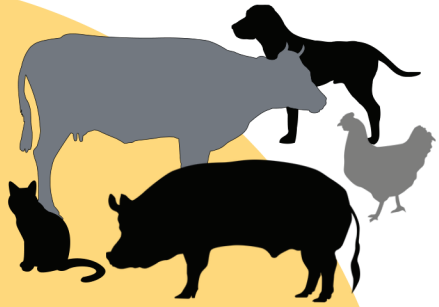
AAVLD 2022 ANNUAL MEETING

# TECHNOLOGICAL ADVANCES IN VETERINARY DIAGNOSTICS:

# WHAT'S HERE AND WHAT'S ON THE HORIZON?

65th Annual Meeting



**06-12**
**OCTOBER 2022**
Minneapolis, MN

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# AAVLD Strategic Plan

***Adopted 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.

# American Association of Veterinary Laboratory Diagnosticians

The American Association of Veterinary Laboratory Diagnosticians (AAVLD) is a nonprofit professional organization.

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# Acknowledgments

Each year many individuals contribute to make the AAVLD/USAHA meeting successful. We want start by recognizing the many students, post-docs, residents, diagnosticians, and researchers for submitting 223 abstracts of which 214 were accepted for presentation and provided rich scientific content for this meeting. We appreciate the efforts of both on-site and virtual attendees whose participation made this meeting possible. We would also like to give special recognition to our invited AAVLD plenary session speakers as well as the USAHA-AAVLD Keynote speaker for sharing their knowledge and experience with meeting participants. The support of all exhibitors and sponsors is gratefully acknowledged.

We give special thanks to the Program Committee members, abstract reviewers, and session moderators for their critical role in planning and successfully executing the meeting program. Their role is so important that their names are listed below for specific recognition. Any individual interested in contributing to next year's annual meeting in one or more of these areas should please reach out to the Executive Board and let them know.

On behalf of entire AAVLD executive team, we are grateful for the excellent administrative support provided by Reda Ozuna, the flawless meeting coordination provided by Kaylin Taylor, and general program oversight offered by Dr. David Zeman. Their efforts have ensured successful planning and execution of this event.

Sincerely,
Eric Burrough, DVM, PhD, DACVP
Keith Poulsen, DVM, PhD, DACVIM

**********

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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

## 2022 Trainee Travel Awardees

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*Oklahoma State University*

Rawal, Gaurav
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Serafini Poeta Silva, Ana Paula
*Iowa State University*

Sykes, Chelsea
*California Animal Health and Food Safety Laboratory*

## 2022 Awards Committee

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Dr. Deepanker Tewari

Dr. Keith Bailey

The AAVLD Foundation with generous contributions received from our members and committees, provides significant monetary support to advance our discipline and the development of the next generation of veterinary laboratory diagnosticians.

Thank you to all donors!

The AAVLD Foundation Committee

# AAVLD AWARDS

## 2021 AAVLD Organizational Awardees

E.P. Pope Award
**Patrick G. Halbur**

Distinguished Service Award
**Christina Loiacono**

Outstanding Performance Award for Diagnostic Services
**Margaret Steinman**

Life Membership Award
**P. Suzanne Carman | Neil Allison | Richard E. Mock**
**Harvey Fisch | Robert W. Fulton | Helen M. Acland**

Best Oral Presentation
**Megan Neveau**

Best Poster Presentation
**Haley Zeliff**

Best Recorded Presentation
**Nadia Saklou**

## 2021 AAVLD Committee Awards

Pioneer in Virology Award
**Edward J. Dubovi**

Richard L. Walker Bacteriology Award
Sponsored by Anaerobe Systems
**Treana Mayer**

J. Lindsay Oaks Best Student Molecular Biology Award
Sponsored by VMRD, Inc.
**Sai Narayanan**

Brenda Love Best Student Bacteriology Poster Presentation Award
Sponsored by bioMerieux
**Daniela Pena Hernandez**

Diagnostic Pathology Slide Seminar Resident/Graduate Student Award
**Michael J. Clayton | Kristen J. Hill-Thimmesch | Jeongha Lee**

## 2021 Joint AAVLD/ACVP Pathology Awards

AAVLD/ACVP Diagnostic Pathology Resident/Graduate Student Award
Awarded by AAVLD
**Rachael Gruenwald**

ACVP/AAVLD Diagnostic Pathology Resident/Graduate Student Award
Awarded by ACVP
**Ryan Yanez**

# 2022 AAVLD Plenary Session

## Technological Advances in Veterinary Diagnostics: What's Here and What's on the Horizon?

**Saturday October 8th, 2022, 8:00 AM-11:30 AM**

**Digital pathology in veterinary diagnostics - still cutting edge or already mainstream?**

**Dr. Aleksandra Zuraw** is a toxicologic pathologist at Charles River Laboratories and an online publisher at the Digital Pathology Place. After finishing her residency and PhD, she worked for a digital pathology company, which started a great passion for this discipline and resulted in the creation of the Digital Pathology Place website – a blog and podcast with digital pathology informational resources.


**Abstract:**

The capability to capture microscopic images digitally made available in the early 1990s, followed by the development of the whole slide scanning technology to digitize glass slides in the early 2000s, opened the door for digital pathology to enter both human and veterinary diagnostic practices. At the beginning, the adoption was very limited due to equipment costs, however with time the technology became faster, less expensive and more institutions started adopting it. Currently most of the reference veterinary laboratories offer digital anatomical and clinical pathology services. Recently a point of care model has been developed where practitioners can place a digital pathology imaging device at their clinics and get access to the expertise of a board certified clinical veterinary pathologist just by sending the scanned images of the microscopic samples they prepared during a patient visit. Because the diagnostic specimens are digitized, image analysis algorithms can be used to streamline and accelerate the evaluation and thus the diagnostic pathologists are equipped with powerful tools helping them do their job faster and more consistently.

The whole slide scanning technology advanced and became more available, but it is just one side of the digital pathology coin – the more expensive and more official one. There is another side – the one that can be done with tools available at every veterinary practice such as a microscope, microscope camera or a smartphone. The combination of those two sides of digital pathology makes it a technology that can meet different needs and reach a great penetration in veterinary diagnostics if the veterinarians are made aware of its benefits and of the different tiers of digital pathology that can be used according to their needs and budget.

**Machine learning for sequence classification and predicting antigenic phenotype**

**Dr. Tavis K. Anderson** is a Research Biologist at the National Animal Disease Center, USDA-ARS. As a computational biologist, he has studied the genetic predictors of influenza host range and virulence, developed methods that use sequence data to understand the genetic and antigenic variability of endemic viruses, and has studied how vaccines can be designed and applied to prevent virus transmission.


**Abstract:**

Genome sequencing has become a common task in veterinary diagnostic laboratories. A subsequent challenge is the integration of the sequences with complex data from public sources and locally inferred secondary data such as phenotypic or epidemiologic information to make informed diagnostic decisions. Under this paradigm, meaningful datasets for inference must be formed and this can be achieved across large volumes of data using in-database machine learning algorithms. After forming context datasets, machine learning approaches can be used to identify genetic variations of diagnostic significance and applied in targeted surveillance or in real-time genomic epidemiology. Analytical pipelines may also be generated that can assign classifications to genetic sequence data alongside visualization of identified sequence variants. These pipelines are intuitive and customizable and can be used by diagnosticians to develop trained prediction models that can run automatically and that provide accurate output faster than alternative methods. An extension of machine learning algorithms are models that predict antigenic diversity and drift from genetic sequence data. Models that predict virus antigenic characteristics from genetic sequence data can provide a fast and accurate method linking diagnostic sequence data to antigenic characteristics. Machine learning approaches can identify genetic features associated with virus classification, predict the antigenic novelty of a virus strain, and can be used to increase researchers' understanding of endemic and emerging viruses by helping them to quickly identify new viral variants.


**Twenty years of mass spectrometry in veterinary diagnostics: what's happened and what's ahead**

**Michael Filigenzi** is the Laboratory Manager for the Toxicology Section of the California Animal Health and Food Safety Laboratory at UC Davis, where he has worked for the last 22 years. Prior to that, Mike worked as a chemist, supervisor, and manager in contract environmental laboratories. Over his 40-year career, Mike has specialized in the analysis of organic chemicals using a wide variety of chromatographs and mass spectrometers.


**Abstract:**

Mass spectrometry is a bedrock technique in analytical chemistry and has a long history of use in veterinary diagnostic toxicology. The last twenty years have seen major advancements in the field which have had significant impacts on toxicological investigations. These include widespread adoption of liquid chromatography-mass spectrometry methods and the commercialization of benchtop high resolution

mass spectrometers. Additionally, the technique has begun to migrate into other disciplines in veterinary diagnostics, particularly with the use of MALDI-TOF instruments in bacteriology. This presentation will provide a short background of historical developments in mass spectrometry followed by the most significant advances of the last twenty years and how they've changed veterinary diagnostics. Some more recent advances in the field and how they might someday apply to the work we do in diagnosing diseases and toxicoses in animals will then be discussed. These include the use of MALDI-TOF systems for identification of botulinum toxin and the use of mass spectrometry imaging to explore the molecular composition of mounted tissue sections through histology-directed imaging. There are even mass spectrometers that are used in real time to identify different tissue types in the (human) operating room, which could have implications for veterinary diagnostics. Mass spectrometry techniques have significantly enhanced veterinary diagnostic capabilities and their continued evolution holds great promise for further improvements in this field.

**The past, present, and future of diagnostic veterinary microbiology: a forty-yearperspective of the field, and its intersection with research**

**Dr. Thomas J. Inzana** is a Professor and Associate Dean for Research at Long Island University College of Veterinary Medicine. He is a Board-Certified Clinical Microbiologist and formerly Professor and Section Head of the Clinical Microbiology Laboratory for the Virginia-Maryland College of Veterinary Medicine Teaching Hospital. He is the recipient of the 2022 Distinguished Microbiologist award from the American College of Veterinary Microbiologists.

**Abstract:**

Diagnostic microbiology, particularly veterinary diagnostic microbiology, has advanced tremendously over the past 40 years, and particularly over the past 10 years. Veterinary diagnostic microbiology, as well as human clinical microbiology, has progressed from most supplies and reagents being made in house, to the development of commercial reagents, biochemical kits and automated equipment, to antigen detection, gene sequencing and hybridization, PCR kits, MALDI-TOF, next generation sequencing, and RNA-sequencing. Nucleic acid sequencing may be particularly useful for identification of predicted antimicrobial susceptibility, as well as for microbial identification. Research has been responsible for the development of these and other advances (such as biosensors), but these innovations also contribute to future research, thus expediting the field logarithmically. During this session we will review how diagnostic veterinary microbiology and antimicrobial susceptibility testing has progressed over the past 40 years, with my perspective and examples of how research has progressed diagnostic microbiology, and also how the availability of clinical samples and needed understanding and innovations have driven research progress. Current and future innovations in diagnostic microbiology will require interdisciplinary, collaborative research with colleagues in other fields. Bioinformatics, artificial intelligence, digital engineering and modeling, sensor technology, and improved amplification and detection methods will advance the field, as the cost/benefit allows.

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## Plenary Session

Saturday, October 8, 2022

Nicollet Grand Ballroom



Symbols at the end of titles indicate the following designations:

§ AAVLD Laboratory Staff Travel Awardee

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**Digital pathology in veterinary diagnostics - still cutting edge or already mainstream?**

*Aleksandra Zuraw*[1,2]

[1]Charles River Laboratories, Frederick, MD; [2]Digital Pathology Place, Fairfield, PA

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*Tavis K. Anderson, Amy Vincent Baker*

Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA

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*Mike Filigenzi*

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

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*Thomas J. Inzana*

Veterinary Biomedical Sciences, Long Island University, Brookville, NY

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## Bacteriology 1

Saturday, October 8, 2022

Greenway DE

**Moderators:** Orhan Sahin and Chien-Che Hung



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**Evaluation of artificial neural network based classifier models to discriminate phenotypes of *Salmonella enterica* using Fourier-transform infrared spectroscopy (FT-IR). * †**

*Macy Rasmussen, Rodney Moxley, John Dustin Loy*

School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE

*Salmonella* spp. are associated with a broad range of infections in veterinary species and are a significant cause of human foodborne illness. The disease-causing potential varies across different serotypes and strains of Salmonella, and the zoonotic potential of emerging multi-drug resistant (MDR) Salmonella strains has caused concern. Fourier-transform infrared spectroscopy (FT-IR) is an emerging tool in clinical microbiology, allowing for rapid and cost-effective generation of spectroscopic fingerprints varying based on composition of bacterial cell wall and outer membrane. Machine learning algorithms can generate reproducible models allowing for classification of unknown strains or phenotypes of Salmonella from FT-IR fingerprints. Spectroscopic profiles were generated from 137 Salmonella strains of known serogroup, serotype, and species of origin. Samples were prepared using the manufacturer's recommended procedures with independent replication. Serogroups were determined by commercial assay using Remel Wellcolex kits, and serotype was determined by NVSL reference lab. A subset (n=100) was characterized by PCR to determine the presence or absence of genes conferring antimicrobial resistance (AMR). Classifier models were generated by selecting a phenotype of interest and using artificial neural networks (ANN) to analyze a training set of isolates and find discriminatory signatures in FT-IR spectrum. The classifier models were evaluated through application to a set of known test isolates and comparing the assigned phenotypes to the true phenotype. A serogroup classifier was generated from 118 strains of varying serogroups, serotypes, and species of origin. The training set contained 60 strains, and the test set contained 58, which closely approximated each other regarding strain diversity. The in-house generated classifier model had 89.6% agreement when predicting serogroups of the test isolates. We compared the in-house classifier to a model provided by the instrument manufacturer. When applied to our test isolates, the manufacturer's model had 94.8% agreement in predicting serogroup. The Kappa statistic for agreement between the two classifiers was 0.40 (fair agreement). Classifier models were also developed to identify MDR Salmonella isolates. These classifiers were developed by specifically analyzing the amide (1800-1500$cm^{-1}$), polysaccharide (1200-900$cm^{-1}$), fingerprint (900-700$cm^{-1}$), and mixed (1500-1200$cm^{-1}$) regions of the FT-IR spectrum. The classifier model generated from the amide and polysaccharide regions identified MDR Salmonella isolates with 86.27% agreement, 53.33% sensitivity, and 100% specificity. The Kappa statistic between the classifier-predicted and true phenotypes was 0.617 (substantial agreement). The results indicate FT-IR has the potential for use as a phenotypic classification tool for Salmonella spp., and further addition of strain diversity may enhance the ability of phenotypic discrimination in the classifier tools.

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

**Genetic Diversity of *Mannheimia haemolytica* isolates from bovine respiratory disease cases in Missouri * † ◊**

*Hatem Kittana[2,1], Anthony J. Ogunbadewa[2], Michael Calcutt[2], Tamara Gull[2], Solomon O (Wole) Odemuyiwa[2]*

[1]Veterinary Pathobiology, Texas A&M University, Canyon, TX; [2]Veterinary Medical Diagnostic Laboratory, University of Missouri-Columbia, Columbia, MO

*Mannheimia haemolytica* (MH) is the most frequently isolated bacterium from the lungs of cattle with bovine respiratory disease complex. Capsular serotype and expression of leukotoxin A (lktA) correlate with pathogenicity in MH isolates. We investigated the genetic diversity of MH isolates in diseased lungs of cattle submitted to the VMDL.

MH isolates were identified using MALDI-TOF and probed with primers specific for MH and lktA genes. Primers specific for serotypes 1,2, and 6 were used to type isolates. Isolates negative for lktA and MH were subjected to whole genome sequencing.

MALDI-TOF identified 95 isolates were identified as *M. haemolytica* and four as *M. varigena* (MV). Two isolates, *Salmonella enterica* and *Pseudomonas gessardii*, served as negative controls. Of 95 isolates identified as MH, 3 (3.2%) were non-hemolytic; two of four MV isolates were hemolytic. MH-specific primers confirmed 83 (90.2%) of hemolytic MH isolates (MH+). LktA gene was confirmed in 73 (88.0%) of MH+ isolates. Serotype-specific primers typed 24 (32.9%), 7(9.6%) and 14 (19.2%) of 73 beta-hemolytic LktA+ MH isolates as serotypes 1, 2, and 6, respectively. While 4 (5.6%) samples were not typable, serotype classification of 24 (32.9%) samples was ambiguous since they tested positive for both serotype 1 and 2. Twenty isolates that could not be unambiguously typed as beta-hemolytic, LktA-expressing, MALDI-TOF positive MH were further characterized using whole genome sequencing. Initial analyses showed that 14 of the isolates were *M. hemolytica* while 3 were *M. varigena*; 3 isolates were unclassifiable. Of the 20 isolates, 5 had multidrug resistance genes for five different groups of antibiotics. We identified plasmids and mobile genetic elements (MGE) that may play a role in horizontal gene transfer.

This study showed the presence of a heterogeneous population of *Mannheimia* species in lung tissues submitted to our laboratory from Missouri cattle with respiratory disease. The presence of MGE is a significant finding that may contribute to the spread of antibiotic resistance to other bacterial species.

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

**Whole genome sequencing analysis of enterotoxigenic *Escherichia coli* isolated from weaned pigs**

*Xiao Hu*

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

Recently, several cases of colibacillosis associated with E. coli F18-fimbrial antigen and leading to edema disease have been reported in swine production systems in the U.S. High morbidity and mortality have been related to these cases, despite the use of antimicrobial treatment and autogenous vaccination. Further characterization of diarrhea-related enterotoxigenic *Escherichia coli* **(**ETEC) is essential for implementing new strategies aiming to reduce the impact of the disease on pig production. In this study, whole-genome sequencing (WGS) analysis was performed for 58 enterotoxigenic ETEC isolates from weaned pigs to better understand the changes in virulence and antimicrobial resistance profiles over time. The isolates were selected from clinical cases submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) from 2016 to 2022. All were hemolytic on blood agar, about 98.3% of isolates were positive for LT, 89.7% for Sta, 96.6% for STb, 81% for Stx-2e, 87.9% for adhesin F18, 77.6% for adhesin gene *tia*, and 10.3% for K88. None of the isolates were positive for K99, 987p, or F41. Phylogenetic analysis based on the core genome SNPs divided the 58 isolates into 4 primary lineages. Lineage 1 included 6 isolates and most isolates from this lineage encoded 4 toxins (EastA, LT, STa, and STb) and adhesin K88; lineage 2 included three isolates, and these all encoded 3 toxins (EastA, LT, and STb) and adhesin F18; lineage 3 included 5 isolates and isolates in this lineage encoded toxin EastA, LT, and STb and F18; and lineage 4 included the other 45 isolates. Most isolates in lineage 4 were from 2021-2022 and encoded LT, STa, STb, Stx2e, adhesins F18, and Tia. Tia is a new adhesin that has not previously been reported in swine ETEC. While most isolates from 2016 to 2019 were clustered into lineages 1 to 3, these isolates did not express toxin Stx2e or adhesin Tia. Six resistance genes, including AAC(3)-IIa (aminoglycoside N-acetyltransferase), AadA (aminoglycoside 3″-O-nucleotidyltransferase), aph(3′)-Ia (aminoglycoside 3′-phosphotransferase), CmlA (chloramphenicol efflux MFS transporter), DfrA (trimethoprim-resistant dihydrofolate reductase), and Sul3 (sulphonamide resistant dihydropteroate synthase), were also mainly present in isolates from lineage 4. These data provide significant and timely insights into the molecular pathogenic makeup of contemporary ETEC isolates of swine-origin from the Midwest U.S. and may facilitate the development of potential means to mitigate severe outbreaks caused by this pathogen.

**Molecular typing of *Avibacterium paragallinarum* using next-generation sequencing * †**

*Meera Surendran Nair[1], Jasmine Morgan[1], Manoj Kumar[1], Lingling Li[1], Deepanker Tewari[2], Suresh Kuchipudi[1]*

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

Infectious Coryza (IC) is an acute upper respiratory tract infection of poultry caused by the gram-negative bacterium *Avibacterium paragallinarum* (AP) and causes significant economic losses to the poultry industry. Inactivated whole-cell vaccines prepared from circulating AP strains are primarily being used to prevent IC. However, there is limited cross-protection among different serovars, and a mismatch between circulating and vaccine strains could be a significant cause of sporadic IC outbreaks. Current diagnostic tools including conventional microbiology and molecular assays are not serovar-specific and do not differentiate the three major serovars (A, B & C) of AP. Additionally, we currently do not have the capability in the US to serotype AP. Therefore, developing methods for characterizing the genetic diversity of circulating AP strains is critical in preventing IC outbreaks.

The study aimed to establish next-generation sequencing methods to identify and characterize the recent outbreak serovars of AP. We performed a comparative whole genome analysis of 41 isolates collected from 2019 Pennsylvania IC outbreaks. Paired end reads (2 × 150 bp) were generated using the Illumina MiniSeq platform. AP genomes were assembled using Tychus and annotated with Prokka. Roary was used to obtain the pan-genome containing the entire gene repertoire shared by all isolates and nine reference genomes. Phylogenetic inferences were performed with the newly generated complete genome sequences and the AP reference and representative genomes obtained from the NCBI GenBank database. BreSeq analysis was performed to predict mutations in isolates compared to the closely related reference serovar genomes.

The genomic size varied among isolates ranging between 2.11 and 2.79Mbp. The total GC content was consistent across isolates, ranging between 40.71% and 41.55%. The three, core-genome, accessory-binary, and SNP-based phylogenetic trees defined three main clades that showed a differential isolate distribution. Four isolates were observed to be belonging to a divergent A Serovar clade. Another four formed the second clade related to, but not identical to the Serovar C reference genomes. Remaining isolates were observed to be 100% identical but grouped in a third clade separated from the known reference serovars. Our data suggest a much higher genetic diversity of AP isolates in PA than previously known. The pangenome analysis revealed a low number of core (7%), and an increased number of accessory genes (93%), indicating a higher-level heterogeneity among the isolates and references considered, highlighting the ‘open’ nature of the pan-genome. We further evaluated the genetic variability in antigenic regions identified using BreSeq among different serovars to develop a targeted amplicon sequencing method. This approach will help rapidly type AP isolates using state-of-art sequencing platforms, including Oxford Nanopore Minion.

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

**Distribution and characterization of *Streptococcus suis* strains of clinical importance within the US swine herd * †**

*Jessica Santos Streauslin[1], Kent Schwartz[1], Rachel Jean Derscheid[1], Drew Magstadt[1], Eric R. Burrough[1], Phillip Gauger[1], Loni L. Schumacher[1], Michael Rahe[1], Alyona Michael[1], Panchan Sitthicharoenchai[1], Christopher Siepker[1], Franco Matias Ferreyra[2], Marcelo Almeida[1], Rodger Main[1], Orhan Sahin[1], Bailey Arruda[1,3]*

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There is limited information concerning the distribution of relevant *Streptococcus suis* strains in the US swine herd. Herein, we compiled and analyzed a large collection of systemic *S. suis* isolates from diagnostic submissions from Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) from January 2015 throughout December 2019 using histopathology, serotyping, and whole-genome sequencing (WGS). Data collected per case included laboratory diagnosis, pig age, production site, state of sample origin, viral infection status, *S. suis* growth quantity, and contamination level. Around 2,100 porcine cases had *S. suis* isolated from the central nervous system (CNS) and histologic evaluation, of which about half of the cases had evidence of bacterial meningitis.

Five serotypes were the putative cause of meningitis: serotype 1 (17%), serotype 7 (14%), serotype 2 (11%), serotype 1/2 (9%) and untypeable by coagglutination (8%). The serotype distribution varied slightly by state: serotype 1 was the leading cause of bacterial meningitis in Missouri, Minnesota, and Indiana, serotypes 1 and 7 predominated in Illinois and serotype 7 predominated in Iowa. Sixty-five percent of the cases occurred prior to 7 weeks of age, where serotype 1 had the highest peak of cases at 3 weeks of age, serotypes 7 and 1/2 at 5 weeks of age, and serotype 2 had a peak at 6 and 8 weeks of age. *S. suis* isolates (146) were selected for WGS based on isolation frequency of the serotype, confirmation that the pig of origin was individually examined, growth quantities of *S. suis* versus contaminating bacteria, absence of other systemic bacterial pathogens, and PRRSV and/or IAV status. Using traditional virulence-associated genes (VAGs), the expected virotype (*epf+/mrp+/sly+*) was only identified in 29% of the isolates, whereas the most recently proposed virotype to predict virulence (*ofs+/srtF+*) was detected in 84% of the isolates. Most of the isolates that lacked the recently proposed VAGs (*ofs-/srtF-*) had an average nucleotide identity (ANI) below 95% when compared to the *S. suis* reference strain P1/7, indicating that these isolates were probably "*S. suis*-like" strains. The most common sequence types (ST) were ST1 (25%), ST28 (9%), and ST977 (7%).

Based on this data, strains associated with bacterial meningitis in US swine herds appear to be more diverse than previously thought. Importantly, the reported variable effectiveness of *S. suis* autogenous vaccines may be in part due to inappropriate strain selection (e.g., around 45% of porcine cases without any histopathologic evidence of bacterial meningitis still had *S. suis* isolation from the CNS). Accurate case definition, selection of appropriate pigs for sampling, sample collection, sample management, and the submission process, along with the proper interpretation of diagnostic assay results (site of isolation, bacterial growth, and histologic lesions), are crucial for accurate identification of true disease-causative strains.

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

**Avian tuberculosis in a trumpeter swan from a local park lake in the U.S. Midwest †**

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Avian tuberculosis (ATB), caused by *Mycobacterium avium* subsp. *avium* (MAA), is endemic globally. Although most avian species are susceptible to the infection, there have not been many case reports of ATB or surveillance for MAA in free-ranging wildlife in the United States. Here we report a case of ATB in a swan.

An adult female Trumpeter swan (*Cygnus buccinator*), found dead at a public park in Iowa, was submitted to Iowa State University Veterinary Diagnostic Laboratory in February 2022 for diagnostic work-up. The swan with inactive ovaries was in fair to poor body condition with fair post-mortem preservation. Gross pathology showed that the majority of normal liver parenchyma was displaced with coalescing granulomas. Similar abscesses were found in the spleen, on serosal surfaces of the intestines and the mesentery, and within the abdominal air sacs. Histopathological findings from the liver, spleen, mesentery and air sacs revealed that the caseous granulomas with a necrotic central core containing rod-shaped bacteria had numerous macrophages rimmed by concentric fibrous connective tissue, multiple lymphocytes, and plasma cells. Both gross and microscopic lesions were suggestive of ATB.

Ziehl-Neelsen staining demonstrated the presence of numerous acid-fast bacteria within the center of necrosis in the liver. PCR-based assays for *M. avium* complex (MAC) and MAA were done on nucleic acid extracts from the liver and cloacal swab. The liver tested positive for MAA DNA, while the swab was negative. For further confirmation, fresh tissue samples were submitted to the USDA National Veterinary Services Laboratories (Ames, IA). Acid-fast bacteria recovered from the gizzard and pool of liver and spleen. All the cultures except one from the gizzard were confirmed as MAA. Partial sequencing of 16s rRNA and RNA polymerase β-subunit (*rpoB*) gene supported that the isolate was phylogenetically close to MAC and MAA, respectively.

Knowing that the bacteria can cause disease in both wild and domestic birds and mammals and imposes a zoonotic risk, the diagnosis of ATB in a swan from a public park underscores the need for awareness of MAA circulation in wild birds and the environment. Further investigations may be desired to determine the prevalence of ATB in the wildlife of the United States.

† Graduate Student Oral Presentation Award Applicant

**Differentiation of *Edwardsiella ictaluri* and *Edwardsiella piscicida* isolates from fish using VITEK® MS System (MALDI-TOF technology)**

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The identification of unusual and/or difficult-to-identify microorganisms isolated from clinical fish specimens is challenging. In most diagnostic laboratories, bacterial pathogens isolated from fish accessions are identified based on phenotypic characterization.

This study evaluated the analytical performance characteristics of Vitek matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Vitek – MS -MALDI-TOF) to identify *Edwardsiella ictaluri* and *Edwardsiella piscicida* isolated from fish. The only species of *Edwarsiella* included in the Vitek MS System database are *E. tarda* and *E. hoshinae*. So, identification of other species requires a complete analysis using particular criteria and specific negative and positive controls.

A total of 96 isolates identified as *E. ictaluri* (n=52) and *E. piscicida* (n=44) kept in the bacterial collection of the MSU Aqua Lab were included in this study. Frozen *Edwardsiella* sp strains were inoculated to blood agar plates and incubated at 28 - 30 centigrades for 48 hours. After incubation, small (1mm), round, translucent white colonies with beta hemolysis grew from the suspected *E. ictaluri* isolates. The suspected *E. piscicida* isolates grew small to big (1-3mm), round white colonies with beta hemolysis even after reisolating colonies on blood agar plates.

All isolates were then run on the Vitek – MS -MALDI-TOF. A known *E. piscicida* and an ATCC *E. ictaluri* strains were used as positive controls. These results were then sent to the RUO/SARAMIS database for analysis. Once the spectra of the isolates were imported, the relative and absolute taxonomy were analyzed. Additionally, the results obtained from morphology characterization were compared with results obtained by bacterial 16S rRNA sequencing.

A total of 45 isolates were confirmed as *E. ictaluri* and 42 as *E. piscicida*. However, five isolates in each group also exhibited identification as *E. hoshinae* (99.9%) by Vitek - MS. Further WGS will be performed in these ten isolates to solve the identification conflicts.

**Evaluation of a lateral flow assay (NGTest® Carba-5) to detect carbapenemases produced by Enterobacterales Isolated from companion animals and in canine and feline feces**

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Carbapenemase-producing Enterobacterales (CPE) can spread rapidly among veterinary patients, as demonstrated by several recent animal hospital outbreaks. Strategies to rapidly detect colonized animals are needed to implement infection control measures. The NGTest Carba-5 (NGBiotech, Paris, FR) lateral flow assay (LFA) has been used to detect 5 major carbapenemase enzyme families (KPC, NDM, VIM, IMP and OXA-48-like) produced by CPE isolated from humans. The NGTest Carba-5 LFA can be combined with a cartridge filter (DetecTool, NGBiotech, Paris, FR) to detect carbapenemases directly in human blood and fecal specimens. This study evaluated the performance of the NGTest Carba-5 to detect carbapenemases produced bacteria isolated from companion animals and assessed its ability to detect carbapenemases directly in companion animal feces.

A collection of 32 CPE strains, that were previously confirmed to produce carbapenemases from 3 of the 5 major families (NDM, KPC and VIM), were evaluated: 15 *Escherichia coli* (13 NDM, 1 KPC, 1 VIM), 9 *Enterobacter cloacae* (6 NDM, 3 KPC), 6 NDM *Klebsiella pneumoniae*, 1 KPC *Citrobacter koseri* and 1 VIM *Morganella morganii*. 490 fecal specimens (379 dog, 111 cat) were tested using the DetecTool plus the NGTest Carba-5 LFA and the results were compared with detection of CPE using chromogenic agar (HardyChrom CRE agar, Hardy Diagnostics, Springboro, OH), following enrichment in 5 mL Brain Heart Infusion broth supplemented with half of a 10ug ertapenem disc. Presumptive CPE colonies from chromogenic agar culture were confirmed to possess a carbapenemase gene by PCR (Carba-R, Cepheid, Sunnyvale, CA). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the NGTest Carba-5 compared to chromogenic agar culture, with PCR confirmation of CPE isolates.

The NGTest Carba-5 LFA detected a carbapenemase in all 32 confirmed CPE isolates (32/32) with 100% agreement to the known carbapenemase type. 25 (5.1%) fecal specimens were positive by chromogenic agar culture and 23/25 (92%) were positive by the NGTest Carba-5 LFA, with 100% agreement of carbapenemase type. A single specimen was positive by LFA and negative by culture. The sensitivity, specificity, PPV and NPV were 92.6%, 99.8%, 96.2% and 99.6% respectively. The NGTest Carba-5 LFA can detect carbapenemases in bacteria isolated from companion animals. It showed high sensitivity and specificity for the direct detection of carbapenemases in feces when compared to chromogenic agar culture. Additional work is needed to validate the NGTest Carba-5 LFA with bacteria isolated from animals that produce IMP and OXA-48 like enzymes. The NGTest Carba-5 LFA is a useful tool for veterinary microbiology laboratories to detect carbapenemases in bacterial isolates and to identify animals colonized with CPE.

## Bacteriology 2

Sunday, October 9, 2022
Great Lakes A2

**Moderators:** Akhilesh Ramachandran and Nicole Ann Aulik

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

## *Ornithobacterium rhinotracheale*-associated extra-respiratory disease in turkeys: retrospective analysis of 13 cases † ◊

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*Ornithobacterium rhinotracheale* (ORT) is well-known as a respiratory pathogen of turkeys. A few reports of systemic ornithobacteriosis are present in the literature, but no focused studies on this clinical presentation are available. This study aimed to describe the clinical, microbiological, and pathologic findings associated with systemic ornithobacteriosis. Through retrospective analysis, 13 cases in which ORT was isolated from non-respiratory tissues in growing meat turkeys were identified. Cases were received at the Heeke Animal Disease Diagnostic Laboratory (ADDL) in southern Indiana and spanned from December 2019 to May 2022. All ORT isolates were initially identified via biochemical methods, with 7/13 confirmed via MALDI-TOF analysis.

The age of the affected flocks varied from 34-104 days old. From the 13 cases, a total of 21 specimen pools yielded ORT when cultured aerobically, including 14 pools of non-respiratory organs. ORT was isolated from joints (7 cases), liver (5), spleen (1), and pericardium (1). ORT was isolated as a pure culture or as the dominant organism in 85.7% of positive specimens. ORT was recovered from mixed growth in two cases -involving liver and pericardial swab- among *E. coli*, *Enterococcus faecalis*, and *Enterobacter* sp. For cases in which respiratory tissues were also submitted for culture, ORT was isolated from respiratory tissues in 5/8 instances. During the analyzed period, a total of one hundred and thirty-eight specimens had positive or suspected ORT culture results. The 13 cases described here represented 9.6% of ORT isolates obtained in the lab during the 29-month period.

From turkeys in which ORT was isolated from non-respiratory tissues, gross findings (6 cases) included swollen hocks, unspecified synovitis, caseous synovitis, hepatomegaly, and necrotizing hepatitis. Histologic lesions (same 6 cases) from affected tissues included lymphohistiocytic hepatitis, hyperplastic tenosynovitis, and heterophilic epicarditis. Comorbidities diagnosed at the time of ORT culture included bordetellosis (3 cases), colibacillosis (3), reoviral tenosynovitis (2), and crop mycosis (1). In 5/13 cases, no other pathogen was diagnosed. Twelve out of 13 flocks had accessions to the ADDL prior to isolation of extra-respiratory ORT. Three flocks were previously diagnosed with respiratory ornithobacteriosis, four with bordetellosis, four with systemic colibacillosis, two with bacterial tracheitis and airsacculitis of unknown etiology, and one with ocular aspergillosis.

ORT was associated with disease outside the respiratory tract in 13 separate instances over the study period, including almost 10% of all ORT isolations. These findings indicate that this pathogen is associated with systemic disease in domestic turkeys.

† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

**Antimicrobial susceptibility trends and genetic diversity of *Brachyspira hyodysenteriae* isolated from U.S. swine farms †**

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The anaerobic spirochete *Brachyspira hyodysenteriae* is one of the primary etiological agents of swine dysentery (SD). The disease is characterized by bloody and mucoid diarrhea that affects the colon and cecum of pigs, causing a substantial economic loss to the swine-rearing countries globally. There have been reports of antimicrobial resistance of the SD agents to the commonly used antimicrobials. However, knowledge regarding the susceptibility trend and molecular epidemiology of *B. hyodysenteriae* is limited, specifically from North America. The objective of this study was to determine the antimicrobial susceptibility trends of U.S. *B. hyodysenteriae* isolates and to investigate their genetic diversity and a possible association between phenotypic and genotypic resistance using whole-genome sequencing (WGS). Antimicrobial susceptibility testing using a custom Sensititre MIC panel (containing tiamulin, tylvalosin, lincomycin, doxycycline, bacitracin, and tylosin) on 93 *B. hyodysenteriae* isolates recovered from clinical submission to Iowa State University Diagnostic Laboratory (ISU VDL) between 2013 to 2022 was performed. In general, the majority of isolates showed high susceptibility to tiamulin and tylvalosin based on clinical breakpoints. However, 7 isolates showed tiamulin resistance (MIC >2 μg/mL). Additionally, 64 isolates showed high level resistance to tylosin (MIC 64 to >128 μg/mL) and all isolates to bacitracin (MIC 128 to >256 μg/mL). From the WGS data, the t*va*(A) gene was detected from all isolates (n = 7) that expressed phenotypic resistance to tiamulin. In addition, point mutations in 23S rRNA (T326A and A2154T) and 16S rRNA (G1039C) were observed, which appeared to be associated with tylosin resistance. Tiamulin resistant isolates originated mainly from the same farm sites and clustered together on the core-genome SNP-based phylogenetic tree. Similarly, isolates from the same farm sites tend to cluster together. Overall, these findings indicated no specific pattern for susceptibility over time in the *B. hyodysenteriae* isolates tested in this study, while a high level of resistance to some of the commonly used antimicrobials (i.e., tylosin and bacitracin) was apparent. WGS analysis indicated that *B. hyodysenteriae* isolates from the same farm sites were closely related, indicating a low level of genetic diversity at the farm level.

† Graduate Student Oral Presentation Award Applicant

**Isolation of *Trueperella abortisuis* in swine herd abortions. Is this an emerging pathogen? ◊**

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*Trueperella abortisuis* (formerly *Arcanobacterium abortisuis*) is a Gram-positive coccobacillus belonging to the *Arcanobacteriae* family and was first isolated in abortion cases of swine in Japan, followed by abortion cases in Spain and the United Kingdom, where this organism is an emerging pathogen. *T. abortisuis* has been identified in the semen of boars in the United States but has never been isolated from porcine abortions in the US. Based on current knowledge, this organism may play a role as an emerging pathogen in swine abortion cases in the US, either as a primary pathogen or in conjunction with other microorganisms of the female reproductive tract. In this report, *T. abortisuis* was consistently identified in uterine samples, placenta, and fetal tissues from multiple swine abortions in three unrelated production systems in Midwest United States (US). *T. abortisuis* was isolated by aerobic bacterial cultures and MALDI-TOF MS, as well as metagenomic analysis. Affected herds showed signs of reproductive failure, characterized by a decrease conception rate of up to 12%, repeated cycles, purulent secretion from vulva, and/or abortions between 27-50 days of gestation. Sows and gilts did not display any other clinical signs. Gross and histopathology examination of placenta, uterus, and fetal lung tissues revealed severe necrotizing and purulent placentitis in numerous sows, and suppurative pneumonia in one aborted fetus, with large numbers of Gram-positive coccobacilli in all cases. In all affected tissues, *T. abortisuis* was isolated with normal flora. Common abortigenic pathogens of swine were ruled out by molecular testing, ELISA testing, and aerobic and anaerobic bacterial cultures. This report suggests *T. abortisuis* should be a differential diagnosis for swine reproductive failure and abortions in which purulent discharge is observed. Further research should be performed to determine the potential role of this pathogen in swine abortions and reproductive failure.

◊ USAHA Paper

## Canine late term abortions due to *Francisella tularensis*

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A 1.5 year old intact female chocolate Labrador retriever had been vaccinated as recommended with DHLP, rabies and received routine deworming, heartworm prevention and one year boosters. On February 11, 2021, the Labrador "Allison" was presented to her veterinarian as the owner suspected she was bred on January 17th, 2021. Ultrasound confirmed the pregnancy with an anticipated delivery on March 21, 2021. but she delivered still born pups early. She was accidently bred again in November and 4-6 pups were detected in radiographs on 12/3/21. On December 13th, 2021 "Allison" delivered one small, non-viable, not full-term puppy. Patient presented with inappetence, fever of 102.7F and a bloody vaginal discharge. Oxytocin was administered and 3 more under-developed pups were delivered. Ciprofloxacin was administered. The placenta and one puppy were submitted to the diagnostic laboratory for necropsy in fair postmortem condition.

Top differentials for late term abortions in canines usually include *Brucella canis,* canine herpes virus-1, and *Leptospira* spp. These agents are often ruled out by traditional PCR or real-time PCR. Negative results often result in an assumption of idiopathic abortion. The referring veterinarian had also requested aerobic and *Brucella* cultures. No significant lesions were identified on histopathology, no significant bacteria were recovered from the placenta after 2 days incubation under aerobic conditions and media. However, moderate numbers of small grey gram-negative rods were recovered on the *Brucella* agar after 48 hours, 5% $CO_2$ incubation. Bruker MALDI-TOF MS identified the isolate as *Francisella tularensis* with a score of 2.54. This pathogen was also detected in fetal liver and lung by PCR. Test results for canine herpesvirus and *Mycoplasma* spp. PCR were negative. Ciprofloxacin treatment was extended for 4 weeks and citrated blood submitted 1 week following treatment tested negative for *F. tularensis* by PCR. The rDVM notified IL Public Health as *F. tularensis* is a select agent. No illness was reported by the owner or any of the veterinary medical staff treating "Allison". The dog had a history of hunting rabbits and tularemia is believed to be the cause of the two late term abortions. Dogs often do not have severe clinical signs as observed in cats. This is a case where culture for fastidious organisms enabled a diagnosis that could have been missed if limited to molecular testing for likely differentials.

**Association between genomic polymorphism and tuberculous lesions in free-ranging white-tailed deer infected with *Mycobacterium bovis*. †**

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Bovine tuberculosis (bTB), mainly caused by *Mycobacterium bovis* (*M. bovis*), is an infectious disease affecting most animal species, including white-tailed deer (*Odocoileus virginianus*). Severity of bTB and its outcome (i.e., resolution, confinement, or dissemination) seem to be influenced by a combination of host- and pathogen-related factors; however, the role played by bacterial factors in determining the differences in pathological findings observed in infected animals has been scarcely analyzed in white-tailed deer. The aim of this study was to assess the association between anatomical distribution of tuberculous lesions with isolate spoligotype and *M. bovis* group assignment based on whole genome sequencing (WGS). We also explored the possibility of identifying the tuberculous lesion dispersal group of each isolate using single nucleotide polymorphisms (SNPs). A total of 606 *M. bovis* genomes sequenced from tuberculous lesions collected from free-ranging white-tailed deer from Michigan between 1994 to 2019 were included in this study. The *M. bovis* genomes were classified into two groups based on the dispersion of the lesions: cranial (n=371), if the lesions occurred in the head only, and disseminated (n=235), if the lesions occurred somewhere else in the body. Our results suggested that the risk of finding cranial and disseminated lesions in free-ranging white-tailed deer was similar among detected spoligotypes and *M. bovis* groups. The predictive regularized logistic regression model showed that the SNPs have some marginal ability to discriminate between the two lesion groups. The *M. bovis* isolates from the cranial group revealed 45 predictable SNPs, whereas the *M. bovis* isolates from the disseminated group revealed 34 predictable SNPs. In conclusion, our results showed that the occurrence of tuberculous lesions (cranial and disseminated) could be predicted by SNPs in the *M. bovis* genomes sequenced from the deer population; however, a clear correlation between *M. bovis* infection phenotype and genomic polymorphisms requires further validation studies to better understand the clinical outcome of *M. bovis* infection in free-range white-tailed deer.

† Graduate Student Oral Presentation Award Applicant

**Retrospective analysis of *Mycoplasma hyorhinis*. Its role in pulmonary and systemic infection and the correlation of quantitative *in situ* detection by RNAscope® and of qPCR for its diagnosis**

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*Mycoplasma hyorhinis* (*Mhr*) disease is characterized by polyserositis and arthritis in nursery pigs. The diagnosis of *Mhr* disease includes the identification of lesions (polyserositis) concurrent with *Mhr* detection by qPCR or bacteriological culture. It has been suggested that *Mhr* could be responsible for lesions similar to those observed during *M. hyopneumoniae* infection in the absence of other pathogens. There is scarce information about direct detection methods that correlate the role of *Mhr* with different lesions. Therefore, this study aims to 1) determine the infection rate of *Mhr* with other bacterial pathogens in clinical samples using retrospective diagnostic data and 2) correlate quantitative *in situ* detection by RNAscope® with the amount of nucleic acid detected by qPCR in naturally infected pigs.

The frequency of *Mhr* and other systemic and respiratory pathogens was evaluated in clinical cases received at the ISU-VDL between 2016 and 2021. Of 7079 cases evaluated by qPCR, *Mhr* was detected in 53%. *Mhr* was detected as a single infection in 5.03% and 12.75% of systemic and respiratory cases, respectively. The most common pathogens isolated in respiratory and systemic coinfection cases included *G. parasuis*, *S. suis*, *P. multocida*, *A. suis*, *Streptococcus* spp., *T. pyogenes*, *M. hyopneumoniae*, and *A. pleuropneumoniae.* The proportion of *Mhr*-*G. parasuis* and *Mhr*-*S. suis* coinfection was higher in systemic than respiratory cases. There were no significant differences in the mean *Mhr* Ct values in lung or fibrin, but the Ct average was significantly lower in systemic single infection cases. No significant differences in the *Mhr* mean Ct was detected between single and coinfections in respiratory cases.

A subset of respiratory (n=19) and systemic (n=28) cases were selected for further *Mhr* RNAscope® *in situ* detection, quantification by digital analysis, and correlation with *Mhr* qPCR Ct values. No difference in the proportion of bronchopneumonia and interstitial pneumonia was observed between systemic and respiratory cases. In contrast, a significantly higher proportion of cases of airway-centric lesions with BALT hyperplasia was observed in respiratory cases. No correlation between the amount of *Mhr* detected *in situ* by RNAscope® and qPCR Ct values was observed in systemic cases, but there was a strong correlation in pulmonary cases.

Detecting *Mhr* without concomitant pathogens supports its role as a primary pathogen in systemic disease. Polymicrobial infections are common in the field, concluding that pathogen interaction may favor *Mhr* infection, colonization, and replication. The high proportion of respiratory cases with BALT hyperplasia in *Mhr*-positive cases supports the potential role of *Mhr* as a pulmonary pathogen. The combination of digital analysis and RNAscope® showed a high correlation with lung qPCR detection. Thus, this technique can add diagnostic value to *Mhr*-respiratory cases and could be an essential tool for investigating the pathogenesis of *Mhr*.

**Comparison of different laboratory techniques to evaluate growth activity *in vitro* of different *Mycoplasma hyopneumoniae* strains # * †**

*Calvin Cheng-Yu Ko, Pablo Pineyro, Chong Wang, Rachel Jean Derscheid*

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*Mycoplasma hyopneumoniae* (*Mhp*) is a fastidious porcine respiratory pathogen that has proven difficult to culture. Due to this limitation, *Mhp* identification and quantitation relies on qPCR detection. However, this technique does not provide information about bacterial viability. Traditionally *Mhp* growth and metabolic activity have been evaluated by measuring colony-forming units (CFU) and color-changing units (CCU) in solid agar and liquid media, respectively. In addition to laboratory challenges for the species, there is also variability between strains. This study aims to compare and correlate different laboratory techniques, including CFU, CCU, flow cytometry (FlCy), and ATP luminometry (ATPlu), to evaluate *Mhp* growth and metabolic activity in reference and field-adapted strains.

Two laboratory stains *Mhp* strain 232 (p21), strain J (p70, ATCC®25934™), and a contemporary field adapted strain 2010 (p11), were cultured in triplicate at 100-fold dilution in a final volume of 12 mL of Friis medium and incubated at 37°C in an aerobic tissue culture rotator. As a negative and background control, 12 mL Friis media were incubated under the same conditions. At 0, 12, 24, 36, 48, 72, 96, 120, and 144 hours post-inoculation (HPI), aliquot from each replicate were collected for CFU, CCU, FlCy, and ATPlu evaluation. CCU was also performed on the original frozen sample before dilution.

Both quantitative methods, CCU and FlCy, equally demonstrated the *Mhp* exponential growth with a detectable plateau at 48 HPI. No significant differences in the number or proportion of live *Mhp* detected by CCU or FlCy were observed among strains regardless of the time-point. Both CCU and ATPlu also demonstrated an incremental growth by 72 HPI and a rapid decline of detectable metabolic activity by 120 and 144 HPI. No strain differences were observed over time, neither for CCU nor for ATPlu. The correlation of CFU and FlCy varied from 0.71 to 0.90, while CCU or ATPlu was 0.88 to 0.97 among strains. Correlation of growth and metabolic detection method range from 0.80 to 0.96 for CFU and ATPlu and 0.67 to 0.83 for CCU and FlCy, among strains. The similar detection rate over time and high correlation between traditional CFU-CCU and FlCy-ATPlu make the latest an interesting alternative for detecting and quantifying *Mhp*. The FlCy-ATPlu approach could reduce *Mhp* detection time from approximately 21 days currently required by CFU/CCU to a few hours.

