP) for sample handling, equipment calibration, etc., to control for these factors. This study found that even very small differences in performing a test can introduce a measureable impact on results. Although the deviation from the SOP did not affect the binary diagnostic results, it underlined the fact that test repeatability is dependent on attention to detail.

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

Poster 145

Whole-Genome Sequencing for detecting serotypes and virulence genes in *Haemophilus parasuis* isolates ◊

Nubia Macedo, Rodger Main, Ganwu Li, Orhan Sahin

VDPAM, Iowa State University, Ames, IA

Glässer's disease is considered one of the most prevalent bacterial diseases of nursery pigs, and it is caused by *Haemophilus parasuis* (HPS). PCR methods have been used to detect serotypes and virulence genes among HPS strains. More recently, considerable progress has been made in implementing whole-genome sequencing (WGS) as a routine diagnostic and typing tool for bacterial pathogens. The objective of this study was to compare the use WGS to detect HPS serotypes and a known HPS virulence factor, the virulence-associated trimeric autotransporter (*vtaA*) gene. Fifty HPS strains isolated between 2014 and 2017 by the Iowa State University Veterinary Laboratory (ISU VDL) were characterized by serotyping PCR and *vtaA* PCR. WGS was also performed to detect both serotype and virulence of strains based on the detection of different capsular genes and *vtaA* genes. These isolates were obtained from porcine clinical cases submitted to the ISU VDL, and were isolated mainly from pericardium (n=15), lung (n=13), pleura (n=7), joint (n=7), brain (n=5), nasal (n=2) and liver (n=1). Both methods yielded one serotype for each strain, which demonstrated a striking reduction in the number of untypeable strains when compared to methods that use standard serotyping antisera. Preliminary results showed that 8 and 11 different serotypes were detected by PCR and WGS, respectively. However, neither PCR nor WGS were capable of discriminating between serotypes 5 and 12, since no capsular polysaccharide gene could be confirmed to differentiate between these serotypes. The serotypes most commonly detected by PCR were serotypes 5/12 (22%), 1 (20%), 4 (14%), 7 (14%), 13 (12%), 2 (8%), 14 (6%) and 6 (4%), while WGS detected serotypes 5/12 (22%), 2 (18%), 4 (14%), 1 (12%), 7 (12%), 13 (10%), 14 (6%), and 6, 11 and 15 (2% each). Overall, there was a very good agreement (kappa coefficient=0.81) between PCR and WGS. There was 100% agreement between PCR and WGS for serotypes 4, 5/12 and 14. However, from 10 isolates classified as serotype 1 by PCR, only five were serotype 1 by WGS, while four were classified as serotype 2 and one as serotype 11. Moreover, three isolates classified as either serotypes 6, 7 or 13 by PCR, were classified as serotypes 1, 15 or 2 by WGS, respectively. When comparing the detection of a known HPS virulence factor (*vtaA*), 100% agreement between PCR and WGS was observed. PCR methods have been widely used to type bacterial pathogens. Nevertheless, these methods only detect particular genes and are unable to uncover new or rare changes in the genome. In this study, we showed that WGS is a powerful tool for identification of serotypes and virulence factors in HPS. Moreover, the comprehensive information provided by WGS will greatly enhance the monitoring of HPS strains and serotypes circulating in the herds. Utility of WGS for prediction of HPS resistance to antimicrobials is currently underway.

◊ USAHA Paper

Integrated security program considerations for Animal Laboratories

Ryan N. Burnette, Bersabeh Tigabu

Merrick & Company, Arlington, VA

Several biosecurity components greatly influence laboratory facilities and programs. The type of biological agents and the level of biosafety required to work with particular infectious agents dictates the architecture and the engineering of laboratory facilities. These include the type of building, airflow and filtration requirements, decontamination protocols, waste management procedures, and the level of security measures during normal and abnormal operations. The presence of animals in laboratory facilities brings unique considerations to the design and operation of laboratories to account for various factors, including biosecurity. These include the behavior, mobility, size, and number of animals, along with accommodations for animal care and housing requirements, in addition to any specific engineering controls. Laboratory animals are assets that present unique elements of laboratory threats and vulnerabilities, and as such, they require additional consideration when implementing a biosecurity program.

Integrated security planning (ISP) ensures protection of laboratory assets and establishes threat management, vulnerability analyses, biorisk assessment and management, and a tangible connection to other laboratory management systems. In designing ISP, lab-specific risk assessments, vulnerability analyses, and threat assessments should be conducted. Furthermore, the goals of ISP should be defined, and any gaps and vulnerabilities should be identified. An ISP implementation team should include supervisory and security staff, IT supervisory and technical staff, and line worker and support personnel. Integrating all these components and personnel is a requirement for success of the mission. This presentation will detail the unique biosecurity attributes of laboratory animal programs and provide attendees with insights into designing a site-specific ISP.