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

**Assessing the MIC breakpoints of nitrofurantoin in canine urine and its antibacterial activity against *Escherichia coli*, *Staphylococcus pseudintermedius*, and *Enterococcus faecium***

*Chien-Che Hung[1,2], Jennifer Marie Reinhart[2], Carol Wolfgang Maddox[1,3], Csaba Varga[3], Ryan Dilger[4], Lauren Forsythe[2], Amy Stevenson[1], Rebecca June Franklin-Guild[5], Narayan C. Paul[6], Akhilesh Ramachandran[7]*

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Nitrofurantoin, a broad spectrum nitrofuran class antibiotic, is applied as a first-line antibiotic in treating human urinary tract infection (UTI) due to its great efficacy against urinary pathogens and high achievable concentration at the site of action. Interest of using this antibiotic in companion animals has increased due to the growing demand for effective antibiotics to treat UTIs caused by multi-drug resistant bacteria. Currently, the susceptibility interpretation for nitrofurantoin is based on the breakpoints set for humans, while the canine-specific breakpoints are still unavailable. In this study, we assessed the concentration of nitrofurantoin reaching dog's urine using the recommended oral dosing regimen. Further, we examined the efficacy of this breakpoint concentration against the common UTI pathogens, *E. coli, Staphylococcus pseudintermedius*, and *Enterococcus faecium*. Eight experimental beagles were treated with 5 mg/kg of nitrofurantoin (Macrodantin®) P.O t.i.d. for 7 days. The urine samples were collected via cystocentesis at 2, 4 and 6 hours after administration on day 2 and day 7 and used to quantify nitrofurantoin concentrations by liquid chromatography. The results showed that 26.1-315 μg/mL nitrofurantoin was detected in the dogs' urine with a mean and median concentration of 104.8 and 92.75 μg/mL, respectively. Additionally, individual dogs presented with urinary nitrofurantoin concentrations greater than 64 μg/mL of nitrofurantoin urine concentration in at least 50 % of the dosing interval. This concentration efficiently killed *E. coli*, and *Staphylococcus pseudintermedius*, but not *Enterococcus faecium* strains: *E. coli* and *S. pseuduintermedius* had a $MIC_{90}$ value of 16 μg/mL, while *E. facecium* had a $MIC_{90}$ value of 128 μg/mL. Taken together, these results suggest that the value of 64 μg/mL may be set as a practical breakpoint against UTI pathogens, and nitrofurantoin could be an effective therapeutic drug for empiric therapy against *E. coli* and *Staphylococcus pseudintermedius* in canine UTIs.

**Exploratory study of methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence in sow-herds and bacterial shedding variations during different reproductive cycle phases * †**

*Christine Harness, Nubia Macedo, Joseph T. Thomas, Pablo Pineyro*

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

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections, and its resistance is attributable to the presence of the mecA gene, which encodes PB2a, a transpeptidase that decreases binding between the bacteria's cell wall and beta-lactam antibiotics. It has been demonstrated that MRSA is spread between farm-workers and livestock. Studies in Danish and Dutch farms indicated a 40% increase in MRSA prevalence among sows from pre-farrowing to 3 weeks post-farrowing. Other studies have confirmed that farm personnel who have regular direct contact with pigs have a greater risk of infection for MRSA. Although the technology in modern swine production systems allows reducing human-animal contact, some activities such as artificial insemination, farrowing, and perinatal piglet care still rely on direct human supervision, making this production phase one of the riskiest for MRSA transmission from swine to farm workers. The objective of this study was to evaluate the within-herd prevalence of MRSA on sow's farms, and its potential variation with different stages of the reproductive cycle. Sixty vaginal swabs from 12 sow farms, located in Iowa were collected at breeding (n=20), within three days pre-farrow [due to farrow] (n=20), and within 1-day post farrowing [just farrowed] (n=20). 454 vaginal swabs from sows at 12 farms were cultured on differential media, and tested for antimicrobial susceptibility by broth microdilution for *S. aureus* before determining the presence of *nuc* and *mecA* genes by PCR to confirm the presence of MRSA. All farms sampled were positive for the presence of *S. aureus*, but the prevalence within farms during farrowing varied from 3.1 to 40%. Phenotypic characterization of the *S. aureus* isolates showed sixteen (17.97%) were Oxacillin resistant, and 14 (87.5% were positive for the Penicillin-binding protein 2 (PBP2a), which is encoded by the mecA gene, using a PBP2a Latex Agglutination Test. Genotypic characterization confirmed that MRSA characteristic genes were present in only one farm. Interestingly, samples remained positive at all reproductive stages, and the proportion of positive samples per stage was 19.4%, 15.2%, and 31.3% in just farrowed, due to farrow, and just bred, respectively. These results support earlier findings that suggest MRSA can be endemic to specific farms, and its prevalence among the population can fluctuate based on different events affecting sows. Determining when sows are more likely to shed higher amounts of MRSA can facilitate producer efforts to mitigate the spread of MRSA among personnel and minimize herd prevalence.

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

**Characterization of *Enterococcus* spp isolated from chickens and their environment in US broiler integrations**

*Martha Pulido-Landinez, Hugo Ramirez, Gunnar Dunnam, Hannah Garvin, Jay kay Thornton*

Mississippi Veterinary Research and Diagnostic Laboratory System, Mississippi State University, Pearl, MS

Bacteria from the genus *Enterococcus* are considered normal inhabitants of the gastrointestinal tract of chickens. These are facultatively anaerobic, gram-positive cocci. Among the most isolated from poultry are *E. faecalis, E. faecium, E. hirae, E. durans*, and *E. cecorum.* Although considered as part of chickens' normal microbiota, over the past two decades pathogenic strains of *E. cecorum* have emerged within the commercial poultry industry causing mainly enterococcal spondylitis (vertebral osteomyelitis - VOA), and more recently, severe systemic disease in young broilers. In recent studies, we made a retrospective analysis of an emerging *E. cecorum* outbreak in a Southern US broiler integration. Multiple questions arose related to the origin of these bacteria in chickens younger than 4 weeks.

The goal of the present study is to characterize *Enterococcus spp* isolates recovered at the Mississippi Veterinary Research and Diagnostic Laboratory of Mississippi State University. Cases from breeders, hatchery, and broilers of vertical integrations in the Southern US were analyzed. Using a convenience sampling method 20 isolates from breeders (pullets, breeders, and their environment), 14 from the hatchery (newborn chickens and hatchery environment), and 20 isolates from cases of broiler chickens exhibiting systemic disease and VOA were selected.

All isolates were analyzed on the Vitek – MS -MALDI-TOF. These results were then sent to the RUO/SARAMIS database for analysis. Antibiotic minimal inhibitory concentrations (MICs) were obtained using Thermo Fisher® Sensititre® Complete Automated AST System following manufacturer recommendations. The MICs obtained by antimicrobial sensitivity test were analyzed using WHONET Microbiology Laboratory Database Software.

From the breeder's samples, *E. cecorum* was isolated from pullets, and *E. cecorum*, *E. faecium*, and *E. faecalis* were isolated from adult breeders and their environment. At the hatchery, *E. faecalis* was isolated from newborn chickens and the hatchery environment. Related to broiler samples, *E. cecorum* and *E. hirae* were isolated from broilers 6 days old, and mainly *E. cecorum* was isolated from broiler chickens older than 3 weeks. These results suggested *Enterococus* spp presence varies according to the age of the birds, and the type of sample. AST results will be discussed during the presentation.

The emergence of *E. cecorum* systemic disease must be considered as important in terms of poultry health, and because it can negatively impact the productive performance of the affected chickens. But the presence of other Enterococcus must be considered important in terms of antimicrobial resistance and its potential implication on public health

**Carriage of antimicrobial resistant flora and enteric pathogens among veterinary professionals in the upper Midwest**

*Kelli Maddock[1], Teckla Webb[2], Paul Carson[2], Gerald Stokka[3]*

[1]Veterinary Diagnostic Laboratory, North Dakota State University, Fargo, ND; [2]College of Health Professions, Department of Public Health, North Dakota State University, Fargo, ND; [3]Extension Veterinary Medicine, North Dakota State University, Fargo, ND

Antimicrobial resistance (AMR) is a significant threat to public health worldwide. Colonization of human patients and healthcare workers with antimicrobial resistant bacteria is a well-known risk for infection and has been extensively described. However, few reports describe livestock and companion-animal associated transmission of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant S. pseudintermedius (MRSP), or other antimicrobial resistant bacteria to veterinary workers. The goal of this study was to determine the carriage rate of antimicrobial-resistant commensal bacteria in veterinarians and veterinary technicians practicing in the upper Midwest (North Dakota, Minnesota, and South Dakota), and to correlate AMR bacterial carriage to professional risk factors such as primary species contact, personal protective equipment (PPE) use, and handwashing behaviors.

Participants answered risk-based survey questions and submitted self-collected rectal and nasal swabs. Swabs were plated to culture medium as appropriate for body site. Bacterial isolates were identified by standard microbiological techniques and through use of Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF). Once identified, antimicrobial susceptibility testing (AST) was performed. All samples were observed for the presence of normal flora or other pathogens not specified in the study aims. Nasal swabs were observed for presence of *S. aureus* and *S. pseudintermedius*. Rectal swabs were analyzed for presence of *Salmonella* species, *Campylobacter* spp., Shiga-toxin producing *Escherichia coli*, extended spectrum beta lactamase-producing (ESBL) Enterobacterales, carbapenemase-producing Enterobacterales (CRE), and vancomycin-resistant *Enterococcus* spp. (VRE).

59 collection kits were returned; 3 participants submitted only nasal swabs while 56 participants submitted both nasal and rectal swabs. 59.3% of participants were veterinarians, 39% were veterinary technicians and 1.7% were unspecified. From the nasal swabs, 10.2% of participants were carriers of MRSA while 6.8% were carriers of MRSP. From the rectal swabs, 1.8% of participants were carriers of VRE; however, no *Salmonella* spp., *Campylobacter* spp., Shiga-toxin producing *E. coli*, CRE, or ESBL-producing Enterobacterales were isolated.

This study demonstrated a high prevalence of MRSA and MRSP carriage among veterinary professionals with rates more than double the estimated rates of carriage for both veterinary professionals and healthcare workers. MRSA carriage was associated with primary contact with beef cattle, whereas MRSP carriage was more strongly associated with primary contact with cats and dogs.

**The canary in the coal mine: why active hospital surveillance is critical and how your laboratory can help! †**

*Christy King[2], Dixie Mollenkopf[2], Madison Tracy[2], Patrick Parker[2], Nicholas Marino[2,1], Mallory Carnes[2], Amanda Hutcheson[2], Dubraska Diaz-Campos[2], Joany Van Balen Rubio[2], Thomas Wittum[2]*

[1]University of Wisconsin, Madison, WI; [2]The Ohio State University, Columbus, OH

In 2018, the Ohio State University College of Veterinary Medicine (OSU CVM) implemented an Antimicrobial Stewardship Program. A periodic environmental surveillance (ES) sampling of the Veterinary Medical Center (VMC) has been a core element of this program. The ES focuses on specific pathogens that have been identified as an urgent threat to public health by the Centers for Disease Control and Prevention. The pathogens targeted in the active surveillance include *Enterobacteriaceae* resistant to extended spectrum cephalosporins (ESC), carbapenemase-producing Enterobacterales (CPE), *Salmonella spp*., methicillin-resistant *Staphylococcus spp.* (MRSs), chloramphenicol resistant *Enterococcus spp.,* and enrofloxacin resistant *Pseudomonas aeruginosa*. Identification of these pathogens allows the hospital to be aware of the local environmental microflora which can act as a sentinel for disease in the hospital, potentially causing nosocomial infections. Therefore, the objective of this program is to identify resistant bacterial pathogens, characterize their resistance profiles, analyze prevalence patterns, and initiate infection control protocols where needed in the OSU VMC.

From January 2018 through May 2022, a total of 4242 samples were collected from approximately 86 locations. Sixty-four percent (n=2872) of samples were collected from the small animal hospital. Areas sampled included shared human and animal space and human-touch only surfaces. Samples were collected using Swiffers® with a clean hands/dirty hands technique and processed through selective culture media.

A total of 4516 isolates were recovered, with half (51%, n=2281) coming from human-touch only surfaces. ESC's accounted for 46% (n=1942) of these isolates with 28% displaying an AmpC phenotype. CPEs were identified in 1% (n=56) of isolates and MRSs contributed to 11% of resistance phenotypes.

As a result of this program, the equine section of the OSU VMC worked with the antimicrobial stewardship team to strengthen their *Salmonella* fecal and ES programs. In 2019, ES was critical in identifying persistent CPE and ESC in the ICU and other key areas of the small animal hospital. Successful intervention strategies were implemented to control the spread of ESC's between patients and the persistent finding of CPE in the environment. The microbiology laboratory works in tandem with the ES program to set up targeted sampling, cleaning and disinfection protocols when concerning pathogens are cultured. Surveillance, along with antimicrobial stewardship and infection control, increases patient safety, health, and well-being, and can act as an important tool to prevent further spread of disease.

† Graduate Student Oral Presentation Award Applicant

## Bacteriology - On Demand



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**Characterization of *Corynebacterium pseudotuberculosis* isolated from free-ranging cervids in Montana**

*Colleen Matzke, Erika Schwarz-Collins*

Montana Veterinary Diagnostic Laboratory, Bozeman, MT

*Corynebacterium pseudotuberculosis* is a well-known livestock pathogen, causing caseous lymphadenitis in small ruminants and pigeon fever in horses. *Corynebacterium pseudotuberculosis* infections in wild cervids have been reported infrequently around the world, with no published reports of infections in wild cervids in North America. The significance of this pathogen in cervids is currently unknown, but assumed to be similarly detrimental to infections in livestock. Recently during chronic wasting disease surveillance testing, we detected *C. pseudotuberculosis* in lymph node specimens collected from hunter-harvested, free ranging cervids in Montana, USA. Bacterial isolates were cultured and morphologically characterized, then speciated using commercially available biochemical identification kits (BD BBL Positive Crystal or Biomeriux Coryne API). A subset of isolates that exhibited atypical characteristics were further characterized using MALDI-TOF mass spectrometry and compared to isolates that had been previously collected from equine and small ruminant patients. Here we present the results of these cervid-origin *Corynebacterium* biochemical characterizations and isolate comparisons.

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## Epidemiology 1

Saturday, October 8, 2022
Greenway FG

**Moderators:** Keith P. Poulsen and Jianfa Bai

**1:00 PM** **Influenza A virus RNA positivity by age category and proportion of specimen submissions in the US swine population * †**
*Daniel Moraes, Giovani Trevisan, Guilherme Arruda Cezar, Andreia Arruda, Phillip Gauger, Gustavo Silva, Panchan Sitthicharoenchai, Edison Magalhães, Bret Crim, Kent Schwartz, Marcelo Almeida, Eric R. Burrough, Alyona Michael, Christopher Siepker, Rodger Main, Paulo Lages, Cesar Corzo, Mary Thurn, Jerry Torrison, Rob McGuaghey, Franco Matias Ferreyra, Jamie Retallick, Jon Greseth, Darren Kersey, Travis Clement, Angela Pillatzki, Jane Hennings, Melanie Prarat, William Hennessy, Ashley Sawyer, Dennis Summers, Daniel Linhares* . . . . . 39

**1:15 PM** **Prevalence of porcine rotaviruses in the Midwest of the United States, 2015-2022**
*Jianfa Bai, Nanyan Lu, Cong Zhu, Wai Ning Tiffany Tsui, Vaughn Hamill, Susan Brown, Lance Wade Noll* . . . . . 40

**1:30 PM** **Evaluation of sampling and laboratory parameters contributing to successful virus isolation of Senecavirus A**
*BoMyoung Kim, Munashe Chigerwe, Beate Crossley* . . . . . 41

**1:45 PM** **PRRSV characterization from outbreaks in a midwestern US production system from 2018 to 2021 and time to low prevalence, time to baseline production, and total losses * †**
*Rodrigo Paiva, Cesar Moura, Pete Thomas, Giovani Trevisan, Gustavo Silva, Daniel Linhares* . . . . . 42

**2:00 PM** **Macroepidemiological aspects of porcine circovirus type 2 detection in the United States. * †**
*Guilherme Arruda Cezar, Giovani Trevisan, Edison Magalhães, Gustavo Silva, Marcelo Almeida, Bret Crim, Eric R. Burrough, Phillip Gauger, Christopher Siepker, Alyona Michael, Panchan Sitthicharoenchai, Rodger Main, Mary Thurn, Paulo Lages, Cesar Corzo, Jerry Torrison, Rob McGuaghey, Franco Matias Ferreyra, Jamie Retallick, Jon Greseth, Darren Kersey, Travis Clement, Angela Pillatzki, Jane Christopher-Hennings, Melanie Prarat, William Hennessy, Ashley Sawyer, Dennis Summers, Andreia Arruda, Daniel Linhares* . . . . . 43

**2:15 PM** **Porcine reproductive and respiratory syndrome virus RNA detection in tongue tips from dead animals * †**
*Isadora Fernanda Machado, Edison Magalhães, Ana Paula Serafini Poeta Silva, Rodrigo Paiva, Mafalda Mil-Homens, Guilherme Arruda Cezar, Onyekachukwu Osemeke, Daniel Moraes, Cesar Moura, Phillip Gauger, Giovani Trevisan, Gustavo Silva, Daniel Linhares* . . . . . 44

**2:30 PM** **Detection of viral and bacterial pathogens in postmortem lung specimens from bovine respiratory disease cases at the University of Missouri Veterinary Medical Diagnostic Laboratory, 2019-2021 ◊**
*Rosalie Ierardi, Tamara Gull, Solomon O (Wole) Odemuyiwa, Morgan Stansell* . . . . . 45

**2:45 PM** **Postmortem diagnoses and factors influencing diagnosis in captive white-tailed deer (*Odocoileus virginianus*) in Wisconsin from 2009-2021**
*Lorelei Lynne Clarke* . . . . . 46

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**Influenza A virus RNA positivity by age category and proportion of specimen submissions in the US swine population * †**

*Daniel Moraes[1], Giovani Trevisan[1], Guilherme Arruda Cezar[1], Andreia Arruda[2], Phillip Gauger[1], Gustavo Silva[1], Panchan Sitthicharoenchai[1], Edison Magalhães[1], Bret Crim[1], Kent Schwartz[1], Marcelo Almeida[1], Eric R. Burrough[1], Alyona Michael[1], Christopher Siepker[3], Rodger Main[3], Paulo Lages[4], Cesar Corzo[5], Mary Thurn[5], Jerry Torrison[5], Rob McGuaghey[6], Franco Matias Ferreyra[6], Jamie Retallick[6], Jon Greseth[7], Darren Kersey[7], Travis Clement[7], Angela Pillatzki[7], Jane Hennings[7], Melanie Prarat[8], William Hennessy[8], Ashley Sawyer[8], Dennis Summers[8], Daniel Linhares[1]*

[1]VDPAM, Iowa State University, Ames, IA; [2]Veterinary Diagnostic and Production Animal Medicine, Ohio State University, Columbus, OH; [3]VDL, Iowa State University, Ames, IA; [4]Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, MN; [5]University of Minnesota, Saint Paul, MN; [6]Kansas State University, Manhattan, KS; [7]South Dakota State University, Brookings, SD; [8]Ohio Animal Disease and Diagnostic Laboratory, Reynoldsburg, OH

Influenza A virus in swine (IAV-S) is an important cause of swine respiratory disease worldwide (1), with a risk for zoonotic transmission. Increasing diversity of IAV currently circulating in pigs challenges control efforts, and frequent incursions of human seasonal viruses into swine have greatly influenced the dynamics of IAV-S evolution (2). An organized information hub enables a better understanding of detection and diversity for IAV-S, thus improving strategic surveillance and control. The purpose of this study was to describe the positivity by age category and proportion of positive specimens of different types in submissions involving the detection of IAV-S RNA using RT-rtPCR over time in porcine samples submitted to five USA National Animal Health Laboratory Network (NAHLN) level 1 accredited Veterinary Diagnostic Laboratories (VDLs) participating in the Swine Disease Reporting System (SDRS) (www.fieldepi.org/SDRS). Results from IAV-S RNA screening by RT-rtPCR along with submission metadata were obtained from diagnostic submissions from January 2010 to December 2021 from the Iowa State University VDL, University of Minnesota VDL, Kansas State University VDL, South Dakota State University Animal Disease Research and Diagnostic Laboratory, and the Ohio Animal Disease Diagnostic Laboratory. RT-rtPCR data were collated and organized into an inter-VDL standardized format at the submission level. Variables included date, geographic region (state), RT-rtPCR test result, age category and specimen. Submissions from wean-to-market samples had higher positivity (38%) than submissions coming from the adult/sow farm age category (29%), and a bi-seasonal trend was observed in Spring and Fall season. Moreover, the difference in percentage of positivity for wean-to-market and adult/sow farm diverged more (15.68%) in the fall. Additionally, there was a preceding increase in detection of IAV in results from wean-to-market samples about a month before the increased detection in samples from breeding herds, matching what had been previously described for porcine reproductive and respiratory syndrome virus (PRRSV) (3). There was a decreased proportion of submissions of lung tissues from 76% in 2010 to 23% in 2021, and a significant increase in oral fluid submissions from 2010 to 2021, from 6% to 55%, respectively. Nasal swab and nasal wipe submissions changed from 9% to 4% from 2010 to 2021. This study described macroepidemiological aspects of IAV-S RNA detection and its positivity distribution by age category and specimen; highlighting the importance of monitoring IAV-S in a standardized method using SDRS to enable timely surveillance in the field.

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

**Prevalence of porcine rotaviruses in the Midwest of the United States, 2015-2022**

*Jianfa Bai[1], Nanyan Lu[1], Cong Zhu[2], Wai Ning Tiffany Tsui[1], Vaughn Hamill[1], Susan Brown[1], Lance Wade Noll[1]*

[1]Kansas State University, Manhattan, KS; [2]University of Minnesota, St. Paul, MN

Rotaviruses A, B and C are major diarrheagenic pathogens in swine. Traditionally, rotavirus A was the primary rotavirus in swine, but rotavirus B, especially rotavirus C have been seen more frequently in swine diarrhea cases in the past two decades. A triplex real-time PCR assay developed at KSVDL was used to monitor the prevalence of the three major rotaviruses in swine. KSVDL has received 1342 samples for molecular diagnosis of pig rotavirus during 2015-2022 (till mid-June). Among these 1342 samples, 607 were positive to rotavirus C, 413 to rotavirus A, and 65 to rotavirus B. The overall percentage of positive for rotavirus A, B, and C were 30.8%, 4.8% and 45.2%, respectively. The number of samples submitted for porcine rotavirus diagnosis varied from 59 to 395 for each year, and positive rate for each virus varied from year to year as well. The highest positive rates for rotavirus A, B and C were 67.1% (2022), 13.6% (2016) and 57.7% (2020), respectively; and the lowest rates were 22.9% (2019), 1.3% (2022) and 10.5% (2022) for rotavirus A, B and C, respectively. Co-positive of more than one rotaviruses has been observed. The most prevalent co-infection was observed in rotavirus A and C combination throughout of the years of investigation. The co-positive rate of rotavirus A and C reached the highest rate of 27.0% in 2016, and the lowest rate of co-infection of 3.9% was observed in the first half of 2022. The overall average of rotavirus A and C was 13.0%. As positive rate for rotavirus B is low, co-infection rates of rotavirus B with rotavirus A or C were also low, which were in between 0.0% and 2.7% for each year. Interestingly, we also observed all three species-positives that were in between 0.0% (2022) and 7.8% (2021), some were higher than two-species positive rates that involved rotavirus B. Accumulated data indicated that rotavirus A has continuously been the predominant species, while rotavirus C has becoming an important species with similar or even higher prevalent rate to that of rotavirus A. Co-infection with rotavirus A and C was also observed. Rotavirus B was seen in pig diarrhea cases, but with a much lower prevalence rate.

**Evaluation of sampling and laboratory parameters contributing to successful virus isolation of Senecavirus A**

*BoMyoung Kim[1], Munashe Chigerwe[1], Beate Crossley[1,2]*

[1]School of Veterinary Medicine, UC Davis, Davis, CA; [2]California Health and Food Safety Laboratory, Davis, CA

Virus isolation(VI) of Seneca Valley virus(SVV), an emerging vesicular disease of swine, is essential for viability testing, reference stock production, and monitoring of virus evolution. This study aimed to evaluate the association between successful SVV isolation and characteristics of samples and laboratory parameters using the California Animal Health and Food Safety(CAHFS) laboratory dataset from 2017 to 2018.

The study population included 1102 samples submitted to CAHFS for SVV testing by 15 regulatory officials from 13 sites in California. Samples included swabs of unspecified(n=953, 86.5%), foot(n=108, 9.8%), nasal(n=24, 2.2%), lung and mammary gland(n=6, 0.5%), mix of either nasal and foot, or nasal and oral samples(n=6, 0.5%), and oral(n=5, 0.5%). Samples were submitted individually(n=1033, 93.7%) or pooled(n=69, 6.3%). All pooled samples were from pigs in Stanislaus from June to September 2017. VI results were determined by cytopathic effect and realtime PCR. Virus isolation was successful in 769 samples(69.8%) and negative in 333 samples(30.2%). The VI negative samples consisted of 305 PCR positive samples(91.6%) and 28 PCR negative samples(8.4%).

Multivariable logistic regression was used to assess 17 variables. Sample characteristics were duration between submission and testing, date and month of specimen collection, test date, submitter, county, collection site, animal species, whether vesicles were sampled, specimen (tissue sampled), and pooling. Laboratory parameters included cell passage number, confluency, and reagent lots.

Diagnostic probability of our final model resulted in less than moderate accuracy(AUC = 0.68, 95% confidence interval(CI): 0.65-0.72). The sampling month, specimen type, pooling, and interaction between month and pooling contributed to predicting VI result. During 2017 to 2018, failure to isolate SVV was 100 times and 17 times, respectively, more likely during August(OR = 0.01 (95% CI: 0.001 to 0.071) p = 0.000004) and July(OR = 0.06 (95% CI: 0.01 to 0.49) p = 0.01) using April as the reference. When foot samples were set as references, unspecified swabs(OR = 1.72 (95% CI: 1.11 to 2.66) p= 0.016) had an ~2-fold increased association with positive VI, likely due to a relatively large sample size compared to other sample types. There were no significant differences between individual and pooled samples; however, during August(OR = 45.21 (95% CI: 6.62 to 308.66) p=0.0001), and July(OR = 8.12 (95% CI: 1.03 to 63.9) p = 0.04), individual samples were 45 and 8 times more likely to generate positive SVV isolations, compared to pooled samples. Laboratory parameters were not significantly associated with VI. This study suggests that high environmental temperatures might negatively affect virus recovery. Use of individual sample seems to improve SVV recovery when environmental temperatures are elevated (August, July). Further studies on causality among ambient temperature, pooling, and sample quality are required.

**PRRSV characterization from outbreaks in a midwestern US production system from 2018 to 2021 and time to low prevalence, time to baseline production, and total losses * †**

*Rodrigo Paiva[1], Cesar Moura[2], Pete Thomas[3], Giovani Trevisan[1], Gustavo Silva[1], Daniel Linhares[1]*

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Porcine Reproductive and Respiratory Syndrome (PRRS) is a major endemic disease affecting the US swine industry. Since the emergence in the late 1980s, various outbreaks characterized by severe reproductive losses, reduction in growth rate, respiratory disease, and increase in mortality were reported in the United States. This study aggregates PRRS virus (PRRSV) ORF-5 sequencing from 35 PRRS outbreaks from a production system located in a high-density swine production area in the midwestern US from 2018 to 2021 and metrics associated with production impact. The track of PRRSV sequencing between herds was performed to characterize the most frequent strain circulating. The strains were compared with the metrics defined as the time in weeks to achieve low prevalence (TTLP), the number of weeks to recover the baseline in productivity (TTBP), and total losses (TL). The objectives of the study are a) describe the changes in terms of the most frequent circulating strain; b) describe the TTLP, TTBP, and TL associated with different strains.

This retrospective study collected sequencing data submitted to Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) from 35 farms and compared the frequency and strain type associated with TTLP, TTBP, and TL. Herds were considered as reaching TTLP when 8 consecutive weeks of PCR-negative results or piglet serum testing (n=30) were obtained. TTBP was calculated based on an exponentially weighted moving average (EWMA), comparing the outbreaks 26 weeks before and after the break, until recovering the production average. The TL was represented by the piglets not weaned below the expected until achieving TTBP, with the total piglets divided by thousands of sows.

The most frequent PRRSV strains detection changed from L1A RFLP 1-7-4 in previous years of the study to L1C variant 1-4-4 in 2021. The TTLP, TTBP, and TL were 77, 34, and 5,420 respectively for PRRSV strains L1C variant RFLP 1-4-4; 62, 22, and 4,850 for PRRSV L1A RFLP 1-7-4; 59, 31, 3,757 for the other strains different than 1-4-4 and 1-7-4. These results revealed the changes in terms of most frequent virus circulation and TTLP, TTBP, and TL associated with the occurrence of these strains in a production system in the Midwestern US. This data supports the importance to keep track and monitoring the virus in the breeding herds to help the producers and veterinarians in their decision-making and aim to eliminate and control PRRSV.

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

**Macroepidemiological aspects of porcine circovirus type 2 detection in the United States. * †**

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At the end of the 1990s, porcine circovirus type 2 (PCV2) was identified as a new strain of the *circoviridae* virus family in North America. The emergence of PCV2 coincided with severe clinical manifestations such as reproductive failures, systemic disease, dermatitis and nephropathy syndrome, and postweaning multisystemic wasting syndrome. Since then, thousands of samples have been submitted for testing at US veterinary diagnostic laboratories (VDLs), generating a robust diagnostic testing and testing results database. This study aggregates PCV2 DNA PCR data from 5 US swine-centric VDLs, unraveling the PCV2 macroepidemiology aspects and detection dynamics.

Historical data from test and testing results were retrieved from participant VDLs and organized at the submission level using PROC DATA, FORMAT, SORT, and SQL scripts available in SAS software. The dataset was connected to Microsoft Power BI®, a data visualization tool, to construct dynamic charts to monitor the number of submissions over time, specimen, age category, and percentage of positive submissions. Final charts were made available on the Swine Disease Reporting System (SDRS) webpage (https://fieldepi.org/SDRS) under the Porcine Circovirus Type 2 (PCV2) dashboard for consultancy.

The final dataset consisted of 126,429 separate submissions tested for PCV2. from 2010 to 2017; the average number of annual submissions was 5,920, and the percentage of PCV2 PCR positive results was 32.39%. The wean-to-finish age category represented 37.71% (47,677) of submissions tested, while the adult/sow farm category represented 26.73% (33,802). After 2018, the average annual percentages of total PCV2 submissions and PCV2 positive submissions increased by 71.03% and 9.76%, respectively (10,125 submissions; 42.15% positive). Such increases may be partly attributed to the rapid adoption and usage of processing fluid samples, a new sample type being used for PCV2 testing. In 2021, processing fluid represented 28.92% (3,051 of 10,549) of all specimens tested for PCV2. Oral fluid represents 9.82% (1,036 of 10,549) of total submissions tested for PCV2, with PCV2 detected by PCR in 60% of these cases. In addition, Ct-values of post mortem tissue samples (e.g., lung, lymph node, and fetus) were lower than antemortem samples (e.g., colostrum, environmental, fecal swab, serum, processing fluid, oral fluid). Lymph nodes had the lowest average Ct (20.7), and the highest average Ct (34.6) was obtained from colostrum/milk samples. These results revealed the megatrends in PCV2 detection, demonstrating the importance of actively monitoring PCV2 activity in the US, aiding veterinarians and producers to make informed animal health decisions for disease prevention and control.

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

**Porcine reproductive and respiratory syndrome virus RNA detection in tongue tips from dead animals * †**

*Isadora Fernanda Machado[1], Edison Magalhães[1], Ana Paula Serafini Poeta Silva[1], Rodrigo Paiva[1], Mafalda Mil-Homens[1], Guilherme Arruda Cezar[1], Onyekachukwu Osemeke[1], Daniel Moraes[1], Cesar Moura[2], Phillip Gauger[1], Giovani Trevisan[1], Gustavo Silva[1], Daniel Linhares[1]*

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most challenging pathogens for swine production, therefore, the ability to estimate the prevalence of infected herds is critical to developing strategies for PRRSV control and elimination programs. Although serum sampling is the traditional method for PRRSV surveillance, populational-based methods are widely implemented, e.g., processing fluids (PF), oral fluids, and family oral fluids (FOF), partly because they can increase herd sensitivity without increasing cost, time, and labor. A recent populational-based method using tongue tips (TT) from dead piglets from farrowing rooms was shown to be capable of detecting PRRSV RNA and can be used as an alternative to other well-known methods.

Three breeding herds (Farms A, B, and C) that are PRRSV RNA-positive unstable were selected for sampling. On each farm, TT (≈2 cm) from dead piglets were collected in disposable bags from three age groups: newborn age (<24h of age), processing age (2-7 days of age), and weaning age (18-21 days of age). From the same room and ages in which TT were collected, blood samples (n≈45) were randomly collected (one piglet per litter). Additionally, PF (n≈3) and FOF (n≈25) were collected from pigs of processing and weaning age, respectively. TT, PF, and FOF were tested individually, and serum samples were tested in pools of five, for PRRSV RNA, and the probability of RNA detection in TT was compared to serum, PF, and FOF for each age group.

In farms A and B, PRRSV RNA was detected in 100% and 95.2% of overall TT, respectively, and pooled serum samples were positive in all age groups in different percentages for these farms. In particular, the cycle threshold (Ct) was lower in TT compared to serum samples for newborn age. Thus, the use of risk-based sampling, e.g., TT, provides a good estimate of the population's health status. Additionally, PRRSV RNA was detected in PF from processing age at farms A and B, while FOF were positive in all three farms, in different percentages. In contrast, at the farm C, no PRRSV RNA was detected in TT, and pooled serum samples were PRRSV RNA-positive only in the weaning age. Further, no PRRSV RNA was detected in TT when serum samples from the same group tested PRRSV RNA-negative. Collecting TT from dead piglets was practical and time-efficient. Taken together, these results suggested the potential diagnostic value of TT for PRRSV monitoring and surveillance.

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

**Detection of viral and bacterial pathogens in postmortem lung specimens from bovine respiratory disease cases at the University of Missouri Veterinary Medical Diagnostic Laboratory, 2019-2021** ◊

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Bovine respiratory disease (BRD) is one of the most important health conditions of beef cattle. Most research on BRD is focused on feedlots, but this disease is an important challenge to cow-calf producers as well. Missouri is one of the top cow-calf producing states in the U.S. This report describes viral and bacterial pathogens recovered from BRD cases diagnosed at the University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL) from January 1, 2019 to December 31, 2021.

During this three-year period, 386 cases of bovine pneumonia were diagnosed by a pathologist or identified as such by the referring veterinarian with subsequent detection of a primary BRD pathogen. Primary BRD pathogens were defined as *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, bovine viral diarrhea virus (BVD), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (PI3), or bovine herpesvirus 1 (BHV-1). Cases were only included if postmortem lung tissue was evaluated. Swabs were not considered in this analysis.

Aerobic culture of lung was performed in 382 of 386 cases. In 27.5% of cultured cases, no significant pathogens were isolated. Significant bacterial isolates included *M. haemolytica* alone in 25.1%, *H. somni* alone in 12.6%, and *P. multocida* alone in 8.9%. In 17.8% of cases, two or more of *M. haemolytica*, *P. multocida*, and/or *H. somni* were isolated concurrently. The remaining 8.1% of cases were consistent with systemic infections (e.g., septic calves with *Salmonella* spp. isolated from multiple organs) or with secondary opportunists such as *Trueperella pyogenes*, *Bibersteinia trehalosi*, and/or *Fusobacterium necrophorum*.

A complete respiratory PCR panel for BVD, BRSV, PI3, and BHV-1 was performed on 340 of 386 cases. In 80.6% of cases, no virus was detected. The most commonly detected viruses, either alone or in combination, were BRSV (12.1%) and BVD (7.6%). Additionally, *Mycoplasma bovis* was detected in 55.4% of the 112 cases in which PCR was performed. This should be interpreted with caution as *M. bovis* PCR is typically requested at the pathologist's discretion and is not automatically included in the standard respiratory panel.

During the same period, the detection rate of BVD persistent infections was 0.3% among 4,371 ear notches tested via immunohistochemistry or antigen capture ELISA, depending on the client's request. These numbers include out-of-state submissions and generally reflect previously reported industry averages.

Etiologies of BRD detected in bovine lung submissions to the VMDL, which overwhelmingly represent cow-calf operations, are broadly similar in relative proportions to those previously reported in feedlot cattle.

◊ USAHA Paper

**Postmortem diagnoses and factors influencing diagnosis in captive white-tailed deer (*Odocoileus virginianus*) in Wisconsin from 2009-2021**

*Lorelei Lynne Clarke*

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

There have been significant changes to Wisconsin agriculture over the past decade, one of which is the emergence of commercial farming of white-tailed deer (WTD; *Odocoileus virginianus*) for meat production and hunting. While ruminant species generally are afflicted by similar pathologies, the development of high-density WTD farming may lead to the emergence of previously unrecognized or under-recognized disease trends. There are no currently available large-scale surveys of disease and pathology from captive WTD herds in Wisconsin or the Upper Midwestern USA. Archived pathology records at the Wisconsin Veterinary Diagnostic Laboratory (WVDL) between 2009-2021 were evaluated for WTD postmortem cases. Information recorded from each case included the type of submission (whole body necropsy or fresh and fixed tissues), signalment (sex, age, species), state of origin, date of submission, whether autolysis was noted in the necropsy report (Y/N), pathologic diagnosis(es) (as reported by the primary pathologist of each case), and specific infectious causes when found. Many cases were indicated to have multifactorial disease, so up to 3 diagnoses and 3 infectious agents were recorded for each case as available. Postmortem records from 277 WTD were included. Diagnoses were found in 81.9% of cases. Submissions received in spring (March, April, May) were 0.31 (CI 0.10-1.03) times as likely to have a diagnosis as the average WTD case. Submissions in the Fawn age group (1 week-6 months of age or indicated as "fawn") were 1.91 (CI 1.01-3.81) times more likely to have a diagnosis than the average. Of those animals where a diagnosis was reached (n=227), 48.5% had comorbidities with an average of 1.34 diagnoses/animal. The most common diagnoses were bronchopneumonia and enteritis/enterocolitis (both 16.2% of total diagnoses). The most common isolates from bronchopneumonia cases were *Pasteurella multocida, Bibersteinia trehalosi*, and *Trueperella pyogenes*. The most identified pathogens in enteritis/enterocolitis cases were rotavirus, *Clostridium perfringens*, and *Escherichia coli*. The most common non-infectious diagnosis was poor nutritional status/inanition (7.0% of total diagnoses) followed by hepatic lipidosis (2.2%) and selenium/vitamin E deficiency (1.9%). Fawns were more likely to be diagnosed with bronchopneumonia, enteritis, septicemia, poor nutritional status, and parasitism when compared to juvenile and adult WTD. Likewise, all these conditions were more frequently diagnosed in submissions received in the summer (June, July, August), which was when most fawn submissions were received. Focusing on fawn diagnostics may be recommended for laboratories expanding their WTD testing.

## Epidemiology 2

Sunday, October 9, 2022
Lake Bemidji

**Moderator:** Giovani Trevisan

**11:15 AM** **Monitoring porcine epidemic diarrhea virus detection using real-time diagnostic data to support animal health decisions * †**
*Guilherme Arruda Cezar, Giovani Trevisan, Edison Magalhães, Gustavo Silva, Marcelo Almeida, Bret Crim, Eric R. Burrough, Phillip Gauger, Christopher Siepker, Alyona Michael, Panchan Sitthicharoenchai, Rodger Main, Mary Thurn, Paulo Lages, Cesar Corzo, Jerry Torrison, Rob McGuaghey, Franco Matias Ferreyra, Jamie Retallick, Jon Greseth, Darren Kersey, Travis Clement, Angela Pillatzki, Jane Hennings, Melanie Prarat, William Hennessy, Ashley Sawyer, Dennis Summers, Andreia Arruda, Daniel Linhares* . . . . . *58*

**11:30 AM** **A retrospective outbreak investigation of *Actinobacillus pleuropneumoniae* serotype 15 in central Iowa * †**
*Isadora Fernanda Machado, Mafalda Mil-Homens, Ana Paula Serafini Poeta Silva, Pete Thomas, Levi Johnson, Lori Feldmann, Lauren Glowzenski, Daniel Boykin, Tyler Bauman, Alyona Michael, Marcelo Almeida, Daniel Linhares, Gustavo Silva, Derald Holtkamp.* . . . . . *59*

**11:45 AM** **Molecular characterization of *Glaesserella parasuis* strains circulating in North American swine production systems**
*Robert Mugabi, Ana Paula Serafini Poeta Silva, Maria Clavijo* . . . . . *60*

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

**PCR detection of SARS-CoV-2 and differentiation of major variants of concern in human and animals**

*Wai Ning Tiffany Tsui, Vaughn Hamill, Lance Wade Noll, Nanyan Lu, Donald Harbidge, Tesfaalem Sebhatu, Susan Brown, Gregg Hanzlicek, Jamie Retallick, Jianfa Bai*

Kansas State University, Manhattan, KS

The SARS-CoV-2 virus, causative agent of COVID-19, has undergone continuous mutations throughout the course of the pandemic. Variants of concern (VOC) have emerged, including the Delta variant, first detected in India in December, 2020, and later in the US by March, 2021. Delta quickly became the predominantly circulating VOC in US, and remained so until the arrival of the more transmissible Omicron variant in November, 2021. By the end of December, 2021, infections from Omicron in the US had surpassed those from Delta. A molecular assay that can detect and differentiate both the Delta and Omicron variants was developed. A collection of 660,035 SARS-CoV-2 full- or near-full genomes, including 169,454 Delta variant strains and 24,202 Omicron variant strains, were used for primer and probe designs. *In silico* data analysis predicated assay coverage of >99% of all strains, including >99% of both Delta and Omicron strains. The differential tests for Delta and Omicron were designed based on the Δ156-157 aa deletion in the S-gene, and the Δ31-33 aa deletion in the N-gene, respectively; the N-gene mutation is also present in the original B.1.1.529 main genotype, and in BA.1, BA.2 and BA.3 subtypes, ensuring coverage of a vast majority of Omicron strains. Standard curves, generated with spiked human clinical samples, indicated PCR amplification efficiencies of 104%, 90.7% and 90.4% for Omicron, Delta, and non-Delta/non-Omicron wildtype genotypes, respectively. Correlation coefficients were all >0.99. The detection limit of the assay was 14.3, 32.0, and 21.5 copies per PCR reaction for Omicron, Delta, and wildtype genotypes, respectively. Selected samples with Omicron, Delta and wildtype genotypes identified by the RT-qPCR assay were also confirmed by sequencing animal samples, previously testing positive for non-SARS-CoV-2 animal coronaviruses, all tested negative for SARS-CoV-2 by this assay, indicating high assay specificity. Human nasal swab samples that previously tested positive (n=182) or negative (n=42) for SARS-CoV-2 by the ThermoFisher TaqPath COVID-19 Combo Kit, produced the same result with the new assay; among positive samples, 55.5 % (101/182), 23.1% (42/182) and 21.4% (39/182) were identified as Omicron, Delta, and non-Omicron/non-Delta wildtype genotypes, respectively. A total of 639 animal respiratory samples were tested by the assay, from sample hosts including 416 canine, 107 feline, 52 bovine, 17 deer, 15 swine, 8 equine, 5 each of goat and sheep, 2 leopard, and 1 each of racoon and tiger. Only the tiger and 1/2 leopard samples, and 2/416 canine samples tested positive for SARS-CoV-2, indicating a very low positive rate for this sample population. To conclude, we have developed a highly sensitive and specific assay that offers detection of the majority of SARS-CoV-2 strains as well as differentiation of Omicron, Delta, and non-Delta/non-Omicron wildtype genotypes.

**Swine lameness diagnosis at Iowa State University Veterinary Diagnostic Laboratory from 2018-2020**

*Panchan Sitthicharoenchai, Michael Rahe, Christopher Siepker, Alyona Michael, Tyler Alan Harm*

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

Lameness is a topic of high concern in the swine industry, as animals with locomotive dysfunction result in a loss of production, an increase in culling and mortality, and a significant negative impact on animal welfare. Here, we present a retrospective evaluation of causes of swine lameness diagnosed at the Iowa State Veterinary Diagnostic Laboratory (ISU VDL) from January 2018 to December 2020 by LIMS search of 124 diagnostic codes related to lameness in swine. A total of 1384 cases were coded for 74 of the 124 selected diagnostic codes. The number of swine case submissions diagnosed under the diagnostic codes related to locomotive dysfunction increased by two-fold in 2020 compared to 2018. The most commonly affected anatomical system contributing to the clinical signs of lameness was the joint, followed by the bone, nervous system, muscle, integument, and hoof. Bacterial arthritis accounted for the most common cause of swine lameness, with *Streptococcus suis* as the most prominent etiology. There were many cases with tissue changes of bacterial arthritis in which specific etiology(ies) could not be identified due to bacterial contamination and limited ancillary testing. Interestingly, there was a high prevalence of metabolic bone disease in 2020 associated with hypovitaminosis D3 resulting in low bone ash and density, accompanied by the development of rickets and pathological fracture. Viral encephalomyelitis (e.g., porcine sapelovirus) was the most common cause of disease diagnosed in the central nervous system, resulting in locomotive dysfunction. Among the 1397 cases, 66 described clinical signs of lameness but were non-diagnostic due to the lack of appropriate samples received upon submission. These data represent recent etiological trends in cases of swine lameness submitted to the ISU DVL and confirm the importance of proper tissue submission and ancillary test selection in diagnosing clinical cases of swine lameness.

**Characterizing the epidemiology of bovine anaplasmosis to inform control efforts in Ohio * †**

*Andreas Eleftheriou[1], DaZané Cole[1], Justin Kieffer[2], Risa Pesapane[1,3]*

[1]Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH; [2]Department of Animal Sciences, The Ohio State University, Columbus, OH; [3]School of Environment and Natural Resources, The Ohio State University, Columbus, OH

*Anaplasma marginale*, the primary agent of bovine anaplasmosis, drives major economic losses in the cattle industry worldwide. In the United States, anaplasmosis is endemic in several southeastern, midwestern and western states. However, there is no published evidence about prevalence in cattle herds from Ohio. Our objective was to characterize the prevalence of anaplasmosis in beef cattle from Ohio and examine associations with farm location and animal age through the combined use of serological and molecular testing.

We intentionally sampled four herds that had a high likelihood of anaplasmosis to characterize circulating genotypes as part of a broader study between December 2020 and December 2021. Serum samples (n = 324) were screened for antibodies to *Anaplasma* spp. using a commercial competitive enzyme-linked immunosorbent assay (VMRD Inc., Pullman, WA, USA) considered to be the most suitable test for this purpose. Seropositive samples were subsequently tested for *A. marginale* using probe-based real-time PCR assays. Based on real-time PCR testing, we estimated a pooled prevalence of 38.53% (95% CI: 33.26-43.81%) with some farms exhibiting higher prevalence than others (range: 19.40-56.86%) and older animals being more likely to test positive (OR: 1.41, 95% CI: 1.28-1.58). Combined serological and molecular testing identified 44 seropositive individuals that tested PCR negative for *A. marginale*.

Our cross-sectional study is the first to provide prevalence estimates of bovine anaplasmosis in Ohio. Older animals were more likely to be infected, a finding that supports previous studies. To understand why some farms exhibited higher prevalence than others will require further investigation. The 44 individuals that tested seropositive but negative for *A. marginale* may have been due to user error or assay limitations. However, it is more likely that they were infected with *Anaplasma phagocytophilum*, which led to positive serology results due to immunological cross-reactivity. An emerging pathogen in Ohio, *A. phagocytophilum* is vectored by the black-legged tick (*Ixodes scapularis),* which will bite cattle. These results suggest that seropositive cattle in geographic locations where *A. phagocytophilum* and *A. marginale* co-circulate, such as in Ohio, will require molecular testing for correct diagnosis, which will become more critical as the range of the black-legged tick expands across North America. Broadly, to better inform local and regional control efforts, we must conduct wider prevalence studies and characterize *A. marginale* genotypes and their modes of transmission within cattle herds in Ohio.

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

**Collection of host-seeking *Dermacentor variabilis*, vector of bovine anaplasmosis, on beef cattle pastures in Missouri ◊**

*Rosalie Ierardi[1,2], Grace Ver Mehren[3], Ram K. Raghavan[1,2,4]*

[1]Veterinary Medical Diagnostic Laboratory, University of Missouri College of Veterinary Medicine, Columbia, MO; [2]Department of Veterinary Pathobiology, University of Missouri College of Veterinary Medicine, Columbia, MO; [3]Division of Animal Sciences, University of Missouri College of Agriculture, Food & Natural Resources, Columbia, MO; [4]Department of Public Health, University of Missouri School of Health Professions, Columbia, MO

Bovine anaplasmosis is an economically significant disease of cattle, with impacts including weight loss, spontaneous abortions, and death. The primary arthropod vector of anaplasmosis in the Midwest is the American dog tick, *Dermacentor variabilis.* Our research seeks to determine the prevalence of *Anaplasma marginale*, the causative agent of bovine anaplasmosis, among host-seeking *D. variabilis* ticks in Missouri. To this end, ticks are collected at regular intervals from pastures on four beef grazing operations, each in a separate geographic region of Missouri. Pastures in our study are actively grazed by cattle and consist primarily of open grassland, with areas of grassland-woodland edge habitat. Ticks are collected with flannel drags over 750-meter transects, according to previously published methods.