Poster 147

Genotypic and phenotypic antimicrobial susceptibility assessment of *Campylobacter jejuni* isolates associated with small ruminant abortions

Kris A. Clothier, Heather Fritz

California Animal Health & Food Safety Lab, U. C. Davis, Fairfield, CA

Campylobacter jejuni is the most common food-borne pathogen in the United States, infecting over 1.3 million people each year and producing protracted clinical symptoms including fever, cramps, and bloody diarrhea. Rare but serious sequellae to infection include arthritis, irritable bowel syndrome, and the autoimmune disease Guillain-Barré Syndrome which can result in long-term paralysis. Antimicrobial resistance in *C. jejuni* presents a serious risk to human patients, and as such CDC includes this organism as part of the annual National Antimicrobial Resistance Monitoring System (NARMS) surveillance program. In recent years, *C. jejuni* has emerged as an important cause of abortion in small ruminants, replacing *C. fetus* as the major abortifacient *Campylobacter sp.* in late-term sheep and goat abortion cases. Sporadic cases of *C. jejuni*-associated gastroenteritis have also been reported in livestock species, and shedding associated with these conditions represent a risk for zoonotic transmission. The purpose of the present study was to assess 21 *C. jejuni* isolates recovered between 2002-2019 from small ruminant abortion cases identified at the Davis branch of the California Animal Health and Food Safety Lab System for evidence of antimicrobial resistance using genotypic and phenotypic methods. Minimum inhibitory concentration (MIC) testing using the NARMS Sensititer panel indicated that resistance was infrequently identified against most antimicrobials, being undetected for azithromycin, clindamycin, erythromycin, florfenicol, gentamicin, and telithromycin; and rare in ciprofloxacin (4.7%) and naladixic acid (9.5%.) The exception was to tetracycline, with 90.5% of isolates identified as resistant. All of these isolates were positive for the *tet*O resistance gene. Additionally, all isolates were positive for the multidrug-resistance efflux complex and its regulatory gene *cme*R. An additional 47.6% of isolates contained the *oxa*-61 gene from the class D β-lactamase family of resistance genes. Results of the present study indicate that resistance to tetracycline is common in isolates associated with abortion in small ruminants and is conferred primarily via the *tet*O gene. Although these isolates represent a convenience sample and are not representative of all *C. jejuni* in small ruminants, the presence of tetracycline and β-lactamase resistance genes are concerning and warrant additional monitoring to assess zoonotic transmission risks from abortion cases.

Poster 148

Epidemiology and pathological characterization of Infectious Bronchitis Virus infection in California backyard chickens + * †

Omar antonio gonzales viera[1], Francisco R. Carvallo[1], Emily R. Blair[1], Rodrigo Gallardo[2],
Beate Crossley[3], Asli Mete[1]

[1]Anatomic Pathology, California Animal Health and Food Safety, Davis, CA; [2]Population Health and Reproduction, UC Davis, Davis, CA; [3]California Animal health and Food Safety, Davis, CA

Infectious bronchitis (IB) is a highly contagious disease of chickens. It is caused by a gamma coronavirus. The virus primarily infects the respiratory system, and infection usually causes increased mortality if complicate by secondary bacterial infection, especially *E. coli*. Nephrotropic strains of the virus infect kidneys and causes nephritis and mortality. Commercial poultry farms in the US and throughout the world have a high prevalence of infectious bronchitis virus (IBV), and vaccination is the primary preventive strategy. While raising backyard chickens has become a common practice, backyard chickens are typically not vaccinated for IBV. IBV has been consistently detected in backyard flocks in California over the years. The lack of information about the prevalence of IBV in backyard chickens in California, locations of affected premises, gross and histologic lesions, and the circulating strains in backyard chickens has instigated this study. Backyard chickens submitted to the California Animal Health and Food Safety Laboratory System (Davis and San Bernardino branches) for postmortem examination from January through March 2019 were included in the study. One set of trachea, kidney, and cecal tonsil from each chicken was fixed in 10% buffered formalin for routine histological examination and IBV immunohistohemistry(IHC). A second set of same tissues was frozen at -80°C for PCR test. Data collected from the diagnostic laboratory system included gender, age, breed, cause of death, necropsy findings, and whether IBV testing was done. The results of histopathology and immunohistochemical staining were recorded for each bird. The two laboratories received 203 chickens, of which 143 chickens were at Davis and 60 at San Bernardino. Initial analysis revealed 154 females, 24 males, and 25 of unspecified gender. Among the 203 chickens, 109 were >1year-old, 40 were <1-year-old, and 54 were of unknown age. The submissions were from 32 different counties. Seven chickens had interstitial nephritis and/or renal gout, two chickens had tracheitis, and two chickens had both nephritis and tracheitis. Histological characterization of the lesions and correlation of histologic findings with the IBV infection status in all the chickens are in progress. PCR and IHC tests are underway on additional suspected cases, based on histopathology findings. Moreover, select PCR positive samples from various counties will be sequenced to determine the IBV genotypes involved in these cases. Understanding the prevalence and epidemiology of IBV infection in backyard chickens, and determining the circulating virus strains will help in establishing a vaccination program against IB in backyard chicken flocks in california.