Following 38 collection attempts on 10 days in May and June 2022, 166 ticks have been collected. The most frequently encountered species during these attempts is the lone star tick, *Amblyomma americanum* (69.9%), followed by *D. variabilis* (30.1%). Collected *A. americanum* include 6 adult females, 12 adult males, and 98 nymphs. Collected *D. variabilis* include 28 adult females and 22 adult males.

Tick host-seeking behavior is influenced by a variety of factors including climatic and weather conditions, land cover, and host density. From each study site, hourly measures of precipitation, air temperature, relative humidity, and wind speed are recorded from onsite data loggers to identify abiotic drivers of tick abundance and phenology at these sites.

In our study, *D. variabilis* represents 30.1% of ticks collected thus far, whereas previous studies in north-central Missouri report *D. variabilis* accounted for only 1% to 14% of ticks collected. This may be an effect of collecting from open grassland, instead of primarily forested areas as in the previous studies. Our findings indicate substantial local variation in tick composition depending on the site sampled. The site in north-central Missouri has yielded almost exclusively *D. variabilis*, while sites in eastern and southwestern Missouri have yielded almost exclusively *A. americanum*.

To determine the prevalence of *A. marginale* in host-seeking *D. variabilis* ticks, our next steps involve nucleic acid extraction from collected ticks and real-time PCR to detect *A. marginale*. Tick collection will continue until a sufficient sample size has been achieved.

◊ USAHA Paper

**A simulation-based evaluation of targeted pre-movement active surveillance protocols for African swine fever**

*Sasidhar Malladi, Peter Bonney, Amos Ssematimba, Benjamin Blair, Cesar Corzo, Marie Culhane*

Secure Food Systems team, University of Minnesota, Saint Paul, MN

African swine fever (ASF) is a highly contagious and deadly disease that has caused extensive global economic losses. Effective pre-movement active surveillance protocols for ASF are critical for outbreak control and to support the permitted movement of pigs from a Control Area. In this work, we evaluated various pre-movement active surveillance protocol options for finisher swine herds with different sample sizes, varying the prioritization order for sick and dead pigs, and for multiple ASF viral strain scenarios.

We used a stochastic within-herd ASF transmission model together with an active surveillance simulation model to predict the probability of detection before movement. The model incorporated multiple transmission mechanisms such as direct contact, nose-to-nose contact between pigs in adjacent pens, and people or fomite-mediated spread. The predicted disease mortality and morbidity on various days post-exposure from the transmission model were used to simulate detection via active surveillance for a given diagnostic sensitivity of the RRT-PCR test. Using North American swine industry data, the estimated rates of normal morbidity and mortality due to routine causes unrelated to ASF was factored in to predict the chances of sampling an ASF virus positive pig. The protocol options were all based on sample collection at 24 hours before movement but varied with respect to the sample size and the prioritization of sampling sick, dead, and apparently healthy pigs for testing. We also considered that a fraction of the pigs with mild clinical signs (e.g., lameness, loss of appetite, and erythema) would not be correctly identified and may not be included in the sampling.

Our results indicated that targeted sampling of pigs with mild clinical signs can enhance detection as these symptoms occur earlier in the disease process relative to mortality. The probability of detection when prioritizing sick, dead, and apparently healthy pigs was greater compared to protocols only prioritizing sick and apparently healthy pigs for highly virulent ASF viral strain scenarios particularly when a lower fraction of pigs with mild clinical signs are correctly identified. However, the probability of detection under these prioritization schemes was comparable under several of the moderately virulent strain scenarios. Overall, the results indicate that targeted sampling protocols that prioritize the collection of specimens from sick and dead pigs are important to develop robust surveillance strategies that are effective across different ASF viral strains. The results on the probability of detection provide beneficial information that can be used for both emergency preparedness and surveillance protocol design.

**Certified Swine Sample Collector Training Program**

*Justin Brown*

VDPAM, Iowa State University, Ames, IA

In 2019, the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) led a series of functional exercises and drills to test the federal, state and industry response to an African Swine Fever virus (ASFV) outbreak. Through these exercises, areas of concern identified include: limited number of people authorized to correctly collect and submit diagnostic samples during a foreign animal disease (FAD) response and limited proficiency of FAD diagnosticians (FADDs) when performing on-farm investigations due to inadequate experience with modern swine production.

Through funding provided by USDA's National Animal Disease Preparedness and Response Program, a collaborative group, with representation from the American Association of Swine Veterinarians, Iowa State University's Swine Medicine Education Center (SMEC) and Center for Food Security & Public Health (CFSPH), National Pork Board, and the Multi-state Partnership for Security in Agriculture, was formed to address these issues.

To address the limited personnel for collecting samples, resources were developed to train workers on proper techniques of various ante-mortem and post-mortem samples. For each sample type, a one-page handout and a video were developed to facilitate training. To determine best methods for delivery of the training, a working group was formed to develop program standards.

The program standards include:

-Trainer is required to contact the animal health officials in the state(s) in which they plan to train or utilize Certified Swine Sample Collectors (CSSCs) to confirm their eligibility to participate in the program

-Training must be conducted by a USDA category II accredited veterinarian

The curriculum consists of a classroom and hands-on portion:

-Classroom portion: trainer reviews training resources and administers a 25-question exam

-Hands-on portion: follows the classroom training. The trainer demonstrates the technique and the trainee practices to demonstrate proficiency

During a foreign animal disease outbreak, state animal health officials (SAHOs) will determine when CSSCs will be authorized to collect samples.

It is the responsibility of the trainer to:

-Schedule and conduct training sessions

-Follow the program standards

-Record and report names and contact information of successful trainees

It is the responsibility of the trainee to:

-Actively participate in training sessions and pass evaluations

-Properly collect, package and submit samples

-Become recertified annually

Following successful completion of the training program, CSSCs will be able to correctly collect, package and ship diagnostic samples for a category II accredited veterinarian or as requested by a SAHO. Certification stays with the CSSC if employment changes. The training resources and programs developed will improve the swine industry's ability to prepare and respond to a FAD outbreak. SAHOs are beginning to roll out the program on a state-by-state basis.

**U.S. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) integrated antimicrobial resistance data dashboards from companion animals**

*Olgica Ceric[1], Amy Merril[1], Beth Harris[2], Jennifer Rodriquez[2], Christine Foxx[3], Christina Loiacono[2], Sarah Peloquin[1], Jake Guag[1], Claudine Kabera[1], Gregory Tyson[1]*

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The FDA's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) and the USDA's National Animal Health Laboratory Network (NAHLN) monitor antimicrobial resistance (AMR) in animal pathogens routinely isolated by veterinary clinics and diagnostic laboratories across the U.S. A cross-agency collaborating group was formed to develop a centralized data collection and reporting process across participating laboratories from both networks. The group consists of members from FDA's Vet-LIRN and National Antimicrobial Resistance Monitoring System (NARMS), and USDA's NAHLN.

More than 40 laboratories from both networks provided antimicrobial sensitivity test (AST) data on *Escherichia coli*, *Salmonella* spp., and *Staphylococcus intermedius* group species in dogs, using a commercially available testing platform. Laboratories sequenced a subset of isolates and submitted the whole genome sequencing data to public sequencing repositories. Both networks followed Clinical and Laboratory Standards Institute (CLSI) AST testing methods.

The first integrated report for 2018 data was released in December 2020 as a part of NARMS's Integrated Report Summary for 2018. The report included dashboards with minimum inhibitory concentration (MIC) data for approximately 2,300 isolates. This was the first time that integrated AMR monitoring data from dogs, collected from FDA and USDA's networks, became available in the U.S. The second joint report for 2019 data was released in March 2022, which includes MIC data for approximately 4,000 isolates. Dashboards for the second report also included resistance mechanisms from genomics data, along with the percent resistance and MIC distributions for each of the antibiotics included in the panels.

By developing a centralized data collection and reporting process across laboratories from FDA and USDA networks, data can be monitored for trends in AMR phenotypes and genotypes to identify new or emerging resistance profiles, to help monitor the continued efficacy of antibiotics over time, and to provide information to all stakeholders regarding these trends.

**Evaluation of the use of processing fluid testing in PRRSV-endemic sow farms**

*Angela Renee Colonga, Michelle LaCasse, Megan Lillehei, Kendra Pachniak, Eryn Stewart, Brittany Stokes, Michele Leiferman, Albert Rovira*

University of Minnesota, St. Paul, MN

Processing fluids (PF) are an excellent tool to detect PRRSV in farrowing rooms after an outbreak, and to monitor its disappearance after implementation of control measures. However, the use of PF to monitor PRRSV in endemic farms has not been studied in detail. The objective of this study was to evaluate PF as a tool to detect PRRSV circulation and strain variability in PRRSV-endemic farms. PRRSV testing data from 58 consecutive weeks from 68 sow farms were extracted from the laboratory information management system and analyzed. These farms routinely tested for PRRSV on PF on a weekly basis. In addition, these farms tested for PRRSV in serum of affected sows every time there was an observed increase in abortions.

Overall, 5,255 PF samples were tested and 2,421 were found positive (46%). However, the percentage of positive samples varied greatly from farm to farm, ranging from 1% of positive weeks in some farms to 98% in others. The median Ct value ranged from 27 to 40 (negative) and the minimum Ct value ranged from 16 to 33. Most farms experienced abortion events diagnosed as clinical PRRSV by PCR in sow serum. On average, there were 1.7 PRRSV abortion events per farm. However, this also varied from farm to farm, with a range of 0-9 PRRSV abortion events. The Ct values of the PF PCR tests performed before and after PRRSV abortion events were compared and found to be significantly different ($p<0.0001$). The median Ct value before a PRRSV abortion event (non-clinical phase) was 40 (negative), while the median Ct value after a PRRSV abortion event (clinical phase) was 27. These results were further analyzed by building a ROC curve to investigate the accuracy of a Ct value to predict a clinical PRRSV event. The cutoff value that maximizes sensitivity (88%) and specificity (71%) is Ct=32.

An average of 1.7 different PRRSV strains were identified by Orf 5 sequencing per farm (ranging from 0 to 4). More than one strain was identified in 49% of the farms. PRRSV Orf 5 sequencing was attempted 389 times and a full Orf 5 gene sequence was obtained in 308 occasions (79% sequencing success). Sequencing success was found to be dependent on the Ct value of the sample and on the specimen. For example, 100% sequencing success was achieved for samples with a Ct <24, independently of the specimen type. In contrast, samples with a Ct of 30-33 had a success rate of 75% for serum and 37% for PF.

In summary, PRRSV detection in PF of PRRSV-endemic farms is common. The Ct value can be used to determine the relevance of a positive result, although with low accuracy. A cutoff value that provides high Se and Sp could not be found. Co-circulation of multiple PRRSV strains in one farm is common. A single PF sample lacks accuracy as a diagnostic test to determine the role of PRRSV in endemic farms with increased abortions. However, weekly monitoring of PRRSV dynamics through PF testing may be a practical and cost effective strategy.

**Using PCR-negative results to enhance the monitoring of pig populations # †**

*Ana Paula Serafini Poeta Silva[1], Guilherme Arruda Cezar[1], Giovani Trevisan[1], Edison Magalhães[1], Gustavo Silva[1], Marcelo Almeida[1], Bret Crim[1], Eric R. Burrough[1], Phillip Gauger[1], Christopher Siepker[1], Alyona Michael[1], Panchan Sitthicharoenchai[1], Rodger Main[1], Mary Thurn[2], Paulo Lages[2], Cesar Corzo[2], Jerry Torrison[2], Rob McGuaghey[3], Franco Matias Ferreyra[3], Jamie Retallick[3], Jon Greseth[4], Darren Kersey[4], Travis Clement[4], Angela Pillatzki[4], Jane Hennings[4], Melanie Prarat[5], William Hennessy[5], Ashley Sawyer[5], Dennis Summers[5], Andreia Arruda[6], Daniel Linhares[1]*

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The early detection of emerging pathogens is often delayed due to the investigation of endemic pathogens that include similar clinical diseases in pig populations. For instance, the introduction of porcine epidemic diarrhea (PEDV) in the US in 2013 resulted in an increased number of porcine submissions for transmissible gastroenteritis virus (TGEV) testing in five swine-centric US Veterinary Diagnostic Laboratories (VDLs), and as reflection, the number of TGEV PCR-negative cases increased substantially. The introduction of a foreign agent, e.g., African swine fever virus, will likely demand testing for endemic agents with similar clinical presentation, e.g., porcine circovirus 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSV), and Salmonellas.

Therefore, this study's goal was to monitor PCR-negative testing results by utilizing the Swine Disease Reporting System database that contains PCR test data and testing results from the seven pathogens derived from diagnostic cases submitted to five VDLs containing (n = 1,264,909 cases). Time series (January 1st, 2010, through June 6th, 2022) for the proportion of PCR-negative cases (logit-transformed) per week were created for all seven pathogens (individually, aggregated in enteric [PEDV, porcine delta coronavirus (PDCoV), and TGEV], respiratory [Influenza A virus (IAV), *Mycoplasma hyopneumoniae* (MHP), PCV2, and PRRSV], and all seven pathogens together) using Seasonal Autoregressive-Integrated Moving-Average (SARIMA) algorithm. After that, residuals from SARIMA were used to detect aberrations over time using Exponential-Weighted Moving-Average (EWMA), e.g., week time-points that were >3-sigma upper control limit.

Individually, aberration time-points were detected for MHP (n = 1; aberration week: 2013-12-23), PDCoV (n = 4; last aberration week: 2021-10-18), PEDV (n = 5; 2014-06-16), PRRSV (n = 3; 2014-02-17), and TGEV (n = 6; 2014-06-16); but none for IAV and PCV2. One aberration time-point was reported for aggregated respiratory (2020-10-26) but 14 for enteric (2016-12-05). When cases contained all seven agents testing PCR-negative, three aberration time-points were detected (last two aberration weeks: 2021-10-11 and 2022-06-06). The aberration observed in enteric pathogens might have reflected the intensive testing done during PDCoV and PEDV epidemics. Further, the increasing number of surveillance programs implemented in high-health pig herds could explain the increase in PCR-negative cases for all pathogens in recent years.

A methodology to monitor PCR-negative cases derived from the main US VDLs was proposed that may support the monitoring of emerging exotic pathogens. Further analyses will entail covariates (sample types and production type) and assess model validation and forecasting.

# AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

**Monitoring porcine epidemic diarrhea virus detection using real-time diagnostic data to support animal health decisions * †**

*Guilherme Arruda Cezar[1], Giovani Trevisan[1], Edison Magalhães[1], Gustavo Silva[1], Marcelo Almeida[1], Bret Crim[1], Eric R. Burrough[1], Phillip Gauger[1], Christopher Siepker[1], Alyona Michael[1], Panchan Sitthicharoenchai[1], Rodger Main[1], Mary Thurn[2], Paulo Lages[2], Cesar Corzo[2], Jerry Torrison[2], Rob McGuaghey[3], Franco Matias Ferreyra[3], Jamie Retallick[3], Jon Greseth[4], Darren Kersey[4], Travis Clement[4], Angela Pillatzki[4], Jane Hennings[4], Melanie Prarat[5], William Hennessy[5], Ashley Sawyer[5], Dennis Summers[5], Andreia Arruda[6], Daniel Linhares[1]*

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Since the introduction of the porcine epidemic diarrhea virus (PEDV) in the United States in 2013, the swine industry has been fighting to control and eliminate PEDV from herds across entire swine production systems. Close monitoring PEDV detection is crucial to understanding its dynamics, distribution, and impacts. Collated veterinary diagnostic data offers a hub for monitoring endemic agents and identifying changes in the detection pattern at the overall and state-specific baseline level. This study aims to demonstrate the need for real-time data monitoring from 5 swine-centric laboratories to rapidly identify changes in the detection patterns at the US state level.

PEDV RT-rtPCR testing data from 2013 to 2022 were retrieved from participant VDLs and organized at the submission (case) level. Regardless of the number of samples submitted per case, one positive specimen designated a positive case. Monthly overall state-level changes in the percentage of positive cases were assessed using exponential smoothing models (ESMs) to forecast the expected 12-month period. Model-recovered residuals (predicted values minus observed values) were scanned by an exponential weighted moving average (EWMA) model to monitor and inform potential alterations from the expected baseline. The state-specific results were reported as no changes, 2-3, or >3 standard deviations (STD) above or below the baseline levels and plotted on a map for visualization.

In January 2022, PEDV was within state-specific baselines for the monitored states; however, an increase in PEDV RT-rtPCR positive submissions were observed initially at the end of January. From February to May 2022, five states (MO, MN, IA, KS, and NC) had PEDV detection >3 STDs above the state-specific baseline. From January to May 2022, 3,010 of 19,011 (15.75%) submissions tested PEDV positive. A higher number of submissions were observed from wean-to-market facilities and represented 7,288 submissions, with 20.8% testing positive. These detection levels were above 2021 values when 1,988 of 18,411 (10.8%) submissions tested PEDV positive. The information generated from monitoring PEDV detection has been included in the SDRS monthly reports (https://www.fieldepi.org/SDRS), providing up-to-date information about changes in PEDV detection patterns. This information supports practitioners and producers in making informed decisions on biosecurity, biocontainment, pig placements, herd exposure, and cleaning/disinfection protocols to prevent the further spread of PEDV. This study demonstrated the importance of closely monitoring aggregated veterinary diagnostic data, supporting the US swine industry with information during crisis events, and providing readily available data to help practitioners and producers make data-driven animal health decisions.

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

**A retrospective outbreak investigation of *Actinobacillus pleuropneumoniae* serotype 15 in central Iowa * †**

*Isadora Fernanda Machado[1], Mafalda Mil-Homens[1], Ana Paula Serafini Poeta Silva[1], Pete Thomas[2], Levi Johnson[4], Lori Feldmann[5], Lauren Glowzenski[3], Daniel Boykin[6], Tyler Bauman[7], Alyona Michael[1], Marcelo Almeida[1], Daniel Linhares[1], Gustavo Silva[1], Derald Holtkamp[1]*

[1]Department of Veterinary Medicine, Iowa State University, Ames, IA; [2]Iowa Select Farms, Iowa Falls, IA; [3]TriOak foods, Oakville, IA; [4]New Fashion Pork, Jackson, MN; [5]Protein Sources, Mapleton, MN; [6]Cactus, Osceola, IA; [7]The Maschhoffs, Carlyle, IL

*Actinobacillus pleuropneumoniae* (APP) is a widely distributed bacteria, causing important impacts on the health and economics of pigs. APP is a gram-negative bacterium classified in biotypes I and II, according to its isolation characteristics. APP serotype 15, classified as biotype I, was first described in Australia, and has been reported in North America, South America, and Japan. The APP transmission between herds occurs mainly through respiratory secretions from infected animals, directly or indirectly. Aerosol transmission is possible but at short distances. Moreover, the APP survival in the environment has a short duration in dry and warm conditions.

Since 2010, APP serotype 15 has been infrequently diagnosed at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). From 2010 to 2020, according to the ISU-VDL data, the total APP serotype 15 cases was 31 in the United States, and from January to October 2021, 11 cases were diagnosed. However, from November 2021 to January 2022, 20 cases were diagnosed in a small geographic cluster in Central Iowa, considered a high hog density area, with a mortality up to 56%.

A retrospective outbreak investigation was conducted by veterinarians at the Iowa State University in collaboration with the herd veterinarians and pork producers of seven growing sites owned by seven different companies, from February to May 2022. Information on other affected sites in the companies was shared during the investigation. The primary objective of the investigation was to assess how the bacteria may have been transmitted to the affected pigs' sites and evaluate risk events over 14 days prior to the outbreak date.

Four of the seven outbreaks investigated reported stress events prior to the outbreak, e.g., energy and water outages. Biosecurity procedures, e.g., shower-in-shower-out and downtime, were generally lacking. Production companies unknowingly shared operational connections with other companies, e.g., loading crews, feed deliveries, and rendering. Interestingly, 16 of the 20 growing pig sites were using rendering, and likely shared a common route offering an explanation for why the outbreaks were confined to a small geographic area. The results of the investigation suggested the lateral spread of the bacteria, with the primary transmission route most likely to be the rendering process and lack of biosecurity procedures to prevent the spread.

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

**Molecular characterization of *Glaesserella parasuis* strains circulating in North American swine production systems**

*Robert Mugabi, Ana Paula Serafini Poeta Silva, Maria Clavijo*

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*Glaesserella parasuis* (*G. parasuis*) is the causative agent of Glässer's disease in pigs. Serotyping is the most common method used to type *G. parasuis* isolates. However, the high number of non-typables and low discriminatory power makes serotyping problematic. In this study, 172 field clinical isolates and 15 *G. parasuis* reference strains were whole-genome sequenced (WGS). Multilocus sequence types, serotypes, core-genome phylogeny, AMR, and putative virulence genes were determined. *In silico* WGS serotyping revealed 13 of 15 serotypes, with 0.79 Simpson's diversity index. The dominant serotypes were 7, 13, 4, and 2. MLST identified 57 STs (53 novel), with 0.94 Simpson's diversity index. The most predominant sequence type (ST) was a novel ST454, corresponding to serotypes 7 and 13. At least one group 1 *vtaA* gene was observed in all isolates, except Serotype 6 (ST114), 8 (ST17 and ST299), 9 (ST25), 10 (ST407), and 15 (ST408). The number of group 1 *vtaA* genes was significantly associated with serotype 7 or ST6, ST454, ST416, and ST478 ($P < 0.05$). This study showed the use of WGS to type *G. parasuis* isolates, and can be considered an alternative to the more labor intensive and traditional serotyping and MLST based on PCR amplification. A combination of serotyping, MLST, and core-genome phylogeny provides better discrimination. These aspects combined with isolate's metadata are critical in disease epidemiology and design of autogenous vaccines.

## Epidemiology - On Demand



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**Pooling series to determine sensitivity loss in *Theileria orientalis* surveillance testing**

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Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA

Surveillance testing for *Theileria orientalis*, an agent of Bovine Infectious Anemia, is conducted using qPCR. Surveillance capacity can be greatly expanded by pooling multiple blood samples for DNA qPCR testing. However, pooling samples can reduce assay sensitivity. The purpose of this study is to determine sensitivity loss for representative positive samples in increasing pool sizes.

The distribution of Ct values for 657 positive *T. orientalis* surveillance samples was determined, with values ranging from 18 to 40, the majority falling between values of 25 and 30. This distribution was subsampled to a 100 sample curve, and blood samples previously confirmed positive by qPCR were selected by their Ct value to replicate this Ct distribution. Each of the 100 blood samples were taken through a pooling series consisting of a re-extraction as an individual sample, as well as its incorporation into pools of 2, 4, 6, 8, and 10 samples, with the remaining samples in each pool being made up of negative *T. orientalis* samples previously confirmed by qPCR. Blood samples were pooled together for a final volume of 150 µl, DNA was extracted using the Qiagen DNeasy Blood &Tissue kit, and run using a *T. orientalis/A. marginale* duplex qPCR assay to determine the Ct value of positive samples in different pool sizes.

Ct values increased from the individual sample value at an average of 0.97, 1.59, 2.17, 2.38, and 2.48 in 2, 4, 6, 8, and 10 sample pools, respectively. With this increase in Ct value we saw a 1% loss in sensitivity with pools of 4 and 6 samples, and a 2% loss in sensitivity with pools of 8 and 10 samples. Both samples that became undetectable when pooled with 3 or more other samples had individual Ct values greater than 38, representing 2% of the last 657 previous samples. Using this information, we can determine optimal pool sizes and retest strategies for different regions based on the prevalence of *T. orientalis* in order to expand surveillance capacity.

**Identifying the optimal age to initiate cancer screening in giant breed dogs**

*Gina Brandstetter, Jill M. Rafalko, Ashley Phelps-Dunn, Michelle C. Rosentel, Kristina M. Kruglyak, Angela L. McCleary-Wheeler, Katherine M. Lytle, Lauren E. Holtvoigt, Todd A. Cohen, Allison L. O'Kell, Jason Chibuk, Ilya Chorny, Daniel S. Grosu, Dana WY Tsui, Andi Flory*

PetDx, La Jolla, CA

Giant and large breed dogs are at higher risk of cancer, particularly osteosarcoma; however, few studies have examined the age at which these breeds typically develop cancer. This study analyzes data from a cohort of approximately 200 giant and very large breed dogs to determine the optimal age to initiate cancer screening for early detection.

The study population comprised 199 cancer-diagnosed dogs representing 12 of the breeds denoted by the American Kennel Club as the "largest dog breeds" (https://www.akc.org/expert-advice/dog-breeds/16-largest-dog-breeds/), including: Anatolian Shepherd, Bernese Mountain Dog, Bullmastiff, Cane Corso, Dogue de Bordeaux, Great Dane, Great Pyrenees, Irish Wolfhound, Leonberger, Mastiff, Newfoundland, and Saint Bernard. Data regarding cancer diagnoses in these dogs were collected as part of a larger research study from three sources: the CANcer Detection in Dogs (CANDiD) study, the National Cancer Institute Division of Cancer Treatment and Diagnosis Biological Testing Tumor Repository, and patients included in a 2021 publication by Hart *et al* from The University of California – Davis. Age at cancer diagnosis was analyzed for the overall study cohort and by breed.

For the 199 subjects in the study population, there were 95 females and 104 males. Most dogs (n=158, 79%) were spayed/neutered. Weight data was available for 73% of subjects (n=146), and the median weight of the population was 52.15 kg. The age at cancer diagnosis ranged from <1 year to 12 years, with a median of 6.0 years. For breeds represented by 10 or more cancer-diagnosed subjects, a breed-based median age at diagnosis was calculated, as follows: Bernese Mountain Dog (n=59; median age at cancer diagnosis 7.0y); Great Dane (n=42; 6.0y); Great Pyrenees (n=15; 8.0y); Irish Wolfhound (n=18; 6.1y); Mastiff (n=16; 5.0y); Saint Bernard (n=25; 6.0y); Remaining breeds (n=24; 5.5y). The most commonly diagnosed cancer in this study population was osteosarcoma (n=100), followed by lymphoma/lymphoid leukemia (n=34), and mast cell tumor (n=22).

In 12 of the largest dog breeds, the median age at cancer diagnosis was approximately 6 years. As cancer is expected to develop over time, it may be prudent to initiate cancer screening (using traditional approaches such as physical exam, CBC, chemistry, urinalysis, and newer methods such as liquid biopsy) two years prior to the peak incidence of disease, in an effort to identify and treat cancer at an early stage. Therefore, as a general recommendation, screening should be considered as early as age 4 in giant and very large breed dogs, with some variability when considering individual breeds. For Mastiffs (median age at diagnosis of 5), it may still be reasonable to initiate screening at age 4 (as an exception to the "two years prior" recommendation), as the breed was represented by a relatively small number of cancer-diagnosed subjects in this study (n=16), and the mean age at diagnosis was 6.2 years.

## Molecular Diagnostics and Bioinformatics 1

Saturday, October 8, 2022
Greenway HI

**Moderators:** Phillip Gauger and Pam Ferro



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**Development and validation of automated high throughput sample processing for SARS-COV2 surveillance**

*Duan Sriyotee Loy, Aaron Sedivy, Bruce Brodersen, John Dustin Loy*

Nebraska Veterinary Diagnostic Center, University of Nebraska-Lincoln, LINCOLN, NE

The SARS-COV2 pandemic caused significant worldwide morbidity and mortality. Different diagnostic methods have been employed to detect SARS—CoV2. The SalivaDirect™ assay, developed by Yale School of Public Health and approved by Food and Drug Administration (FDA) for use under Emergency Use Authorization (EUA), is an extraction free real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay run on saliva. Two liquid handlers: the Tecan Fluent® 780 (Fixed tips) and 480, have been validated for saliva sample processing and pre-rRT-PCR steps. A comparative analysis of cross-contamination, Limit of Detection (LoD), and Limit of Detection Confirmation (LoDC) was conducted to assess the technical performance of the automated Tecan Fluent® 780 vs manual protocol, for sample cellular lysis and heat inactivation of saliva samples. The program script used by the Fluent® 780 is capable of processing 372 samples per run in under one hour. A similar comparative analysis was conducted for Tecan Fluent® 480 with a manual protocol to transfer treated samples into PCR reaction plates, in both 96 and 384-well formats. The script used by the Fluent® 480 is capable of transferring 93 samples onto a 96-well formatted PCR reaction plate in 5 minutes, and 372 samples onto one 384-well formatted PCR reaction plate in under 30 minutes, per run. Sample tracking capabilities of the Tecan Fluent® 480 and 780 can track sample IDs and generate files for use by the thermocycler and for laboratory documentation.

Cross-contamination analysis was performed using 10 negative and 10 positive samples using both manual and automated protocols for the 96 well plate format, with an alternating order of positive/negative samples and samples tested in triplicate. Additionally, LoD was conducted by testing SARS-CoV-2 Full Genome RNA spiked into saliva negative for SARS-CoV-2 at concentrations of 50, 25, 12.5, 6.25, 3.125 and 1.563 copies/µl. Following, 20 replicates (LoDC) at 0.5x, 1x, and 2x of the preliminary LoD (6 copies/µl) were also tested in the same workflows.

Results from the cross contamination test demonstrate there is no cross-contamination caused by the automated procedure for both Tecan Fluent® 480 and 780. The LoD of the SalivaDirect Assay using the automated protocol was confirmed to be fewer than 6 copies/µl. These procedures have subsequently been approved as part of the EUA protocol for the SalivaDirect™ assay.

In summary, the high throughput Tecan Fluent® 480 and 780 are a robust automation approach to utilize for surveillance testing. Automation of laboratory testing can reduce test error and reduce the need for additional personnel while increasing testing capacity, and potentially can reduce the need for disposable tips. Integration of these instruments into laboratories has the potential to enhance capacity for future emerging or high consequence pathogen surveillance testing.

**Development of an Ion Torrent targeted NGS panel for detection of feline respiratory pathogens with the inclusion of SARS-CoV-2**

*Jobin Kattoor, Rebecca P. Wilkes*

Animal Disease Diagnostic Lab, Purdue University, West Lafayette, IN

A wide array of pathogens can cause respiratory diseases in cats. In 2019, when the pandemic struck the human population, it was found that the cat family, including domestic cats, can be infected by SARS-CoV-2 and suffer from respiratory disease. Hence, in this study, we incorporated primers to detect SARS-CoV-2 into a targeted NGS panel that was developed to detect and sequence known pathogens of cats. Addition of these primers allows surviellence for SARS-CoV-2 with routine testing for feline pathogens. The test validation focused on SARS-CoV-2 and other pathogens known to cause respiratory disease in cats including Feline herpesvirus, Feline calicivirus, *Chlamydia felis, Mycoplasma felis, and Bordetella bronchiseptica.* Nucleic acids were extracted from the samples with the Applied Biosystems MagMAX Viral/Pathogen II kit and cDNA libraries were prepared using an automatic Ion Chef DL8 system and were sequenced in an Ion S5 sequencer using the manufacturer protocols. Data were assembled using SPAdes and mapped to a reference file containing sequences from the pathogens. Geneious software was used to evaluate the reads, and BLAST analysis was performed to confirm the results. Feasibility was evaluated with known positive samples and type strains for each agent. For SARS-CoV-2, the primers were synthesized against conserved regions in the genome to successfully identify all known variants. LOD of the assay was evaluated with dilutions of positive clinical samples, and based on comparison to qRT-PCR/qPCR for each agent, the LOD was similar to a Ct value of 36-37 for all the pathogens except *Mycoplsma felis*. The analytical specificity of the primer sets was tested using known positive samples containing respiratory pathogens of cats, spiked with SARS-CoV-2 RNA, and *in-silico* evaluation using BLAST. The targeted NGS panel exclusively identified the specific agents. When compared to known positive and negative clinical samples from cats based on qRT-PCR results, the diagnostic sensitivity and specificity of the targeted NGS assay were both 100% for SARS-CoV-2. We found some variability in the results with different conditions, including extraction methods and sample storage conditions. Based on the results, inclusion of SARS-CoV-2 primers into a targeted NGS panel for detection of feline pathogens is an effective means of incorporating surveillence for this organism into the diagnostic laboratory.

**Identification of highly pathogenic avian influenza A type H5N1 (clade 2.3.4.4) in red foxes (*Vulpes vulpes*)**

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Highly pathogenic Avian Influenza A (HPAI) is highly transmissible among birds and can cause high mortality in domestic poultry. The current HPAI epizootic in the United States has been confirmed in 35 states, affecting more than 37.9 million domestic birds as of June, 2022. Influenza adapts to its host(s) through variation in hemagglutinin (HA) and neuraminidase (NA) binding. In this study, we have identified and characterized HPAI H5N1 clade 2.3.4.4 viruses in Red Foxes (*Vulpes vulpes*) from New York.

Between April and May 2022, 24 Red Foxes were submitted to the NYS Department of Environmental Conservation Wildlife Health Program or Janet Swanson Wildlife Hospital at Cornell and brought to the Animal Health Diagnostic Center (AHDC) at Cornell University for Influenza PCR. Some of the observed fox kits showed signs of distress and veterinary reports included respiratory and neurologic signs including seizures.

Tissue samples were extracted using a modified version of the MagMAX CORE Nucleic Acid Purification Kit. Lung and brain samples from ten of the 24 fox kits were found to be positive for Influenza matrix PCR and "non-negative" (presumptive positive) for Avian Influenza PCR (NAHLN) and H5PCR (NVSL). The purified nucleic acid was then subjected to multi-segment reverse-transcription PCR (M-RT-PCR) for whole genome amplification, a method that takes advantage of the highly conserved 5' and 3' termini of the 8 RNA segments of Influenza A viruses. Libraries were prepared from the M-RT-PCR product using Illumina DNA Prep and pooled using a concentration-based normalization. Pooled libraries were sequenced on the Illumina iSeq (2x150bp chemistry). Preliminary typing was performed on the raw reads by using INSaFLU and found to be of the subtype H5N1. Read quality was assessed using FastQC. Sequencing reads were error-corrected using Lighter and trimmed with Trimmomatic to remove the Illumina adapters and bases with poor quality. Reference-based assemblies were made for each sample using Snippy with the H5N1 reference genome A/Thailand/1(KAN-1)/2004. Segment 4, hemagglutinin (HA) assemblies were analyzed using the H5 Clade Classification tool available on the Influenza Research Database, and all assigned to the clade 2.3.4.4. Phylogenetic analysis was conducted by performing alignments with published genomes in GISAID using MAFFT and Geneious Prime Tree Builder.

It is likely that the fox kits acquired HPAI, subtype H5N1, clade 2.3.4.4, through contact with infected wild or domesticated birds. HPAI has recently been also reported in fox kits from Canada, the Netherlands, Japan, and the US (in the states of Iowa, Michigan, Minnesota, and Wisconsin). In addition to red foxes described here, HPAI has also been detected in bobcats, coyote, and skunks in North America, which highlights the relatively broad host range of the current HPAI H5N1 strain and indicate that all carnivorous mammals should be considered at risk of infection.

**Analysis of IAV transmission in large U.S. swine production systems using active surveillance †**

*Megan Neveau[1], Aditi Sharma[1], Phillip Gauger[1], Amy Vincent Baker[2], Tavis K. Anderson[2]*

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Influenza A virus (IAV) is one of the three most frequently detected causes of respiratory disease in swine. The passive USDA swine surveillance system provides aggregated metrics to quantify spatial and temporal changes in genetic diversity; however, swine production is not homogenous. Production systems vary in size and management strategies that may affect the transmission and evolution of IAV. We conducted active surveillance on selected sow farms and their linked downstream nurseries from 4 large US production systems for at least 12 monthly collections. From IAV positive samples, we obtained 85 complete HA sequences, and of these, we successfully assembled 61 whole genomes with linked production system metadata. To infer transmission and evolution, we conducted Bayesian phylodynamic analyses on the active surveillance data combined with control sequences sourced from passive swine and human surveillance. We detected 6 genetic clades from four HA lineages: the H1 1A classical swine, the H1 1B human-seasonal, and the H3 2010.1 and 1990.4 lineages. The 1B and H3 1990.4 strains showed evidence of transmission from sow farm to downstream nurseries. In contrast, 1A and H3 2010.1 viruses were detected in nurseries without upstream detection in linked sow farms. We also detected seven unique human-to-swine transmission events in the H1N1 pandemic clade (1A.3.3.2) in sow and nursery sites. These data demonstrated that nursery sites were also infected with IAV not linked to the sow farm, possibly due to subclinical IAV below detection levels in the breeding herd, mixing of sow farm sources at the nursery, regional spread of new strains, or human-to-swine transmission. Detection of IAV in sow farms was predictive of transmission to nurseries in 3 out of 4 systems in our study, which can inform measures to decrease IAV virus movement among these production sites. Additional investigation is needed to understand nursery detections not linked to the sow farm.

† Graduate Student Oral Presentation Award Applicant

**Comparative genome analysis of group B rotaviruses from a goat kid and foals with diarrhea**

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Rotaviruses (RVs) are among the significant enteric pathogens of animals and humans. In 2021, a novel Equine Rotavirus Group B (ERVB) in Kentucky was identified from young foals with diarrhea and suggested ERVB originated from ruminants based on their genome analysis. Interestingly, in March 2021 (around the similar time point of ERVB outbreak), University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) received a fecal sample from a 1-week-old goat kid with diarrhea in a farm with 5 other diarrheic kids. The fecal sample was tested for *Bovine coronavirus*, *Bovine rotavirus* Group A, *E. coli* K99+, *Cryptosporidium parvum* and *Salmonella* spp by multiplex real-time PCR assays and none of them were detected. Once the ERVB-specific real-time PCR became available, the fecal sample from the goat kid was tested for ERVB and found positive. Genome sequence of RVB from the fecal sample of the goat kid was obtained using metagenomic testing by Illumina MiSeq. Contigs were assembled de novo using CLC Genomics and analyzed by BLASTX using the BLAST2Go plugin. Analysis of contigs revealed that 9 viral segments (out of 11) of RVB were sequenced either completely or partially. BLAST analysis indicated that the new RVB identified from the goat kid in the current study has overall higher nucleotide identity to ERVB than previously sequenced goat RVBs. These findings suggest that the recent ERVB outbreaks in foals and the RVB infection in the goat kids in Kentucky may be related.

**RNA next generation sequencing of FTA card-spotted clinical samples from commercial poultry farms in Mexico**

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Shipment of diagnostic samples from foreign countries is increasingly difficult due to high shipping costs, and import/export controls on shipping live viral or bacterial samples. These challenges can be overcome by using FTA cards, which inactivate pathogens in the samples and allow transport at room temperature. The cards are commonly used for pathogen identification by PCR and Sanger sequencing. With the greater availability, reduced costs, and improved protocols, Next Generation Sequencing (NGS) is becoming a more practical tool for routine diagnostics. As part of a collaborative effort, clinical samples (oropharyngeal, cloacal and tissue swabs) from Mexican commercial chicken flocks were collected (100 birds per flock), pooled (25 samples per pool), spotted on FTA cards, and shipped to our BSL-3 laboratory for untargeted microbial pathogen identification by NGS. Using an in-house RNaseH-mediated host/bacterial rRNA depletion to increase NGS sensitivity) and sequence-independent single primer amplification (SISPA) with the Illumina MiSeq sequencing of Nextera libraries, members of several avian viral families and bacterial pathogens were identified. Full-genome sequences were obtained for several viral pathogens, including infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV), avian metapneumovirus subtype A (AmPV-A), low pathogenic H5N2 avian influenza, and multiple picornaviruses. Although some of these pathogens were known to circulate in Mexican poultry flocks, in-depth analysis of full-genome sequences provided more insights into their subtype/genotype/pathotypes. Some of the bacterial pathogens identified using 16S rRNA analysis included *Mycoplasma synoviae*, *M. gallisepticum*, *Ornithobacterium rhinotracheale*, and *Bordetella avium*. Although many known or potential pathogens were identified, their roles in clinical disease remain undetermined because of the lack of metadata from the samples flocks. Although more expensive than real-time RT-PCR, direct RNA NGS of field-collected clinical samples embedded on FTA card is an advantageous agnostic diagnostic tool with potential for full-genome analysis of avian viral and bacterial pathogens.

**Validation of an automated workflow using Hamilton liquid handlers in a molecular diagnostics laboratory**

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Accuracy and efficiency are key factors in the timely detection and management of infectious diseases. In the agriculture industry, multi-million dollar decisions are often made based on the results of diagnostic testing for such diseases. PCR has long been the gold standard for detection of viral and bacterial pathogens, but this sensitive form of testing is often subject to the possibility of human error from technicians performing the assays. The MVDL Molecular Diagnostics section has operated with traditional, manual methods for nucleic acid extraction and PCR setup for many years. The lab proposed a workflow overhaul that included the implementation of automated liquid handlers to improve accuracy of results, lab efficiency, and testing capacity. Two Hamilton Microlab STAR and a Hamilton Microlab STARlet automated liquid handlers were purchased, along with NucleoLIS automated reporting software. Method programs were designed for these instruments to prepare clean reagent plates for nucleic acid extractions on the MagMax CORE Nucleic Acid Purification Kit, load samples and lysis into extraction plates, and load nucleic acid templates into PCR plates. A side-by-side comparison was performed for each Hamilton method compared to manual protocols currently used by the lab. Clean reagent, extraction, and PCR plates were weighed to ensure the proper liquid volume was loaded by the instruments. Both manually-loaded and STAR-loaded extracted material were tested using real-time PCR. Results demonstrated that the STARs load appropriate clean reagent volumes into plates. The STARs also load a variety of sample types at appropriate volumes into the correct positions on a plate to yield similar Ct values compared to manual extraction and PCR. Barcoded samples allowed NucleoLIS software to track each sample through the extraction, PCR, and reporting processes. Tracking of samples and results in this manner improved accuracy and confidence in results reporting. Utilization of the STARs proved to increase lab efficiency in many aspects. Nucleic acid extraction plates could be loaded two times faster than the average PCR technician loading by hand. Weekly clean reagent plate preparation time was reduced by almost two-thirds. Testing capacity was also improved dramatically. At maximum extraction capacity, the lab would be able to extract 768 samples per hour with the STARs. Increased capacity will be incredibly beneficial in future outbreak scenarios, when the lab will be able to extract and test over 4,500 samples in an 8-hour shift if necessary.

**Exploration of mucin 5B (MUC5B) in swine oral fluid * †**

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Mucins are large glycoproteins present in all mucosal surfaces of the body. The 22 mucins identified to date have a variety of functions. Among other functions, mucins of the upper respiratory tract and oral cavity (MUC1, MUC4, MUC5AC, MUC5B, MUC7, MUC16, MUC19, and MUC20) have the capacity to capture and clear microorganisms using a variety of mechanisms.

Despite their importance in homeostasis, mucins are largely understudied in veterinary medicine. This study focuses on mucin 5B (MUC5B) in swine oral fluids with the specific aim of evaluating its possible use as a measure of oral fluid sample quality in PCR testing.

Among 51 swine oral fluid samples submitted for routine testing to the Iowa State University Veterinary Diagnostic Laboratory, Next Generation Sequencing (NGS) detected MUC5B sequence fragments in 7 samples. Based on the NGS information, three qPCR MUC5B primer sets were designed using Primer Express™ Software v3.0.1 (Applied Biosystems). Primers were evaluated in oral fluid and serum samples (serum used as an expected negative sample), and the optimal primer was selected based on dissociation curve analysis.

The final MUC5B qPCR was based on extraction with E.Z.N.A.® Viral RNA Kit (Omega Bio-tek, USA) and amplification with a primer concentration of 500nM, probe concentration of 100nM, and TaqPath™ (Thermo Fisher Scientific, Inc.) master mix. All qPCRs were run on the Applied Biosystems™ 7500 Fast Real-Time PCR System (Applied Biosystems).

Swine oral fluids (n = 20) and sera (n = 20) collected from commercial production sites were tested with the MUC5B qPCR. Oral fluid samples (n = 2) known to be positive for MUC5B by NGS were used as positive controls. Cq responses were compared among specimens, with samples producing Cqs ≥ 37 considered negative.

In this study, 11 of 20 oral fluids tested with the MUC5B qPCR had Cqs < 37 (range: 34.9 to 36.3), and 19 of 20 sera were < 37 (range: 29.7 to 36). Although the investigators had expected serum samples to be negative for MUC5B, the mean MUC5B Cq was lower in serum (mean: 32.5) than oral fluid (mean: 36.5).

MUC5B is a gel-forming mucin that provides lubrication, enhances the viscoelastic properties of oral fluids, and interacts with infectious agents, e.g., MUC5B in saliva is able to completely inactivate HIV (Habte et al., 2006). In pigs, an understanding of the physiological role of MUC5B in serum is lacking, but in humans some reports suggested that higher levels of mucins in serum may be associated with non-infectious diseases, e.g., cancer and auto-immune diseases (Boonla et al., 2003; Agha-Hosseini et al., 2017; Bademler et al., 2019). Overall, the results of this study eliminated the possibility of using MUC5B as a sample quality indicator for swine oral fluids and raised questions regarding the significance of MUC5B in serum.

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

## Molecular Diagnostics and Bioinformatics 2

Sunday, October 9, 2022
Lake Minnetonka

**Moderators:** Solomon O (Wole) Odemuyiwa and Ying Fang

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

**Genome sequence of *Neorickettsia risticii* from an aborted equine fetus**

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*Neorickettsia risticii* and the recently described *N. findlayensis* are obligate intracellular bacteria of digenean trematodes and mammals that cause Potomac horse fever (PHF), a severe systemic febrile diarrheal disease in horses. *N. risticii* has also been sporadically reported to cause abortion in pregnant mares. Complete genome sequences of *N. risticii* and *N. findlayensis* have been previously determined from cell cultures inoculated with the blood of infected horses. However, the genome sequence of a *Neorickettsia* species from an aborted equine fetus has never been reported before.

A 5.5-month-old aborted equine fetus with presumptive lymphohistiocytic enteritis and colitis tested positive for *N. risticii* by a real-time PCR assay. Metagenomic sequencing from the archived and frozen fetal colon sample was used to obtain the genome sequence of the pathogen. Libraries were prepared using Nextera XT library preparation kit after reverse transcription of the total nucleic acid extraction of the sample. Pooled libraries with unique indexed adapters were sequenced using a MiSeq instrument. 5.63% of the 2,100,333 total reads corresponded to *N. risticii* genome. Reads were trimmed using the TrimmomaticPE v0.39 tool and assembled *de novo* using SPAdes v3.15.4. A total of 136 of the 191 contigs >2 kbp mapped to the *N. risticii* genome (GenBank accession CP001431) using BlastN, equivalent to 94.5 % coverage. Taxonomy assignation using OrthoANI against reference genomes of *N. risticii*, *N. findlayensis*, *N. helminthoeca* and *N. sennetsu* unambiguously determined *N. risticii* as the species with 99.62% average nucleotide identity. This was further confirmed using the tax-check module of the NCBI PGAP tool (build6021). Finally, a reference-guided assembly was performed using the SPAdes trusted-contig option with the *N. risticii* CP001431 chromosome. This resulted in a near-complete *N. risticii* genome in a large contig of 816.33 kbp and five smaller contigs totaling 99.8 % coverage of the reference. This work reports the first *N. risticii* genome from a case of equine abortion by this pathogen.

**Phenotypic resistance prediction in *Escherichia coli* isolates from the National Animal Health Laboratory Network Antimicrobial Resistance Pilot Project, 2018-2022 †**

*Henri Chung[3], Christine Foxx[2], Karin Dorman[3], Iddo Friedberg[3], Christina Loiacono[1], Beth Harris[1]*

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Antimicrobial resistance (AMR) is a pressing global health threat affecting humans and animals. The USDA launched this pilot to monitor AMR trends in pathogens routinely isolated from sick animals by U.S. veterinary clinics and diagnostic laboratories. One of the pathogens we monitor is *Escherichia coli*, a zoonotic pathogen that can accumulate AMR genes via horizontal gene transfer. We evaluated AMR-related phenotypes in *E. coli* isolates from cats, chickens, cows, dogs, horses, pigs, and turkeys (N = 980) using host-specific, commercially available Sensititre™ broth microdilution plates. Results were split into two datasets, based on minimum inhibitory concentration (MIC) data for antibiotics with species-specific breakpoints in the *Vet01S* (*clsi;* CLSI, 2020) and without CLSI breakpoints (*type*: re-categorized into greater than [wild type, *wt*] or less than the 75$^{th}$ percentile of values [non-*wt*]), respectively. *E. coli* isolates were also sequenced using either the Illumina iSeq500 or MiSeq platforms to produce AMR genotypes; AMR genes and associated plasmid replicons were detected using >70% identity and >95% reference gene coverage thresholds using the NCBI AMRFinder and ABRicate toolkits. In total, 276 [GM1] unique AMR genes were used to predict AMR phenotype in three logistic regression models: (A) presence/absence of a single gene against individual antibiotic drugs within a class, (B) presence/absence of a single gene within groups (based on expected antibiotic class resistance conferred), and (C) number of genes within each group as in (B). In model (A), the presence of *aac(3)-IId* in the *clsi* data increased the odds ratio (OR) of gentamicin resistance by 220-fold compared to gene absence ($p < 0.0001$). *blaCMY-2* presence had >75-fold OR against absence for 3$^{rd}$-generation cephalosporins in both datasets. *blaTEM-1, dfrA17, florR, sul1* and *sul2,* and *tet(A)* and *tet(B)* increased the OR of predictive resistance over 75-fold against amoxicillin, trimethoprim/sulfamethoxazole, florfenicol, sulfonamides, and tetracyclines in the *type* data, respectively. In model (B), the presence of genes conferring sulfonamide and folate pathway antagonist resistance separately increased the OR of sulfathiazole and trimethoprim/sulfamethoxazole resistance by 824- and 179-fold, respectively ($p < 0.0001$ in both cases). In model (C), increased numbers of beta-lactam resistance-associated AMR genes only affected the likelihood of cefovecin resistance (31-fold, $p < 0.01$). Overall, we show that AMR genotype information from whole-genome sequencing can be used to predict antibiotic resistance with varying accuracy: individual AMR genes are strong predictors of antimicrobial resistance, followed by presence/absence of any gene within groups based on expected antibiotic class resistance conferred. Interestingly, we show for the first time that different alleles of the same gene, e.g. *blaEC-5* and *blaEC-18*, may have varying effects on resistance phenotype prediction in *E. coli*.

† Graduate Student Oral Presentation Award Applicant

**Feline upper respiratory infection: a retrospective analysis of clinical cases submitted to Georgia Veterinary Diagnostic Laboratories**

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Several viral and bacterial agents can cause feline upper respiratory infections. The Georgia Veterinary Diagnostic Laboratories provide ocular or respiratory PCR panels to aid disease diagnosis. While the ocular panel targets feline herpesvirus (FHV), feline calicivirus (FCV), and *Chlamydophila felis* (*C. felis*), the respiratory panel detects FHV, FCV, *C. felis*, *Mycoplasma spp*., *Bordetella bronchiseptica* (*B. bronchiseptica*) and Influenza A virus. The objective of this study is to conduct a retrospective assessment of the test results from these two panels.

A total of 63 submissions were made for the ocular panel during 2016 to 2021. The positivity rate was 55.56% with 35 cases positive for at least one pathogen (35/63). There were 9 cases with co-infections of 2 agents (7 cases) or all 3 agents (2 cases) resulting in a co-infection rate of 25.71% among positives. The most common detection was a single positive of FCV or *C. felis* (9 cases each), followed by a single infection of FHV (8 cases).

A total of 388 submissions were made for the respiratory panel during 2012 to 2021. The positivity rate was 70.10% with 272 cases positive for at least one pathogen (272/388). There were 115 cases with co-infection of 2 agents (92 cases), 3 agents (21 cases), or 4 agents (2 cases) resulting in a total multi-infection rate of 42.28% among positives. The most frequent detection was single positive of *Mycoplasma* (95 cases), followed by co-infection of FCV and *Mycoplasma* (56 cases). The most common detection among co-infections of two pathogens was from FCV and *Mycoplasma* (60.87%), followed by FHV and *Mycoplasma* (18.48%). The most common detection among co-infections of three pathogens was from FCV, FHV, and *Mycoplasma* (61.90%), followed by FCV, *Mycoplasma*, and *C. felis* (23.81%). Interestingly, there was no detection of Influenza A virus during these 10 years.

Given the high incidence of *Mycoplasma* in upper respiratory infections from our current study, we propose adding *Mycoplasma* in the feline ocular panel to better cover the potential agents. With the possibility of COVID-19 becoming an endemic disease, adding SARS-CoV-2 to the panel is also under consideration. Information obtained from this study will also provide insights for Georgia clinicians of prevention and treatment suggestions when communicating with the pet owners.

**Whole genome sequencing for genetic, antigenic and pathotypic characterization of turkey arthritis and hepatitis reoviruses**

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Avian reoviruses continue to cause disease in chickens and turkeys with varied pathogenicity and tissue tropism. Turkey arthritis reovirus (TARV) has been associated with lameness in turkey flocks older than 10 weeks of age resulting in significant economic losses since 2011. Starting in January 2019, we have discovered new variants of reoviruses causing hepatitis and mortality in turkey poults with median age of 15.5 days. The change from causing mild enteric disease to economically important arthritis and hepatitis highlights the need for genetic, antigenic, and pathotypic characterization of TARV and newly emerging turkey hepatitis reoviruses (THRVs). The selection of vaccine strains of reoviruses is challenging due to the ability of their genomes to mutate and recombine, substantial heterogeneity in the spatial distribution and spread of different strains, and potential turnover in dominant strain across time.

Understanding the evolutionary and epidemiological characteristics of TARVs and THRVs is critical for designing effective vaccination campaigns at national level. Sequence data and epidemiological metadata were integrated in a Bayesian phylodynamic framework to quantify evolutionary change in the virus across time, differentiate between endemic and emerging strains, and ultimately help identify appropriate strains for manufacturing effective vaccines. The 200 TARVs and 70 THRVs representing different states, age of turkeys, and year of isolation were selected for whole genome sequence analysis. Selected isolates were submitted to AviServe for serotyping and *in vivo* pathotyping. Analysis of all 10 gene segments grouped THRVs with TARVs with no specific grouping of THRVs indicating that THRVs are probably variants of TARVs. The segments encoding outer capsid proteins are evolving/changing at a faster rate as compared to the segments encoding inner core proteins. The average substitution rate across all segments was 0.00188 (range: 0.00124 – 0.00278) per site per year. Segments encoding outer capsid proteins (sigma B, sigma C, and mu B) showed the highest substitution rates (0.00191, 0.00227, and 0.00278 substitutions/site/year, respectively). The time-tree for the mu B segment showed more clustering that could represent 4 or 5 unique serotypes. The time-trees suggest little spatial structuring of the TRV population. The results of genotyping, phylodynamic analysis, serotyping and pathotyping will be discussed in detail.

**Use of a porcine endogenous reference gene (internal sample control) in a PRRSV RT-qPCR * †**

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Endogenous reference genes, i.e., housekeeping genes inherent to a specimen, have been used extensively as internal sample controls (ISC) in PCR applied to gene expression research and, with increasing frequency, in veterinary diagnostic PCR. This study evaluated the detection of a porcine endogenous reference control in a commercial PRRSV RT-qPCR (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., Westbrook, Maine, USA) using samples of known PRRSV status collected under ideal (research) conditions.

Oral fluids (n = 130) and serum (n = 132) were collected from 12 14-week-old pigs individually housed under experimental conditions. Pigs were vaccinated with a PRRSV modified-live vaccine (MLV) (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc., Duluth, Georgia). Samples were collected on day post vaccination (DPV) -7, 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 17, 21, 28, 35, and 42 and then tested using a commercial RT-qPCR that detects PRRSV and the ISC simultaneously. ISC results were analyzed in terms of the frequency of detection and distribution of Cq values. Thereafter, the 95th, 97.5th, and 99th percentiles of the ISC Cqs were calculated per specimen using R 4.1.0 (R core team, 2020) to establish the upper limits of the expected response.

All oral fluid and serum samples were negative for PRRSV RNA at -7 and 0 DPV, with the first positive result at 3 DPV for both sample types. The ISC was detected in all samples (n = 262), with a mean Cq value of 26.6 in oral fluid, and 27.1 in serum. The ISC upper limits (95th, 97.5th, and 99th percentiles) in oral fluids were 29.5, 30.1, and 30.7 Cqs, and in serum were 29.1, 29.3, and 29.5 Cqs.

Although not commonly described in veterinary diagnostics, the use of ISCs could be a useful addition to quality management in routine PCR testing. In this study, the ISC response in samples collected under the "best-case scenario" (i.e., under experimental conditions and immediately stored) was uniform over the time and unaffected by PRRSV replication. Thus, preliminary data suggest that failure to detect the ISC presents an irregularity either with the sample or the testing process. Work in progress will evaluate the effect of sample "mishandling", e.g., adverse storage conditions on ISC detection.

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

**PRRSView: An analytical platform for the assessment of PRRSV ORF5 genetic sequences**

*Anugrah Saxena[1], Michael Zeller[2], Giovani Trevisan[1], Aditi Sharma[3], Daniel Linhares[1], Karen Krueger[1], Jianqiang Zhang[1], Phillip Gauger[1]*

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the most economically important swine pathogen in North America and is second globally only to African swine fever virus. PRRSV is a positive-sense, single-stranded RNA virus associated with reproductive disorder of sows and respiratory disease of pigs at all ages. Diagnostic tests are commonly used to monitor the presence of PRRSV in swine populations including sequencing the open reading frame 5 (ORF5) gene to track the epidemiology of the virus and lateral introductions into a farm. PRRSView is a web portal created at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) to host analytical and phylogenetic tools related to PRRSV ORF5 sequences with the goal of assisting veterinarians and producers in evaluating the genetic diversity, spatial, and temporal aspects of ORF5 sequences maintained in the ISU VDL database.

PRRSView works in conjunction with the broader Swine Disease Reporting System (SDRS) project to contextualize the ever-changing patterns of PRRSV diversity, and supports interactive tools for veterinarians to analyze their sequence data compared to other sequences detected throughout the United States. The PRRSView homepage provides a phylogenetic overview of the sequences generated by the ISU VDL within the previous month, indicating the current strains detected in circulation. There are currently three ORF5 analytical tools available on PRRSView: a genetic sequence BLAST tool, a vaccine identity tool, and an RFLP tool. The ORF5 BLAST tool allows the users to submit their ORF5 gene sequences and returns up to 10 closely related sequences from the ISU VDL database, with metadata that includes the state, genetic lineage, RFLP, and identity to the query sequence. The vaccine identity tool allows users to quickly calculate the percent homology of their sequence(s) to five different PRRSV vaccines: Ingelvac PRRS ATP, Ingelvac PRRS MLV, Prime Pac PRRS, Fostera PRRS, and Prevacent PRRS, as well as the distance to the Lelystad European strain. This tool also builds a neighbor-joining tree with a set of curated strains to estimate the genetic lineage of the sequence, which is rendered in the web browser for viewing. Additionally, this tool will calculate the RFLP of the sequence, and the exact positions of the cut sites are shown when hovering over the RFLP value. The last analytical tool provided is the ORF5 RFLP tool, which quickly calculates the RFLP pattern of the input sequences.

These analytical tools are designed to allow veterinarians and researchers to easily analyze their PRRSV ORF5 sequences against the expansive ISU VDL database to gain valuable epidemiologic information and comparative data regarding the genetic lineages and related metadata of the PRRSV circulating in a production system, while lowering the barrier of entry for use.

**A real-time PCR assay for detection of three major canine respiratory bacteria: *Bordetella bronchiseptica, Mycoplasma cynos* and *Mycoplasma canis* †**

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Canine infectious respiratory disease (CIRD) is one of the most common diseases in dogs. *Bordetella bronchiseptica* has been identified as the most common causative agent of CIRD, however *Mycoplasma cynos* and *Mycoplasma canis* have also been associated with the disease, with the former species observed to be more pathogenic than the latter. A unique genomic segment of *B. bronchiseptica* encoding for a hypothetical protein with unknown function was selected as the detection target for this assay; a BLAST search of this target did not result in high homology to other non-target bacterial genomes. Although the *Mycoplasma* genus is genetically diverse, causing infections in humans and in many animal species, interestingly, the 16S rRNA gene in different *Mycoplasma* species is genetically divergent, and can be used as a target for differential detection. All available target sequences were used for primer and probe designs. Larger segments of the genomes, encompassing the primer binding regions, were cloned to serve as positive amplification controls (PACs), and used for analytical analysis of the assay. Standard curves using cloned PACs for *B. bronchiseptica, M. cynos and M. canis*, resulted in PCR amplification efficiencies of 95.8%, 97.0% and 94.5%, respectively, with correlation coefficients all >0.99; limit of detection for these targets was observed at endpoint Ct of 37, which corresponded to 15, 10 and 25 copies per PCR reaction for the three bacteria, respectively. Screening of 740 canine respiratory samples submitted to KSVDL during 2019-2021 identified 111 that were positive to at least one of the three pathogens, among which 22, 73 and 57 were positive to *B. bronchiseptica, M. cynos and M. canis,* respectively. Selected positive samples were sequence confirmed. Interestingly, 37.0% (27/73) of *M cynos* positives were also positive to *M. canis*, and 58.3% (14/24) of *B. bronchiseptica* positives were also positive to one of the two *Mycoplasma* species, indicating potential co-infection may have occurred in these animals. A majority of samples tested were nasal swabs with some lung tissues submitted. To evaluate whether these specimen types may impact assay performance, two 100-fold dilutions were prepared from highly concentrated samples of B. *bronchiseptica, M. cynos and M. canis*. The original sample and both dilutions, representing high, medium and low concentration range, were spiked into CIRD-negative swab and lung tissues, then extracted and tested by the PCR assay. All target Ct values among like dilutions were nearly identical between both specimen types, indicating that nasal swabs and lung tissue do not affect assay sensitivity.

† Graduate Student Oral Presentation Award Applicant

**Development of a sensitive and reliable qPCR assay for detection and differentiation of *Streptococcus equi* and *Streptococcus zooepidemicus***

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*Streptococcus equi* subspecies *equi* (SEE) causes a highly contagious upper respiratory disease in horses known as strangles and is the most frequently reported equine infectious disease in the USA. Strangles outbreaks are associated with high morbidity and extensive costs associated with diagnostic testing, treatment, biosecurity measures, and lost revenue from deaths and event cancellations. The ancestor of SEE, *Streptococcus equi* subsp. *zooepidemicus* (SEZ), is considered an opportunistic commensal organism that may cause pneumonia, endometritis, and abortion in horses. Laboratory diagnostics are critical for control and eradication of streptococcal outbreaks, but current tests have limitations. Classically, diagnosis of SEE and SEZ infections relied on microbiologic culture, but this method is slow and has low detection sensitivity. Multiple qPCR assays have been developed to facilitate detection of both SEE and SEZ. Some assays, however, cannot differentiate concurrent infection with SEE and SEZ, and some target genes of SEE are found in the genomes of some SEZ leading to false-positive results for SEE. Conversely, known truncations or deletions of the target sequence in SEE may result in false-negative results. Thus, improved PCR assays for SEE and SEZ are needed.

We present a reliable qPCR assay capable of differentiating between, and simultaneously identifying, SEE and SEZ. Primers and probes were designed from whole-genome sequencing of SEE (n=50) and SEZ (n=50) isolated from the respiratory tract of horses in Texas. Primers for SEE aligned *in silico* with all publicly-available genomes of SEE (n=288) and additional SEE genomes from the United Kingdom (n=356), but not with publicly-available SEZ (n=23); SEZ primers aligned *in silico* with all available SEZ genomes but not SEE. This assay, which includes an exogenous internal control, underwent validation per AAVLD guidelines and tested with routine diagnostic respiratory tract samples submitted for culture and qPCR testing from 85 Texas horses. Twenty of 21 (95%) SEE culture-positive samples were positive for SEE by multiplex qPCR, and 22 of 23 (96%) SEZ culture-positive samples were positive for SEZ by multiplex qPCR. Of 32 culture-negative samples tested, SEZ was identified by qPCR in three samples (9%) and SEE was identified in two samples (6%). Nine samples were culture-positive for Streptococci other than SEE or SEZ: all tested negative by qPCR except one sample identified by culture as *S. dysagalactiae* subsp. *equisimilis* that was identified as SEZ by qPCR. Of the 42 samples culture-positive for SEE or SEZ, 20 (48%) were positive by qPCR for both organisms, including three samples that yielded only culture growth of SEZ. The multiplex qPCR test appears to have high sensitivity and specificity relative to culture and enables simultaneous identification of SEE and SEZ.

## *Salmonella bongori* from a pet bearded dragon as a source of pediatric salmonellosis * †

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We report the bacteriological and molecular typing of *Salmonella* isolates associated with paediatric salmonellosis from South Dakota, USA. Department of Health and the Animal Disease Research and Diagnostic Laboratory, South Dakota jointly investigated two cases of *Salmonella* infection from children. Both the patients were found to be infected by *Salmonella bongori* but the source of infection was not known. In both the cases, the common factor was a pet- bearded dragon. Bearded dragon is a reptile that has been linked to *Salmonella* outbreaks in United States previously. We collected and analysed samples from patient's pet bearded dragons to check for the presence of *Salmonella* and to check whether it is the source of infection. The Salmonella isolates were identified using MALDI-TOF and were typed using Illumina 2 x 300 paired end whole genome sequencing (WGS) based chemistry. The serotype of the isolates were predicted from the WGS data using SeqSero tool. We identified several *Salmonella* serotypes from the Bearded Dragon isolates. Core genome SNP based clustering of the WGS data revealed that the *Salmonella bongori* isolates from pet Bearded Dragons and the infected children contained less than 10 SNPs, indicating that the isolates were highly similar. This established that the source of infection in children were the pet Bearded Dragons. We further compared the publicly available genomes of outbreak associated *Salmonella* isolates linked to bearded dragon, reported previously from United States with the 18 *Salmonella* isolates from this study. The phylogenetic analysis revealed division of isolates into two major clusters. To further characterize the *Salmonella* strains, we performed three phenotypic assays- cell invasion assay, biofilm assay and serum response assay on the representative set off isolates to investigate the ability of dragon isolates to invade and infect the human host. The results showed that all the isolates have the ability of cell invasion and biofilm formation. Overall, these observations indicate that pet Bearded Dragons could be a zoonotic reservoir of invasive *Salmonella*.

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

**A simplified purpose-built platform and improved diagnostic tool for testing of endemic and foreign animal vesicular diseases †**

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Foreign animal diseases (FADs) such as African swine fever, classical swine fever, and the vesicular foot and mouth disease (FMD) pose significant threats to the stability of US agriculture. In particular, foot and mouth disease virus (FMDV) presents an increased challenge as all suspected vesicular disease cases must be addressed, and discerning between various vesicular diseases requires advanced diagnostic platforms. The rapid and real-time sequencing brought about by nanopore technology has allowed molecular diagnostics to emerge as a viable tool for performing quick rule outs of vesicular diseases, while also enabling real-time tracking of mutations in the viral transcriptome.

This study evaluated a semi-automated, field deployable system for the rapid handling of swine samples including viral RNA extraction, library preparation, and sequencing using the Oxford Nanopore MinION device. The endemic Seneca virus A (SVA) was used to model FMDV. Loop mediated isothermal amplification (LAMP) was utilized to increase starting quantity of RNA recovered from nucleic acid extraction protocols in order to meet minimum requirements of tagmentation based library preparation. A multiplex LAMP primer set (6 total primer sets) was designed to evenly amplify 3 distinct regions covering 16% of the 7300bp genome without necessitating a thermocycler.

Rapid tagmentation-based libraries prepared with multiplex LAMP system demonstrated a barcoding success rate of 92.9%, and successful alignment rate of 69.2%. Our device achieves LAMP production rates of 331 times starting material, and generates libraries of up to 119ng from 1.1ng extracted SVA RNA spiked into TE buffer. Our LAMP sequencing system allowed for detection of SNPs within the primer bounds at up to 98%. We are currently evaluating the device efficacy on vesicular fluid samples extracted from SVA positive pigs, as well as expanding the device to simultaneously process multiple samples in order to reduce reagent cost and library production time.

† Graduate Student Oral Presentation Award Applicant

**ThermaStop™ and ThermaStop-RT™ - a solution to miss-priming in diagnostic PCR applications**

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Polymerase chain reaction (PCR) technology is a versatile and ubiquitous process in animal diagnostic testing. PCR is used for a broad range of applications including simple pathogen detection, viral and bacterial strain characterization and sequencing. Improvements in thermocyclers, reagent kits, enzymes and buffers have reduced the time needed to design and optimize primers, probes, and reaction conditions. However, the problem of mis-priming prior to amplification remains, especially in diagnostic settings with extremely complex specimen matrices. The Iowa State University Veterinary Diagnostic Laboratory evaluated ThermaStop™ and ThermaStop-RT™ reagents, which affect the activity of polymerase and reverse transcriptase. ThermaStop™ acts on the polymerase to suppress PCR mis-priming errors at low temperatures, functioning like a hot-start that binds and inhibits the activity of DNA polymerases. Unlike antibody and chemical hot-start reagents, ThermaStop™ rebinds to the polymerase upon cooling and resumes its inhibitory function. Likewise, ThermaStop-RT™ interacts with reverse transcriptase at low temperatures to reduce priming errors that lead to non-specific products when gene-specific primers are used. These products promote more specific and efficient primer use and improved singleplex and multiplex amplification sensitivity and quantitative accuracy. We evaluated these products in two different applications, target amplification prior to Sanger sequencing and bacterial sero/toxin typing assays. Initially, we chose our porcine reproductive and respiratory syndrome virus (PRRSV) open reading frame 5 (ORF5) Sanger sequencing assay to test the ThermaStop-RT™ reagent. The electropherograms frequently contained long stretches of unresolved or broad peaks and double peaks. Thirty clinical samples of PRRSV infection were tested. With the addition of ThermaStop-RT™, a complete ORF5 sequence was obtained from 93.3 % (28/30) of the cases. Without ThermaStop-RT™, the success rate fell to only 63.3% (19/30). Additionally, any bases that were unidentifiable without ThermaStop-RT™ were resolved when the reagent was used. We used our *Escherichia coli* (*E. coli*) toxin/pilus typing assay to evaluate ThermaStop™. This is a conventional PCR assay which targets 15 different toxin and pilus genes. With this high level of multiplexing, non-specific amplification is observed a majority of the time. Twenty three *E. coli* isolates were evaluated in the presence and absence of ThermaStop™. Non-specific amplification was observed with 39.1% (9/23) of the isolates without ThermaStop™. All non-specific amplification was either completely blocked or significantly reduced with the addition of ThermaStop™ in all isolates. In conclusion, the addition of ThermaStop™ and ThermaStop-RT™ to diagnostic PCR assays can greatly improve the quality of the results over a wide range of applications. Further evaluation of these reagents in other PCR applications is needed.

**Heating or diluting swine oral fluid samples does not improve qPCR detection †**

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Reports in the literature describe improved detection of nucleic acids (NA) in human oral fluids (OF) by heating and/or diluting the sample (Ranoa et al., 2020). In this study, we tested the effect of heating (95°C × 30 m) or diluting (tris borate EDTA; TBE) swine oral fluid samples on the detection of RNA, i.e., porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) or DNA, i.e., *Mycoplasma hyopneumoniae* (*MHP*).

In Experiment 1, OF samples containing PRRSV (n = 8), IAV (n = 8), or *MHP* (n = 8) were 2-fold serially diluted (neat, 1:2, 1:4, 1:8) using OF free of PRRSV, IAV and *MHP* as diluent (n = 32 aliquots per pathogen). Each aliquot was then split into 4 and randomized to one of 4 procedures: (P1) heat (95°C × 30 m) and direct qPCR; (P2) heat, cool (25°C × 20 m) and direct qPCR; (P3) heat, cool, NA extraction, and direct qPCR; (P4) NA extraction and qPCR (control).

In Experiment 2, OF samples known to contain PRRSV (n = 9), IAV (n = 10), or *MHP* (n = 10) were split into three aliquots: (D1) undiluted; (D2) diluted 1:2 with OF free of PRRSV, IAV and *MHP*; (D3) diluted 1:2 with TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Aliquots were completely randomized and then tested in consecutive random number order.

NA extraction and qPCRs were performed using commercial kits (IDEXX Laboratories, Inc., Westbrook, Maine, USA) and a magnetic induction thermocycler (MIC PCR™, Bio Molecular Systems, Australia). Results were reported as quantification cycles (Cq) and samples with Cq values < 40 were considered positive.

In Experiment 1, testing using P4 (control) produced 32/32 aliquots positive for PRRSV, 32/32 for IAV, and 31/32 for *MHP*. Aggregating the results for P1, P2, and P3, 1/96 aliquots were positive for PRRSV, 5/96 for IAV, and 47/96 for *MHP*. For all pathogens, Cqs for P4 (control) were consistently lower than P1, P2, or P3 positives.

The results from Experiment 2 showed no gain with D2 or D3:

PRRSV (means) - undiluted Cq = 32.3; D2 Cq = 34.2; D3 Cq = 36.8.

IAV (means) - undiluted Cq = 29.0; D2 Cq = 30.0; D3 Cq = 29.9.

*MHP* (means) - undiluted Cq = 33.0; D2 Cq = 33.6; D3 Cq = 33.9.

In this study, the heat and dilution treatments described in the literature were detrimental to the detection of PRRSV, IAV, and *MHP* nucleic acids in oral fluid samples by qPCR. While seemingly contrary to these reports, examination of the literature showed that investigators reporting the use of these treatments generally did not include comparisons with standard methods. That is, quantitative measures of the gain or loss in performance achieved by alternative methods was typically lacking. In this study, comparisons showed that the best results were obtained using standard extraction and amplification methods.

† Graduate Student Oral Presentation Award Applicant

## Molecular Diagnostics - On Demand



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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**Characterization of a contemporary porcine respiratory coronavirus isolate * †**

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Porcine respiratory coronavirus (PRCV), first identified in 1984 in Belgium, is a mutant of transmissible gastroenteritis virus (TGEV) with a large deletion in the N-terminal region of spike (S) protein. This large deletion accounts for the alteration of enteric (TGEV) to respiratory (PRCV) tissue tropism. In the past ~30 years, PRCV has rarely been studied and most cited information is on historical isolates obtained from the 1980s to 1990s. The main objective of this study was to obtain a contemporary PRCV isolate and compare it with historical PRCV isolates. We first screened 1245 lung homogenate samples from pigs with a history of respiratory disease submitted to the Iowa State University Veterinary Diagnostic Laboratory during November and December 2020 using a PRCV/TGEV duplex real-time RT-PCR. All of the tested samples were negative for TGEV ($C_T$ ≥40) and 0.48% (6 out of 1245) were positive for PRCV with $C_T$ values 18.5-33.6. The PRCV PCR-positive samples were subjected to virus isolation (VI) for three passages in Swine Testicle (ST) cells. The cell culture supernatants at each passage were tested by PCR to verify the VI outcomes. The passage 3 cell culture superanants were PRCV PCR positive for three of the six samples with $C_T$ ranges of 20.4-28.1. The three PRCV isolates were tested by next-generation sequencing and the complete genome sequence was obtained from PRCV isolate ISU20-92330 that was derived from a 80-day-old pig in Indiana, USA. Only partial sequences were obtained from the other two isolates and they were not used for further analyses. Compared to the S gene of TGEV strains, the S gene of PRCVs ISU20-92330/2020, OH7269/2014, Minnesota-46140/2016, ISU-1/1990, LEPP/1994, AR310/1993, and 1894X/1994 had 648, 648, 681, 681, 621, 621, and 678 nucleotide (nt) deletions at the N-terminal, respectively. At the whole-genome level, ISU20-92330 had 98% nt identity to OH7269, 96.7% nt identity to Minnesota-46140, and 96.6-97.4% nt identities to the historical isolates 1894X, ISU-1 and AR310. For the spike gene and protein, ISU20-92330 had 97.9% nt (98.3% aa) identity to OH7269, 95.9% nt (95.4% aa) identity to Minnesota-46140, and 95.4-96.5% nt (95.9-97.2% aa) identities to the historical isolates 1894X, ISU-1 and AR310. In addition, PRCV ISU20-92330 had different deletion patterns compared to other PRCV strains in the non-coding region between S and ORF3a gene as well as ORF3a gene. For the two relatively recent PRCV strains OH7269 and Minnesota-46140, only genetic sequence data from clinical samples are reported and no cell culture isolates are available, limiting further characterizations. Availability of the isolate ISU20-92330 will allow for further characterization of the growth charateristics and pathogenesis of contemporary PRCV in comparison with historical PRCV isolates. PRCV infection of pigs could also be a model to study pathogenesis and immune response of human respiratory coronaviruses such as SARS-CoV-2.

* Graduate Student Poster Presentation Award Applicant
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**Update on whole genome sequencing of IAV-S in Ohio**

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Influenza A virus (IAV) is one of the most important respiratory pathogens in humans and swine. Belonging to the family *Orthomyxoviridae*, IAVs are capable of interspecies transmission due to the presence of the same sialic acid receptors in humans, swine, and some avian species. This makes surveillance of IAVs a critical factor in the "One Health" approach and monitoring zoonoses and reverse zoonoses crucial for developing pandemic preparedness and response plans.

Here we provide an update on IAV-S viruses circulating in Ohio pig farms between 2020 and 2022. Samples submitted to the Ohio ADDL that test positive for IAV-S matrix real-time RT-PCR are then subtyped by PCR, and whole genome sequencing (WGS) is attempted if the matrix PCR Ct is below 30. IAV-S viral RNA is sequenced using an amplicon-based library prep strategy and the Illumina DNA prep kit on an Illumina MiSeq instrument. Consensus sequences corresponding to the 8 different IAV-S gene segments are obtained by aligning the paired-end sequence reads to a reference sequence and trimming to the coding regions of the reference sequence. These consensus sequences are then further characterized by US and global classification using phylogenetic analysis and the OctoFLU pipeline. Of note, after transitioning from the MagMAX Pathogen RNA/DNA Kit to the MagMAX CORE Nucleic Acid Purification Kit, we noticed that our IAV-S WGS efficiency decreased significantly. This was determined to be caused by residual inhibitors present in the nucleic acid used for sequencing after isolation with the MagMAX CORE Kit.

Our findings indicate a decline in H3N2 prevalence and increase in H1N1 prevalence of approximately 30% over the last 3 years with the predominant H1N1 constellation being H1-gamma/N1-classical and the predominant H3N2 constellation being H3-IV_A/N2-2002B. Interestingly, after detecting ≤ 1 H1-alpha subtypes per year from 2018 to 2020, there were 10 H1-alpha subtypes detected from April 2021 to January 2022. Novel IAVs can emerge as a result of bidirectional transmission between humans and swine (swine are considered to be "mixing vessels" for IAV) coupled with antigenic drift and shift. WGS of IAV-S is a great epidemiological tool to monitor circulating IAVs and identify novel influenza viruses.

**Clinical validation of a multi-cancer early detection blood-based "liquid biopsy" test in dogs using next-generation sequencing**

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Cancer is the leading cause of death in adult dogs; however, no established screening paradigms exist for early detection. Liquid biopsy methods that interrogate cancer-derived genomic alterations in cell-free DNA fragments in blood are being adopted for early cancer detection in human medicine and are now available for use in veterinary medicine.

Blood samples from an all-comers cohort of 352 cancer-diagnosed dogs and 524 presumably cancer-free dogs were subjected to DNA extraction, proprietary library preparation, and next-generation sequencing. Sequencing data were analyzed using an internally developed bioinformatics pipeline, previously established in an independent cohort, to detect genomic alterations associated with the presence of cancer.

For three of the most aggressive canine cancers (LSA, HSA, OSA), the detection rate of liquid biopsy was 85.4%; for eight of the most common canine cancers (LSA, HSA, OSA, MCT, STS, AGASACA, mammary gland carcinoma, and malignant melanoma) detection rate was 61.9%; and the overall 'multi-cancer' detection rate (across all cancer types represented) was 54.7%. In total, liquid biopsy detected cancer signal in 30 different cancer types. The test specificity was 98.5%, corresponding to a 1.5% false positive rate. At least 2 presumably-cancer free dogs received a *Cancer Signal Detected* (positive) result and were diagnosed with cancer after undergoing confirmatory cancer evaluations. One of these cases involved a 7yo FS mixed-breed dog. Blood was collected and frozen until testing (per study protocol) 4 months later, at which point a *Cancer Signal Detected* result was issued. Despite a normal physical exam, lung nodules and a cavitated heart mass were identified via imaging, and FNA cytology of a lung nodule was consistent with probable sarcoma (suspect hemangiosarcoma). The patient remained asymptomatic, and the family elected palliative care while continuing close monitoring. Additional liquid biopsy samples collected at 5- and 7-months following the initial blood draw confirmed the previously detected genomic alterations. Mild clinical signs developed at month 7, and the patient was euthanized due to progressive disease, avoiding potential progression to a life-threatening bleeding event. Necropsy confirmed masses of the spleen, rib, lung, and heart; hemangiosarcoma was confirmed via histopathology in all tissue sites. Genomic analysis of the tissue samples confirmed the presence of the genomic alterations previously identified in plasma.

A novel, multi-cancer early detection (MCED) liquid biopsy test has demonstrated the ability to identify cancer-associated genomic markers (in some cases months prior to the onset of clinical signs) in canine patients. The case study presented represents the first known reported case in which cancer-associated genomic alterations were shown to be present and detectable in blood several months prior to the development of clinical signs of cancer in a canine patient.

**Identification and detection of bovine *Boosepivirus A* and *B* in the US diarrheal cattle**

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Next generation sequencing (NGS) is commonly used in clinical diagnostics and can be applied for sequencing both a single virus and multiple viruses. NGS is an excellent tool for case investigation of cattle diarrhea commonly caused by multiple known and unknown pathogens. In 2019, through metagenomic sequencing, a bovine picornavirus named bovine boosepivirus (BooV) IL41203-19 was identified in the fecal sample of a 10-day-old calf with diarrhea which was also positive for bovine kobuvirus and cryptosporidium. Genomic characterization revealed that BooV IL41203-19 shared the highest identity with the Japan BooV strain (Bo-12-7/2009/JPN) at both the complete nucleotide and amino acid levels, belonging to *Boosepivirus B* species. Similarly, metagenomic sequencing identified the presence of a BooV strain IL33712-22 in a fecal sample of a cattle in 2022 which was also positive for bovine coronavirus, bovine torovirus, and bovine calicivirus. Interestingly, genomic analysis showed that IL33712-22 shared a much higher nucleotide identity 77.8% with the Japan BooV strain (Bo-11-39/2009/JPN) than with four boosepivirus B strains including IL41203-19 (54%) at the complete genome level, indicating that IL33712-22 belongs to *Boosepivirus A* species. Further real-time RT-PCR screening for*Boosepivirus B* in 84 clinical samples submitted for a diarrheal testing panel showed that 5 were positive for BooV and all were coinfected with one to four other enteric pathogens. Our data showed that both *Boosepivirus A* and *B* were present in US cattle and provide further evidence that BooV might contribute to cattle diarrhea observed in different states. Future studies on epidemiology and pathogenesis of bovine BooV are warranted.

**Role of inactivating molecular transport medium in zoonotic disease surveillance**

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Speed and accuracy of zoonotic disease surveillance sampling and testing is vital for rapidly emerging viruses that, as we have just seen with SARS-CoV-2, can kill millions of people and greatly disrupt the world's economy. The global food animal supply faces similar threats as HPAI and ASF continue to spread around the world. While sequencing and qPCR technologies have improved speed and sensitivity of results, the continuing challenge for molecular diagnostics is a well-collected and preserved sample.

This presentation will provide a literature survey of previously published studies from the past 10 years featuring PrimeStore® MTM sample collection for molecular detection of human and animal pathogens. Specific review of sequencing studies and PCR pathogen detection in challenging sample types will be presented, as well as an overview of the key inactivation studies leading to PrimeStore® MTM's FDA-clearance for influenza and *M. tuberculosis* samples.

Proven inactivating molecular transport mediums (IMTM) can be used to collect and preserve samples from the field, lysing and stabilizing nucleic acids for safe transportation to laboratories without the cold chain and even at high ambient temperatures. PrimeStore® MTM is the original IMTM that has been proven globally at multiple reference laboratories and can be used to collect a wide range of sample types (blood, urine, feces, oral fluids, "meat juices", homogenized tissue, swabs of all types including oral, nasal, rectal, environmental) and pathogens (ASF, CSF, FMD, AI, ND, SARS-CoV-2, Influenza, MTB, *M.Bovis*, Cryptosporidium). The "saturated swab delivery principle" in tandem with IMTM creates superior ease of use when collecting single or pooled samples on farms, in meat processing facilities, or in the wild, and provides high quality nucleic acids for qPCR and sequencing on many well-known testing platforms.

Safety in transit and safety for scientists in laboratories were additional driving forces behind the invention of IMTM. Pathogen inactivation by IMTM eliminates the need for biocontainment in testing facilities, thus creating surge capacity for emergency outbreaks. Using PrimeStore® MTM as part of proper surveillance with regular ongoing testing is the early warning system the world needs against animal and zoonotic pandemics.

**Interlaboratory comparison exercise (ILC) of SARS-CoV-2 molecular detection assays used by veterinary diagnostic laboratories in the United States and Canada**

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To help veterinary diagnostic laboratories evaluate their existing methods to detect SARS-CoV-2, we sought to establish an interlaboratory comparison exercise (ILC) to determine the methods' level of detection, evaluate variant detection, and specificity. This work is critical to ensure laboratories can effectively identify SARS-CoV-2 in animals and humans. In this ILC, 23 blind-coded RNA samples were analyzed by 57 participants located in 45 laboratories in the North America. Samples included various spiking levels of SARS-CoV-2 (B.1 variant) RNA in preservative solution, Alpha (B.1.1.7) and Beta (B.1.351) variants to assess sensitivity, and Feline Infectious Peritonitis Virus (FIPV) as a potential confounder to assess specificity of methods. The participants used their routine diagnostic procedures for RNA extraction and quantitative RT-PCR to analyze the samples. Results were received and analyzed according to the principles of International Organization for Standardization (ISO) 16140 - 2:2016. Qualitative assessment suggests that >95% sensitivity was confirmed for the detection of SARS-CoV2 RNA at 500 copies or higher for all variants. This ILC also revealed that 81% and 92% of the analysts obtained $LOD95_{eff.\ volume}$ values below 20 copies in the N1 and N2 assay respectively. Specificity was >99%, with only one false positive result for blank samples. The specificity of the methods was further confirmed using FIPV samples. All participants were able to detect the Alpha and Beta variants with their routine methods. Overall, the ILC successfully verified the sensitivity and specificity of methods and determined the level of detection of viral RNA. The study allowed participants to compare their performance to each other, assess their current method performance, identify possible limitations/areas for improvement, and recognize method strengths as part of a continuous learning environment.

The study supports the need for reliable diagnosis of COVID-19 in potentially infected animals and humans.

**Whole genome sequencing of African swine fever virus detected at a "backyard" pig holding in Mongolia.**

*Dong-Hun Lee[1], Erdene-Ochir Tseren-Ochir[3], Douglas P. Gladue[4], Manuel V. Borca[4], Guillermo R. Risatti[1,2]*

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African Swine Fever (ASF) is a highly contagious and fatal viral disease affecting both domestic and wild suids. In Mongolia, ASF was first reported in Bulgan Province in January 2018. Outbreaks were later reported in Orkhon, Darkhan-ul, Tuv, Dundgovi, Selenge provinces and Songinokhairkhan District of Ulaanbaatar City.

We sequenced an ASF virus (ASFV) detected by conventional PCR in tissues obtained from pig housed in a non-commercial backyard holding using Illumina next-generation sequencing (NGS, short-reads sequencing). Maximum-likelihood phylogenetic analysis and NCBI BLAST search results of the whole genome sequence suggested that the virus belongs to the Georgia-07-like genotype II ASF virus and share high sequence identity with viruses recently detected in Eastern Europe and Asia.

During the process, we observed that it was difficult to effectively sequence, and assemble de novo repetitive regions commonly present in ASFV genomes using short-read NGS. To address this problem, we compared reference-based genome assembly tools to find an approach that improve the assembly quality of repetitive sequences. Nonetheless, the first whole genome of ASF virus detected in Mongolia was generated. This kind of study would provide further insights for tracing evolution and spread of the Georgia-07-like ASFVs emerging in Central Asia.

**Whole genome sequencing and phylogenetic analysis of rabies viruses from bats in Connecticut, USA, 2018-2019.**

*Ji-Yeon Hyeon[1,2], Guillermo R. Risatti[1,2], Zeinab Helal[1,2], holly McGinnis[2], Maureen Sims[2], Amelia Wheeler[2], David Chung[1], Junwon Kim[1], Julia Desiato[1], Dong-Hun Lee[1]*

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We performed whole genome sequencing and genetic characterization of rabies viruses (RABV) detected in bats submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) during 2018-2019. Among 88 bats submitted to CVMDL, six brain samples (6.8%, 95% confidence interval: 1.6% to 12.1%) tested positive by direct fluorescent antibody test. RABVs were detected in big brown bats (*Eptesicus fuscus, n* = 4), a hoary bat (*Lasiurus cinereus, n* = 1), and an unidentified bat species (*n* = 1). Complete coding sequences of four out of six detected RABVs were obtained. In phylogenetic analysis, the RABVs (18-62, 18-4347, and 19-2274) from big brown bats belong to the bats EF-E1 clade, clustering with RABVs detected from the same bat species in Pennsylvania and New Jersey. The bat RABV (19-2898) detected from the migratory hoary bat belongs to the bats LC clade, clustering with the eleven viruses detected from the same species in Arizona, Washington, Idaho, and Tennessee. The approach used in this study generated novel data regarding genetic relationships of RABV variants, including their reservoirs, and their spatial origin and it would be useful as reference data for future investigations on RABV in North America. Continued surveillance and genome sequencing of bat RABV would be needed to monitor virus evolution and transmission, and to assess the emergence of genetic mutations that may be relevant for public health.

**Antibiotic susceptibility of pre-antibiotic era *Streptococcus* spp. isolated from animals**

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To improve the understanding of the emergence of antimicrobial resistance (AMR) and evolution of bacterial pathogens, the whole genome sequences (WGS) of *Streptococcus* spp. isolated from animals before the widespread clinical use of antimicrobials were generated and analyzed.

A total of 53 *Streptococcus* spp. isolates were recovered from lyophilized bacteria that were obtained from animal samples submitted to the diagnostic laboratory at the University of Connecticut in the 1940s. The16S rRNA gene sequences were used to genetically identify these isolates (BLASTn). Streptococcus species were then further confirmed by whole genome phylogenetic analysis. Recovered isolates were identified as *S. agalactiae* (n=15), *S. dysgalactiae* (n=15)*, S. uberis* (n=10), *S. pyogenes* (n=7), *S. equi sub.zooepidemicus* (n=4), *S. oralis* (n=1), and *S. pseudoporcinus* (n=1).

AMR of these isolates was assessed using Sensititre™ Streptococcus STP6F AST Plates (phenotype) and by genetic analysis of assembled contigs (genotypes). It was observed that *S. agalactiae, S. pyogenes*, *S. oralis*, and *S. pseudoporcinus* isolates (n=24) showed sensitivity to all the antibiotics. Meanwhile, *S. dysgalactiae, S. uberis and S. equi sub.zooepidemicus* isolates had heterogeneous patterns of sensitivity. For instance, *S. dysgalactiae* isolates showed intermediate resistance (n=4) and resistance (n=4) to tetracycline or were sensitive to all antibiotics tested (n=7). Only one of the *S. uberis* isolates (n=10) showed multi-antibiotic resistance to ceftriaxone, chloramphenicol, and vancomycin. *S. equi sub.zooepidemicus* isolates (n=4) showed intermediate resistance (n=3) or were resistant (n=1) to tetracycline, in addition 3 of these isolates showed intermediate resistance to clindamycin.

The genetic analysis revealed that *S. agalactiae* isolates carried the *mre(A)* gene, macrolide efflux gene whereas none of the other *Streptococcus* spp. carried genes associated with antimicrobial resistance.

This study of *Streptococcus* spp. isolates from the pre-antibiotic era is aimed to provide insights into the evolution of antimicrobial resistance.

**All laboratories are not created equal – reassessing laboratory models**

*Kristina M. Peterman, Lauren Richardson*

Life Sciences, Merrick & Company, Arlington, VA

During the COVID pandemic many existing laboratories were called upon to change their focus to support COVID sample processing or vaccine and therapeutic development. Laboratories that were previously established for the sole purpose of animal diagnostics were called into action to supplement the needs of the human diagnostic laboratories. This change was more than the origin of samples: it was a significant change to the operating model. As we look to programs that demonstrate resilience and ability to adjust to meet changing mission needs, robust risk assessment infrastructure provides a strong foundation for an adaptable organization.

Laboratories with robust risk assessment programs have established procedures that require any change to a laboratory's processes, procedures, or materials, undergoing a reassessment to identify potential risks. The risk assessment for changing an animal diagnostic laboratory to a human diagnostic laboratory at minimum identifies the needs for additional measures in the areas of sample handling, decontamination and cleaning, personnel safety, occupational health, equipment and consumables, waste management, and training. The reassessment also evaluates business continuity planning and dependencies on other areas, including staffing, operational support, maintenance, and expectations for the continuity of service for animal diagnostics.

The establishment of even a basic risk assessment template that looks at the areas of people, materials, equipment, and facilities can assist with developing a plan to shift your laboratory model. The laboratory can then more readily perform risk assessments and reassess processes and procedures as needed and on a recurring basis to quickly identify risks.

## Pathology 1

Saturday, October 8, 2022

Greenway BC

**Moderators:** Marta Mainenti and Panchan Sitthicharoenchai

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**1:45 PM** **Necropsies are required for all racehorse deaths in most U.S. racetracks**
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**2:45 PM** **Congenital neurologic and ocular lesions in calves as a result of *in utero* epizootic hemorrhagic disease virus infection # + * †**
*Jessica A. Kendziorski, Bonnie K. Harrington, Steven R. Bolin, Dodd Sledge, Stephanie French* . . . . . *110*

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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**Postmortem investigation of exercise-associated sudden death in Thoroughbred racehorses**

*Laura Kennedy*

University of Kentucky, Lexington, KY

Postmortem investigation of exercise-associated sudden death (EASD) is the bane of the racehorse pathologist. While the event is dramatic and generally within full view of backstretch workers, the media, or the general public, the postmortem examination is often underwhelming. Recent media scrutiny following the sudden death of a prominent horse has further complicated the picture, as absence of a diagnosis can quickly lead to wild speculation and conspiracy theories.

EASD is divided into two broad categories: those with significant postmortem examination findings and those without, termed autopsy-negative. From March 2017 through May of 2022, 32 EASD examinations have been performed at the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL). Significant lesions were identified in 14 cases (44%) while 18 cases were considered autopsy-negative. Diagnoses related to the cardiac system included myocarditis (5 cases), myocardial fibrosis (1 case), and chronic pericarditis and pleuritis (1 case). Four cases involved rupture of a blood vessel, including one case of aortic rupture and hemopericardium, and three cases of mesenteric vessel rupture and hemoperitoneum. Two cases were attributed to severe exercise-induced pulmonary hemorrhage (EIPH) and one case presented with massive body wall hemorrhage of undetermined cause.

In 2008 the Jockey Club (the official registry and governing body of American Thoroughbreds) and industry stakeholders including racetrack management, horsemen, private and regulatory veterinarians, and Thoroughbred breeders initiated a coordinated effort to address musculoskeletal injuries in racehorses. Through these and other efforts, the rate of catastrophic musculoskeletal injuries (CMI) has decreased significantly. With the decreased rate of CMI, EASD has become a more prominent cause of exercise-associated mortality. As occurred in 2008, a focused approach to EASD and associated risk factors is being undertaken through multi-institutional research groups and regulatory bodies.

**Disseminated fungal disease in a cat caused by *Cystofilobasidium macerans* (*Cryptococcus macerans*). + * †**

*Scott Mitchell[1,2], A. Giselle Cino Ozuna[2], Andrew Hanzlicek[3], Aline Rodrigues Hoffmann[4], Lara Sypniewski[5], Brianne Marie Taylor[2]*

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A four-year-old, castrated male, domestic shorthair cat presented to the Oklahoma State University Boren Veterinary Medical Hospital with a history of progressive weight loss and icterus. Serum chemistry revealed persistently elevated liver ALKP, ALT, and total bilirubin. On abdominal ultrasound, splenomegaly and mild to moderate lymphadenomegaly were appreciated. No significant abnormalities were observed cytologically on aspirates of the liver. Molecular and serologic testing was negative for *Toxoplasma gondii,* FeLV, FIV, and *Dirofilaria immitis*. Over six months, liver enzyme values and icterus failed to improve with medical management, and humane euthanasia was ultimately elected. *Histoplasma* quantitative antigen testing on urine was twice negative prior to euthanasia. On necropsy examination there was severe icterus. The liver was diffusely, moderately pale and friable. Numerous, 1-3 mm diameter, pale white foci were scattered on the renal capsules and extended throughout the cortex and medulla. Histologically, there was severe, disseminated pyogranulomatous and lymphoplasmacytic hepatitis, interstitial nephritis, and interstitial pneumonia with splenic histiocytosis. Scattered throughout these tissues were variable numbers of 3-5 μm diameter, narrow-based budding yeasts characterized by a 1-2 μm diameter, basophilic and argyrophilic nucleus surrounded by a 1-2 μm thick clear capsule. Morphologic characteristics of yeasts were highly suspicious for *Histoplasma* sp. Due to previously negative antigen testing of urine, formalin-fixed paraffin-embedded samples of liver and kidney were submitted for panfungal PCR and sequencing at the University of Florida CVM Molecular Fungal ID Laboratory. PCR and sequencing results matched *Cystofilobasidium* sp. with 99.85% identity. *Cystofilobasidium macerans* is the sexual stage of *Cryptococcus macerans*. Cases of clinical disease caused by infection with *Cystofilobasidium* or *Cryptococcus macerans* have scarcely been reported in human medical literature, and to the authors' knowledge no cases have previously been described in veterinary species. A single case report of meningoencephalitis caused by *Cryptococcus macerans* has been reported in man that resulted in epilepsy, and antifungal therapy was successful. In conclusion, this is the first description of disseminated *C. macerans* infection in feline species, and the organism should be considered a differential for disseminated granulomatous inflammation in cats. Final diagnosis *in vivo* presented a challenge in this case due to the lack of yeast organisms and inflammation on multiple cytologic examination of liver aspirates, despite the severity of lesions. Molecular testing for fungal organisms on liver biopsies could represent a better sampling for the detection of *C. macerans*.