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

Poster 149

Implementation of automated procedures for whole genome sequencing

Melanie Prarat, Katherine Shiplett, Yan Zhang

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

In the past few years, next generation sequencing (NGS) technologies have become available for whole genome sequencing (WGS) analysis of bacterial isolates. WGS is increasingly used to support investigations of outbreaks in humans and animals. Whole genome sequencing uses a single test to yield information about antibiotic resistance patterns, serotype, virulence factors, quasispecies, etc. The accurate subtyping and subsequent clustering of isolates of a pathogen associated with an outbreak event is essential for successful investigation and eventual traceback to a specific source. The ADDL WGS laboratory is a State-funded laboratory and has been a member of FDA's Vet-LIRN and GenomeTrakr Networks since April 2016. In this capacity, the ADDL supports FDA foodborne investigations by performing WGS to determine which illnesses are part of an outbreak, differentiate sources of contamination, and link small numbers of illnesses, including geographically distinct illnesses that might not otherwise have a common source of the outbreak identified. WGS is performed on a weekly basis, with up to 36 bacterial isolates per run. Several automated nucleic acid extraction instruments are used in veterinary diagnostic laboratories, and many commercial high-throughput purification kits are available to produce high quality nucleic acid that can immediately be used for NGS library preparation. High throughput liquid handlers allow for hands-free library preparation. At the Ohio ADDL, we have experience using the QIAcube and ThermoFisher FLEX for automated nucleic acid extraction. Early evaluations of several high-throughput nucleic extractions kits are underway. Recently, an opentrons liquid handler was installed and is currently being validated for use. Automation of sample preparation and library preparation prior to sequencing can significantly improve efficiency while reducing human error and allowing analysts to perform other laboratory tasks concurrently.

Poster 150

Respiratory viruses identified in Western Canadian beef cattle by high throughput sequencing and their association with bovine respiratory disease # † ◊

Maodong Zhang[1]*, Janet E. Hill*[3]*, Yanyun Huang*[1,2]

[1]Veterinary Pathology, University of Saskatchewan, Saskatoon, SK, Canada; [2]Veterinary Pathology, Prairie Diagnostic Devices Inc., Saskatoon, SK, Canada; [3]Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada

Bovine respiratory disease (BRD) causes significant economic losses in western Canada despite viral vaccination and massive antimicrobial treatment. The pathogenesis involves interactions between bacteria, viruses, environment and management factors. Primary viral infection can greatly increase susceptibility of beef cattle to bacterial infection, and is thus a vital part of BRD pathogenesis. The objective of this study was to use metagenomic sequencing to characterize the respiratory viromes of paired nasal swabs and tracheal washes from western Canadian feedlot cattle, with or without BRD. A total of 116 cattle (116 nasal swabs and 116 tracheal washes) were analyzed. Based on results generated from MiSeq, Illumina, the presence of influenza D virus (IDV), bovine rhinitis A virus (BRAV), bovine rhinitis B virus (BRBV), bovine coronavirus (BCV) and bovine respiratory syncytial virus (BRSV) was associated with BRD. Agreement between identification of viruses in nasal swabs and tracheal washes was generally weak, indicating that sampling location may affect detection of infection. Subsequently, qRT-PCR performed on IDV for all 232 samples, the sensitivity and specificity of Miseq was 58.06% and 98.88%, respectively. Furthermore, Nanopore sequencing was applied to 19 selected IDV positive samples and the results showed 74% agreement of virus detection with those by Miseq and 84% with those by qRT-PCR. This study reported several viruses for the first time in Canadian beef cattle, providing a basis for further studies investigating candidate viruses important to the prevention of BRD. We also demonstrated that next generation sequencing is a powerful non-targeted diagnostic tool for virus detection.

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