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

## *Actinobacillus seminis* abortion in a United States Boer goat herd

*Alyona Michael[1], Rachel Desotel[2], Karen Krueger[1], Orhan Sahin[1], Phillip Gauger[1]*

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

*Actinobacillus seminis* is a gram-negative coccobacillus capable of colonizing the genital tract of male small ruminants and undergoing ascending infection in times of stress. It is considered a major cause of ovine orchitis and epididymitis in sheep-producing countries, including Australia and New Zealand. Transmission occurs primarily via direct contact between infected rams, and can result in persistent infection and depressed fertility. Placentitis and fetal loss is documented following experimental infection of pregnant ewes, however, they are largely considered to be subclinical carriers. Reports of naturally occurring abortion due to *A. seminis* are rare. In goats, natural infection has been reported in bucks co-housed with infected rams, and experimental inoculation induces analogous reproductive lesions. *A. seminis* has also been isolated from bovine semen and vaginal discharge, with rare reports of epididymo-orchitis.

In December 2021, Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) received quadruplet fetuses and fetal membranes aborted mid-gestation by a Boer doe. Fetuses were uniform in size (25-29cm) with minimal (2 of 4) to moderate (2 of 4) gross evidence of in-utero autolysis. Suppurative fetal bronchopneumonia and necrosuppurative placentitis with intralesional bacterial colonies were observed histologically. Pooled fetal stomach contents yielded a high pure growth of *Actinobacillus seminis* after 24 hours of incubation on bovine blood agar at 37C.

Following the diagnosis of *A. seminis*-mediated abortion, preputial and seminal cultures were performed on breeding males at the affected site. These consisted of three Boer (bucks A-C) and one vasectomized Pygmy teaser-buck (D). Of the four, only buck A, the sire of the aborted litter, tested positive by culture for A. seminis, yielding high and moderate growth of A. seminis on preputial and seminal cultures, respectively. Cytologic examination of his semen documented presence of neutrophils with intracytoplasmic bacteria. Buck A was home-raised, individually housed since weaning, and in his first breeding season, having only been sexually exposed to two does. The sole other doe bred by buck A also aborted mid-gestation; however, no abortifacient etiology could be determined.

Isolation of *A. seminis* in the U.S.A was last reported in association with ovine epididymitis in 1964. Digitized records at ISUVDL from 2010-2022 yielded only 7 instances of A. seminis isolation: 4 from bovine abortion submissions, 1 urine and 1 seminal culture from bulls, and 1 ovine uterine swab. *A. seminis* was not etiologically implicated in reproductive morbidity in any of these 7 cases due to lack of confirmatory lesions. To our knowledge, the diagnostic case summarized above represents the first report of naturally occurring caprine abortion, and only the second report of *A. seminis*-associated reproductive disease, in the United States.

**Necropsies are required for all racehorse deaths in most U.S. racetracks**

*Susan Stover, Francisco A. Uzal*

UCDavis, Davis, CA

The Horseracing Integrity and Safety Authority (HISA) was established when the Horseracing Integrity and Safety Act was signed into federal law in 2020. The HISA is overseen by the Federal Trade Commission (FTC) and is responsible for drafting and enforcing uniform safety and integrity rules in Thoroughbred racing in the U.S. An important FTC-recently-approved rule is the mandatory necropsy of all horses that die or are euthanized at a covered racetrack facility. The necropsy rule became effective July 1, 2022 and will most likely have an impact on many AAVLD-accredited laboratories in the US, as it dictates that "necropsies should be performed at facilities and by personnel with capabilities and expertise to perform necropsy examination of racehorses….and field necropsies are strongly discouraged." The goals of the necropsy examinations are to identify the cause of death and pre-existing pathology that predisposed to death. The necropsy findings will be used in a review of factors surrounding the incident by the Racetrack Safety and Welfare Committees, will be highly educational to the racing community, and will assist in prevention of similar injuries or illness in other racehorses. Paramount to the goal is understanding common pathologies that predispose to death in racehorses, how to efficiently perform the necropsy to observe pathologic changes, and understanding the relevance of the findings to racing industry participants. Several AAVLD accredited laboratories have been performing necropsies of racehorses for many years (e.g. California, Kentucky, New York). Most fatal musculoskeletal injuries are associated with bone- and site-specific pre-existing stress fractures or subchondral bone stress remodeling. Knowledge of features associated with stress fractures (e.g., periosteal callus) and subchondral bone remodeling (e.g., focal discoloration and osteopenia) can facilitate observing key findings. Dissemination of necropsy findings in reports is instrumental in educating racing industry participants who can relate findings to previous clinical signs for the detection of stress fractures and subchondral lesions in live racehorses. Detection of horses with mild injuries allows for rehabilitation and recovery. Information that will potentially help preventing sudden death and other medical causes of death is also a useful outcome of the racehorse post-mortem programs.

**Pathogenicity evaluation of different *Glaesserella (Haemophilus) parasuis* serotype**

*Haley McClure[1], Eduardo Fano[2], Molly Elizabeth Kroeger[1], Christine Harness[1], Adthakorn Madapong[3], Kepalee Saeng-chuto[3], Pablo Pineyro[1]*

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The gram-negative bacterium *Glaeserella parasuis* (GPS), formerly known as *Haemophilus parasuis*, colonizes the upper respiratory tract of pigs and is responsible for Glasser's disease causing polyserositis. Kielstein and Rapp-Gabrielson classification defined serovars 1, 5, 10, 12, 13, and 14 as virulent, serovars 2, 4, 8, and 15 as mesogenic, and serovars 3, 6, 7, 9, and 11 as attenuated strains. However, GPS virulence does not necessarily correlate with the serovar. Therefore, this study aims to evaluate the virulence of three different serovars in a swine challenge model.

Four treatment groups of 5-week-old pigs [GPS-serotype 4 (S4) (n=15), GPS-serotype 7 (S7) (n=14); GPS-serotype 12 (S12) (n=14) and negative controls (NCtrl) (n=15)] were challenged intravenously and intraperitoneally with 1ml 10^7 cells/ml of each strain on each treatment group and 1 ml of PBS for the NCtrl group. Mortality was recorded daily and all pigs were necropsied at 10 days post infection unless pigs experienced sudden death, then a necropsy was performed the same day. The severity of gross lesions (pericarditis, pleuritis, peritonitis, and arthritis) was scored on a scale from 0 (no lesion) to 5 (severe lesion) and presented as a cumulative score of 20. Fresh samples for molecular detection and bacteriological culture and tissues fixed in 10% buffered formalin for histopathological evaluation were collected during necropsy.

At the end of the study, the cumulative mortality was 50%, 30%, 0%, and 0% for S12, S4, S7, and NCtrl groups, respectively. The gross lesion average score was 8.8, 7.1, 2.4, and 0 for the S12, S4, S7 and NCtrl groups, respectively. Histological lesions include fibrinous to fibrinous suppurative pleuritis, pericarditis, and peritonitis with no difference in the severity among groups. No fibrinous arthritis was observed in the S7 group. GPS was detected by PCR on joint swabs on S4 (21.4%) and S12 (28.5%), with no significant difference in Ct values. The detection rate and bacterial load on spleen was significantly higher in S4 (50%; Ct 30) and S12 (50%; Ct 30) compared with S7 (7.1%; Ct 33). The detection rate on tonsil was significantly higher on S4 and S12 (85%; 92%) than on S7 (57%), although the average Ct values amongst group was 31±1.

In conclusion, experimental inoculation with serotype 7 resulted in lower mortality rate and less severe gross lesions. Although serotype 12 is considered more virulent than serotype 4, in this study both present similar mortality, lesion severity, and detection rate in multiple tissues. Despite the inoculation route, GPS was detected at the same rate and Ct value in tonsil in all subtypes, therefore detection on this tissue should be evaluated carefully to determine causation.

**Retrospective of equine paranasal sinus pathology**

*Jennifer Janes*

University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY

The equine paranasal sinuses are a complex network of paired air-filled structures including the frontal, maxillary (rostral and caudal), conchal (dorsal and ventral) and sphenopalatine sinuses. Pathology can occur via two main routes, either the nasal passages or communication of the upper cheek teeth to the maxillary sinus. An 8-year retrospective identified 79 cases submitted to the University of Kentucky Veterinary Diagnostic Laboratory with significant equine paranasal sinus pathology. Case submissions were as follows: 60 (biopsy), 17 (postmortem examination), 2 (cytology). Where reported, the average age was 11 years of age (1 day – 25 yrs), gender breakdown (36 males and 37 females) and most common breeds included Thoroughbreds (n=42) and Warmbloods (n=9). Lesions were localized to the left (n= 28), right (n=33) or both sides (n=2). Forty-two cases involved one sinus and 21 cases involved multiple sinuses. Distribution of sinuses was as follows: frontal (n=33), maxillary (n=32), rostral maxillary (n=8), caudal maxillary (n=7), conchal (n=5), sphenopalatine (n=6) and ethmoid (n=1). Disease processes were categorized as sinusitis (n= 39), paranasal sinus cyst (n=18), ethmoid hematomas (n=9), secondary to ongoing dental disease (n=6), neoplasia (n=4), aneurysmal bone cyst (n=2) or other (n=1).

Sinusitis comprised the majority of cases. Infectious agents were isolated on culture or identified microscopically in 10 cases. Seven cases identified bacterial pathogens (*Escherichia coli, Streptococcus zooepidemicus, Fusobacterium necrophorum*) and three cases identified *Aspergillus sp*. Most cases submitted only fixed tissue and often had a long history of antimicrobial treatment. Case histories tended to be chronic extending months to years. Five cases reported tooth removal 4 months to 1 year prior with subsequent development of sinusitis. The frontal and maxillary sinuses were the most reported location.

Paranasal sinus cysts occurred most in horses under 4 years of age. Sixteen cases were diagnosed on biopsy submissions and two on postmortem examination. Postmortem cases indicated a chronic history refractory to treatment or invasion of surrounding bone and dental tissue. Frontal and maxillary paranasal sinuses were the most common locations.

Ethmoid hematomas are a well-documented entity composed of proliferative granulation tissue with variable inflammation and hemosiderin. All horses were over 6 years of age except one yearling.

In 6 cases, oral pathology with extension to the paranasal sinuses was reported. These included odontomas (n=3), odontogenic hamartoma (n=1) and orosinus diastemas (n=2).

Finally, neoplastic cases comprised a neurogenic carcinoma, osteoma, poorly differentiated sarcoma and adenocarcinoma. Aneurysmal bone cysts were identified in a yearling and perinate. One case was determined to be a chronic reactive process with the inciting cause no longer apparent.

**Salmonellosis in captive elephants: report of two cases**

*Melissa Macías-Rioseco, Jennine Ochoa, Javier Asin, Robert B. Moeller, Francisco A. Uzal*

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Salmonellosis is caused by *Salmonella* spp., a gram-negative, rod-shaped, bacterium. The clinical presentations of salmonellosis in animals are enteritis and/or septicemia. Individuals of different species may carry the organism without showing clinical signs, thus acting as reservoirs. Reports of salmonellosis in elephants are rare and limited to *Salmonella enterica* serovars Dublin, Typhimurium, or Enteritidis. This case report presents two cases of salmonellosis in captive elephants, caused *Salmonella enterica* serovar Muenchen and *Salmonella enterica* serovar Montevideo, respectively. Animal A was a 48 years old Asian elephant, euthanized due to degenerative joint disease and gastrointestinal hemorrhage. Animal B was a 48 years old, African elephant with chronic, recurrent colic, followed by death. Grossly, the carcass of animal A was markedly pale, the small intestine was congested and the intestinal mucosa was multifocally ulcerated and covered by a yellow pseudomembrane. The wall of the stomach was severely thickened, the mucosa was multifocally ulcerated and the gastric serosa close to the greater curvature was covered by yellow exudate. Animal B had a reticular pattern in the liver, the mucosa of the stomach was multifocally congested, and the mucosa of the jejunum was multifocally effaced by small 0.5 to 2.5 cm diameter, well-demarcated, dark red foci. The cecum also had marked submucosal edema that extended to the proximal colon, and severe congestion. The ileocolic lymph nodes were mildly enlarged. Microscopically, animal A had multifocal, necrosuppurative enterocolitis and necrotizing and pleocellular gastritis. Animal B had necrotizing typhlocolitis. *Salmonella enterica* serovar Muenchen and *Salmonella enterica* serovar Montevideo were isolated from animals A and B, respectively. These elephants came from different unrelated premises and did not have a common source of feed. For animals in captivity, there should be a high standard of cleanness and husbandry to minimize the risk of salmonellosis both in animals and humans.

**Congenital neurologic and ocular lesions in calves as a result of *in utero* epizootic hemorrhagic disease virus infection # + * †**

*Jessica A. Kendziorski[1,2], Bonnie K. Harrington[1], Steven R. Bolin[1], Dodd Sledge[1], Stephanie French[1]*

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Congenital malformations in calves can be associated with an *in utero* infection of a teratogenic virus, among other potential infectious and non-infectious causes. Bovine viral diarrhea virus (BVDV) is considered one of the most common infectious agents leading to congenital malformations in the nervous and ocular systems in cattle. However, in absence of evidence for an infection with BVDV, other viral etiologies should be considered. Herein, we report a herd outbreak of congenital neurologic and ocular lesions caused by epizootic hemorrhagic disease virus (EHDV) in a 30,000-head dairy cattle farm in Michigan. From November to December 2020, multiple newborn calves at one farm displayed various neurologic signs, including inability to stand, ataxia, and inappropriate mentation. Tissues from two calves were submitted to the Michigan State University Veterinary Diagnostic Laboratory for histopathologic examination and ancillary testing. Gross and histologic lesions included severe hydrancephaly and dystrophic mineralization in the brain and subretinal scarring with synechia of the retina to a band of fibrosis in the eye. PCR testing of calf tissues for EHDV was positive, while a general pestivirus screen and bluetongue virus were negative. Additionally, antigen for BVDV was not detected in the eye via immunohistochemistry. Blood was submitted with one calf, and serology testing performed by the National Veterinary Services Laboratories revealed positive antibody titers for EHDV serotype 1 (EHDV-1) at 1:40, EHDV-2 at greater than 1:640, and EHDV-3 at 1:80. Antibody titers for Cache Valley virus and bluetongue virus were not detected. Antibody titers were also performed on serum collected from cows with calves exhibiting neurologic signs and heifers and milk samples collected from the abomasum of affected calves, all of which had antibodies for EHDV-2, providing additional evidence of widespread exposure to EHDV-2 on this farm. One cow did have high antibody titers for bluetongue virus. Combining the strong evidence for calf and cow exposure to EHDV-2 and associated congenital lesions and a lack of evidence supporting other differentials, a diagnosis of an *in utero* teratogenic EHDV-2 infection was made. This report is the first presentation from the United States of calves infected *in utero* with EHDV expressing congenital neurologic and ocular lesions.

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## Pathology 2

Sunday, October 9, 2022

Great Lakes A1

**Moderators:** Christopher Siepker and Drew Magstadt

Symbols at the end of titles indicate the following designations:

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**A novel chuvirus causing meningoencephalitis in alligator snapping turtle, the first in-situ evidence of chuviral disease in vertebrates. # * †**

*Weerapong Laovechprasit[1], Kelsey Young[1], Brian Stacy[3], Dalen Agnew[2], James B. Stanton[1]*

[1]Department of Pathology, University of Georgia, Athens, GA; [2]Department of Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI; [3]Office of Protected Resources, NOAA Fisheries, Gainesville, FL

Chuviruses, which are in the recently discovered family *Chuviridae*, were first identified in arthropods and have been detected through metagenomics in several animal groups. In chordates, few chuviruses have been characterized, including in fish (Wenling fish chuvirus and hardyhead chuvirus) and in snakes (Guangdong red-banded snake chuvirus and Herr Frank virus). Still, their pathological significance is yet to be determined.

This study demonstrated the first in-situ evidence of chuvirus pathogenicity in animals. A novel chuvirus was identified in a lethargic wild alligator snapping turtle (*Macrochelys temminckii*) on the shore of a Newnans Lake (Alachua County, Florida) and later euthanized in a moribund state. The principal necropsy finding was moderate to severe, diffuse, lymphoplasmacytic meningoencephalomyelitis with numerous perivascular cuffs. Transmission electron microscopy of the brain demonstrated 85-nm diameter spherical, enveloped viral particles. Moreover, randomly primed MinION sequencing resulted in the assembly of a complete viral genome that best aligned to chuviruses. The genome sequence was subsequently used to create a custom RNAscope® probe for in-situ hybridization, which confirmed the presence of chuviral RNA within several areas of the central nervous system, co-localizing with multiple areas of necrosis and non-suppurative inflammation. All other tissues lacked viral staining. The phylogenetic analysis illustrated that this virus clustered with other vertebrate chuviruses.

Based on pairwise amino acid identities, this chuvirus belongs to a novel species, putatively named *Piscichuvirus macrochelydis*. This study suggests strong evidence of the pathogenicity of not only this novel chuvirus but potentially for the entire family *Chuviridae*. Therefore, additional studies of these viruses are needed to elucidate their role in animal disease.

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**Psittacid herpesvirus-5 infection in Indian ringneck parakeets in Southern California**

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Four Indian ringneck parakeets (*Psittacula krameri;* also commonly referred to as ringneck parrots or rose-ringed parakeets) were submitted by two private owners for autopsy following a history of difficulty breathing and death. Gross findings were variable and included thickening of the left posterior thoracic air sac, pale spots throughout the liver, mild dilation of the proventriculus, coelomic effusion, enlargement of the spleen, and pulmonary congestion and edema. Microscopically, the submitted parakeets had significant lower respiratory tract lesions including necrotizing bronchitis, parabronchitis, and interstitial pneumonia with numerous syncytia containing eosinophilic intranuclear inclusions. Electron microscopy of the lungs was compatible with infection of a virus of the family Herpesviridae, and psittacid herpesvirus-5 (PsHV-5) was detected via PCR and sequencing. PsHV-5 is a novel alphaherpesvirus that has been recently associated with pneumonia in Indian ringneck parrots from Australia and Europe. To the authors' knowledge, this is the first confirmed case of PsHV-5 infection in birds in the Americas.

**Development of small-molecule inexpensive fluorescent in-situ hybridization (smiFISH) for direct detection of porcine hemagglutinating encephalomyelitis virus (PHEV)**

*Trevor Tiarn Arunsiripate, Christine Harness, Molly Elizabeth Kroeger, Jennifer Groeltz-Thrush, Eric R. Burrough, Pablo Pineyro*

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

Porcine hemagglutinating encephalomyelitis virus (PHEV) is the only described betacoronavirus affecting pigs. Commonly, PHEV causes encephalitis and ganglioneuritis of the myenteric plexus resulting in tremors, muscle fasciculations, and vomiting. In 2015, an outbreak of influenza-like respiratory cases was reported in show pigs. PHEV was the only pathogen confirmed by PCR. Serological studies have demonstrated that PHEV is endemic in sow herds, detection is not unexpected. In a retrospective exploratory study, we demonstrated by PCR and confirmed by direct detection (RNAScope) that PHEV can be implicated in cases of interstitial pneumonia. Thus, direct detection is imperative to confirm causation and accurately interpret detection of this endemic pathogen. Current commercial molecular techniques for direct detection have cost limitation that restricts their routine application in veterinary medicine. Here we propose to use PHEV as a model to design small-molecule inexpensive fluorescent in-situ hybridization (smiFISH). A consensus spike (S) gene sequence was generated based on five PHEV sequences detected in respiratory cases. The smiFISH probe design was completed with Oligominerapp with a restricted size of 36-41 bp in length, guanine and cytosine nucleotide (nt) content ranging from 20-80%, and a restricted sequence of 3 identical consecutive nt. Further analysis removed probes with affinity to other porcine respiratory epitheliotropic viruses, other betacoronaviruses, and the swine transcriptome. Negative (n=9) and positive (n=9) strand unique sequences of the PHEV RNA (primary probes) were selected and a 28 nt sequence identical to all primary probes was incorporated at the 5' end of each probe (FLAP sequence), allowing the annealing of a secondary 3' and 5' Cy3 fluorescent probe complementary to the FLAP sequence. Pooled negative and positive sense probes were used for PHEV detection in experimentally infected primary porcine kidney cells, and sections of lung from clinical cases confirmed positive by PCR. The staining protocol included overnight hybridization at 55C, followed by a nuclear counter stain with DAPI. The fluorescent signal was evaluated with a fluorescent microscope with an excitation and emission range of 535-555nm and 570-625nm, respectively. In PHEV infected cells, a large proportion of cells showed strong and localized perinuclear signal restricted to the cytoplasm. The fluorescent signal for the positive strand was remarkably strong compared to the uninfected control samples. In positive lungs, the fluorescent signal was observed in the respiratory epithelium and interstitial macrophages, with similar cellular mRNA distribution observed on cell culture. Our results show that smiFISH probes can detect the positive and negative strands of PHEV in cell culture and clinical specimens. This allows development of a fast and inexpensive method for direct detection necessary to confirm causation of veterinary endemic pathogens.

**Retrospective study of brain and spinal cord histologic lesions in commercial pigs naturally infected with porcine reproductive and respiratory syndrome virus**

*Marta Mainenti, Michael Rahe, Christopher Siepker, Alyona Michael, Pablo Pineyro, Marcelo Almeida, Phillip Gauger, Drew Magstadt*

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Porcine reproductive and respiratory syndrome virus (PRRSV) is among the most economically significant swine diseases worldwide. The most common presentations are respiratory disease (interstitial pneumonia) in growing pigs and reproductive failure in sows or gilts. Non-suppurative encephalitis is also reported sporadically in both natural and experimental infections.

Recently, the Iowa State University Veterinary Diagnostic Laboratory has detected an increased number of PRRSV encephalitis or myelitis diagnoses, with a total of 17 cases in 2021 compared to an average of 1.8 cases per year from 2007 to 2020. Thirty-four diagnostic cases of PRRSV encephalitis, with 74 pigs ranging from 1 to 24-week-old, were retrospectively evaluated to characterize the histologic lesions caused by PRRSV in the central nervous system (CNS). All included animals (74) had histologic lesions in the CNS and PRRSV was detected by rRT-PCR in either lung, brain, or spinal cord, with direct detection by immunohistochemistry in the CNS in all cases. Clinical signs included lateral recumbency, paddling, ataxia, head tilt, stargazing, nystagmus, paralysis, high fever, respiratory signs, and high mortality.

Histologic sections of cerebrum, cerebellum, brainstem, and spinal cord were evaluated in 100, 85, 65, and 39% of animals, respectively, and were affected in 100, 97, 88, and 90%, when present. Lesions affected either the gray matter, white matter, or meninges, with mild predilection for the white matter in brainstem and spinal cord. Primary histologic changes consisted of non-suppurative perivascular inflammation (100%), followed by gliosis (77%) and neuronal necrosis (31%). In the spinal cord, large foci of axonal degeneration in the funiculi were also common (59%). The perivascular inflammation was mainly lymphoplasmacytic in all animals, with frequently admixed histiocytes (93% of animals) and occasional eosinophils (33%). In addition, variable degrees of cell lysis with karyorrhexis of perivascular mononuclear cells were observed in 93% of animals. A concurrent fibrinosuppurative meningoencephalitis or myelitis was present in 8 animals (11%), consistent with bacterial involvement. Interstitial pneumonia consistent with PRRSV infection was present in all cases where lung was available (31 out of 34 cases).

Sequencing of the ORF5 region was performed in 30 out of 34 cases. Only lineage 1 PRRSV was detected consisting of 3 sublineages (L1A, L1C, and L1E) and 6 different RFLP patterns. Approximately 60% of the sequences were 1-7-4 L1A (33%) or 1-4-4 L1C variant strain (27%).

In conclusion, PRRSV infection of the CNS should be considered in pigs with concurrent respiratory and neurologic clinical signs. Based on this study, lymphoplasmacytic and histiocytic perivascular inflammation with cell lysis of mononuclear cells was the most common and consistent feature of PRRSV infection in the CNS and may help differentiate from other causes of encephalitis and myelitis in pigs.

**Highly pathogenic avian influenza virus infection of raptors in the upper Midwest, USA**

*Arno Wünschmann[1], Dana Franzen-Klein[2], Victoria Hall[2], Michele Leiferman[1], Michelle Carstensen[3], Mia Torchetti[4]*

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An outbreak of highly pathogenic avian influenza (HPAI) virus (H5 clade 2.3.4.4b) occurred in North America in 2022 affecting poultry and free-ranging birds as well as red fox kits. The objective of the current study was to describe the gross and histologic lesions in birds of prey that presented alive to The Raptor Center of the University of Minnesota during the outbreak and that were euthanatized on admission due to a poor prognosis. A total of 6 bald eagles (BAEA), 7 great horned owls (GHOW) and 9 red tailed hawks (RTHA) were submitted for necropsy. Gross lesions were uncommon. The pancreas of 4 BAEAs and 3 GHOWs had more or less distinct areas of reddening. Hemorrhage was present in the cerebrum of 2 BAEAs. The myocardium of one RTHA had white streaks. Subtle to severe histologic lesions were detected in the brains of all birds. The inflammation was most prominent in the cerebrum and affected the neuroparenchyma and ventricular system. The infiltration was frequently mixed cellular (including mononuclear cells and heterophils). Pannecrosis and/or selective neuronal necrosis as well as ependymitis were distinct features of the infection. The heart of 5 of 6 BAEAs, 5 of 7 GHOWs and 7 of 9 RTHAs had areas of cardiomyocyte necrosis usually with a histiocytic infiltration. Histologic detectable pancreas necrosis was common in GHOWs (6 out of 7) and occasionally detected in BAEAs (2 of 6) and RTHAs (3 of 9). Adrenal necrosis was present in 3 of 6 BAEAs and 3 of 8 RTHAs. Splenic necrosis and histiocytic splenitis was common in GHOWs and fairly uncommon in BAEAs and RTHAs. The liver of 2 BAEAs, 3 GHOWs and 2 RTHAs had subtle foci of lytic necrosis. Pectenitis and/or anterior uveitis were detected in 2 BAEAs, 2 GHOWs and 4 RTHAs. Oropharyngeal swabs of all birds tested positive for HPAI virus (H5 clade 2.3.4.4b). In conclusion, a variety of birds of prey infected with H5 2.3.4.4b virus have histological lesions in the brains of BAEAs, GHOWs and RTHA that allow for a suspect diagnosis of influenza virus infection. Nevertheless, confirmation of the diagnosis by molecular (and/or virologic) techniques is recommended mainly to rule out other viral and protozoal infections.

**Sudden death of racehorses in California: what is new?**

*Francisco A. Uzal[1], Susan Stover[1], Jessica Morgan[1], Carrie Fino[1], Santiago Diab[2], Federico Giannitti[3], Ashley E. Hill[1], Rick Arthur[1]*

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Sudden death of racehorses in California and elsewhere adversely affects racehorse welfare, jockey safety and public perception of horseracing. A definitive cause of death could not be established in approximately 50% of the cases of sudden death historically, although heart failure is suspected to be responsible for a large number of these deaths. Two separate studies were performed to determine if i) there is an association between sudden death and microscopic heart lesions in racehorses, ii) these lesions and/or death are associated with high values of cobalt, vitamin B12 and/or levothyroxine (T4) and iii) there is a difference in exercise history between sudden death and control horses. In the first study, microscopic examination of the heart of 27 horses with sudden death and 19 control horses revealed that non-inflammatory myofiber injury was present, with almost twice the prevalence in horses with sudden death than in controls. No significant differences in cobalt, vitamin B12 or T4 concentrations were found between study and control horses. Although the cause of sudden death in these horses was not determined, possible causes include inherited or spontaneous cardiac arrythmias, which may be exacerbated by exercise. In a second study, an analysis of exercise history of sudden death cases and matched controls found that sudden death horses had been exercised less intensely in the month prior to death. These horses may have shown prodromal signs, indicating that they were less tolerant to strenuous exercise. Determining those signs could help prevent sudden death.

**Approach to adding digital pathology for routine diagnostics in a high throughput veterinary diagnostic laboratory**

*Rachel Jean Derscheid[1,2], Jennifer Groeltz-Thrush[2], Randy Berghefer[2], Eric R. Burrough[1,2]*

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

In January of 2020, the Iowa State Veterinary Diagnostic Laboratory undertook an investigation of digital imaging options for our routine diagnostic histopathology cases. One of our obstacles was the dearth of veterinary diagnostic laboratories utilizing digital for comparison. The purpose of this is to present our key consideration points including our processes for evaluation, identification of stakeholders, and obstacles, foreseen and unforeseen, encountered.

After an initial investigation by a pathologist and the laboratory manager, it was determined that dedicated personnel would be required. A Diagnostic Associate was hired to manage the research, selection, set-up, and logistics of digital pathology. This began with an analysis of requirements and capabilities, leading to a request for proposals (RFP). Of the proposals received, 4 systems were evaluated, including remote and on-site visits and demonstrations. Two systems stood out, but one was selected, with the intent to purchase a second machine to accommodate our daily caseload with minimal impact on turnaround time for pathologists reading slides.

A number of factors were considered including: ease of use of hardware for scanning, quality of digital images, scanning speed, compatibility with current Laboratory Information Management System (LIMS), technical support and customer service, maintenance and longevity, and user-friendliness of slide navigation software. Key stakeholder groups included the histology technician group, the pathologists, and our information technology group.

Since getting our scanner onsite in January of 2021, we have worked through the logistics of barcoding, LIMS integration, daily casework management, and glass slide maintenance. We are now partially digital, with all pathologists having the option of slide scanning for initial case reading or scanning after reading glass for slide analysis, image capture, or teaching. Our process is ongoing, with the planned addition of a second scanner and continued troubleshooting of our image viewing software.

**SARS-CoV-2 infection in two farmed minks (*Neovison vison*)**

*Ryan Yanez, Dodd Sledge, Matti Kiupel*

Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI

A facility with 17,000 mink (Neovison vison) had 12 mink die on 9/27/2020 and 7 die on 9/28/2020. The facility owner reported coughing, anorexia, and bloody nasal discharge for several weeks in a large percentage of the group prior to death of these animals. Two male minks were submitted for necropsy to Michigan State University Veterinary Diagnostic Laboratory. Gross findings were unremarkable. Histologically, the nasal epithelium was eroded to ulcerated and had a lymphoplasmacytic to suppurative infiltrate. The turbinates were coated by mucus, hemorrhage and numerous degenerate neutrophils and foamy macrophages. In the lungs, large to small sized veins and arteries had prominent lymphoplasmacytic cuffing with vasculitis and fibrinoid necrosis. Extensive loss of terminal bronchiolar epithelium was a prominent feature. The alveolar interstitium contained lymphoplasmacytic to neutrophilic infiltrates and fibrin thrombi were within alveolar capillaries. Hyaline membranes were not observed. PCR for SARS-CoV-2 on nasal turbinate and lung samples from both minks was positive and confirmatory testing performed by the National Veterinary Services Laboratory (NVSL) in Ames, Iowa was also positive. Immunohistochemistry for the SARS-CoV-2 nucleoprotein antigen showed strong cytoplasmic immunoreactivity within mononuclear inflammatory cells, the nasal epithelium, tracheal epithelium, and bronchial and terminal bronchiolar epithelium. Rarely, there was cytoplasmic immunoreactivity of endothelial cells in the subepithelial stroma of the turbinates. This report documents unique histologic lesions and positive endothelial immunohistochemical labeling in farmed minks diagnosed with SARS-CoV-2.

**Vasculitis and generalized disease associated with systemic bovine adenovirus type 7 infection in a calf from the United States**

*Tyler Alan Harm, Hanjun Kim, Kyoung-Jin Yoon*

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

Bovine adenovirus type 7 (BAdV-7), a member of the *Atadenovirus* genus, is a reported pathogen in cattle worldwide. BAdV-7 is reported to cause enteric and respiratory disease, however, its significance as a pathogen remains unclear. In the United States, BAdV-7 has been detected or isolated from both healthy and diseased cattle, particularly those with respiratory disease. Systemic illness and death as a result of BAdV-7 infection are less commonly reported despite a potentially high prevalence of the virus reported in cattle herds worldwide. Here we report a laboratory investigation of a case associated with BAdV-7 infection which resulted in systemic clinical signs, lesions, and death observed in a single calf. Tissues from a 2-week-old beef calf were submitted to the Iowa State University Veterinary Diagnostic Laboratory for an enteric workup. The calf originated from southeast South Dakota and presented with bloody diarrhea and lethargy. At the time of presentation, no other calves or cows were affected in the herd. The calf rapidly declined after initial presentation, failed to respond to treatment, and died. Gross examination identified pulmonary edema, hepatic pallor, renal infarcts, serosal hemorrhages, and ulcerative enterocolitis. Microscopic postmortem examination identified necrotizing vasculitis with thrombi and necrosis in all examined organs. Smudgy, amphophilic, 8-10 μm in diameter intranuclear inclusion bodies were observed in all submitted organs, specifically in the areas of necrosis. Intranuclear inclusions were predominately found in endothelial cells (endotheliotropic) but occasionally observed in renal tubular epithelium and hepatocytes. Following postmortem examination, systemic adenoviral infection was the leading differential based on the morphology of the intranuclear inclusions. A nested polymerase chain reaction test performed on homogenates of kidney, liver, lung, and spleen and an intestinal content using degenerated universal adenovirus primers amplified a 318-324 bp portion of adenoviral DNA polymerase gene. While the PCR result confirmed systemic infection of BAdV-7 in the animal, the PCR amplicons had a varying degree of identity (96.2%-99.1%) with the corresponding sequence of BAdV-7 strain SD18-74 (MN901942), suggesting genetic variation among viruses in different tissues. Whole-genome sequencing for the virus in different tissues and virus isolation attempts are in progress to follow up on this observation and evaluate its significance. Co-infections with other viruses or bacteria have been ruled out using nucleic acid-, bacterial-, and immunohistochemical-based testing. Few disease-causing strains of BAdV-7 have been reported and characterized. Characterization of additional strains is important to defining BAdV-7's role as a bovine pathogen.

**Out-of-field toxic effects of radiation therapy + * †**

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Treatment-related toxic effects are commonly reported in cancer patients undergoing radiation therapy (RT). Radiation-induced toxicity typically affects tissue within and near the irradiated field. However, tissue changes are increasingly recognized in tissues that are distant from the irradiation field, such as the development of neurological signs despite undergoing hindlimb RT alone. Precise mechanisms for distant, out-of-field, radiation toxicity are unknown. The objective of the study was to investigate the underlying mechanisms by which localized RT induces out-of-field effects. Nine-to-thirteen week old SKH1 mice were treated with a single dose of 20Gy or 30Gy radiation to the right hindlimb. Mice were euthanized at [6 hours (h), 24h, 5 days (d), 12d, 25d] post treatment. Plasma and irradiated tissues (skin, muscle, femur) were collected, along with left femur, brain, and spleen. All tissue except brain were processed for cytokine and immune cell analysis by flow cytometry and immunohistochemistry. Within brain, glial cell activation and neurogenesis were assessed by immunohistochemistry. Hypocellularity and increased reticular fibers in the irradiated bone marrow was noted 5d post treatment compared to unirradiated mice. Distant effects including decreased splenic weight and lymphocyte populations and widespread microgliosis and astrocytosis in the brain. This study is the first to evaluate temporal toxic effects in unirradiated tissues following local RT in mice. It provides evidence of clinically relevant distant tissue effects following focal RT and supports further work is needed to identify potential strategies to minimize the side effects in cancer patients.

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**Postmortem examination of 13 elk (*Cervus canadensis*) calves after radio collar application * †**

*Elise Hennessy[1,2], Kyle Garrison[3], William Moore[3], Mark Vekasy[3], Paul Wik[3], Kristin Mansfield[3], Colleen Lynch[1,2], Laura Williams[1,2], Elis A. Fisk[1,2], Gerald Dykstra[1,2], Jeffrey Abbott[1,2], Joshua D. Ramsay[1,2], Laura White[1,2], Ashley Warren[1,2], Chrissy Eckstrand[1,2], Steven Edmonds[1,2], Holly Drankhan[1,2], Kyle Taylor[1,2]*

[1]Veterinary Microbiology & Pathology, Washington State University, Pullman, WA; [2]Washington Animal Disease Diagnostic Laboratory, Pullman, WA; [3]Washington Department of Fish and Wildlife, Spokane Valley, WA

Thirteen elk (*Cervus canadensis*) calves were submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL) as part of a multi-year survival/mortality investigation by the Washington Department of Fish & Wildlife involving capture and fitting with radio collars for monitoring. Submitted calves were classified as non-predation mortalities and represented a subset of a total of 99 calves from the Blue Mountains elk herd in southeastern Washington that had died within the first year of life in June 2021. The time elapsed from application of radio collars to calf death ranged from 4 days to 4 months, and causes of death were myriad. Findings of note include one case of viral bronchopneumonia attributed to a novel *Mastadenovirus* and one case of interstitial pneumonia and encephalitis attributed to *Sarcocystis alceslatrans*. Other findings and diagnoses are also reported and discussed. The detection of novel and little reported diseases in necropsy findings highlights the value in performing routine postmortem examination of monitored wildlife populations, as it broadens our knowledge and understanding of population diseases and health. The study will be repeated in 2022, with the potential for recognition of patterns and novel findings.

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

**Evaluation of the susceptibility and pathology of human ACE-2 knock-in transgenic rats to SARS-CoV-2.**

*Jessie Trujillo[1], Igor Morozov[1], Konner R. Cool[1], Dashzeveg Bold[1], Hongmei Jiang[4], Viktoria Hyddmark[4], Angela Bartels[4], Yumei Wu[4], Joe Warren[4], Helmet Ehall[4], Guojun Zhao[4], Jamie Retallick[1], William Wilson[2], Michael Schotsaert[3], Adolfo García-Sastre[3], Juergen Richt[1], Natasha Gaudreault[1]*

[1]Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; [2]Foreign Arthropod-Borne Animal Diseases Research Unit (FABADRU), National Bio and Agro-Defense Facility, USDA Agricultural Research Service (ARS), Manhattan, KS; [3]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY; [4]Genetically Engineered Models and Services, Envigo RMS, St Louis, MO

Distinguishing features of coronavirus disease 2019 (COVID-19) in humans that present in animal models facilitates investigation into SARS-CoV-2 pathogenesis and therapeutic discovery. In this study, a codon-optimized human ACE2 cDNA was integrated into the first coding exon of the rat *Ace2* gene locus using a CRISPR-based strategy. Rats expressing human angiotensin-converting enzyme 2 (hACE2) were evaluated as a preclinical model for SARS-CoV-2. The susceptibility of hemizygous and homozygous knock-in rats to the ancestral Wuhan-like SARS-CoV-2 USA-WA1/2020 strain was evaluated for 14 days following intranasal infection, with *post mortem* evaluations performed at 3, 6, and 14 days post challenge (DPC). Both hemizygous and homozygous knock-in rats were highly permissive to SARS-CoV-2 infection and demonstrated moderate weight loss by 3DPC. Viral RNA was detected by RT-qPCR in both, nasal washes and oral swabs at high levels (>10e9 copy number [CN] viral RNA/ml), and in significantly less amounts (~10e5 CN viral RNA/ml) in rectal swabs. There was no significant difference in the amounts and kinetics of viral RNA shedding between the two rat genotypes. The rats shed viral RNA from the upper respiratory tract for less than 10 days (tested positive up to 7DPC), while rectal swabs remained positive only until 5DPC. Fresh tissues (lung, forebrain, and hindbrain) collected at necropsy contained high levels of viral RNA in the lung at 3DPC (>10e10 CN/ml), with slightly lesser amounts at 6DPC (>10e9 CN/ml), and significantly less RNA at 14 DPC (10e4 CN/ml), with no discernable differences between genotypes. In addition, viral RNA (10e2-10e3 CN/ml) was recovered from either the forebrain and/or hindbrain at 3 DPC and 6DPC, suggestive of viral neuroinvasion. Pathological evaluation demonstrated moderate, multifocal, necrotizing rhinitis involving the respiratory and olfactory sensory epithelium and moderate to severe, necrotizing, bronchointerstitial pneumonia at 3DPC with persistent and progressive pneumonia at 6DPC. Additional investigations of SARS-CoV-2 tropism within the respiratory system and systemically, including the central nervous system are underway. In summary, the present work describes the development and characterization of a preclinical COVID-19 animal model using hACE2 knock-in rats. We conclude the hACE2 knock-in rat is highly susceptible to SARS-CoV-2 infection and exhibits similar pathology as COVID-19 in humans, and could be relevant for COVID-19 countermeasure development.

## Pathology - On Demand



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**Systemic toxoplasmosis in an adult dog with pancreatic atrophy**

*Erin A. Graham, Marcia RS Ilha*

Tifton Veterinary Diagnostic and Investigational Laboratory, University of Georgia, Tifton, GA

*Toxoplasma gondii* is an apicomplexan protozoan known to affect many animal species. Felids, including domestic cats, are the definitive host. Toxoplasmosis can cause central nervous system disease, radiculoneuritis, interstitial pneumonia, abortion, and systemic infection. Systemic toxoplasmosis is most common in young animals and is often associated with immunosuppression.

A one-year-old intact female American pit bull was evaluated for a two-month history of weight loss and diarrhea despite normal appetite. The dog was euthanized and submitted for postmortem examination after a one-week history of blindness followed by acute onset of seizures. Antemortem diagnostics provided by the referring veterinarian included normal complete blood count, serum chemistry profile, and bile acids.

On necropsy, the dog was emaciated with generalized muscle atrophy and scant adipose stores. The pancreas was small and described as narrow, linear, red-grey lobules spanning the mesentery along the proximal duodenum. Feces were soft and pale green. There was mild dilation of the lateral ventricles of the brain. Histopathology demonstrated systemic inflammation and necrosis associated with numerous protozoal zoites and cysts in the brain, cardiac and skeletal muscle, adrenal glands, urinary bladder, liver, and digestive tract. Vascular necrosis was infrequently observed. Inflammation in the brain was characterized by necrotizing and histiocytic to lymphoplasmacytic meningoencephalitis and ventriculitis with protozoal cysts and zoites. Ocular lesions included bilateral endophthalmitis with retinal necrosis and detachment. Other pertinent pathologic findings included interstitial pneumonia and necrotizing cortical adrenalitis. These histologic changes were attributed to systemic protozoal infection, of which *Toxoplasma gondii* and *Neospora caninum* were considered differentials. PCR testing for *Toxoplasma gondii* was positive on fresh samples of brain (Ct value: 25.4) and heart (Ct value: 21.4). *Neospora caninum* PCR testing was negative.

Pancreatic histopathology revealed severe atrophy of the exocrine pancreas with a few nodular remnants of acinar cells surrounded by prominent ducts, clusters of islet cells, and mild lymphoplasmacytic inflammation. These changes were considered consistent with chronic canine juvenile pancreatic atrophy. Taken together, the histologic finding of severe pancreatic atrophy with reported clinical signs are strongly suggestive of exocrine pancreatic insufficiency (EPI). Known sequela of EPI in dogs include chronic malnutrition and small intestinal bacterial overgrowth. These factors are suspected to have contributed to impaired immune function in this individual, allowing for the development of an unusual manifestation of systemic toxoplasmosis in an adult dog.

**Proliferative parathyroid lesions in captive bred American bullfrogs (*Lithobates catesbeianus*) with metabolic bone disease ***

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Parathyroid lesions in anurans are infrequently reported and most often in relation to experimental interventions. Husbandry-related parathyroid changes have yet to be documented in this order of Amphibia. Three American bullfrogs (*Lithobates catesbeianus*) living in a captive colony were euthanized due clinical concern for metabolic bone disease secondary to inadequate housing and UVB light. Antemortem, three individuals within the colony had an oral ranula containing slightly cloudy, teal fluid. On cytologic evaluation via fine needle aspiration, the fluid was of low cellularity with occasional clusters of moderately sized, uniform epithelial cells which had ovoid to elongate or occasionally irregular nuclei with clumped chromatin and small to moderate amounts of mid-blue cytoplasm. The findings were consistent with epithelial proliferation. Postmortem examination revealed cystic dilation and variable proliferation of unidentified structures within the cranial coelom corresponding to the anatomic location of anuran parathyroid glands. Histologically, the structures consisted of sheets and whorls of elongated cells. Immunohistochemistry (IHC) for pan-cytokeratin demonstrated strong cytoplasmic staining in the elongated cells of these structures, supportive of parathyroid origin. With a Grimelius special stain, occasional elongated cells contained dark brown to black cytoplasmic neuroendocrine granules, further supporting parathyroid origin. To our knowledge, this is the first report attempting to characterize anuran parathyroid glands with immunohistochemistry and special staining and the first report of parathyroid proliferation secondary to UVB light deficiency and metabolic bone disease in bullfrogs.

* Graduate Student Poster Presentation Award Applicant

**Obese pet pig with pancreatic necrosis and dilated cardiomyopathy**

*Kianna Dean[2], Anne B. Lichtenwalner[2], Nanny Wenzlow[1]*

[1]Pathobiology, Louisiana State University, Baton Rouge, LA; [2]UMaine, VDL, Orono, ME

A 1-year-old, intact, overweight pet sow with a history of sluggish behavior, weakness, lack of appetite and shortness of breath, was submitted deceased for a postmortem examination to the UMaine-VDL.

On necropsy, the animal presented severe, diffuse, pancreatic necrosis and hemorrhage, a markedly dilated right atrium and pulmonary artery, and mild mitral valve endocardiosis. Less significant findings were: moderate fibrinous polyserositis (*Streptococcus, spp., Staphylococcus, spp*. and *Bacillus, spp*. positive) involving the peritoneum and the epicardium; moderate fibrinosuppurative pneumonia with marked septal edema (*Streptococcus, spp*. positive); generalized mild lymphadenopathy; mild focal chronic hepatic fibrous serositis; mild strongyle-associated typhlocolitis; and mild cystitis.

Microscopic findings confirmed severe pancreatic necrosis with associated adipose tissue saponification, moderate myocardial interstitial fibrosis, myofiber hypertrophy with disarray, moderate degeneration and loss of cardiomyocytes, mild interstitial pneumonia, and mild centrilobular to midzonal hepatic necrosis.

Trace mineral evaluation on liver tissue revealed mild zinc and severe manganese deficiency, marginal selenium, and excessive iron.

Cardiomyopathies are uncommonly observed in pigs and are usually of congenital or genetic origin, or develop secondarily to nutritional deficiencies, such as carnitine or taurine as described in dogs and cats for dilated or hypertrophic cardiomyopathies respectively. A calorie-rich diet composed of ground barley with peas, wheat and vitamins was fed to this pig and illustrates how sub-optimal management of pet pigs can cause obesity, which in this case was associated with pancreatic necrosis similar to that observed in overweight dogs and cats fed high-fat diets.

**Concurrent parvoviral myocarditis and enteritis in a Rottweiler puppy**

*Heidi H. Rose*

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A five-week-old, female Rottweiler was presented for necropsy at the Mississippi Veterinary Research and Diagnostic Laboratory. Limited clinical history indicated that the puppy died acutely and had recovered from parvovirus four weeks previously. Generalized subcutaneous edema, ascites, hydrothorax, and hydropericardium, as well as pulmonary edema, were observed at necropsy. The myocardium of the left ventricle was pale. The serosal surface of the small intestine was mildly granular; and the mucosa was mildly reddened, with red-tinged intestinal content. Peripheral and mesenteric lymphadenopathy was moderate.

Histologically, there was multifocal infiltration of low to moderate numbers of histiocytes, lymphocytes, plasma cells, and neutrophils in the myocardium. Within inflammatory foci, cardiomyocytes often had pale, disrupted sarcoplasm and occasional loss of nuclei. Rarely, nuclei were enlarged with marginated chromatin and a central, amphophilic intranuclear viral inclusion body. Few fibroblasts were present within the interstitium adjacent to inflammatory foci. In sections of small intestine, variable numbers of lymphocytes and plasma cells infiltrated the lamina propria. Few to moderate numbers of crypts were dilated with fluid and cellular debris and there were sparse intraluminal neutrophils. Crypt epithelial cells were multifocally attenuated. In a section of ileum, lymphocytes were decreased in submucosal lymphoid follicles. Canine parvovirus PCR on pooled sections of small intestine was positive. Immunohistochemistry for canine parvovirus, performed at the Michigan State University Veterinary Diagnostic Laboratory, identified parvovirus antigen within inclusion- containing cardiomyocytes. The gross and histologic lesions, positive parvovirus PCR results, and positive parvovirus IHC of affected cardiomyocytes are consistent with concurrent parvoviral myocarditis and enteritis in this Rottweiler puppy. Bicavitary effusion and pulmonary edema are indicative of congestive heart failure secondary to myocarditis.

Parvoviruses infect mitotically active cells. Lymphoid tissues are first affected, and virus is disseminated via lymphocytes. In the gastrointestinal tract, crypt enterocytes are primarily affected. *In utero* or early neonatal infection can also result in parvoviral infection of cardiomyocytes while these cells are undergoing rapid replication. Puppies with parvoviral myocarditis may die acutely or later develop myocardial fibrosis and congestive heart failure as a sequela of parvoviral infection. While parvoviral hemorrhagic enteritis is well-recognized, parvovirus-associated myocarditis in young puppies is now uncommon because of widespread vaccination and transfer of maternal antibodies. Concurrent lesions of myocarditis and enteritis are atypical, and reports of generalized parvoviral infection are rare.

**Necrosuppurative meningomyelitis and perineuritis in a lamb with presumptive in utero *Parelaphostrongylus tenuis* infection**

*Rachel White[2], Nanny Wenzlow[1,3], Anne B. Lichtenwalner[1,4]*

[1]Cooperative Extension, University of Maine, Orono, ME; [2]Ecology and Environmental Sciences, University of Maine, Orono, ME; [3]Animal Disease Diagnostic Laboratory, Louisiana State University, Baton Rouge, LA; [4]School of Food and Agriculture, University of Maine, Orono, ME

Gastropods act as intermediate hosts and vectors of important parasites of livestock (e.g. the meningeal worm *Parelaphostrongylus tenuis; P.tenuis*). Where white-tailed deer (*Odocoileus virginianus* [WTD]; definitive host for *P. tenuis*) habitat overlaps with gastropod-infested (intermediate host of *P. tenuis*) pastures, risk for *P. tenuis* transmission to livestock (accidental host of *P. tenuis*) increases. While *P. tenuis* infections in WTD usually remain subclinical, infections in livestock are often fatal. Neurologic signs usually appear in the accidental host 1 to 6 months after ingestion of the infective larvae in gastropod-infested pastures. In this field study of 6 sheep farms at high risk of *P. tenuis* related losses, a Katahdin lamb from a farm with high numbers of both WTD and gastropods was submitted for necropsy after a clinical episode of ascending hindlimb paralysis followed by unexpected death at 2.5 weeks of age. On gross examination, the hindlimbs were flaccid with marked skeletal muscle atrophy. The corresponding joints and bones as well as the front legs were unremarkable. Mild subcutaneous and fascial erythema was seen in the right lumbar region over the epaxial muscles, at the level of the 5th lumbar vertebra. Large, pus-filled (~20 ml) abscesses were present bilaterally, subtending the epaxial lumbar muscles. Moderate amounts of yellow-tan pasty pus-like material overlaid the lumbar spinal cord and expanded the corresponding meninges of the spinal canal. Histology of the lumbar spinal cord revealed severe extensive necro-suppurative meningo-myelitis with numerous cross-sections of nematode structures (presumptive *P. tenuis*) and associated severe extensive necro-suppurative peri-neuritis of the lumbar spinal nerves. Bacterial culture of the abscesses isolated primarily *Staphylococcus aureus*, with a few *E. coli* colonies. Deep intramuscular abscesses, necro-suppurative meningo-myelitis with peri-neuritis are unusual manifestations of *P. tenuis* infection in domestic or wild animals. Finding nematode forms in a lamb younger than 1 month of age suggests transplacental transmission of *P. tenuis* during gestation. Confirmatory neural tissue *P. tenuis* PCR results are in progress.

**Endoscopic digestive biopsies and their clinical usefulness in horses**

*Emmie Vuillier[1], Nanny Wenzlow[2], Daniel Jean[1]*

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Endoscopic digestive biopsies are commonly taken in horses with a typical clinical context including one or more of weight loss, malabsorption, hypoproteinemia, and recurrent abdominal pain. The purpose of this study was to: 1) describe the clinical characteristics of patients that had endoscopic duodenal and rectal biopsies taken, 2) describe the histological findings of the digestive biopsies and identify potential correlations between histological results and clinical data. This study includes horses with complete clinical records that also had endoscopic digestive biopsies taken at our equine hospital (CHUV) between 2017 and 2021. Over the past 5 years, we observed an increasing trend of endoscopic duodenal/rectal sampling at our Equine Hospital and identified 36 horses with such biopsy samples. Ten of those horses had no significant histological changes and 26 had increased immune cell infiltrations. Duodenitis/proctitis were most often described as lymphoplasmacytic (54% and 37% from duodenal and rectal tissues respectively). The infiltration intensity ranged in most cases from minimal to mild (82%) with low correlation between duodenal and rectal changes (28%) in the same patient. Horses with increased duodenal infiltrates were also more likely to have an additional diagnosis of gastric glandular ulcers (46%), compared to horse without increased duodenal infiltrates (20%). Overall, there is no correlation between clinical and paraclinical findings with regards to immune cell type or their intensity in the duodenum or rectum. The clinical usefulness for endoscopic digestive biopsies in horses is likely more important than previously described in the literature and lymphoplasmacytic enteritis is the most frequent histological finding.The histological interpretation of both duodenal and rectal biopsies in an equine patient provides a more complete evaluation of the infiltrating immune cells and their intensity.

**Recurrent coinfection of infectious laryngotracheitis virus and multidrug resistant *Gallibacterium anatis* in commercial laying pullets.**

*Emily Reinhardt, Natalie Tocco, Linnette Vasquez, Kirklyn Kerr, Zeinab Helal, Guillermo R. Risatti*

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

Over a four month period in the winter and spring of 2022, the Connecticut Veterinary Medical Diagnostic Laboratory received 30 whole chickens and 12 tracheas collected on site from white Loman Selected Longhorn and Loman Brown pullets, respectively. These pullets were submitted during two separate episodes of severe respiratory disease at a commercial poultry unit with over 1.7 million birds maintained on two sites located in Connecticut. Over the period of 4 weeks, 6% of birds were lost from a house containing 100,000 birds aged 9 weeks and 8% of birds were lost from a house of 87,000 birds aged 35 weeks. On gross anatomic examination, 90% of the 30 birds submitted and 11 of the 12 tracheal samples had the following lesions; there was blood mixed with varying amounts of exudate along the length of the trachea, the larynx and tracheal mucosa were coated in a pseudomembrane, and the tracheal mucosa was dark red. Several birds also had caseous exudate within the coelomic cavity, both free or lightly adherent to the coelomic wall and viscera. Microscopically, there was severe, fibrinonecrotizing and heterophilic laryngotracheitis, with epithelial erosion and ulceration, and luminal hemorrhage and exudate containing innumerable bacteria. Along the apical epithelium and within sloughed epithelial cells in the tracheal lumen were epithelial syncytia with intranuclear inclusion bodies. In the coelomic cavity of many birds there was serositis and mesenteritis formed around yolk lipoproteins, consistent with egg yolk peritonitis. Based off the histopathologic findings, a diagnosis of Infectious Laryngotracheitis (ILT) was made, which was confirmed in both disease events by PCR testing of pooled tracheal swabs. Bacterial culture of a pooled tracheal swab isolated *Gallibacterium* sp., which was further sequenced and identified as *Gallibacterium anatis*. Culture and sensitivity completed on samples from the 30 birds revealed antibiotic resistance to four separate antibiotic classes, i.e. multidrug resistance. Infectious laryngotracheitis is a reportable respiratory disease of poultry caused by the highly contagious Gallid-herpesvirus-1, characterized by high morbidity and considerable mortality. *G. anatis* is a globally distributed, opportunistic pathogen of intensively reared poultry and is considered an emerging pathogen with increasing reports of multidrug resistant strains. In this poster presentation we describe repeated cases of an intercurrent infection of ILT and multidrug resistant *G. anatis* in an egg-laying poultry unit.

**Histologic findings in non-cervids associated with the 2021 outbreak of epizootic hemorrhagic disease in North Dakota**

*Quynn Steichen[1], Brianna Stenger[1], Liam Broughton-Neiswanger[2], Heidi Pecoraro[1]*

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During the summer and fall of 2021, there was an increase of epizootic hemorrhagic disease (EHD) cases in North Dakota over previous years. All of the 2021 ND EHD cases followed the distribution of *Culicoides sonorensis*, the EHD midge vector, along the upper Missouri River.

Cases were initially identified in wild and farmed white tail deer populations and, as the outbreak progressed, began spilling over into elk, cattle and bison herds, and even small ruminants. Of 26 total confirmed EHD positive cases that were submitted to the North Dakota State University Veterinary Diagnostic Laboratory (NDSU VDL), 50% were non-cervids, including eight cattle, four bison, and one goat.

Histologic findings were variable among the non-cervids and included lesions in the lung, liver, and vasculature of multiple organs. Pulmonary edema and hemorrhage were most common amongst all species, including the goat. Additionally, there was myocardial perivasculitis and cerebral and brain stem hemorrhage in one gravid beef cow, acute hepatitis in two other beef cows, focal vasculitis in the omentum and centrilobular hepatocellular degeneration in one bison cow, and fibrinoid necrosis and vasculitis in the rumen of another bison cow.

All cases were identified as serotype 2. Thus far, sequencing has not identified mutations in the EHD virus isolates to suggest increased virulence. Instead, the increase of cases has been attributed to two consecutive summers of drought conditions, creating an ideal environment for proliferation of the midge vector.

This report aims to highlight the panoply of histologic lesions associated with the recent EHD outbreak in North Dakota and to raise awareness of the need to have EHD as a potential differential for unexpected death in a diverse array of species during the late summer and fall months.

**Outbreak of canine distemper virus (CDV) of America-5 lineage in an animal shelter in Alabama ***

*Jack Stucky[1,3], Michael Luther[1], Alicia Wise[1], Rebecca P. Wilkes[4], Frances Kendrick[2], Heather Walz[1,3], Jenny Pope[1,3]*

[1]AVDL, Auburn, AL; [2]Valley Creek Veterinary Hospital, Selma, AL; [3]AUCVM, Auburn, AL; [4]Purdue Animal Disease Diagnostic Laboratory, Lafayette, IN

Canine distemper virus (CDV) is a widespread viral disease present in dogs and wild mammals throughout the United States. It is a systemic disease and dogs often present with neurologic or respiratory signs and secondary infections are frequent. Vaccinations against CDV are a common practice, but it remains a major cause of disease in dogs. An outbreak of CDV at a shelter in Alabama was investigated following submission of a 6-week-old puppy for necropsy. This puppy presented on emergency with chewing gum seizures after being treated for parvoviral infection at another veterinary hospital and was subsequently euthanized. Two other puppies in the litter had already died.

Gross findings were consistent with interstitial pneumonia and hyperkeratotic skin lesions were present on the abdomen and pawpads. RT-PCR for CDV on lung was performed at the Alabama Veterinary Diagnostic Lab and was positive. Additionally, histologic findings were consistent with CDV infection. Intracytoplasmic viral inclusions were seen in neurons and epithelial cells lining the trachea, bronchioles, common bile duct/pancreatic duct at the duodenal papilla, urinary bladder, and skin. The brainstem also had vacuolation and spheroids in the subventricular white matter along with gemistocytic astrocytes. Bronchointerstitial pneumonia and edema were present throughout the lungs as well as rare syncytia. Hyperkeratosis and epidermal hyperplasia was observed on the paw pads and the ventral abdomen, the latter of which also featured multifocal pustules with bacterial cocci. Concurrent infections included canine parvovirus, canine enteric coronavirus, rotavirus, and *Cystoisospora* sp.

Lung and spleen were sent to the Purdue University Animal Disease Diagnostic Laboratory for viral gene sequencing and this strain of CDV was identified to group in the America-5 lineage. RT-PCR on urine samples from 6 puppies at the shelter including the remaining littermates was performed and all were positive for CDV albeit with high CT values. Multiple of these puppies were later euthanized due to various clinical signs, but no additional puppies were submitted for necropsy. Adult vaccinated dogs exposed to these puppies did not develop clinical signs.

* Graduate Student Poster Presentation Award Applicant

**Natural infection with highly pathogenic avian influenza H5N1 in wild red foxes (*Vulpes vulpes*) and bobcats (*Lynx rufus*) in Wisconsin**

*Betsy Elsmo[1], Shawna Hawkins[3], Erin Lemley[3], Mia Torchetti[2], Mary Lea Killian[2], Lindsey Long[4], Ailam L. Lim[1]*

[1]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI; [2]NVSL, Ames, IA; [3]UW Madison School of Veterinary Medicine, Madison, WI; [4]Wisconsin Department of Natural Resources, Madison, WI

Natural infection with highly pathogenic avian influenza virus (HPAI) H5N1 belonging to Eurasian lineage, clade 2.3.4.4b was detected by PCR in 11 wild fox kits (*Vulpes vulpes*) and 2 bobcats (*Lynx rufus*) in Wisconsin, United States, concurrent with outbreaks of highly pathogenic avian influenza virus in wild birds and domestic poultry in the region. Affected fox kits and bobcats displayed neurological signs and had evidence of systemic viral infection, primarily affecting the brain and respiratory system. Genomic analysis of these isolates confirms the presence of multiple virus strains commonly circulating in the midwestern United States in wild birds and domestic poultry, consistent with independent spill over events. These findings highlight the importance of HPAI surveillance in wild mammals that share the landscape with or consume wild birds, particularly during active HPAI outbreaks.

## Serology

Saturday, October 8, 2022

Greenway J

**Moderators:** Michael Rahe and Luis G. Gimenez-Lirola

**1:00 PM** **Detection of *Mannheimia haemolytica*-specific IgG, IgM and IgA in serum samples and their relationship to respiratory disease in cattle**
*Korakrit Poonsuk, Carita Kordik, Matthew Hille, Ting-Yu Cheng, William B. Crosby, Amelia Woolums, John Dustin Loy, Bruce Brodersen* . . . . . *139*

**1:15 PM** **Validation of a new competitiv ELISA for the detection of EHDV antibodies**
*Loïc Comtet, Mickael Roche, Muriel Malzac, Philippe Pourquier*. . . . . *140*

**1:30 PM** **Dynamics of antibody response and bacterial shedding of *Mycoplasma hyorhinis (Mhr)* and *Mycoplasma hyosynoviae (Mhs)* in oral fluids of experimentally challenged CDCD pigs**
*Precy Dizon Magtoto, Bailey Arruda, Ronaldo L. Magtoto, Mingxi Guo, Henrique Meiroz-De-Souza-Almeida, Juan Carlos Mora-Diaz, David Baum, Jeffrey Zimmerman, Rina Opulencia, Luis G. Gimenez-Lirola* . . . . . *141*

**1:45 PM** **A prospective seroprevalence survey of *Anaplasma marginale* among Missouri beef cows, 2021-2022 ◊**
*Rosalie Ierardi, Michael Z. Zhang, Shuping Zhang, Ram K. Raghavan*. . . . . *142*

**2:00 PM** **A novel double antigen ELISA for the species independant detection of CCHFV antibodies**
*Loïc Comtet, Fabien Donnet, Philippe Pourquier* . . . . . *143*

**2:15 PM** **Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian Influenza A**
*Stéphanie Lesceu, Marina Gaimard, Chloe Redal, Jean-Emmanuel Drus, Catherine Lefebvre, Philippe Pourquier*. . . . . *144*

**2:30 PM** **Performant competitive and indirect ELISAs for African swine fever diagnosis in domestic swine and wild boar**
*Loïc Comtet, Fabien Donnet, Philippe Pourquier* . . . . . *145*

**2:45 PM** **A microfluidic biosensor architecture for the rapid and accurate detection of chronic wasting disease prion**
*Sura Muhsin, Amjed Abdullah, Estela Haverstick Kobashigawa, Muthana Al-Amidie, Sherri Russell, Michael Z. Zhang, Shuping Zhang, Mahmoud Almasri* . . . . . *146*

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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**Detection of *Mannheimia haemolytica*-specific IgG, IgM and IgA in serum samples and their relationship to respiratory disease in cattle**

*Korakrit Poonsuk[1], Carita Kordik[1], Matthew Hille[1], Ting-Yu Cheng[2], William B. Crosby[3], Amelia Woolums[3], John Dustin Loy[1], Bruce Brodersen[1]*

[1]Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, NE; [2]Veterinary Preventive Medicine, Ohio State University, Columbus, OH; [3]Department of Pathobiology and Population Medicine, Mississippi State University, Mississippi State, MS

*Mannheimia haemolytica* is one of the major causes of bovine respiratory disease in cattle. The organism is the primary bacterium isolated from calves and young cattle affected with enzootic pneumonia (Rice et al., 2008). Due to the fact that *M. haemolytica* is an opportunistic pathogen, capable of causing severe bronchopneumonia in some circumstances, serology results can be difficult to interpret as *M. haemolytica*-specific antibodies may be apparent in clinically normal cattle (Quinn et al., 2011). There is limited information regarding the relationship between the serum antibody levels and *M. haemolytica* susceptibility in feedlot cattle, and a lack of testing options to assess these associations. Therefore, a novel indirect ELISA was developed and evaluated to enable quantification of antibody responses to whole cell antigen using *M. haemolytica* A1 strain P1148 (ATCC 14003).

Serum and nasopharyngeal swab samples (n = 324) were collected from feedlot calves (n = 163) at the day of arrival (day 0) and the last day of the experiment (day 20 or 21). Nasopharyngeal swabs were cultured for *M. haemolytica*. All animals were observed for clinical signs of respiratory disease. Sick calves received antibiotic treatments during the study. Additional swab and serum were collected abefore antimicrobial administration. Time and duration of the clinical disease observed and all treatments were recorded. Serum samples from cattle negative to *M. haemolytica* culture at day 0 were used as true antibody negative specimens. All serum samples collected from cattle which were culture-positive for *M. haemolytica* at least 14 days after the culture-positive sampling date were used as true antibody positive specimens for test validation. All serum samples were tested by *M. haemolytica* antibody-specific IgG, IgM, and IgA indirect ELISAs. The test cutoffs were analyzed based on the true status of the specimens. Serology results were analyzed with respiratory clinical signs and *M. haemolytica* isolation data from both clinically normal and diseased animals.

Diagnostic sensitivity and specificity were estimated at 91% and 87% for IgG at a cutoff of S/P ≥ 0.8. IgM diagnostic sensitivity and specificity were 91% and 81% at a cutoff of S/P ≥ 0.8. IgA diagnostic sensitivity was 89% whereas specificity was 78% at a cutoff of S/P ≥ 0.2. Antibody results of all isotypes were related to the respiratory disease and isolation of *M. haemolytica*. These data suggest that *M. haemolytica* ELISA can be adapted to the detection and quantification of antibody in serum specimens and support the use of these tests for the disease surveillance and disease prevention research in feedlot cattle.

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**Validation of a new competitiv ELISA for the detection of EHDV antibodies**

*Loïc Comtet, Mickael Roche, Muriel Malzac, Philippe Pourquier*

Innovative Diagnostics, Grabels, France

Epizootic hemorrhagic disease virus (EHDV) of deer is an arthropod-borne *Orbivirus* that causes infection in wild and domestic ruminants. In domestic ruminants, EHDV may induce clinical and pathologic signs similar to those of bluetongue virus (BTV), but can also be asymptomatic. Laboratory diagnosis is most often based on the detection of anti-EHDV antibodies in serum. This study summarizes validation data obtained for the ID Screen® EHDV. Competition *(for research use only)*. It is a competitive ELISA based on the use of a VP7 recombinant protein as coated antigen and an anti-VP7 monoclonal antibody, HRP labelled, as conjugate.

Specificity: serum samples from cattle (n=383), domestic deer (n=172), sheep (186) and goats (124), were tested. These samples originated from France and Europe and were collected before 2004. As a result, they are considered free of antibodies to *Orbivirus*. Samples had also tested negative for BTV using the IDScreen® Blue Tongue Competition ELISA kit. All samples were found negative with the ID Screen® EHDV ELISA, giving a measured specificity of 100.0% ($CI_{95\%:}$ bovine [99,0 – 100%]; domestic deer: [97.8 -100%]; sheep [98.0 – 100%] ; goat [97.0 ; 100]).

Sensitivity: 42 samples from deer, experimentally-infected with EHDV US serotypes 1 or 2, were tested. All samples were found positive with the ID Screen® EHDV Competition ELISA, including samples classified as weak positive by the Agar Gel Immuno Diffusion technique. Samples from a reference panel (The Pirbright Institute, UK) comprised of 5 samples from serotypes 1, 2, 7, 8 (EHDV1 RR91, EHDV1 PJ70, EHDV 7, EHDV 8, EHDV 318 (2)) were tested, as were 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.

Exclusivity: 20 samples from a reference panel (The Pirbright Institute, UK) raised again different BTV serotypes (3, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 16 strain UN95 and 2 OBP) were tested. All samples were positive for BTV antibodies. 19 samples were found negative by the EDHV ELISA, and only one sample was found 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 specificity 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 can be observed, especially with samples with high VP7 BTV titers.

Conclusion: the ID Screen® EDHV Competition ELISA is a robust, easy-to-use, highly sensitive and specific ELISA for the detection of EDHV antbodies, even in the context of high BTV seroprevalence.

**Dynamics of antibody response and bacterial shedding of *Mycoplasma hyorhinis (Mhr)* and *Mycoplasma hyosynoviae (Mhs)* in oral fluids of experimentally challenged CDCD pigs**

*Precy Dizon Magtoto[3,4], Bailey Arruda[1], Ronaldo L. Magtoto[1], Mingxi Guo[1], Henrique Meiroz-De-Souza-Almeida[2], Juan Carlos Mora-Diaz[1], David Baum[1], Jeffrey Zimmerman[1], Rina Opulencia[4], Luis G. Gimenez-Lirola[1]*

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*Mycoplasma hyorhinis (Mhr)* and *Mycoplasma hyosynoviae (Mhs*) are commensal organisms in the upper respiratory tract and tonsils of pigs and cause arthritis in post-weaned pigs. Mycoplasma-associated arthritis leading to lameness has emerged as a growing problem in the recent years. This result in significant economic losses due to removal and culling of lame pigs. Thus, early detection for timely and targeted interventions is critical; however, antemortem diagnostic tools for *Mhr* and *Mhs* are scarce. Oral fluid (OF) has been gradually implemented as an effective tool for swine health monitoring. This study evaluated the dynamics of antibody response and bacterial shedding in pen-based OF collected over 56 days post-inoculation (DPI) from 8-week-old cesarean-derived, colostrum-deprived (CDCD) pigs ($n$=30) experimentally inoculated with *Mhr, Mhs* and mock-inoculated with culture medium. Pigs were randomly allocated in three groups (10 pigs per group housed in five pens with two pigs per pen). Group 1 was inoculated with *Mhr* via tonsillar swabbing and intraperitoneal route*;* Group 2 with *Mhs* via tonsillar swabbing, intranasal and intravenous routes; and Control Group via intranasal with culture medium. Infection status and bacterial DNA shedding in OF was assessed by quantitative real-time PCR (qPCR). Results of qPCR revealed *Mhr* shedding in OF between DPI 5 to 52, whereas *Mhs* shedding was detected between DPI 5 to 15. The OF isotype-specific (IgG, IgA, IgM) antibody response was evaluated using indirect enzyme linked immunosorbent assays (ELISAs) based on a recombinant chimeric polypeptide of variable lipoproteins A to G for *Mhr*, and Tween 20-extracted surface proteins for *Mhs*. Receiver operating characteristic (ROC) analysis was performed to assess the diagnostic performance of the *Mhr* and *Mhs* ELISAs associated to different sample-to-positive (S/P) values. The first antibody response in Group 1 was an IgA observed at DPI 7. However, compared to the Control Group, significant ($p < 0.05$) antibody responses were detected in Group 1 between DPI 12-15 for IgM, and DPI 36-56 for both IgA and IgG isotypes. A weak IgM response in Group 2 was detected only at DPI 10. Significant ($p < 0.05$) IgG and IgA responses were detected at DPI 32-56 and DPI 44-56, respectively, in Group 2 pigs compared to the Control Group. This study demonstrates that OF can be used as a non-invasive antemortem specimen for practical early detection and surveillance of *Mhr* and *Mhs*.

**A prospective seroprevalence survey of *Anaplasma marginale* among Missouri beef cows, 2021-2022 ◊**

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Bovine anaplasmosis is an economically significant disease of cattle, with impacts including weight loss, spontaneous abortions, and death. Contemporary information about the epidemiology of this disease in Missouri is limited. Here, we present the preliminary results of an ongoing prospective serosurvey to evaluate the period prevalence of antibodies against *Anaplasma marginale* in serum from apparently healthy adult beef cows in Missouri. Given the lifelong persistence of most *A. marginale* infections, seroprevalence is a reliable estimate of the proportion of carrier cattle within a population.

Samples were collected from November 2021 through June 2022. Serum was collected with client consent by each herd's local veterinarian during a routine visit. Samples were tested at the University of Missouri Veterinary Medical Diagnostic Laboratory using a commercially available *A. marginale* competitive ELISA (cELISA). A positive result was defined as ≥30% inhibition. Of the 509 individual cattle that were tested, 243 (47.7%) were seropositive. Thirty-two of 33 tested herds (97.0%) had at least one positive animal. Herds are located in 19 counties throughout Missouri.

Participating clients completed a questionnaire which included 38 questions on herd characteristics, management practices, and client perceptions of anaplasmosis disease risk. Questionnaire responses indicated that most producers own cow-calf operations (93.5%) and describe their cattle as "commercial" or "a mixture of commercial and purebred" (90.3%). The median herd size in the survey was 58 cows.

Of the 29 producers with open herds, 27.6% reported testing new cattle for infectious diseases before they were introduced to the herd. None reported testing for anaplasmosis. Eighteen producers (58%) reported seasonal feeding of chlortetracycline in a medicated mineral mixture for the control of anaplasmosis. One producer reported current use of the anaplasmosis vaccine, with a perceived reduction in clinical cases; however, only 4 of 15 cows tested in this herd had a positive antibody titer according to the cELISA. Eight producers (25.8%) reported changing needles between every animal, which is higher than expected and likely reflects selection bias (i.e., producers who volunteer for the study are likely to be concerned about anaplasmosis and may be attempting to reduce transmission in their herds).

Results from the serosurvey confirm that bovine anaplasmosis is prevalent and widespread in Missouri. This serosurvey will continue through the fall of 2022.

◊ USAHA Paper

**A novel double antigen ELISA for the species independant detection of CCHFV antibodies**

*Loïc Comtet, Fabien Donnet, Philippe Pourquier*

Innovative Diagnostics, Grabels, France

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by the Crimean-Congo hemorrhagic fever virus (CCHFV). The virus causes an often-fatal hemorrhagic illness in humans. Animals do not develop clinical symptoms upon infection, but viremia and CCHFV-specific antibodies can be observed. The detection of anti-CCHFV antibodies in livestock is therefore used to reveal CCHFV infection risk areas.

This work presents the development and validation of a novel CCHF double antigen ELISA for the detection of anti-CCHFV nucleoprotein (NP) antibodies *(for research use only)*. As the ELISA is based on recombinant protein it can be run within 90 minutes under standard biosafety conditions.

For assay validation, 95 cattle and 176 small ruminant sera from animals from CCHF endemic regions served as a CCHFV positive reference serum panel. 402 cattle and 808 small ruminant sera from Germany and France served as negative serum panel, as both countries are considered outside of the CCHFV endemic zone. Moreover, sera from monkeys, camels, rats, ferrets, raccoon dogs, raccoons, foxes, hares, pigs and humans were tested, in order to determine the suitability of this novel ELISA for these species.

All negative reference sera (n= 2136) were confirmed by the novel CCHF double antigen ELISA indicating a specificity of 100% ($CI_{95\%}$: 99.8% - 100%). 268 of 271 positive reference sera were tested positive for CCHFV-specific antibodies which means a sensitivity of 98.9% ($CI_{95\%}$: 96.8% - 99.8%).

**Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for H5 avian Influenza A**

*Stéphanie Lesceu, Marina Gaimard, Chloe Redal, Jean-Emmanuel Drus, Catherine Lefebvre, Philippe Pourquier*

Innovative Diagnostics, Grabels, France

Influenza viruses belong to the family *Orthomyxoviridae* and infect a variety of human and animal hosts. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin (H) and Neuraminidase (N) antigens. Eighteen H antigens (H1 to H18) and eleven N antigens (N1 to N11) have been isolated. Most avian influenza viruses (H1 to 18 subtypes) are low pathogenic, such as H9, and are generally involved in co-infection with other avian viruses, which can lead to important losses in poultry flocks. Some subtypes containing H5 and H7 are associated with highly pathogenic forms of the disease, with high rate of mortality. Vaccination is an essential tool for poultry disease control. For many years, conventional vaccines were used. Today, innovation in poultry vaccinology include immune-complex vaccines and vector vaccines. Vector vaccines are made from a vector microorganism of which the genome has been genetically modified to encode an immunogenic protein of the disease of interest. Vectors in poultry vaccines are commonly the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT). One or more genes may be inserted to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with this technology include bio-security, efficiency, ability to breakthrough passive immunity, and long-lasting immunity. In addition, vector vaccines may be used to as part of DIVA (Differentiation between Infected and Vaccinated Animals) strategies. IDvet has developed new tools to monitor vaccination with vector vaccines for Avian Influenza (AI) and implement DIVA strategy. Especially, to monitor vaccination uptake for H5 recombinant vaccine, IDvet developed a new ELISA test based on H5 protein. Given that vaccinated animals will only develop antibodies against the H5 protein, IDvet has also developed a DIVA strategy in which vaccinated animals may be monitored using the previous kit, and naturally infected animals may be detected using the ID Screen® Influenza A Nucleoprotein Indirect. (Only naturally infected animals will develop antibodies against the AI nucleoprotein).

**Performant competitive and indirect ELISAs for African swine fever diagnosis in domestic swine and wild boar**

*Loïc Comtet, Fabien Donnet, Philippe Pourquier*

Innovative Diagnostics, Grabels, France

African Swine Fever Virus (ASFV) control programs require reliable diagnostic tests. IDvet offers an indirect ELISA (code product: ASFS) and a competitive ELISA (code product: ASFC) for the detection of antibodies. This work presents validation data obtained on these ELISA. 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. Panels of known positive and negative samples, as describe below, were tested.

Indirect ELISA ASFS

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 ASFC

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.

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). 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.

**A microfluidic biosensor architecture for the rapid and accurate detection of chronic wasting disease prion**

*Sura Muhsin[1], Amjed Abdullah[1], Estela Haverstick Kobashigawa[2], Muthana Al-Amidie[1], Sherri Russell[3], Michael Z. Zhang[2], Shuping Zhang[2], Mahmoud Almasri[1]*

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Chronic Wasting Disease (CWD) is a fatal neurologic disease of captive and free ranging cervids. The disease development involves a conformational conversion of the cellular prion protein to a protease-resistant and infectious conformer. Current CWD diagnosis includes ELISA and immunohistochemistry for confirming positive results. This project developed a microfluidic biosensor for rapid and accurate detection of CWD pathologic prion using the positive antigen control of the IDEXX CWD ELISA kit and retropharyngeal lymph node (RPLN) tissue homogenates of hunter-harvested white-tailed deer. The detection is based on impedance change which occurs when the prion antigen in the sample binds to its specific antibody on the electrode surface.

The biosensor was designed with unique abilities to concentrate low quantities of CWD prions in the samples to a detectable level, trap, capture, and detect the CWD prions with high selectivity and sensitivity in less than 40 minutes. One of the two sets of sensing electrodes was precoated with a mixture of anti-prion mAB (F89/160.1.5) and cross-linker (Sulfo-LC-SPDP) while the other set of electrodes was coated with mAb against bovine coronavirus or uncoated to serve as negative control. The biosensors were able to focus and trap microbeads with diameter of less than 0.5μm and CWD prions. The AC voltage and frequency were determined (focusing 4 Vp-p, 5 MHz, trapping 5 Vp-p at 6 MHz). The CWD positive control antigen was subjected to 2-fold dilution (1:4 – 1:24) and RPLN homogenates (30 ELISA-positive and 30-ELISA negative) were subjected to 10-fold serial dilutions ($10^{-1}$ to $10^{-4}$). The test specificity for pathogenic prion was confirmed by proteinase K treatment of RPLN homogenates prior to loading the samples into the biosensor.

The results indicated that the optimum antibody concentration and precoating time were 2 μg/ml and 1 hour, respectively. With the positive control antigen, the biosensor had a relative limit of detection (rLOD) of 1:24 dilution while ELISA had a rLOD of 1:4 dilution. With RPLN homogenates, rLOD of was 1:1,000 dilution for the biosensor and <1:10 for ELISA. All ELISA-positive samples were tested positive by the biosensor and all ELISA-negative samples were tested negative by the biosensor. Pretreatment of RPLN homogenates did not affect the impedance change, suggesting that the biosensor detected pathogenic prions in the samples. In summary, the biosensor developed in this project offers a rapid, sensitive, specific testing tool for the detection of CWD prion in RPLN samples.

## Serology - On Demand



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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**Evaluation of ASR1 peptoid-conjugated beads for the detection of chronic wasting disease (CWD) prion**

*Estela Haverstick Kobashigawa[1], Michael Zhang[1], Sherri Russell[2], Jasmine Batten[2], Shuping Zhang[1]*

[1]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO; [2]Missouri Department of Conservation, Jefferson City, MO

Chronic wasting disease (CWD) is a fatal, neurological disease of cervids. The mechanism of disease involves the conversion of a native, protease-sensitive cellular protein ($PrP^{C}$) to a protease-resistant, self-replicating conformation, called $PRP^{Sc}$. Immunohistochemistry (IHC) on obex and retropharyngeal lymph node (RPLN) or ELISA screening followed by IHC confirmation of positive results are widely used for CWD diagnosis. ASR1 reagent consists of a panel of peptides containing N-substituted glycine which bind to misfolded proteins, including $PrP^{Sc}$ and discriminate $PrP^{Sc}$ from $PrP^{C}$. This study evaluated ASR1 reagent as an enrichment reagent for RPLN ELISA and blood RT-QuIC.

RPLN tissue samples, including 30 positives (ELISA/IHC+), 30 suspects (ELISA+/IHC-), and 30 negatives (ELISA-), were obtained from CWD diagnostic cases. Postmortem blood samples were collected from 13 CWD IHC positive. Platelet-rich plasma (PRP) was subsequently prepared by centrifugation. RPLN and PRP samples were incubated with ASR1 reagent for 1 hour at room temperature prior to ELISA and RT-QuIC, respectively. ELISA was performed according to the manufacture's instructions. RT-QuIC was carried in FLUOstar Omega Microplate Reader under the following conditions: 95 μL master mix (320 mM NaI, 20mM $Na_2HPO_4$, 1mM EDTA and 0.1 mg/mL rPrP), 3μL sample at $10^{-1}$ to $10^{-3}$ dilutions, 188 cycles at 42°C, reading every 15 min, gain at 1800, 1 min shaking at 700 rpm and 1 min resting.

Analysis of RPLN samples by ARS1-ELISA indicated that all positive samples were positive by ASR1-ELISA, all negative samples were negative by ASR1-ELISA, and all suspect samples also negative by ASR1-ELISA. RT-QuIC positive rate on blood samples from CWD positive animals were 82.05% (ASR1/NaI), 74.36% (ARS1/NaCl), 66.67% (NaI) and 51.28% (NaCl). RT-QuIC positive rate on blood samples from CWD negative animals was 4.25% using NaI without ASR1 pre-concentration.

In conclusion, ASR1 improves the specificity of ELISA and sensitivity of RT-QuIC.

**Validation of a bead-based multiplex assay for detection of *Trypanosoma cruzi* antibodies in dogs**

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[1]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX; [2]College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX; [3]Department of Cellular Biology, University of Georgia, Athens, GA; [4]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA

The vector-borne protozoan parasite *Trypanosoma cruzi* causes Chagas disease in humans, dogs, and many other mammalian hosts. Dogs are the most frequent host of veterinary concern in the United States, as they experience similar disease progression to humans and are considered a sentinel species (i.e., an indicator that people may be at risk of exposure to the parasite and its vector). Due to only brief windows of parasitemia during the *T. cruzi* lifecycle, and thus limited opportunity to detect it using molecular or hematological methods, serology is often the primary tool used to diagnose Chagas disease in dogs. Even in the absence of direct parasite identification, seropositive dogs are typically considered infected as there is currently no evidence of self-clearing the parasite. This study evaluated a bead based (Luminex) assay for detecting antibodies against *T. cruzi* and compared with existing serological methods to establish cut-off values and relative sensitivity/specificity.

Serum samples were originally collected from hunting dogs from large kennel environments in south Texas where triatomine vectors occur, as part of a longitudinal study of Chagas disease. Samples (n = 133) which were previously characterized using the indirect fluorescent antibody (IFA) test and two commercial rapid assays were tested on the Luminex assay using beads coupled with 12 different antigens: 9 antigens from *T. cruzi,* a negative control recombinant protein (green fluorescent protein, GFP), a *Leishmania* antigen to explore cross-reactivity, and a canine parvovirus antigen which served as an antibody control. For each sample, the ratio of mean fluorescence intensity (MFI) for each *T. cruzi* antigen to that of GFP was calculated. A subset of samples (n = 25) which were previously negative by IFA and both rapid tests was used to establish positive cut-off values for each antigen; samples with an antigen/GFP ratio greater than 4 standard deviations above the mean of these negative sera were considered positive on that antigen.

Samples testing positive on 2 or more antigens were considered positive for *T. cruzi* antibodies. Compared to the IFA, the Luminex assay demonstrated a relative sensitivity of 100% and specificity of 96.97% using this cutoff criterion. Given the high sensitivity and specificity of this assay when compared to the existing IFA, along with the advantages associated with its precision, high-throughput format, potential for automation, and lack of subjective interpretation, the Luminex platform should be considered a valid and improved alternative to existing methods for detection of *T. cruzi* antibodies in dogs.

## Toxicology

Sunday, October 9, 2022

Great Lakes A3

**Moderators:** Cat Barr and Scott Radke

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

**Presumptive raw food-associated thyrotoxicosis in 2 dogs with clinical signs of hyperthyroidism**

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[1]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada; [2]Bowmanville Veterinary Clinic, Bowmanville, ON, Canada

Two dogs with clinical signs consisting of weight loss, PU/PD, anxiety and diarrhea were presented to the same veterinarian in Ontario, Canada. The dogs belonged to unrelated owners but had been fed a similar raw food diet supplied for several years by a common local manufacturer.

Dog #1: 9-year-old, male (neutered), 41 kg Mastiff cross. Laboratory testing showed elevated total T4 (78 nmol/L; RI: 13-51 nmol/L) and free T4 (>77.2 pmol/L; RI: 8-32 pmol/L).

Dog #2: 3-year-old, male (neutered), 40.7 kg Bernese Mountain dog. On blood work, ALT was mildly elevated (241 U/L; RI: 10-125 U/L), and total T4 was also increased (87 nmol/L; RI: 13-51 nmol/L).

Based on clinical presentation and elevated thyroid hormone levels, a clinical diagnosis of hyperthyroidism was made. Hyperthyroidism may be caused by thyroid hormone-secretory neoplasms, which are relatively infrequent in dogs (non-functional carcinomas are most common); however, there was no clinical evidence of thyroid neoplasia in these patients. Total T4 may also be elevated in the presence of thyroglobulin autoantibodies (TGAA), as an *in vitro* phenomenon with some laboratory assays; but TGAA may cause thyroiditis, tissue damage and subsequent hypothyroidism, not hyperthyroidism. Furthermore, free T4 by equilibrium dialysis was also elevated in dog #1, and this test is unaffected by the presence of TGAA.

Pet foods have rarely been linked to cases of thyrotoxicosis in dogs when animal tissues containing thyroid glands (e.g. chicken necks and bovine tracheas) have been included as ingredients in their preparation. Since iodine is stored in the thyroid gland, determination of iodine levels provides an opportunity for screening pet foods when thyrotoxicosis is suspected. We tested samples of the raw foods and identified iodine levels as high as 20.5 mg/kg (ww) by ICP-MS. This represents an iodine intake significantly higher than the maximum level recommended to be safe for dogs (11 mg/kg [AAFCO]; 4 mg/kg [EFSA]). To further investigate "contamination" of the food with thyroid gland tissue (as opposed to another source of iodine), ethanol extracts from these foods were tested for free T4 by equilibrium dialysis. The results mimicked those of the iodine levels.

Following the veterinarian's recommendation, the raw food diets were discontinued and the owners reported improvement of overall clinical signs. Unfortunately, dog #1 developed splenic neoplasia and was humanely euthanized. Although serum from dog #2 was not available for thyroid hormone monitoring, the owner did report that the dog started to gain weight, and the diarrhea, PU/PD and anxiety resolved after transitioning to dry food.

## *Nerium oleander* intoxication in striped skunks (*Mephitis mephitis*)

*Robert H. Poppenga[1], Lisa Shender[2], Terry Spraker[3], Bryan Moore[4], Samuel Stump[1], Rebecca Shepard[1], James Langston[1]*

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Over an approximately two-month period (September to November), nine striped skunks (*Mephitis mephitis*) were found sick or dead at Katherine Landing, Lake Mead National Recreation Area in SE Nevada. Foraging opportunities (e.g., dumpster access, intentional feeding by Trailer Village residents) at the site led to easily observed semi-habituated skunks. Six of the skunks were found deceased, but three skunks were observed with "odd and lethargic" behavior prior to dying. One of the three skunks had a stiff rear limb gait, and another had dug a hole in lake beach sand immediately prior to its death. Four skunks (A-D) in various post-mortem condition were submitted to the CSU Veterinary Diagnostic Laboratory for necropsy. In all four skunks, brain tissue was negative for rabies antigen via fluorescent antibody stains. Canine distemper virus rtPCR results were negative on pooled lung, kidney, and spleen of skunk C and for brain of skunk D. All animals were in good body condition with abundant fat. Skunk C had retrievable stomach contents that included grain. Severe, bilateral pulmonary edema and congestion were consistent gross findings and were confirmed on histopathology when not precluded by tissue autolysis. Stomach contents from skunk C and liver samples from skunks A and B were submitted to the Toxicology Section of CAHFS for unknown toxicant screening by gas chromatography- mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS). Unexpectedly, oleandrin was "positive" in a pooled liver sample.

After reporting the positive oleandrin result, additional history obtained from recreation area personnel indicated that oleander hedges provide privacy barriers between campsites. Skunks may have been exposed to oleandrin from consuming water containing oleander debris. Additional samples, two urine (skunks A and B) and one liver (skunk C), were submitted for oleandrin specific analysis by LC-MS. One urine sample was positive (> 4.5 ppb spiked into urine control) and one liver sample had a trace amount (signal < 5 ppb spiked into liver control).

Combined results for animals A, B and C were: pooled liver +/urine negative, pooled liver +/urine +, and stomach contents -/urine +, respectively. This confirmed exposure to *Nerium oleander* in at least two of the animals. The relatively poor post-mortem condition of the hearts precluded identification of compatible lesions, but consistent pulmonary edema and congestion is compatible with a primary cardiac insult.

To determine if oleandrin can be leached into water from oleander leaves, five leaves were allowed to stand in 800 mL water at ambient temperature. Results after 8 days showed oleandrin present with a signal equal to that of 1 ppb spiked into water. By 16 days the signal had decreased to <5% of this, and by 23 days it was not detected. This shows that oleandrin can be extracted into water from leaves and that the concentration declines over time.

**Carbofuran intoxication in four dogs**

*Megan C. Romano*

University of Kentucky, Lexington, KY

A referring veterinarian contacted the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) toxicologist to discuss a case of four large-breed dogs from the same household who had all developed tremors, seizures, and hypersalivation over the course of 24 hours.

The first dog developed hypersalivation and seizures around 11:00 Wednesday morning, and died at home without veterinary intervention. Around 4:00 Wednesday afternoon, two more dogs, a 2.5-year-old intact male Labrador and a 7-year-old neutered male pit bull mix, developed intermittent tremors and hypersalivation. On presentation to the veterinary hospital, neither dog was symptomatic, with relatively unremarkable physical examinations, CBCs, and chemistry panels. Both dogs were treated as outpatients with activated charcoal gel and subcutaneous fluids.

Thursday morning at around 10:00, the Labrador and a previously unaffected housemate (a 10-year-old neutered male Belgian Malinois), were presented to the clinic with severe tremors, convulsions, hypersalivation, and hyperthermia. Rectal temperatures were 106° and 107°F respectively. The dogs were administered intravenous methocarbamol, diazepam, and fluids. Tremors in both dogs responded to repeated doses of anticonvulsants, and the Labrador's hyperthermia resolved. Both dogs also vomited and developed diarrhea while hospitalized. The Labrador improved significantly overnight, and was discharged on Friday morning.

Despite initiation of active cooling, the Belgian Malinois remained markedly hyperthermic for over eight hours. The dog became hypoglycemic, with transient responses to dextrose administration. Friday morning, the dog appeared to improve briefly and was able to stand with assistance, but died shortly afterward with no obvious struggle or distress.

The Malinois was submitted to the UKVDL for postmortem examination. The cause of clinical signs was not apparent based on either gross or histologic examination, with findings being non-specific indicators of a seizure disorder.

Toxicosis was suspected due to the history of multiple dogs within the same household being affected with similar clinical signs over a short time period. The primary differentials based on the clinical signs (tremors, seizures, hypersalivation, vomiting, and diarrhea) were tremorgenic mycotoxins (i.e., penitrem A and roquefortine C) and cholinesterase inhibitors (e.g., organophosphate or carbamate insecticides). Other neurotoxic pesticides, a variety of therapeutic and illicit drugs, and methylxanthines (e.g., theobromine), and other toxicants were considered less likely.

Analysis of the gastrointestinal contents by gas chromatography-mass spectrometry revealed carbofuran, a cholinesterase-inhibiting insecticide (trade name Furadan). Although the EPA announced in 2006 that no uses of carbofuran were eligible for re-registration, stockpiled carbofuran remains a cause of accidental and malicious poisoning in animals.

**Acute inhalation toxicity in nine American white ibis (*Eudocimus albus*) following exposure to triethylene glycol and propylene glycol containing theatrical fog + * †**

*Jayne Summer Ellis[1,2], Richard Fulton[1], Birgit Puschner[2], John Buchweitz[2]*

[1]Pathobiology and Disease Investigation, Michigan State University, Veterinary Diagnostic Laboratory, Lansing, MI; [2]Toxicology Laboratory, Michigan State University, College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Lansing, MI

Nine wild American white ibis (*Eudocimus albus*) at a zoological institution were found deceased or were moribund and euthanized within 24 hours following exposure to theatrical fog at a halloween event. Propylene glycol and triethylene glycol, the primary active ingredients in the fogging agents utilized, were detected in the lung and kidney tissue from these birds by gas chromatography-tandem mass spectrometry (GC-MS/MS). Histopathology confirmed the gross findings in the lungs of pulmonary edema, congestion, and hemorrhage. Further indicative of acute respiratory challenge, all birds exhibited goblet cell hyperplasia alternating with segmental epithelial attenuation and individual cell necrosis of the epithelium lining the trachea and bronchi, along with apical cytoplasmic blebbing and hypereosinophilia of the bronchial epithelium. Three birds that survived to the day after exposure developed granulomatous pneumonia with lymphocytic to heterophilic perivascular cuffing. The proximity of this mortality event to exposure to glycol-containing theatrical fog, isolation of these compounds from lung and kidney tissue, and correlation with gross and histologic lesions are consistent with toxicosis from inhalation of propylene glycol and triethylene glycol. While exposure to aerosolized glycols has been shown to cause irritation and minor degenerative changes of the respiratory epithelium in laboratory animals and humans, there are no reports of birds exposed to aerosolized propylene glycol and triethylene glycol. This case suggests that birds, likely in part by their unique respiratory anatomy and physiology, are at risk of inhalation toxicity and death following exposure to aerosolized propylene glycol and triethylene glycol.

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

**Tebupirimfos poisoning in adult cows and nursing calves following accidental insecticide exposure: A case review**

*Scott Radke, Drew Magstadt, Laura Burns, Dwayne Edward Schrunk, Tyler Alan Harm*

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

Two adult cows and five calves were submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) in April 2022 for postmortem evaluation following acute mortality in a cow-calf operation. Calves were reported to exhibit ataxia, hypersalivation, and dyspnea approximately twelve hours before death. Ninety-six hours after the first calves died, cows within the herd were observed exhibiting similar clinical signs to those of affected calves. Upon postmortem examination, all cows and calves demonstrated adequate body condition with moderate autolysis. Significant postmortem changes included pulmonary edema/congestion, atelectasis, abundant tracheal froth, watery intestinal content, and a completely voided bladder. Lesions suggestive of an infectious cause of death were not observed either grossly or microscopically. Considering the clinical presentation and lack of significant lesions, toxicologic testing was conducted. The caudate nucleus from one cow and one calf were analyzed for cholinesterase activity by the Michel method. Testing demonstrated greater than 90% inhibition of cholinesterase (0.01 delta pH/hr) in the cow and complete inhibition of cholinesterase (0.0 delta pH/hr) in the calf. The rumen content of the same cow was analyzed by a combined gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry analysis. Tebupirimfos and beta-cyfluthrin were detected in the rumen content. Tebupirimfos and beta-cyfluthrin are organothiophosphate and pyrethroid insecticide compounds, respectively. The fungicide tridemorph was also detected. Following detection of the insecticide and fungicide compounds, it was recognized that the producer had recently cleaned product boxes on a row-crop planter containing insecticide and placed the waste material into an unlabeled bucket. Further investigation revealed that the product had then been mistaken for mineral and added into the adult cow ration. Calves were reported to not have access to any of the cows' ration. This was further supported by the finding of only some hay in the rumen and curdled milk in the abomasum of affected calves. Exposure to the calves is thought to have occurred through the milk. Analytical testing demonstrated both tebupirimfos and tridemorph in a milk sample collected postmortem from one of the cows. The similarity in the history and clinical presentation observed in the calves and cows and ancillary testing results provide evidence of organothiophosphate poisoning. This case highlights the potential risk of organothiophosphate exposure through the milk and the increased vulnerability of calves to organothiophosphate toxicity.

**Grape toxicosis in dogs: *in vitro* studies with tartaric acid in Madin-Darby canine kidney cells**

*Alaunie M. Smiley[1,2], Arthur Armstrong[1,2], Stephen B. Hooser[1,2]*

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

In 2001, the ASPCA Animal Poison Control Center (APCC) reported on uncommon cases of acute renal failure in dogs that ingested grapes or raisins. Subsequently, grapes/raisins were analyzed but no renal toxins were identified. In 2021, the APCC reported two cases in which dogs developed acute renal failure following ingestion of large amounts of tartaric acid (cream of tartar). Tartaric acid can be present in some grapes/raisins in widely varying amounts which may help to explain the variability in toxicity.

The goal of this study was to evaluate if canine kidneys could be adversely affected by tartaric acid. Our hypothesis was that tartaric acid would damage Madin-Darby canine kidney (MDCK) cells in culture. MDCK cells were plated in 96-well culture plates. The cells were dosed with PBS (vehicle), tartaric acid, or related chemicals malic acid or valproic acid (at 10 or 100 mM), and incubated at 37°C. At 24 hrs, the cells were examined microscopically, and an LDH cytotoxicity assay and an MTT cell viability assay were performed for each well. In a single subsequent study, the effects of tartaric acid were compared in cortical renal feline kidney (CRFK) cells and MDCK cells.

Results of replicate studies indicated that at a concentration of 10 mM, MDCK cells were minimally affected by tartaric acid, malic acid or valproic acid. However, at 100 mM, increased cellular toxicity was seen microscopically. These changes were related to greatly increased cytotoxicity demonstrated by LDH release (increases in cytotoxicity compared to PBS controls: tartaric acid = 67%, malic acid = near 100%, valproic acid = 53%), and decreased cell metabolism (viability) based on decreased cellular formazan production (MTT assay) in acid treated (100mM) versus PBS control cells (as a percentage of formazan production in PBS-treated cells: tartaric acid = 63%, malic acid = 27%, valproic acid = 85%). Comparison of MDCK cells to CRFK cells at 24hrs revealed minimal toxicity at 10 mM tartaric acid in both. However, at a concentration of 100 mM, for LDH, 67% cytotoxicity was seen in MDCK cells compared to only 10% in CRFK cells.

In culture, MDCK cells are sensitive to the toxic effects of tartaric acid. If present in sufficient amounts in grapes/raisins, or cream of tartar, it is possible that it could contribute to acute renal failure in dogs following its ingestion.

Student support was provided by Boehringer Ingelheim and the Purdue University College of Veterinary Medicine

**Tetrodotoxin toxicosis and fatality in a great horned owl # †**

*Chelsea Sykes[1,2], Krysta Rogers[3], Anibal G. Armien[4], James Langston[1], Samuel Stump[1], Robert H. Poppenga[1]*

[1]Toxicology, California Animal Health and Food Safety Laboratory, Davis, CA; [2]Pharmacology and Toxicology Graduate Group, University of California, Davis, Davis, CA; [3]Wildlife Health Laboratory, Calfiornia Department of Fish and Wildlife, Rancho Cordova, CA; [4]Diagnostic Pathology/Electron Microscopy, California Animal Health and Food Safety Laboratory, Davis, CA

A juvenile great horned owl (*Bubo virginianus*) was found dead in Contra Costa County, California, in October 2021 and presented to the California Department of Fish and Wildlife's Wildlife Health Laboratory for postmortem examination. Gross findings included good nutritional condition, unequal pupils, generalized subcutaneous edema along the chest, and a mostly intact newt (suspected *Taricha* spp.) in the stomach. Formalin-fixed tissues and the stomach contents (the newt), were submitted to the California Animal Health and Food Safety Laboratory (CAHFS). Histopathology identified marked congestion in the organs and mild to moderate lymphocytic, multifocal myocarditis. Tetrodotoxin (TTX) toxicosis was suspected as four TTX-carrying *Taricha* spp. newts occur in California.

The CAHFS Toxicology Section extracted TTX from a 1 gram sample of the newt by mixing on a GenoGrinder with 70% acetonitrile with 0.1% formic acid. After centrifugation and filtering, the extract was analyzed by Ultra High Pressure Liquid Chromatography Mass Spectrometry (UHPLC-MS/MS) on a SciEx QTrap mass spectrometer using MRM. TTX was identified by comparison to a reference standard spiked into a comparable matrix. TTX was detected at high concentrations in the newt. CDFW then submitted liver and kidney tissue from the owl for analysis and TTX was detected in both tissues at lower concentrations than the concentrations detected in the newt. The detection of TTX in the newt and the tissues from the owl confirmed the diagnosis of TTX toxicosis as the cause of death for the owl.

Tetrodotoxin is a neurotoxin that acts by blocking voltage-gated sodium channels and preventing transmission of action potentials along nerves. This blocks signals from nerves to muscles and results in progressive numbness and paresis. Severe toxicosis can quickly progress to generalized paralysis, respiratory failure, hypotension, and death. In mice, an acute lethal dose (LD50) of 232 μg TTX/kg body weight has been reported.

Pufferfish are the most recognized sources of TTX, however many other species can harbor TTX, including blue ringed octopi, horseshoe crabs, and some newts. These species use TTX for different purposes including defense, hunting, and communication. Found primarily on the Pacific Coast, the rough-skinned newt (*T. granulosa*) stores TTX in their skin for defense as an antipredator mechanism against the common garter snake (*Thamnophis sirtailis*). The evolution of these newts and garter snakes to increase the amount of TTX that they can tolerate in their body in an attempt to overcome the fitness pressure from each other has been well documented. Rough-skinned newts carry the largest amount of TTX of any vertebrate species, and populations with high predation pressure from garter snakes can accumulate up to 14 mg TTX per individual. The ranges of rough-skinned newts and California newts (*T. torosa*) overlap in Contra Costa County; either may have been ingested by the owl in this case.

# AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

**Cholecalciferol toxicity in a dog**

*Scott D. Fitzgerald, Chaunte Lewis, John Buchweitz*

Veterinary Diagnostic Laboratory, Michigan State University, Lansing, MI

A 1-year-old female-spayed American Pit Bull Terrier was presented to an emergency clinic for vomiting and lethargy since being spayed one week earlier. Clinical signs included tense painful abdomen, markedly elevated BUN and creatinine. An abdominal exploratory surgery revealed minimal retroperitoneal blood clots, but no active hemorrhage. The dog did not improve with treatment and was euthanized and submitted for necropsy. This 24.7kg spayed female dog was presented in good body condition. The pleural cavity contained 50ml of serosanguineous fluid, and all lung lobes were congested. The heart was subjectively enlarged, with 2ml of serosanguineous pericardial effusion. The peritoneum contained 30ml of serosanguineous effusion, and a small blot clot was adhered to the right ovarian pedicle. 50ml of dark red-brown partially digested blood was present in the stomach lumen, and multifocal hemorrhages were scattered throughout the gastric and small intestinal mucosa linings. Differentials after necropsy included bleeding disorder and anti-coagulant toxicity.

Histopathologic examination revealed multifocal partial mineralization of alveolar septa and pulmonary vessel walls, as well as moderate hemorrhage, fibrin, and neutrophils within alveolar lumens. The heart had multifocal myocardial degeneration and mineralization scattered throughout the myocardium, but most extensively in the left ventricular free wall and epicardial areas. Within the corticomedullary region of both kidneys many renal tubules had partial mineralization of basement membranes, and mineralization of some glomerular Bowman's capsules. Within the mucosal lamina propria and muscularis layers scattered vessel walls were mineralized. These findings led us to consider vitamin D/cholecalciferol toxicity. So, fresh samples of kidney were submitted to the MSU VDL Toxicology Laboratory for vitamin D analysis by liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS). 25-hydroxyvitamin D2 was present in normal amounts (<1ng/g), but 25-hydroxyvitamin D3 was markedly elevated (43.2ng/g) with respect to reference canine kidneys (<10ng/g).

Sources of excess vitamin D include over-supplementation by the dog owners, contamination of commercial dog feed, and exposure to cholecalciferol rodenticides. The owners claimed not to be supplementing this dog's diet, and the commercial dog food had not been recalled as contaminated, so rodenticide exposure was the presumptive source in this case. There are not many cases of cholecalciferol rodenticide poisoning of domestic dogs in the veterinary literature, however, this is known to be quite toxic to dogs and should be included in your differential diagnosis for dogs with appropriate clinical signs and excessive tissue mineralization without evidence of chronic renal failure.

**Development of reference ranges for bone density, percent calcium, phosphorus, bone ash, and bone marrow fat percent in young commercial turkeys**

*Abigail J. Props[1], Hilary Richards[1], Carter J. Stephenson[1], Grant N. Burcham[1,2], Christina R. Wilson-Frank[1,3]*

[1]Indiana Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN; [2]Heeke Animal Disease Diagnostic Laboratory, Southern Indiana Purdue Agricultural Center, Dubois, IN; [3]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN

Previous investigations have evaluated various reasons for the cause of increased leg deformities in poultry, specifically in turkey production. Some of these causes include increased weight; vitamin $D_3$, calcium, and phosphorus levels in the feed; and various infectious diseases. Also, due to the increase in alternative production methods and backyard poultry farms, there is a need to establish normal reference values for these trace nutrients in order to assess nutritional status in these flocks. This study investigated bone density, calcium, phosphorus, bone ash, and bone marrow fat in femurs from 11 healthy 24-29-day-old turkeys and femurs from 7 healthy 10-week-old turkeys to create a normal range for healthy commercial turkeys. The percent calcium and percent phosphorus were determined by 10,000-fold diluted bone ash solution ICP-MS method. The bone density and bone marrow fat percent were determined gravimetrically. The mean bone density was 1.28 g/mL (1.10-1.32 g/mL) for the 24-29-day-old turkeys and 1.15 g/mL (1.08-1.21 g/mL) in the 10-week-old turkeys. Mean percent calcium and phosphorus were 36.31% (32.27-38.4%) and 18.56% (14.39-21.07%) for the 24-29-days-old turkeys, respectively. The mean percent calcium and phosphorus for the 10-week-old turkeys was 33.82% (32.59-37.36%) and 18.29% (17.22-19.81%), respectively. The calcium-to-phosphorus ratio in the turkey femurs was found to be 1.96:1 in the 24-29-day-old turkeys and 1.85:1 in the 10-week-old turkeys. The mean percent bone ash in the turkey femurs was 54.72% (51.56-60.37%) for the 24-29-day-old turkeys and 68.13% (67.32-69.09%) for the 10-week-old turkeys.

Within a diagnostic setting, bone marrow fat analysis is used to quantify malnutrition in suspected neglect cases. Malnutrition or starvation is not typically a concern within the commercial poultry production; however, ascertaining bone marrow fat standard reference ranges can offer another diagnostic tool to measure the nutritional status in a flock. Preliminary results revealed the mean bone marrow fat percent in the turkey femurs was 23.36% (8.20-51.23%) for the 24-29-day-old turkeys and 61.48% (17.22-78.78%) for the 10-week-old turkeys. These results will lay the foundation for determining normal reference values for bone density, calcium, phosphorus, bone ash, and bone marrow fat in femurs from turkeys and other poultry in order to assess nutritional status and aid in diagnosing the cause of leg deformities that can occur in these flocks.

**Development of a simple novel quantitative LC-MS/MS method for diagnosis of sodium monofluoroacetate (compound 1080) in animal tissues**

*James Langston[1], Samuel Stump[1], Mike Filigenzi[1], Robert H. Poppenga[1], Wilson Kiiza Rumbeiha[2]*

[1]California Animal Health and Food Safety Laboratory System, Davis, CA; [2]University of California Davis, Davis, CA

Sodium monofluoroacetate (Compound 1080) is a potent, category I toxicant which interferes with the Krebs cycle leading to acute death. Widely used as a pesticide around the world, it was introduced to the US as a rodenticide in 1946. However, it was banned by the EPA in 1972 for public use and is only available to trained licensed applicators as bait collars to protect small ruminants from predators. Despite the restrictions, animal deaths from Compound 1080 intoxications continue to be reported in the US. The brain and the heart are the primary target organs and affected animals die from convulsions and cardiovascular collapse a few hours post-ingestion, to as long as 4 days post exposure. There is a pressing need in the veterinary diagnostic community for validated diagnostic test methods to quickly detect and quantitate this toxicant in animal tissues and urine. Currently available test methods are for analysis of environmental matrices such as bait, feeds and water. However, testing of environmental samples can only provide circumstantial evidence of intoxication. The goal of this collaborative study was to develop and validate a simple tissue-based method for quantitative determination of Compound 1080 using kidneys for diagnostic purposes. This is important because animal foods may be contaminated with compound 1080, so having reliable diagnostic methods is important for understanding potential animal intoxication.

We chose an LC-MS/MS approach to this analysis, given the potential for high selectivity and sensitivity. Extraction of Compound 1080 from a one gram kidney sample was done using water and mechanical shaking. Protein was removed by passing the extract through a protein concentration membrane. Additional sample clean-up was achieved using solid phase extraction (SPE) with mixed-mode anion exchange resin. This SPE step was critical for removing matrix interference. The LC-MS/MS analysis was done using a SciEx 7500 QTrap mass spectrometer employing multiple reaction monitoring. Accurate and precise quantitation was attained with external standards and inclusion of an isotopically labelled internal standard: $^{13}C$, $d_2$-fluoroacetate. We were able to obtain quantitative results down to 5 ppb for Compound 1080, with qualitative detection down to 2 ppb. Analysis of canine kidney tissue submitted to the California Animal Health and Food Safety (CAHFS) Toxicology Laboratory showed Compound 1080 present at 120 ppb using this new method. Previous analysis of stomach contents from the same animal using a different method showed trace levels of compound 1080 at levels below 250 ppb. These results show the diagnostic utility of this new method.

This novel analytical method offers a simple, straightforward approach to analysis of Compound 1080 in kidney tissue at clinically relevant concentrations, with high sensitivity and specificity. The method also offers quick turnaround times, with next-day results.

**Amanitin detection in spiked canine kidney, and an Indiana *Amanita phalloides* mushroom using Amatox Lateral Flow Immunoassay Strips with MALDI-TOF-MS confirmation**

*Stephen B. Hooser[1], Carley Knosp[1], Christina R. Wilson-Frank[1], Mike Filigenzi[2], Jacob Seay[1], Candace Bever[3]*

[1]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN; [2]CAHFS, University of California, Davis, Davis, CA; [3]AMATOXtest, LLC, Ann Arbor, MI

Ingestion of *A. phalloides* mushrooms can result in fatal poisoning from amatoxins. Detection of α-amanitin in urine or in tissues has utilized LC-MS/MS. Recent development of a specific and sensitive lateral flow immunoassay (LFIA, Amatox) has facilitated rapid detection of amatoxins in urine. The goal of this study was to determine if Amatox LFIA test strips could be used for α-amanitin detection in spiked canine kidney, and determine whether an *A. phalloides* mushroom harvested in Indiana had detectable α-amanitin.

A dried specimen of *A. phalloides* from Indiana was provided by the Purdue Herbarium. Edible *Agaricus bisporus* mushrooms were purchased locally. Each mushroom was minced, placed in PBS, vortexed and extracted for 3 hrs. Cast-off kidney from non-intoxicated dogs was obtained following necropsy and homogenized. Alpha-amanitin was from Sigma Aldrich. LFIA test strips were obtained from AMATOXtest, LLC (Ann Arbor, MI). Alpha-amanitin was spiked into 1 gram aliquots of kidney achieving concentrations of 0.01, 0.1, 1, 10, or 100 ug/mL. Negative controls were fortified with PBS. Each was extracted with 5 mL acetonitrile, 5 mL PBS followed by methylene chloride. Extracts were applied to conditioned Xtrackt XRDAH C-8/benzenesulfonic acid SPE cartridges. Cartridges were washed with water, acetic acid, and methylene chloride/methanol then eluted with methanol. Extracts were evaporated under nitrogen to approximately 1 mL. Ten uL from each were collected for MALDI-TOF MS. For LFIA, the remaining volume of extract was evaporated to near dryness and reconstituted in PBS.

Amatox LFIA test strips were used according to manufacturer's instructions with minor modifications. Briefly, 100 uL of extract was placed in a glass tube with an Amatox test strip. The strips were incubated at room temperature for 10 min, evaluated and photographed.

MALDI-TOF MS: 10 uL aliquots from each extract were evaporated to dryness and reconstituted in 10 uL of acetonitrile. One uL of extract was placed on a target plate, allowed to dry and overlayed with 1 uL α-CHCA matrix. The spots were dried before obtaining mass spectra via MALDI-TOF MS.

Negative control and *A. bisporus* mushroom extracts resulted in no detectable amanitins using Amatox LFIA test strips. Amanitin spiked samples and *A. phalloides* extract were positive using LFIA strips. Alpha-amanitin spiked kidney homogenates were positive at concentrations as low as 0.01 ug/mL. MALDI-TOF MS analysis confirmed detection of α-amanitin in *A. phalloides* extract and each α-amanitin spiked tissue extract at 941 m/z $[M+Na]^+$.

Using Amatox LFIA test strips, α-amanitin is detectable in homogenized kidney as low as 10 ng/mL. MALDI-TOF MS can be used for confirmatory analysis. An *A. phalloides* mushroom from Indiana was positive for the presence of amanitin.

Student participation (CK) was supported by HHS FDA Vet-LIRN grant 1U18FD007499-01.

**Diagnostic methods for the assessment of metabolic bone disease in response to dietary phosphorus, phytase, and vitamin D in nursery pigs**

*Michael Rahe[1], Panchan Sitthicharoenchai[1], Christopher Siepker[1], Hadley Williams[2], Mike Tokach[2], Jason Woodworth[2], Joel DeRouchey[2], Robert Goodband[2], Jon Bergstrom[3], Steve Ensley[2], Jordan Gebhardt[2]*

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Kansas State University, Manhattan, KS; [3]DSM Nutritional Products, Parsippany, NJ

Metabolic bone disease is an important cause of lameness in swine production most commonly caused by inappropriate dietary levels of phosphorous or vitamin D. The diagnosis of this disease is challenging with significant questions involving the effectiveness and interpretation of available assays and which samples/bones are most sensitive for diagnosis. Three hundred fifty pigs (initially 26.2 ± 1.23 lb) were used to evaluate the effects of different bones and analytical methods on the assessment of bone mineralization response to dietary P and vitamin D in nursery pigs. Pens of 44-day-old pigs (5 pigs/pen) were randomized to 6 dietary treatments in a randomized complete block design with 10 pens/treatment. Treatments were: 1) 0.19% standard total tract digestibility (STTD) P (deficient), 2) 0.33% STTD P (NRC requirement) using monocalcium phosphate, 3) 0.33% STTD P including phytase, 4) 0.44% STTD P (industry level) using monocalcium phosphate, phytase, no vitamin D, 5) diet 4 with vitamin D (1,653 IU/kg), 6) diet 5 with additional 2,000 IU/kg 25(OH)D3 (HyD). After feeding diets for 28 d, eight pigs/treatment were harvested for fresh and fixed bone (metacarpal, 2nd rib, 10th rib, and fibula), serum chemistry, and urine Ca, P, and creatinine analysis. Histologic evaluation of H&E stained sections of 2nd rib, 10th rib, and fibula was performed by in a blinded manner by three diagnostic pathologists. Bones were scored for lesions of failure of endochondral ossification of the physis and microscopic fractures (infractions). Medullary trabecular and cortical bone thickness was measured. The 10th rib had more lesions of endochondral ossification and infraction than the 2nd rib or fibula. Pigs fed a P deficient diet had significantly higher scores of failure of endochondral ossification, more infractions, and thinner medullary trabecular bone compared to other treatment groups. Bone ash and bone density were evaluated utilizing both de-fatted and non-defatted methods of the 3rd metacarpal, fibula, 2nd rib, and 10th rib. DXA scans for bone density and content were performed on the same bones. Bone density and ash responses varied depending on bone. Differences in bone density and ash in response to vitamin D and P were most apparent with fibulas and 2nd ribs. The difference between treatments is smaller than the difference between the two ashing methods. For bone Ca and P, the grams of Ca and P differed between P deficient and non-deficient treatments, but the percentage of Ca and P does not differ between treatments.

## Toxicology - On Demand



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**Novel diagnostic biomarkers of acute hydrogen sulfide poisoning**

*Wilson Kiiza Rumbeiha, Cristina Santana, Dongsuk Kim, Abigail Weir*

Molecular Biosciences, University of California, Davis, Davis, CA

Hydrogen sulfide ($H_2S$) is a colorless, highly lethal gas which targets the respiratory, cardiovascular, and central nervous systems. It is a byproduct of organic matter decomposition, a common source of hazard to animal caretakers and livestock in intensive swine and cattle production industries. Clinical signs of acute $H_2S$ poisoning can vary from a simple eye irritation to dyspnea, acute collapse, convulsions, and sudden death. Tissue-based diagnosis of $H_2S$ poisoning in livestock remains a challenge because $H_2S$ is rapidly metabolized and excreted. Previous work in our lab showed that $H_2S$ significantly suppresses cytochrome c oxidase enzymatic activity ($p<0.01$) in the brain. Using HPLC we also showed that thiosulfate, a metabolite of $H_2S$ as a promising biomarker of $H_2S$ exposure.

The goal of this study was to further identify novel diagnostic biomarkers of acute $H_2S$ poisoning in animals using a mouse model. C57BL/6j mice were exposed to 1000 ppm $H_2S$ once by whole body inhalation for 40 minutes ($LD_{50}$) on day 0 and observed for up-to 28 days. Control mice were exposed to normal breathing air from a cylinder. Serum, lungs, and brain (thalamus) samples were collected for metabolomic and lipidomic analyses for discovery of novel reliable diagnostic biomarkers of acute $H_2S$ poisoning.

From > 200 biomarkers examined acute $H_2S$ exposure significantly reduced thalamic arachidonic acid in a time dependent manner. Thalamic 10(11)-EpDPE (docosahexaenoic acid), an esterified oxylipin was significantly increased on day 7 post-acute $H_2S$ exposure. In both lungs and serum several biomarkers were increased in a time specific manner. For example, there was a 6-fold increase in lung glucose concentration in mice exposed to $H_2S$ and euthanized within 5 mins of $H_2S$ exposure compared to control, suggesting a dysregulation of glucose metabolism. Linoleic acid was significantly increased in serum at intermediate time points. There was also a significant elevation in trans-4-hydroxyproline, a biomarker of collagen on day 28, suggesting lung fibrosis is a delayed effect of acute $H_2S$ poisoning. Yet, orotic acid, N-acetylglycine, and 2-deoxypentitol were significantly elevated throughout the 28 day observation period. These results complement inhibition of cytochrome C oxidase and elevation of thiosulfate in ocular fluid, serum, and urine as potential diagnostic biomarkers of acute $H_2S$ poisoning. These results also indicate that a single exposure to acute $H_2S$ poisoning induces both short-term and long-term effects in sugar and lipid metabolism.

**Diagnostic and tissue bromine residue levels in inorganic bromide intoxicated cows and calves**

*Wilson Kiiza Rumbeiha[1], Lisa Tell[2], Bret McNabb[2], Fabio Lima[2], Tara Urbano[2], Kate Watson[2], Dwayne Edward Schrunk[3], Scott Radke[3], Dongsuk Kim[2]*

[1]Molecular Biosciences, University of California, Davis, Davis, CA; [2]University of California, Davis, Davis, CA; [3]VDL, Iowa State University, Ames, IA

Bromine is a natural element commonly found in the environment. Sources of inorganic bromide in the environment include pesticides and fire retardant compounds. Environmental contamination of feed and water has caused bromide intoxication in livestock. Presence of inorganic bromide residues in food animal products is of significant public health concern.

Diagnosis of bromide intoxication in cattle currently depends on clinical observations and serum bromide analysis but there is still a data gap regarding the diagnostic levels of bromide in serum and other tissues. Using samples collected from a controlled study in which pregnant cows were exposed to bromide concentrations ranging from 0 to 3200 ppm in feed in their third trimester of pregnancy and euthanized 30 days post calving, we analyzed various tissues to determine the diagnostic samples of choice and diagnostic levels. We also determined normal reference values for different tissues in cows and calves. Samples evaluated included blood plasma, ocular fluid, thyroid glands, kidneys, liver, skeletal muscle, and perirenal fat. Tissue bromide analysis was done by ICP-MS.

Results indicated that for tissue to tissue comparisons, calf tissues had significantly higher bromide residue concentrations than cow tissues. Tissue bromide residue concentrations in cows fed 800 ppm bromide or less exhibited a similar pattern to that of the group fed 3200 ppm bromide. In the lower dose groups, ocular fluid had the highest bromine concentrations followed by blood plasma and thyroid glands. In all groups, fat had the least bromide concentration. Surprisingly, in the 3200 ppm dose group, the liver and skeletal muscle tissues had the highest bromide residue concentrations. Normal plasma bromide levels were found to be in the range of 20-50 ppm compared to 48-160 ppm reported in the literature.

From this study, tissue bromide levels > 100 ppm are suggestive of bromide intoxication.

These results also suggest that bromide intoxicated cattle have bromide tissue residues > 100 ppm at least 30 days after withdrawal. In conclusion, this study has provided useful data for diagnosis of bromide intoxication in cattle. These results also have significant implications both from a public health perspective and for carcass disposal.

## Virology

Saturday, October 8, 2022

Greenway A

**Moderators:** Jianqiang Zhang and Roman M. Pogranichniy

**1:00 PM** **Detection of human-seasonal H3 subtype influenza A virus spillovers in swine from 2007-2020 at the Iowa State University Veterinary Diagnostic Laboratory**
*Phillip Gauger, Yusuf Shehata, Megan Neveau* . . . . . *171*

**1:15 PM** **Characterization of the homologous and heterologous PCV2 antibody neutralizing activity in vaccinated animals followed by a PCV2d/PRRSV challenge * †**
*Molly Elizabeth Kroeger, Pablo Pineyro* . . . . . *172*

**1:30 PM** **Development of a TaqMan® allelic discrimination PCR assay for rapid detection of equine *CXCL16* allelic variants associated with the establishment of long-term equine arteritis virus carrier state in stallions. †**
*Come Julian Maxence Thieulent, Mariano Carossino, Udeni B B R Balasuriya, Kathryn Graves, Ernest Bailey, John Eberth, Igor Canisso, Frank M. Andrews, Michael Keowen, Yun Young Go.* . . . . . *173*

**1:45 PM** **Characterization of virulence phenotype of a recently emerged PRRSV 1-4-4 L1C variant strain in comparison with other Lineage 1 PRRSVs under experimental conditions # †**
*Gaurav Rawal, Marcelo Almeida, Phillip Gauger, Jeffrey Zimmerman, Fangshu Ye, Christopher J. Rademacher, Betsy Armenta-Leyva, Berenice Munguia-Ramirez, Grzegorz Tarasiuk, Loni L. Schumacher, Ethan Aljets, Joseph T. Thomas, Jinhui Zhu, Wannarat Yim-im, Vivian Flores, Jolie L. Frenier, Jianqiang Zhang* . . . . . *174*

**2:00 PM** **Marek's disease virus early and late gene expression during temperature-induced reactivation + †**
*Yung-Tien Tien, Keith Jarosinski* . . . . . *175*

**2:15 PM** **Characterization of SARS-CoV-2- specific neutralizing monoclonal antibodies *in vitro* and *in vivo* † ◊**
*Dashzeveg Bold, Ebony Gary, Natasha Gaudreault, Konner R. Cool, Jessie Trujillo, Igor Morozov, David B. Weiner, Juergen Richt* . . . . . *176*

**2:30 PM** **White-tailed deer (*Odocoileus virginianus*) may serve as a wildlife reservoir for nearly extinct SARS-CoV-2 variants of concern**
*Leonardo Cardia Caserta, Mathias Martins, Salman Latif Butt, Md Sohel Ahmed, Nicholas Hollingshead, Lina Maria Covaleda, Mia Renee Rowe Everts, Krysten L. Schuler, Diego G. Diel* . . . . . *177*

**2:45 PM** **Evaluating bluetongue virus at different coinfection ratios in *Culicoides sonorensis* * †**
*Molly J. Carpenter, Jennifer H. Kopanke, Case Rodgers, Justin Lee, Mark Stenglein, Christie Mayo* . . . . . *178*

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**Detection of human-seasonal H3 subtype influenza A virus spillovers in swine from 2007-2020 at the Iowa State University Veterinary Diagnostic Laboratory**

*Phillip Gauger[1], Yusuf Shehata[2], Megan Neveau[1]*

[1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Bioinformatics and Computational Biology, Iowa State University, Ames, IA

Influenza A virus (IAV) causes respiratory disease in both humans and swine. Human-seasonal IAV has been demonstrated to transmit to swine, adapt to the new host, and act as a source of zoonotic infections in people. The objective of this study was to evaluate the frequency of H3 subtype human-seasonal IAV detected in swine using hemagglutinin (HA) sequence data generated at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL).

To identify potential human-to-swine spillovers, we first gathered swine H3 sequences sampled from 2007 to 2020 (n=2,825) from the ISU VDL database. We also collected all publically available human H3 sequences (n=17,100) and used a representative 10% subsample (n=1,710) to capture the diversity of human-seasonal IAV in the United States from 2005 to 2020. The swine and human data sets were aligned and divided into 4 approximately equal-sized phylogenetic trees based on sampling date. Swine viruses that clustered with human HA sequences were suspected to be human-to-swine transmission events and confirmed using NCBI BLAST and ISU FLUture (https://influenza.cvm.iastate.edu).

Sixteen independent human-to-swine transmission events were detected from HA sequences generated at the ISU VDL from 2007-2020. In addition, two of these spillover IAV became endemic in swine during the 2010 decade. The transmissions occurred during the following human influenza seasons: two in 2012-2013, one in 2013-2014, three in 2016-2017, three in 2017-2018, five in 2018-2019, one in 2019-2020, and one in 2020-2021. A swine-origin sequence sampled from a barn in Missouri during 2013 was the first ISU VDL detection of a human spillover that became endemic in swine, now known as the H3 2010.1 clade. Another swine-origin detection from Oklahoma in 2017 was the first ISU VDL sample of the now endemic H3 2010.2 clade. Both sequences are similar to human H3 viruses from the same time frame, with 98.94% and 99.94% nucleotide identity, respectively. The higher identity of the 2010.2 clade may be reflective of the increased IAV surveillance efforts in the second-half of the 2010 decade.

This study demonstrates the frequency of human-to-swine transmissions that may occur when swine are exposed to infected farm employees. There is a risk that any human-like IAV transmitted to swine may undergo reassortment and emerge as a zoonotic IAV that may cause a human pandemic in the future. This emphasizes the importance of veterinary diagnostic labs in monitoring human-like spillover IAV in swine.

**Characterization of the homologous and heterologous PCV2 antibody neutralizing activity in vaccinated animals followed by a PCV2d/PRRSV challenge * †**

*Molly Elizabeth Kroeger, Pablo Pineyro*

VDPAM, Iowa State University, Ames, IA

Porcine circovirus type 2 (PCV2) is economically significant, highly prevalent, and nearly ubiquitous globally and causes porcine circovirus-associated disease (PCVAD). Since its first description in the US, three major antigenic shifts have been observed leading to changes in the subtype prevalence in the field from PCV2a in the late 90s to PCV2b during the early 2000s and lately PCV2d. PCV2 vaccines became commercially in 2006 in North America, and most vaccines relay in a single PCV2 subtype. Overall, PCV2 vaccines are effective in preventing clinical disease and lesions. However, vaccination does not prevent viral replication and infection, potentially leading to gaps in immunity caused by heterologous infection and coinfection. While cross-protection amongst different PCV2 subtypes has been demonstrated, questions remain regarding the full immunological efficacy of commercial vaccines against homologous and heterologous challenges. Therefore, this study aims to assess the serum neutralizing activity in PCV2 vaccinated animals followed by homologous and heterologous infectious challenges. On study day 0 (D0), 20 pigs from a PRRSV/Mhp negative, PCV2 low prevalence, commercial sow farm were weaned and vaccinated with Ingelvac PRRSV MLV. Additionally, pigs received a PCV2a vaccine (n=5), PCV2d vaccine (n=5), a PCV2a/PCV2d vaccine (n=5), or no PCV2 vaccine (n=5). On D28, all 20 pigs were inoculated with 1 mL IM and 1 mL IN of PCV2d (5 log10/2ml dose) and 2 mL IM of 1-7-4 PRRSV (4.0 TCID50/mL). Serum samples were collected on D0, 28, and D56 and were analyzed by a serum neutralization assay against PCV2a, PCV2b, and PCV1-PCV2d chimeric viruses. Antibody neutralizing activity generated in each treatment group was evaluated against PCV2a, PCV2b, and PCV1-PCV2d chimeric viruses. The PCV2a vaccine group consistently displayed the highest VN antibody titers against PCV2a but had significantly lower titers against PCV2b and PCV1-PCV2d chimeric virus. Similarly, the PCV2d vaccine group had the highest VN antibody titers against PCV1-PCV2d, up to 4-fold lower than PCV2a and PCV2b. The PCV2a/PCV2d group showed the best VN antibody response against all PCV2 subtypes. Delayed neutralizing antibody response was observed in nonvaccinated pigs, but at D28, we observed different levels of antibody titers against all PCV2 subtypes. In conclusion, we observed a differential subtype-dependent neutralizing antibodies activity measured by VN assay in this study. Other components of the systemic immune response, including the T-cell response, should be evaluated to assess the efficacy of PCV2 vaccines in homologous and heterologous challenges.

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

**Development of a TaqMan® allelic discrimination PCR assay for rapid detection of equine *CXCL16* allelic variants associated with the establishment of long-term equine arteritis virus carrier state in stallions. †**

*Come Julian Maxence Thieulent[1,2], Mariano Carossino[1,2], Udeni B B R Balasuriya[1,2], Kathryn Graves[3], Ernest Bailey[3], John Eberth[3], Igor Canisso[4], Frank M. Andrews[5], Michael Keowen[5], Yun Young Go[6]*

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Equine arteritis virus (EAV; *Alphaarterivirus equid*) is the causative agent of equine viral arteritis (EVA), a respiratory, systemic, and reproductive disease of equids. Following natural infection, up to 70% of the infected stallions can remain persistently infected, some of which can shed EAV in their semen for over one year (long-term persistent infection [LTPI]). Thus, LTPI stallions play a pivotal role in maintaining and perpetuating EAV in the equine population. Equine C-X-C motif chemokine ligand 16 (CXCL16) has been identified as a critical host cell factor determining LTPI in the stallion's reproductive tract. Two alleles (*CXCL16*$^{S}$ and *CXCL16*$^{r}$) were identified in the equine population and correlated with the susceptibility or resistance of a CD3$^{+}$ T cell subpopulation in peripheral blood to *in vitro* EAV infection, respectively. Interestingly, *CXCL16*$^{S}$ has been linked to the establishment of LTPI in stallions, and thus, genotyping stallions based on *CXCL16*$^{S/r}$ would allow identification of those at the highest risk of establishing LTPI. Thus, we developed a TaqMan® allelic discrimination assay for the genotyping of the equine *CXCL16* gene based on the identification of a single nucleotide polymorphism located in exon 2.

Blood samples from 160 horses, including four different breeds, were collected. Samples were screened for the CD3$^{+}$ T cell susceptibility or resistance phenotype to EAV infection by flow cytometry and subsequently sequenced to determine *CXCL16* allelic composition. Genotyping by Sanger sequencing determined that all horses with the resistant CD3$^{+}$ T cell phenotype were homozygous for *CXCL16*$^{r}$. In contrast, horses with the susceptible CD3$^{+}$ T cell phenotype were homozygous or heterozygous for the *CXCL16*$^{S}$ allele. In addition, genotypification with the new TaqMan® allelic discrimination assay showed perfect agreement (100%) with Sanger sequencing and flow cytometric analysis.

In conclusion, the newly established TaqMan® allelic discrimination PCR genotyping assay described here provides a new diagnostic method for medium to high-throughput genotyping of the equine *CXCL16* alleles with perfect agreement compared to Sanger sequencing allowing accurate identification of stallions at the most significant risk of becoming LTPI EAV carriers. Thus, it will assist with targeted vaccination practices with particular emphasis on stallions carrying the *CXCL16*$^{S}$ allele to prevent the occurrence of the LTPI carrier state. In addition, this test also opens avenues for selective breeding, which is critical for equine breeding enterprises worldwide and from a disease control perspective.

† Graduate Student Oral Presentation Award Applicant

**Characterization of virulence phenotype of a recently emerged PRRSV 1-4-4 L1C variant strain in comparison with other Lineage 1 PRRSVs under experimental conditions # †**

*Gaurav Rawal[1], Marcelo Almeida[1], Phillip Gauger[1], Jeffrey Zimmerman[1], Fangshu Ye[2], Christopher J. Rademacher[1], Betsy Armenta-Leyva[1], Berenice Munguia-Ramirez[1], Grzegorz Tarasiuk[1], Loni L. Schumacher[1], Ethan Aljets[1], Joseph T. Thomas[1], Jinhui Zhu[1], Wannarat Yim-im[1], Vivian Flores[1], Jolie L. Frenier[1], Jianqiang Zhang[1]*

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PRRSV is an RNA virus with a high rate of mutation and recombination. Consequently, novel PRRSV variants with unknown virulence phenotypes periodically emerge. A PRRSV 1-4-4 L1C variant strain emerged in the USA in October 2020 and has spread widely, causing high production losses based on field observations. However, no unequivocal experimental data is available to determine the virulence phenotype of the 1-4-4 L1C variant.

In this experimental pig study, the virulence and transmissibility of the 1-4-4 L1C variant strain was characterized in comparison with three other circulating 1-4-4 strains (L1C non-variant, L1A, and L1H) and one highly virulent 1-7-4 L1A strain. Seventy-two 3-week-old PRRSV-naive pigs were divided into 6 groups with 12 pigs per group. Forty-eight pigs (8/group) were for inoculation and 24 pigs (4/group) served as contact pigs. After one-week acclimation, pigs were inoculated with the corresponding virus or negative media intramuscularly and intranasally. At 2 days post inoculation (DPI), contact pigs were added to the pen adjacent to the inoculated pigs in each room. Daily temperature and clinical signs were recorded. Serum and oral fluid samples were collected at 0, 2, 4, 7, 10, 14, 21 and 28 DPI. Pigs were necropsied at 10 & 28 DPI. Fresh and formalin-fixed tissues were collected at necropsy for examinations. For all analyses, SAS was used and a p-value ≤ 0.05 was considered significant.

The 1-4-4 L1C variant-inoculated pigs became more anorectic and lethargic, had higher mortality, had higher percentage of pigs with fever (>40°C) during 0-10 DPI, and had significantly higher average body temperature than other virus-inoculated groups at several DPIs. The 1-4-4 L1C variant-inoculated group had significantly higher viremia levels compared to all other groups at 2 DPI. Collectively, 4/4, 2/4, 2/4, 0/4, and 2/4 contact pigs in the L1C variant, L1C non-variant, L1A, L1H, and 1-7-4 L1A groups became viremic at 2 DPC. There were more severe gross lung lesions in the 1-4-4 L1C variant-inoculated group compared to others except the 1-7-4 L1A group at 10 DPI. The differences of ADG, microscopic lung lesion score, IHC score, and RNA level in different tissues at 10 DPI were not statistically significant between virus-inoculated groups. Serum antibodies in all virus-inoculated groups were readily detected by the commercial PRRS X3 ELISA and the ISUP virus strain-based IFA antibody assays during 7-28 DPI.

This study provides experimental data in weaned pigs regarding the clinical impact, pathogenicity, transmissibility, and antibody detection of the newly emergent 1-4-4 L1C variant strain, along with comparisons with other PRRSV strains. The findings confirm 1-4-4 L1C variant is highly virulent in weaned pigs. The higher number of contact pigs becoming viremic at 2 days post contact implies the L1C variant strain may have higher transmissibility than other PRRSV strains although it needs to be confirmed with a study involving more pigs.

# AAVLD Trainee Travel Awardee
† Graduate Student Oral Presentation Award Applicant

**Marek's disease virus early and late gene expression during temperature-induced reactivation + †**

*Yung-Tien Tien, Keith Jarosinski*

Pathobiology, University of illinois.edu, Urbana, IL

Marek's disease (MD) is a viral disease in chickens caused by *Gallid alphaherpesvirus* 2, better known as Marek's disease virus (MDV). Herpesviruses are known for their ability to remain latent in the host. Latent infection is an important part of the herpesviral life cycle to escape the host's immune response following primary infection. During the life of the host, herpesviruses often reactivate under stress that can cause symptomatic disease. For MDV, the virus remains latent in $CD4^+$ T lymphocytes which ultimately can result in neoplastic transformation through the viral oncogene, *Meq*. MDV-transformed cell cultures (MDCCs) can be passaged *ex vivo* and used to study herpesvirus-induced transformation, latency, and reactivation. Here, we generated MDCCs expressing monomeric red fluorescent protein (mRFP) fused to the early viral gene, RLORF4 and enhanced green fluorescent protein (eGFP) fused to the late viral gene, UL47. We have formerly established that incubation of MDCCs at low temperature results in reactivation of latent MDV to lytic infection. Using this reactivation model, we are able to distinguish between cells in the early and late stages of replication. We found that, although a significant portion of MDCCs reactivate MDV as indicated by expression of mRFP, only a very small proportion reach the late stages of replication, indicated by eGFP expression. Further studies will reveal the cellular and viral pathways involved in inhibition of replication at the cellular level, as well as viral genes required for this transition to fully productive late gene expression. The work here will provide a better understanding of the "switch" in which herpesviruses reactivate from latency that can provide a basis for therapies that block this event.

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

**Characterization of SARS-CoV-2- specific neutralizing monoclonal antibodies *in vitro* and *in vivo* † ◊**

*Dashzeveg Bold[1], Ebony Gary[2], Natasha Gaudreault[1], Konner R. Cool[1], Jessie Trujillo[1], Igor Morozov[1], David B. Weiner[2], Juergen Richt[1]*

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS; [2]Vaccine and Immunotherapy Center, Wistar, Philadelphia, PA

The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the main target for neutralizing antibodies. Neutralizing antibodies can be induced via natural infection or immunization; they can also be passively transferred as therapeutics using hyperimmune sera or monoclonal antibodies (MAbs). Here, we demonstrate that the 31F4 and 15G1 mouse MAbs specific for the SARS-CoV-2 spike receptor binding domain (RBD) have high neutralizing activity against the ancestral Wuhan-like USA-WA1/2020 SARS-CoV-2 strain and several SARS-CoV-2 variants of concern (VOCs) *in vitro*. Next, we tested their therapeutic potential for SARS-CoV-2 in a COVID-19 Syrian golden hamster model. For this purpose, groups of hamsters (n=6) were treated intraperitoneally with 2 mg each of the respective MAbs on -1 day post challenge (DPC) and +1 DPC. Animals were challenged intranasally with $1x10^5$ $TCID_{50}$ of SARS-CoV-2 (USA-WA1/2020). Three of the hamsters from each group were humanely euthanized for postmortem analysis at 3 DPC and the remaining hamsters at 5 DPC.
The two groups of MAb-treated hamsters did not lose weight and only showed minor lung lesions on days 3 and 5 after SARS-CoV-2 infection. Furthermore, most (11/12) of the Mab-treated hamsters did not shed virus on 3 DPC, and significantly lower levels of virus were detected in nasal washes of 4/6 hamsters at 5 DPC compared to the control group. In contrast, hamsters in the placebo control group lost about 20% body weight within five days of infection, and all control hamsters shed virus at 3 and 5 DPC.

Our results show the RBD-specific MAbs 31F4 and 15G1 are able to effectively neutralize several SARS-CoV-2 strains including VOCs. In addition, they are able to protect Syrian golden hamsters from SARS-CoV-2 associated clinical signs and significantly reduced virus shedding. This indicates the RBD-specific MAbs 31F4 and 15G1 might have therapeutic potential to protect humans and animals from SARS-CoV-2 infections.

† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

**White-tailed deer (*Odocoileus virginianus*) may serve as a wildlife reservoir for nearly extinct SARS-CoV-2 variants of concern**

*Leonardo Cardia Caserta[1], Mathias Martins[1], Salman Latif Butt[1], Md Sohel Ahmed[2], Nicholas Hollingshead[3], Lina Maria Covaleda[1], Mia Renee Rowe Everts[3], Krysten L. Schuler[2], Diego G. Diel[1,3]*

[1]Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY; [2]Public & Ecosystem Health, Cornell University, Ithaca, NY; [3]Animal Health Diagnostic Center, Cornell University, Ithaca, NY

The spillover of SARS-CoV-2 from humans into white-tailed deer (WTD) and its ability to efficiently transmit from deer-to-deer causes concerns due to the potential of this animal species to become a new wildlife reservoir for the virus. Here, we conducted a comprehensive investigation to assess the prevalence, genetic diversity, and evolution of SARS-CoV-2 in WTD in New York State. We tested 5419 retropharyngeal lymph node (RPLN) samples collected from free-ranging hunter-harvested WTD during New York's hunting seasons: Season 1 (October of 2020 through February 2021), and Season 2 (October of 2021 through February 2022) by SARS-CoV-2 real-time RT-PCR.

We detected SARS-CoV-2 RNA in 17 of 2682 (0.6%) samples from Season 1 and in 580 of 2737 (21.1%) from Season 2, from which infectious virus was recovered from 7 samples by virus isolation in cell culture. Analysis of SARS-CoV-2 genome sequences obtained from 164 samples demonstrated the circulation of multiple classical SARS-CoV-2 variants of concern (VOC) including Alpha, Gamma and Delta in WTD in NY. Our analysis suggests the occurrence of multiple spillover events within the Alpha and Delta VOCs, whereas all the Gamma sequences recovered from WTD formed a large monophyletic cluster, concentrated in the region of Allegany County.

While the Alpha and Gamma VOCs were completely replaced by Delta in humans during mid-2021, our data reveals that these variants were circulating in wildlife WTD several months after the last reported detection in humans. Interestingly, the Alpha and Gamma viral sequences recovered from WTD were highly divergent and contained several mutations that distinguished them from SARS-CoV-2 sequences recovered from humans, demonstrating significant evolution of these lineages as they circulated in WTD. In contrast, the Delta SARS-CoV-2 sequences recovered from deer were less divergent from sequences recovered from humans. This finding is consistent with the overlapping time frame in which the Delta VOC was circulating in the human population and sampling of WTD in Season 2 occurred and with multiple recent spillover events of the Delta VOC from humans to deer in NY.

Our results demonstrate sustained circulation of SARS-CoV-2 VOCs in the free-ranging WTD population in New York. These results support the notion that WTD may serve as a new wildlife reservoir for SARS-CoV-2, harboring SARS-CoV-2 variants containing mutations of unknown phenotypes in humans. Multiple potential avenues for contact between humans and WTD in North America (e.g., hunting, wildlife rehabilitation, captive cervid ownership, feeding/baiting) provide opportunities of potential spillback of the virus to humans. Thus, implementation of continuous surveillance programs to assess the extension of WTD as a SARS-CoV-2 reservoir are warranted. Moreover, management actions to minimize virus transmission between humans and animals need to be urgently discussed and implemented.

**Evaluating bluetongue virus at different coinfection ratios in *Culicoides sonorensis* * †**

*Molly J. Carpenter[1], Jennifer H. Kopanke[3], Case Rodgers[1], Justin Lee[2], Mark Stenglein[1], Christie Mayo[1]*

[1]Colorado State University, Fort Collins, CO; [2]CDC, Atlanta, GA; [3]Oregon Health & Science University, Portland, OR

Bluetongue virus (BTV) can result in devastating disease in susceptible ruminants with significant economic losses. Transmission of BTV occurs via the *Culicoides* biting midge and recent outbreaks illustrate the geographical expansion of BTV and increased incursions of novel serotypes. However, determinants of BTV's expansion are not clearly defined. BTV is a segmented double stranded RNA virus capable of reassortment. Reassortment between BTV strains may increase genetic diversity which can alter BTV transmission dynamics and generate epizootic events. In prior in vitro BTV coinfection and modeling studies, progeny genotypes were dominated by the parental strain with the higher initial MOI or higher relative fitness, highlighting the importance of dose, timing, and relative fitness to reassortment potential.

The objective of this study was to evaluate in vivo replication dynamics and reassortment frequency of progeny virus in *Culicoides sonorensis* midges coinfected with different ratios of BTV-10 ATCC and BTV-17 Colorado. To establish single and co-infections, midges were fed a blood meal containing BTV-10 ATCC, BTV-17 Colorado, or both BTV strains with contributing titers of BTV-10 ATCC: BTV-17 Colorado at either 90:10, 75:25, 50:50, 25:75, or 10:90 ratios. Pools of five midges were collected in triplicate every other day and processed for pan BTV qRT-PCR to track virogenesis over time. Day ten post-infection midges were collected in pools of ten and processed for plaque isolation and propagation. The complete genotypes of isolated plaques were identified using shogtun next generation sequencing.

Plaque genotyping indicated that most plaques fully aligned with one of the parental strains. However, a few plaques at a subset of co-infection ratios demonstrated that coinfection in the midge vector can result in reassortment events. Identifying the factors of BTV reassortment and its biological consequences will add an important dimension to the modeling of viral expansion and evolution. Understanding determinants for the emergence of viruses can inform predictive and mitigative tools.

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

## Virology - On Demand



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**Detection of a piscine reovirus-2-like virus in coho salmon fingerlings in the Pacific Northwest**

*Chrissy Eckstrand, Brandi Kenette Torrevillas, Rebecca Marie Wolking, Daniel Strode Bradway, Kevin R. Snekvik*

Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA

A diagnostic investigation was performed to investigate the cause of morbidity and mortality in a cohort of coho salmon fingerlings in the Pacific Northwest. Animals were reported to be clinically anorexic, have pale gills and low hematocrits (<10%), gaping mouths, and increased mortality. No parasites were observed on skin scrapes and pathogenic bacteria were not identified from systemic organs when performed by the referring veterinarian. Histological examination revealed mild to severe myocardial degeneration and lymphohistiocytic myocarditis within both the spongy and solid ventricular myocardium, moderate splenic histiocytosis, and mild splenic histiocytosis. Piscine reovirus-1 and 3 were not detected by real time PCR from FFPE heart and kidney tissue, or fresh tissue pools. Viral culture of tissue pools containing spleen, kidney, heart did not detect the presence of infectious pancreatic necrosis virus, infectious hematopoietic necrosis virus, viral hemorrhagic septicemia virus or spring viremia of carp on CHSE-214, EPC and FHM cell lines.

Total nucleic acids were extracted from filtered heart, spleen, and kidney tissue pool homogenates and cDNA synthesized by sequence-independent single primer amplification. (SISPA) which was used for library preparation. Prepared libraries from each tissue pool were run on the ONT GridION platform for up to 72 hours. Data were analyzed in real-time by the metagenomic classification workflow WIMP with the most significant number of viral reads as Aquareovirus A; however, heavily soft-clipped raw reads only aligned to a short region of the Chum Salmon Reovirus genome (GCA_000866805.1) which is likely a conserved region among Reoviridae. Raw reads were sorted by homology to viral proteins including 3355 reads encompassing all 11 proteins of PRV-2. Based on this result, all raw reads were mapped to PRV-2, producing good coverage and concordance. *De novo* assembly produced a draft genome with 10 segments closely related to PRV-2.

An *in situ* probe against 238 nucleotides in segment L1 using RNAscope technology revealed viral genome in multiple organs including heart, spleen, gill, kidney, liver, blood, and the lamina propria of the intestines. The viral probe was determined to be relatively specific to the PRV-2-like virus, as it did not detect the presence of viral genome in tissues of PRV-1-infected fish.

In conclusion, the cause of morbidity and mortality in these coho salmon fingerlings was identified to be a likely novel piscine reovirus most closely related to PRV-2.

**Retrospective surveillance of SARS-CoV-2 in white-tailed deer using formalin fixed paraffin embedded tissues collected for chronic wasting disease surveillance**

*Isaac David Fitz[1], Antoinette Lona[1], Mariano Carossino[4], Udeni B B R Balasuriya[4], Jamie Retallick[3], William Wilson[2], Juergen Richt[1], Jessie Trujillo[1]*

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 (COVID-19) pandemic. Historical evidence has shown that interactions between humans and spillover animal species play an important role in transmitting SARS-like coronaviruses. SARS-CoV-2 has been shown to naturally and experimentally infect a variety of animal species, including white-tailed deer (WTD, *Odocoileus virginianus*). WTD are an abundant and widespread wild ruminant species in the US, and their health can have an impact on the ecosystem and economies across the country. WOAH and the USDA recommend surveillance of SARS-CoV-2 in spillover species throughout the US; thus, identifying convenient and reliable ways for surveillance of SARS-CoV-2 in wild animal populations is a critical need.

Formalin fixation, followed by paraffin embedding, is a well-established method for tissue preservation/archiving, making them an excellent resource for retrospectively analyzing samples collected over extended periods of time. In this study, we evaluated the suitability of formalin-fixed, paraffin-embedded tissues (FFPET) originally prepared for chronic wasting disease (CWD) surveillance as a sample source for the surveillance of SARS-CoV-2 in deer populations. Through collaboration with AAVLD-accredited diagnostic laboratories in Louisiana (LADDL) and Kansas (KSVDL), a collection of 690 FFPET representing retropharyngeal lymph nodes and tonsils were obtained, and tested for the presence of SARS-CoV-2-specific RNA by RT-qPCR. The samples were obtained between 2019 and 2021, which allowed for data collection both before and during the COVID-19 pandemic. In the present study, viral RNA was isolated from FFPET using a previously validated protocol that utilizes a single tube de-paraffinization and tissue digestion protocol, followed by a rapid automated magnetic bead nucleic acid extraction. Using the CDC RT-qPCR for SARS-CoV-2, we detected viral RNA in 2 out of 470 (0.426%) blocks from Louisiana and 5 out of 220 (2.272%) from Kansas. In conclusion, here we demonstrate that FFPET samples can be used to retrospectively study the prevalence of SARS-CoV-2 in white-tailed deer populations.

**A One-Health approach to Japanese encephalitis surveillance and control in the Northern Territory (Australia)**

*Vidya Bhardwaj[1], Cathy Shilton[1], Ayrial Foster[1], Richard Weir[1], Rachel De Araujo[1], Nina Kurucz[2], David Williams[3], Susanne Fitzpatrick[4], Hayley Pearson[4], Josef Schmidt[5], Skye Fruean[5], Guyan Weerasinghe[5]*

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Japanese encephalitis (JE) was declared a disease of national significance in Australia on 4th March 2022 after confirmed cases were detected in piggeries as well as humans in several states in Australia. The first (fatal) human case of locally acquired JE was in the Northern Territory (NT) in February 2021. Japanese encephalitis virus (JEV) is a mosquito-borne pathogen that has a transmission cycle involving Ardeid birds and pigs, where waterbirds are the primary reservoir hosts, pigs the amplifier hosts and humans and other animal species the dead-end hosts. A surveillance study was conducted looking at the feral pig population as well as mosquitoes to detect the presence of JEV in the NT.

The Northern Australia Quarantine Strategy conducted several aerial surveillance operations during 2021 and 2022, focussed on the coastal regions and adjoining islands in the NT. Gross post mortem examination was performed on every pig with tissues (tonsil, placenta, testes, foetus) submitted to Berrimah Veterinary Laboratory for microscopic examination. Whole blood, sera, tissues and swabs were submitted for PCR and serology.

PCR testing was performed on all tissue samples as well as on blood samples (EDTA). Primers and a fluorogenic probe (Shao *et al.* 2018) were complementary to a nucleotide region for the virus with the qPCR targeting the non-structural protein NS-1 region of the 'Universal' JEV genome. The virus was detected in over 50 animals throughout the surveyed regions with detection being higher in tonsillar tissue compared to other tissues or blood samples (EDTA).

Vector surveillance was performed by PCR testing of mosquitoes collected from carbon dioxide-baited Encephalitis Virus Surveillance (EVS) traps as well as JEV surveillance using FTA cards collected from carbon-dioxide baited Sentinel Mosquito Arbovirus Capture Kit (SMACK) traps. Mosquitoes were either identified to species level or bulk PCR tested to obtain presence/absence data and to perform possible virus isolation, with no positive result to date.

These results show that JEV is present in the feral pig population in the areas sampled with further surveillance required to ascertain the limits of JEV distribution in the NT. It is expected that that surveillance results will help support the public health sector in mitigating risks to human health.

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# POSTER SESSION

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## Posters

Saturday October 8 – Sunday October 9

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## Poster 110

### Emerging and endemic diseases of farmed mink in the upper Midwest USA 2017-2021

*Brittney N. Moore[2], Andrea Pohly[1], John Easley[3], Beth Angell[1], Nicole Ann Aulik[1], Audrey Dikkeboom[1], Ailam L. Lim[1], Kathleen M. Deering[1], Betsy Elsmo[1], Philip N. Bochsler[1], Lorelei Lynne Clarke[1]*

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American mink (*Neovison vison*) have been used for domestic fur production for centuries, but there is little published literature regarding pathology in this species. To better characterize common and novel mink pathology seen at a regional diagnostic laboratory, we performed a retrospective search for farmed mink necropsy reports on archived pathology records from 2017-2021 at the Wisconsin Veterinary Diagnostic Laboratory (WVDL). Information recorded from each case included the signalment (sex, age), number of affected animals, state of origin, date of submission, pathologic diagnosis(es) (as reported by the primary pathologist of each case), and specific causes of disease when found. Many cases were indicated to have multifactorial disease, so up to 3 diagnoses and 3 infectious agents were recorded for each case as available. A total of 72 necropsy reports were identified with a total of 181 animals examined. Bacterial diseases were the most frequently identified cause of disease and/or death and *Streptococcus* spp. were the most frequently implicated organisms. There was a total of 15 diagnoses of pododermatitis or dermatitis, 10 of which presented with associated streptococcal septicemia. Pododermatitis and streptococcal septicemia cases were identified primarily between the months of October and February in all years examined. Viral diseases identified included Aleutian disease enteritis, SARS-CoV-2 pneumonia, canine distemper viral pneumonia, and astroviral meningoencephalitis. The most frequent nutritional disease identified was selenium/vitamin E deficiency. Several cases had pathologic lesions of enteritis and/or septicemia, but no causative organism was identified. Disease and pathology in mink appears to be closely related to management, as would be expected for any closely confined, dense population. Awareness of these typical disease conditions will aid pathologists in providing high quality diagnostics for mink producers and can guide clinical recommendations for mink producers in the Midwest USA.

## Poster 111

### Characterization of *Salmonella enterica* isolates derived from equine environment in a large animal veterinary teaching hospital: 2018-2022 *

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*Salmonella enterica* is the most commonly reported cause of nosocomial outbreaks in large animal veterinary teaching hospitals (LA-VTH) that not only endangers animal patients, but also threatens the safety of staff and students. The objective of this study was to characterize *S. enterica* isolated from environmental samples collected at the University of Georgia's LA-VTH from April 2018 to April 2022. *S. enterica* isolates were characterized by determining their serogroups, serotypes and antimicrobial susceptibility testing profiles. The multiple antibiotic resistance operon *(marRAB)* was examined in all isolates by PCR. In addition, the prevalence of antimicrobial resistance genes (ARG) were determined in selected six isolates using the 87 antibiotic resistance genes microbial DNA qPCR array kit (Qiagen). 29 *S. enterica* isolates were recovered from 683 (4.2%) environmental samples. The serogroups were B & C2 (28%), C1 (17%), E (14%), D (7%) and G (3%). The serovars were Typhimurium (24%), Newport (17%), Anatum (10%), Bareilly, Braenderup, Javiana, Muenchen (7%) and Altona, Mbandaka, Meleagridis, Poona, Saintpaul & III_16: z10: e, n, x, z15 (3.5%), respectively. None of the isolates were susceptible to all of the 22 antimicrobial agents that represented 12 classes of antibiotics. 83%, 14% and 3% of the isolates were resistant to 4, 5 and 6 different classes of tested antibiotics, respectively. Six distinct antimicrobial resistance patterns were detected. 100% resistance was observed to antibiotics screened for the following classes: cephem, lincosamide, macrolide and aminoglycoside. There was 100% susceptibility seen with phenicol, fluoroquinolones, carbapenem and furan classes of tested antibiotics. About one sixth of the isolates (n=5) were multi-drug resistant by excluding antimicrobials to which *Salmonella* spp. are considered intrinsically resistant. The *marRAB* operon was detected from 72% of the 29 isolates. No ARG was detected in 2 of the 6 tested isolates, one ARG was identified in 2 isolates, and 1 isolate each harbored three or four ARGs. The TetB tetracycline efflux pump was amplified from all the isolates that were resistant to tetracycline. Genes encoding resistance to fluoroquinolones such as QnrB-1, QnrB-5 and QnrB-8 groups were detected in the isolate with no susceptibility to orbifloxacin. Resistant genes: *LAT* (class C beta-lactamase), *ermC* & *msrA* (macrolide lincosamide streptogramin_b) and *mecA* (methicillin resistance) were identified in a single isolate that showed resistance to 6 different classes of antibiotics. Although the equine environment in LA-VTH can be frequently contaminated with antibiotic resistant *S. enterica,* our consistently low recovery of this pathogen indicates the effectiveness of our existing disinfection protocols and biosecurity measures.

* Graduate Student Poster Presentation Award Applicant

## Poster 112

### Bovine respiratory syncytial virus infection: report of feedlot cattle cases + *

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BRSV is an enveloped, single negative-stranded RNA virus belonging to the Pneumoviridae family. BRSV infection is endemic in most countries; morbidity could be as high as 60-80% and mortality can reach up to 20%, particularly in young calves. The incubation period ranges from 2 to 5 days. BRSV infects primarily epithelial cells of the respiratory tract, causing epithelial damage and immune cells chemotaxis, leading to inflammation and alteration of the immune system. These changes are usually complicated by secondary bacterial and/or mycoplasma infections.

Although bovine respiratory syncytial virus (BRSV) infection has been reported in cattle in Argentina, it has not been associated before with pneumonia in this species and geographical area. We report here 5 cases of bovine pneumonia associated with BRSV infection.

Autopsies were performed on 35 beef cattle with gross and/or microscopic lesions of pneumonia from three commercial feedlots. Lung samples in five of the animals were BRSV-positive by RT-nested PCR. The lungs of two out of these five animals were co-infected with *Mannheimia haemolytica*, and one with bovine viral diarrhea virus. Microscopically, the lungs of the BRSV-positive animals had fibrinosuppurative bronchopneumonia (3/5) with or without pleuritis, or interstitial pneumonia (2/5).

These results of this study indicate that BRSV not only is part of the bovine respiratory disease complex in Argentina but also may act in conjunction with other viruses and bacteria enhancing the pathogenesis of the disease.

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## Poster 113

### Animals exposed to *Leptospira* spp. in the United States and Puerto Rico during 2018-2020

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Leptospirosis is a worldwide zoonosis. Pathogenic leptospires colonize the renal tubules of reservoir hosts of infection and are excreted via urine. Transmission to humans and animals occurs via direct contact or through environmental exposure to water or contaminated soil. The microscopic agglutination test (MAT) is the historically routine standard for the serodiagnosis of leptospirosis. The MAT identifies animals exposed to *Leptospira* spp. The major disadvantage of the MAT is that it does not differentiate antibody response due to natural infection from a vaccine response. The present study aims to evaluate animal exposure to *Leptospira* in the U.S and Puerto Rico during 2018-2020.

The presence of antibodies against pathogenic *Leptospira* spp. was assessed with MAT according to the standards of the World Organisation for Animal Health (OIE). A total of 568 sera were submitted for *Leptospira* testing from different species and locations in the United States and Puerto Rico during 2018- 2020.

Seropositivity was 49% (278/568) with agglutinating antibodies found in 99 (35.6%) cattle, 82 (29.5%) wildlife, 38 (13.7%) horses, 23 (8.3%) goats, 16 (5.7%) dogs, 11 (4.0%) swine and 9 (3.2%) sheep. The most detected serogroups were Australis, Grippotyphosa, Ballum, Sejroe, Icterohaemorrhagiae, Pomona, Autumnalis, Pyrogenes, Szwajizak, Tarassovi, Hebdomadis, and Canicola. The results showed that animals were exposed to serovars not included in commercial bacterin vaccines such as Ballum, Bratislava and Tarassovi. Our findings suggest that more studies should include culture with concomitant genotyping, and efficacious vaccine and diagnostic strategies to reduce animal disease and zoonotic risk.

## Poster 114

### INgezim COVID 19 S VET: a multi-species ELISA for detection of specific antibodies against S protein of the SARS-CoV-2 virus

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Coronavirus disease (COVID-19) is a zoonotic disease caused by the SARS-CoV-2 coronavirus. It was declared a public health emergency outbreak by WHO in January 2020. Since then, cases of natural infection have been reported in companion (cats, dogs and hamsters), production (mink), wildlife (white-tailed deer) and zoo (monkeys, gorillas, tigers and lions) animals, showing different degree of susceptibility to infection and a wide range of clinical signs, from none to very severe signs. To date, some cases of human-to-animal, animal-to-animal and animal-to-human transmission have been reported. Although animals do not seem to play an important role in the spread of the virus among humans, all those facts have led the OIE to promote studies on the prevalence of infection in animals, and the European Food Safety Authority (EFSA) to issue instructions for surveillance of mink farms.

In this work, we have evaluated the performance of the nucleoprotein (N) and the receptor binding domain (RBD) of the S protein of SARS-CoV-2, as antigenic markers for veterinary serological diagnosis, using mink sera as proof of concept. To achieve this goal, we developed two ELISAs and a duplex immunoassay microarray all in double recognition (DR) format, to detect N-specific and RBD-specific antibodies in mink serum. Both RBD-based ELISA and MI had a sensitivity and specificity of 100%, discriminating accurately between farmed mink exposed (n=101) and unexposed (n=163) to SARS-CoV2. In contrast, we found a worse performance of N in DR-ELISA, not only for mink, but also, for feline and canine sera.

Based on these results, we developed and commercialized INgezim COVID 19 S VET, a multispecies indirect ELISA kit for detection of S-specific antibodies in plasma and serum samples. Our test showed a sensitivity and specificity higher than 98.1% in mustelid (n=454), feline (n=234) and canine (n=362) sera, showing no cross-reactivity with dog and cat sera positive for antibodies to canine coronavirus and feline coronavirus, respectively. The assay was able to detect specific antibodies in two sera of experimentally infected cats from day 10 post-infection. INgezim COVID 19 S VET also showed an optimal concordance with seroneutralization assays for 21 canine (95% concordance) and 17 feline (99.9%) samples. The use of Dried Blood Spot (DBS) technology, collected on a filter paper card, has been also evaluated as a microsampling alternative.

## Poster 115

### Evaluation of a commercial PCV2 kit for detection of antibodies to porcine circovirus type 2 in pig serum

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Post weaning multisystemic wasting syndrome is a disease of growing pigs caused by Porcine circovirus Type 2 (PCV2). The disease causes low morbidity but high mortality with serious economic impact on the swine industry. As a control measure of the disease, specific detection of antibodies by a suitable serological test is essential. There are various serological assays e.g. IPMA, IFA, ELISA available. However, since the IPMA and IFA rely on subjective interpretation, ELISA is suitable for large-scale diagnosis and can be automated.

At the Minnesota Veterinary Diagnostic laboratory, we evaluated the commercial kit, INGEZIM CIRCO IgG available through Ingenasa, Spain for detection of antibodies in pig serum. The samples used in this evaluation were from pigs vaccinated at different time points e.g. 3 weeks and 6 weeks prior to sampling. Also, samples were from pigs vaccinated at weaning and sampled 24 weeks after vaccination. The test detected 100% seroconversion in all the pigs indicating this ELISA could be used to monitor antibody response to PCV2 vaccination and/or infection. The test was also 100% specific as no antibodies were detected in PCV2 free pigs.

## Poster 116

### Determination of seroprevalence for SARS-CoV-2 in farmed and wild white-tailed deer using different antibody detection assays *

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded, enveloped RNA virus belonging to the *Coronaviridae* family, genus Betacoronavirus. A broad host range has been described for SARS-CoV-2, creating concern for future zoonotic outbreaks and further global economic losses. White-tailed deer (WTD) are known to be highly susceptible to SARS-CoV-2 and are widely distributed across the United States with high population density. The popularity for hunting and farming of white-tailed deer creates opportunities for spillback of SARS-CoV-2 from humans to animals. It is therefore important to monitor the seroprevalence of SARS-CoV-2 antibodies in white tailed deer spatially and temporally. Improved serological detection of antibodies against both, the nucleocapsid (N) and spike (S) proteins of SARS-CoV-2 are important to estimate the prevalence of infection in animal populations and improve capabilities for effective screening of different animal species. Accordingly, in the present study, we used two commercial ELISA assays based on the SARS-CoV-2 receptor binding domain (RBD) and the nucleocapsid protein to detect the presence of SARS-CoV-2 antibodies in wild and farmed WTD serum samples obtained from three U.S. states. Also, a surrogate virus neutralization ELISA kit developed for human sera that detects antibodies to RBD in the serum samples was used. A conventional virus neutralization test (VNT) was used as a reference assay. In the present study, 312 deer serum samples (wild and farmed WTD) were collected between 2018 - 2022 from Ohio, Indiana, and Kansas. Our results indicated that 38.5% and 20.8% of deer tested positive by the RBD and N indirect ELISAs, respectively. The highest seropositivity was observed in farmed animals in the state of Kansas. No antibodies to SARS-CoV-2 were detected by any of the tests in deer serum samples collected in 2018. Further research and assay validation is necessary for accurate serosurveillance for SARS-CoV-2 in different animal species.

* Graduate Student Poster Presentation Award Applicant

## Poster 117

### Poultry vector vaccines: innovative serological assays for vaccination monitoring and DIVA testing for avian infectious laryngotracheitis

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Avian infectious laryngotracheitis (ILT) is a respiratory disease of chickens caused by the infectious laryngotracheitis virus called Gallid herpesvirus 1. ILT leads to major losses as a result of mortality and/or decreased egg production. Vaccination is an essential tool for poultry disease control. Different types of vaccines are commercially available. Conventional vaccines (TCO and CEO) based on native virus (partially or totally inactivated) offer good protection but can produce latent infections and reactivation of the virus in the field. Vector vaccines are created by genetic modification(s) of vector microorganisms and the integration into their genomes of exogenous gene(s) encoding for immunogenic protein(s) from viruses responsible of diseases of interest. In the case of poultry vector vaccines, the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT) are commonly used as vector virus. One or more exogenous genes may be inserted to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with this technology include biosecurity, efficiency, ability to breakthrough passive immunity, and long-lasting immunity. Additionally, vector vaccines may be used as part of DIVA strategies (Differentiation between Infected and Vaccinated Animals). In the case of ILT, three types of vaccines exist, based on the gI, the gB or the gD protein. Given that the conventional serological kits do not efficiently detect seroconversion to vector vaccines, the ID Screen® ILT gB Indirect, the ID Screen® ILT gI Indirect and the ID Screen® ILT gD Indirect innovative ELISA's were developed to monitor respectively FP-ILTgB, HVT-ILTgI and HVT-ILT gD vaccines.

## Poster 118

### Cannabinoids screen in animal specimens by UPLC-MS/MS: In-house validation and single-laboratory blinded method test (BMT)

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*Cannabis sativa*, including hemp and cannabis plants, contains more than 113 cannabinoids. Among them, D9-tetrahydrocannabinol (9-THC) is the major psychoactive cannabinoid found in cannabis plants. Hemp contains predominantly the non-psychoactive cannabidiol (CDB) and less than 0.3% of 9-THC (dry weight). Because of the increased human consumption of cannabis products for therapeutic or recreational uses, pets are more susceptible to the exposure of 9-THC toxicity. In addition, following the 2018 Farm Bill, industrial hemp uses as an agricultural commodity to feed livestock has increased. Very little is known about the pharmacokinetic and tissue residues of cannabinoids following oral administration in bovine. So, whether it is for diagnostic purpose or research, there is a need to have a sensitive screen for cannabinoids in animal specimens. A new sensitive analytical method was developed to screen for cannabinoids in bovine specimens, including plasma, urine, and tissues. Sample clean-up procedures, analysis by UPLC-MS/MS, the in-house validation, and the single-laboratory blinded method test (BMT) will be presented. BMT data on bovine plasma confirmed that out of 21 cannabinoids, 17 can be quantitated and 4 can be semi-quantitated with a high degree of confidence at concentrations up to 100 ng/mL.

## Poster 119

### Development of an anticoagulant rodenticide panel in liver and whole blood using UHPLC tandem triple quadrupole mass spectrometry

*Thomas Olsen, Laura Burns, Dwayne Edward Schrunk*

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Traditional methodology for testing anticoagulant rodenticides (ACR) in tissue and whole blood requires time consuming solid phase extraction, followed by long gradient elution using buffered mobile phases. In this method an in-house dispersive solid phase extraction (dSPE) method is employed along with a simplified mobile phase and shorter analytical run time. This new UHPLC-MS/MS method provides a simple and quick way to quantify nine ACR compounds in multiple matrices in less than 15 minutes.

One gram of homogenized liver (or 1 mL of whole blood) was extracted into 5 mL of acetonitrile. The sample extract was cleaned using an in-house prepared dSPE tube containing 1 g alumina basic and 0.2 g C18. A 2 mL portion of the cleaned extract was concentrated to dryness, and dissolved in 50/50 methanol/water. A 5 µL injection was made onto a Thermo Accucore C18 column, 100 x 2.1 mm with 2.6 µm packing. The mobile phase consisted of A: 0.02% ammonium hydroxide in water and B: 0.02% ammonium hydroxide in acetonitrile. Gradient elution was accomplished starting at 2% MPB ramping linearly to 75% MPB from 0.5 to 7.5 min. A wash of 100% MPB is held for 2.0 min before returning to 2% MPB for 3.5 minutes. Anticoagulant rodenticides were detected using an MRM scan mode on a TSQ Altis triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Samples were quantified using a linear regression model with an effective linear range of 20-2000 ppb.

This analytical method was validated based on the FDA bioanalytical method guidelines and meets AAVLD validation standards. This poster will discuss the results of a method validation performed for this assay, including accuracy, precision, limit of quantification, linearity of dilution, and reinjection reproducibility. This robust analytical method employs a novel extraction protocol which allows for quantitative diagnostic results within 24 hours of sample receipt.

## Poster 120

### Development and validation of a cyanobacterial harmful algal bloom toxin panel using liquid chromatography with triple quadrupole mass spectrometry and orbitrap mass spectrometry for the routine screening of environmental waters

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Exposures to cyanobacterial harmful algal bloom (CHAB) toxins are of increasing concern to human and animal health, including livestock, pets, and wildlife. Climate change and eutrophication of streams, small lakes and ponds has led to an increase in the production of CHAB worldwide in recent years, and particularly in the Midwest section of the United States. Common toxins produced by CHABs are microcystins, anatoxin-a, and cylindrospermopsin. The purpose of this research is to develop and validate an analytical method for the determination of microcystin LR, RR, YR, and LA, anatoxin-a and cylindrospermopsin concentrations in water consumed by livestock, companion animals and mammalian and avian wildlife. The main purpose of the method is to determine if the concentrations of CHAB toxins in a water source are safe for animal consumption or at levels that would likely be acutely lethal. The secondary purpose of the method is to determine if concentrations of CHAB toxins are at levels that would negatively affect livestock due to chronic exposure. For veterinary diagnostics, the needed level of quantification is 1ng/mL for anatoxin-a, cylindrospermopsin, microcystin RR, microcystin LA, and 2ng/mL for microcystin LR and microcystin YR.

To develop and validate a novel analytical method for the detection and quantification of CHAB toxins at aqueous concentrations of concern, several methodological issues were addressed. Since CHAB toxins are present in the water, as well as in the cyanobacteria cells, an accurate assessment of the total CHAB toxin exposure requires cyanobacteria cells be lysed prior to analysis. Several cell lysis techniques were investigated to determine the most effective and efficient approach to releasing the toxins. Water samples from streams and ponds in and around Ames, Iowa were analyzed to determine matrix effects and selectivity. The analytical method was validated using two liquid chromatography mass spectrometry (LC-MS) platforms: triple quadrupole mass spectrometry and high-resolution accurate mass (HRAM) orbitrap mass spectrometry. Validation parameters included limit of quantification (LOQ), accuracy, precision, linearity of dilution, re-injection reproducibility, and blinded sample analysis. Parameters were analyzed on both mass spectrometry platforms. The method allows for quick, accurate, and precise analysis of water samples in support of veterinary diagnostics. The limit of quantification for all toxins is in the low ng/mL range, with average bias and % CV < 10%. Improved cell lysis facilitates quicker sample preparation for same day results. Results from water samples surveyed through the fall of 2022 will be presented.

## Poster 121

### Comparison of contemporary and historical porcine respiratory coronavirus isolates in pigs with and without subsequent influenza A virus infection * †

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*Porcine respiratory coronavirus* (PRCV), first reported in 1984 in Belgium, is a deletion mutant of *transmissible gastroenteritis virus* (TGEV). PRCV has a respiratory tract tropism commonly associated with respiratory disease in young pigs. The primary objective of this study was to compare the infection dynamics of strains isolated in 1991 and 2020. The impact of PRCV on a pig can be two-fold: induction of disease and/or potentiation of other respiratory pathogens. Hence the secondary objective was to investigate the impact of PRCV on subsequent influenza A virus (IAV) infection.

In brief, 35 4-week-old pigs were divided into six groups: PRCV-2020 (n=5), PRCV-1991 (n=5), IAV (n=5), PRCV-2020/IAV (n=5), PRCV-1991/IAV (n=5) and negative control pigs (n=10). On day 0 of the study, pigs were inoculated with 4ml of PRCV-1991 or PRCV-2020 strain intranasally. PRCV-2020, PRCV-1991 and five negative controls were necropsied at D3. Except for the negative controls, all remaining pigs werechallenged at D5 with a contemporary IAV H1N1 isolate. Respiratory scores and rectal temperatures were recorded daily. Nasal swabs were taken daily until D10 and were tested by both PRCV and IAV PCR. Serum samples were collected at D3 and D10 and were tested by IAV NP ELISA and TGEV/PRCV differential ELISA. The second necropsy was done at D10, corresponding to 5 days post IAV challenge. Gross lung lesion scores were assessed and tissues were collected for histopathology and immunohistochemistry.

Elevated respiratory scores were observed in the PRCV-infected pigs on D1, D2 and D3. Until D5, PRCV-2020 pigs had significantly higher RNA shedding compared to the PRCV-1991 pigs. Pyrexia was observed at D6 (D1 post-IAV inoculation) in all IAV infected groups, with no significant differences between IAV-infected groups. IAV shedding was uniform across IAV positive control group and both PRCV co-infected groups. Negative controls remained negative over time by both PCRs. Higher ELISA antibody titers were observed in pigs infected with PRCV-2020 compared to PRCV-1991, which correlates with clinical signs and PCR data. Necrosis and inflammation of nasal turbinate were detected in PRCV inoculated pigs at 5 DPI. Necrotizing bronchitis and severe interstitial pneumonia were present in both groups of pigs co-infected with PRCV and IAV.

Compared to the 1991 PRCV, the 2020 PRCV caused slightly more severe clinical respiratory disease and increased amount and length of shedding. Pre-infection of pigs with PRCV did not enhance disease or lesions caused by IAV infection 5 days later.

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

## Poster 122

### Detection of SARS-CoV-2-specific antibodies in domestic cats

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 (COVID-19) and the present pandemic. There is clear evidence that rather large number of wildlife and domestic animals can be naturally or experimentally infected with SARS-CoV-2. In the United States there are approximately 45 million domestic cats and between 35-40 million feral cats. As companion animals, domestic cats live in close proximity to their owners; therefore, the risk of cross-species transmission of pathogens is real. In the present study, a total of 216 serum samples from different North American (US and Canada) locations, collected between 2020 and 2022, were tested using two commercial enzyme-linked immunosorbent assays (ELISAs) based on SARS-CoV-2 nucleocapsid (N) protein and the receptor binding domain (RBD) of the spike protein. A cut-off value was used to identify seropositive and seronegative samples for each assay. For the present work, seropositive and seronegative samples were first tested by the two commercial ELISAs before being analyzed for the presence of neutralizing antibodies by a conventional virus neutralization test using live SARS-CoV-2. Overall, 4.17% (9/216) and 6.48% (14/216) of serum samples were found to be positive for antibodies against SARS-CoV-2 by the commercial N and RBD ELISAs, respectively. Five of the 216 serum samples (2.3%) testing positive in the ELISA assays also displayed neutralizing antibody titers against SARS-CoV-2. Depending on the criteria chosen for positive cut-off for the commercial RBD ELISA, it was observed that those samples with the highest cut-off values were consistent with the presence of neutralizing antibody titers. Conversely, the samples with values close to the cut-off showed no neutralizing antibody titers against SARS-CoV-2. Further studies exploring cross-reactivity with feline coronaviruses need to be done in order to rule out cross-reactivity in the assays used in this study.

## Poster 123

### Rapid detection of African swine fever virus DNA using a colorimetric LAMP PCR

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African swine fever virus (ASFV) currently represents the biggest threat to the porcine industry worldwide, with high economic impact and severe animal health and welfare concerns. Since its reintroduction into the Eurasian continent in 2007, ASFV has spread both east and west-ward, leading to a series of outbreaks during the 2010s, that culminated in the introduction of the virus in large pork-producing countries, such as China and Germany. Notably, in 2021, ASFV was detected in the Dominican Republic and Haiti, raising alarm about the reemergence of the virus in the Americas. Due to the lack of approved vaccines against ASFV, control of the virus relies heavily on molecular surveillance. Real-time PCR, while highly sensitive and specific, requires specialized equipment, the infrastructure to support it, and cannot be implemented in field. The shipment of samples increases the time for results, delaying appropriate responses. Isothermal PCR techniques, such as LAMP, have become increasingly important for point-of-care diagnosis given the minimal material expenses, equipment, and training required.

The present study led to development of a LAMP assay for the detection of ASFV with potential to be used in production or slaughter facilities, with results comparable in sensitivity and specificity to current USDA qPCR methods. A consensus sequence, obtained from all available full-length and partial sequences for the p72 target gene, was used for LAMP primer design. Four different primer sets were synthesized and tested, using a synthetic plasmid containing the cloned ASFV p72 target gene sequence as a positive control. Following initial testing, one of the primer sets, named ASFV-LAMP-BG3, containing 6 oligomers, was selected for validation. The ASFV-LAMP-BG3 primer set showed good thermal stability, amplifying the ASFV DNA at temperatures between 60-70°C. The time to detection, using ASFV-LAMP-BG3, was as fast as 5 minutes in the fluorometric test and 20 min with the colorimetric test. This primer set also proved to be highly sensitive, detecting as few as one ASFV-plasmid DNA copy/μL, in both fluorometric and colorimetric reactions. It also showed high specificity when tested against DNA and RNA from 25 different pathogens affecting swine, or genetically related to those of relevance in swine health. LAMP amplification also did not appear to be affected by the nature of the matrices, including oral fluids, tonsils, blood, or rectal swabs. Taken together, the results show that ASFV-LAMP-BG3 would be a useful tool for rapid, highly sensitive on-site diagnostic testing.

## Poster 124

### First report of porcine parvovirus 2 (PPV2) in pigs with porcine respiratory disease complex (PRDC) and stillbirth in Colombia

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Porcine Parvovirus 2 (PPV2) is a single-stranded DNA virus belonging to the *tetraparvovirus* genus of the *Parvoviridae* family. Since it was identified in 2001, it has been detected in healthy and clinically ill pigs in Asia, Europe, Africa, and the Americas. Current information regarding PPV2 cellular tropism, pathogenesis, clinical manifestations and diagnosis is scarce. Based on recent reports, PPV2 has been suggested as a putative agent or contributor to the porcine respiratory disease complex (PRDC). It has also been suggested that PPV2 could have a role in reproductive failure in sows.

The present study reports the first detection of PPV2 from the lungs of grower-finisher pigs with clinical respiratory signs and stillborn in Colombia. Out of 165 cases, PPV2 was detected by PCR in 62 cases (38%), comprising 57 pigs with clinical respiratory signs and five stillborn. Seven partial sequences (95.5% of 5215 nt; nucleotides 233 to 5131) were obtained from the positive samples. Based on phylogenetic analysis of 109 genomes (7 from Colombia and 102 from all over the world) reported in GenBank, PPV2 strains are distributed in two clades: clade one host's sequences from Asia (China and Myanmar); clade two hosts sequences from China and other countries. Clade 2 is further subdivided into 7-8 subclades depending on the sequenced region (ORF1 or ORF2). Regardless of the sequenced region, Colombian sequences are in the same subclade as those reported in Japan, South Korea, the US, and Brazil.

PPV2 was commonly detected in coinfection with porcine circovirus 2 (PCV2) (49/62, 79%) and porcine reproductive and respiratory syndrome virus (PRRSV) (44/62, 71%). Specifically, PCV2 was detected in all stillborns (5/5, 100%) and 44 pigs with respiratory signs (44/57, 77%); PRRSV was detected in 4 stillborns (4/5, 80%) and 40 respiratory cases (40/57, 70.1%). Histologic evaluation of 10 PPV2-positive animals with respiratory signs and one stillborn showed that variable degrees of histiocytic or histiocytic and lymphocytic interstitial pneumonia was present in 9/10 (90%) of the respiratory cases, and no significant lesions were observed in the stillborn. PRRSV and PCV2 were also detected by PCR in 6/10 (60%) and 8/10 (80%) of the stillborn and respiratory cases, respectively. RNAscope *in situ* hybridization (ISH) for PPV2 corroborated the presence of PPV2 in affected alveolar septa in 2 animals. PCV2 and PRRSV were not detected by immunohistochemistry (0/11).

These results suggest that PPV2 infection is widely prevalent in Colombia and is most commonly associated with other viral agents that are part of the PRDC. In addition, PPV2 detection in stillborns suggests its vertical transmission. Further studies are needed to elucidate its pathogenesis. Based on our findings, a combination of diagnostic techniques for PPV2 detection, including PCR, ISH, and histology, is warranted in the routine diagnosis of respiratory and reproductive cases to further elucidate its role.

## Poster 125

### Canine parvovirus variants in Missouri: 2012 – 2022 * † ◊

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Genetic changes in canine parvovirus (CPV-2) may result in escape from vaccine-induced immunity. Spatial and temporal distribution of CPV-2 genotypes can be used to monitor the epidemiology of canine parvoviral enteritis.

Stored nucleic acid samples from 2012 to 2022 were probed for *carnivore protoparvovirus 1* (FPV and CPV-2) using a TaqMan quantitative PCR system. Positive samples were tested using specific probes to differentiate FPV from CPV-2. Finally, three primer-probe sets were employed to specifically identify CPV-2 genotypes 2a, 2b and 2c.

Of 312 samples that tested positive for CPV-2, 136 (43.6%) had a Ct value <20, 108 (34.3%) had Ct >20<30, and 72 (23.08%) had Ct >30. Of 244 genotyped samples with Ct values <30, 119 (48.8%), 76 (31.2%), and 49 (20.1%) were typed as genotype 2a, 2b, and 2c, respectively. The results of PCR genotyping showed a 100% concordance with genotyping results obtained using Sanger's sequencing. Of 60 samples collected from 2012 – 2015, 21(35.0%) were typed as genotype 2a, 29(48.3%) genotype 2b and 10 (16.7%) genotype 2c. Similarly, 42(47.7%), 36 (40.9), and 10(11.4%) of 88 samples collected between 2016 and 2018 were typed as genotype 2a, 2b and 2c, respectively. From 2019 – 2022, 55(57.3), 19(19.8) and 22 (22.9%) of 96 samples collected were typed as genotypes 2a, 2b and 2c, respectively. There is no significant difference in the prevalence of the different genotypes over the different time periods. Conversely, a spatial heat map of genotype distribution showed distinct spotty localization of genotype 2c in specific counties while genotypes 2a and 2b were diffusely co-localized across the state of Missouri.

These findings showed that all current genotypes of CPV-2 are found in Missouri. Genotype 2c may be a more recent entrant into some counties within the state of Missouri.

* Graduate Student Poster Presentation Award Applicant
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◊ USAHA Paper

## Poster 126

### Attenuation phenotypes and protective efficacy of cell culture adapted PEDV non-S INDEL strain * †

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Porcine epidemic diarrhea virus (PEDV) continues to be an important driver of neonatal disease and economic loss to the US pork industry. However, a safe and effective live attenuated vaccine is lacking. In the present research, US non-S INDEL PEDV strain USA/IN19338/2013 was serially propagated in Vero cells. Virus growth kinetics in cell culture, virulence in suckling piglets, antibody response, protective efficacy, and whole-genome sequences were evaluated in two studies. In Study 1, 5-day-old PEDV naïve piglets (n= 60) were divided into six groups (10 pigs/group) and orogastrically inoculated with PEDV P7, P25, P50, P75, P100, or virus-negative culture medium, respectively (10 mL per piglet; all isolates had a titer of $10^4$ $TCID_{50}$/mL) for the duration of 0-7 days post inoculation (DPI). Clinical observations, virus shedding in rectal swabs, histopathologic lesions, and IHC staining data suggested that the P75 and P100 viruses are more attenuated than the P7, P25 and P50 viruses. However, the P75 and P100 viruses still caused severe diarrhea, suggesting insufficient attenuation. The virus was further passaged in Vero cells up to P200. In Study 2, twelve PEDV negative pregnant sows were divided into 4 groups (3 sows/group) to farrow piglets. The piglets (3 to 7-day-old) were orogastrically inoculated with PEDV P7 ($10^3$ $TCID_{50}$/piglet), PEDV P100 ($10^4$ $TCID_{50}$/piglet), PEDV P200 ($10^4$ $TCID_{50}$/piglet), or virus-negative culture medium, respectively. Approximately 5 piglets from each group were randomly necropsied at 4 DPI for pathological examinations. At 21 DPI (4-week-old), remaining piglets were weaned, and sows euthanized. At 28 DPI, all piglets were orogastrically challenged with PEDV P8 ($10^5$ $TCID_{50}$/piglet) and necropsied one week later (35 DPI). P7-inoculated piglets had severe diarrhea, lethargy, and body condition as well as 75% mortality. P100- and P200-inoculated piglets had almost normal body condition and no lethargy; however, P100 inoculation caused severe diarrhea whereas P200-inoculated piglets had minimal diarrhea. P200-inoculated piglets shed significantly lower level of virus in fecal swabs at 1-3 DPI compared to P7- and at 1 DPI compared to P100-inoculated pigs. P200-, P100-, and P7-inoculation all significantly reduced or prevented fecal virus shedding after challenge at 28 DPI. P200- and P100-inoculated piglets developed similar levels of anti-PEDV IgG antibody (PEDV S1-based FMIA assay) and neutralizing antibody in serum although the antibody levels were lower than that induced by P7-inoculation at 28 DPI. Whole-genome analysis of PEDV P7, P25, P50, P75, P100 and P200 revealed some amino acid mutations and truncation of ORF3 which may be associated with virus attenuation during serial passages in cell culture. Overall, PEDV P200 is a safe and promising vaccine candidate. These data warrants further investigation for vaccination and passive protection in pregnant sows and their suckling pigs.

* Graduate Student Poster Presentation Award Applicant
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## Poster 127

### Potential target antigens for development of a DIVA assay accompanying a prototype marker vaccine for ASFV.

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African swine fever (ASF) is an infectious disease that causes high mortality rates in domestic pigs and wild boar and in consequence, important economic losses in the swine industry. ASF virus (ASFV) is a very complex virus with around 50 structural proteins, some of which are used as target antigens in the available commercial diagnostic tests. At the moment, there are no specific treatment or DIVA (differentiation of infected from vaccinated animals) vaccine available against ASFV.

Under the VACDIVA European project, several promising prototype marker vaccines based on Lv17/WB/Rie11, a modified live attenuated genotype II ASFV field isolate, have been produced. The candidate vaccines have gene deletions replaced by a cassette containing a reporter gene to improve their DIVA characteristics.

In the present study, we have evaluated the immunogenicity of the proteins codified by the deleted and the reporter genes and their potential as DIVA targets for the further development of a serological DIVA assay. Moreover, the highly immunogenic viral antigen p72, was used as control for the detection of infection and monitoring immunity in vaccinated animals. In this context, the target and the reporter antigens have been recombinantly produced in mammalian and insect cells, respectively, and their immunogenicity were evaluated by indirect ELISA.

A total of 62 serum samples from 7 domestic pigs (DP), experimentally inoculated with the parental virus and collected between 0- and 54-days post-infection (dpi) were analysed: 100 % of the pigs seroconverted against the target antigen after 24 ± 5 dpi and against protein p72, after 15 ± 2 dpi. All pigs resulted negative against the reporter antigen.

In the case of vaccinated pigs, a panel of 193 serum samples from 14 DP vaccinated with VACDIVA candidate vaccines and collected between 0- and 64- dpi were analysed. All animals resulted negative against the target antigen and positive against protein p72 since 15 ± 6 days post-vaccination (dpv). Moreover, concerning immunogenicity of the reporter antigen, the 14 animals tested seroconverted at different times after 16 dpv. Currently, serum samples from wild boar have been tested and the data is under analysis.

These preliminary results of the evaluation of the selected proteins are promising and they seem to be good candidates for the development of a companion serological DIVA assay for the prototype vaccines.

**References:**

Gallardo, C, Soler, A, Rodze, I, et al. Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017. Transbound Emerg Dis. 2019; 66: 1399– 1404. https://doi.org/10.1111/tbed.13132

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## Poster 128

### A new high-performance ELISA assay as an alternative to CFT for glanders diagnosis

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Glanders is a zoonotic disease caused by *Burkholderia mallei*. The complement fixation test (CFT) for testing of equine sera is a fastidious method which may not be used for high-throughput testing. Moreover, its standardization is not facilitated by the diversity of antigens used and protocols, which lead to differences in diagnostic sensitivity. False positive results pose a problem for diagnosticians, animal health authorities and owners, while false-negative results can turn a risk into a possible threat. This study summarizes validation data obtained for the ID Screen® Glanders Double Antigen Multispecies ELISA. It is a double antigen enzyme-linked immunosorbent assay (ELISA) based on a recombinant protein.

Specificity was evaluated with equid sera from disease-free and non-vaccinated regions (n=680), and with sera from human blood donors from France (n=100).

Sensitivity was evaluated with CFT positive samples from infected horses, donkeys and mules from endemic areas (India and Pakistan; n=86).

Measured specificity was found to be 100 % in equids ($CI_{95\%}$: 99.4 – 100, n=680) and 100 % in humans ($CI_{95\%}$: 97.9 – 100, n=100). Measured sensitivity on CFT positive samples was 100 % ($CI_{95\%}$: 95.7 – 100, n=86), regardless of the species tested.

This excellent diagnostic performance was confirmed by a published independent study (Mandy C. Elschner et al. 2021, Germany, FL. Measured specificity evaluated with 400 sera was respectively 99.8% ($CI_{95\%}$: 98.6– 100), 99.8% ($CI_{95\%}$: 94.8– 98.4) and 99.2% ($CI_{95\%}$: 97.8– 99.8) for the ID Screen® Glanders Double Antigen Multispecies ELISA, CFT and Western Blot. Measured sensitivity evaluated with 370 sera was respectively 98.1% ($CI_{95\%}$: 96.1– 99.2), 96.5% ($CI_{95\%}$: 94.1.– 98.1) and 97.3% ($CI_{95\%}$: 95.1– 98.7) for the ID Screen® Glanders Double Antigen Multispecies ELISA, CFT and Western Blot. These results present an excellent specificity and sensitivity for the ID Screen® ELISA, in comparison to CFT and Western blot.

## Poster 129

### A new ASF triplex qPCR, with ambient temperature shipping, offering ultra-rapid results

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African Swine Fever (ASF) is a highly contagious hemorrhagic disease and one of the most important infectious viral diseases of swine pigs and warthogs, caused by a double-stranded DNA virus (ASFV) belonging to the family *Asfarviridae*, genus *Asfivirus*. Control and eradication programs require accurate and reliable diagnostic tests. IDvet developed a new triplex qPCR kit for ASF diagnosis, ID GeneTM African Swine Fever Triplex (cat. IDASFTRI), which offers ultra-rapid (35 min) or rapid (55 min) protocols, and both exogenous and endogenous internal controls to ensure accurate results. The kit is ready-to-use, and can be shipped at ambient temperature worldwide, reducing shipping costs and the environmental footprint.

The ID Gene™ African Swine fever Triplex (IDASFTRI) qPCR simultaneously amplifies VP72 gene target DNA as well as endogenous and exogenous non-target positive controls.

Analytical specificity was evaluated with 40 reference ASFV DNAs provided by the European Union reference Laboratory (EURL, CISA-INIA, SPAIN) and the National Reference Laboratory (ANSES Ploufragan, France) and 22 other pathogens involved in animal diseases.

Analytical sensitivity was evaluated with a synthetic nucleic acid and the limit of detection of the PCR (LDPCR) was determined. The Method Detection Limit (MDL) was determined by using negative swine blood, bone marrow, and oropharyngeal fluids samples spiked with the genotype I ASFV Georgia 2007/1 strain at 107,8 HAU/mL (NRL laboratory for ASF, ANSES Ploufragan Laboratory). The spiked samples were extracted with IDvet's nucleic acid purification columns (SPIN) and magnetic beads (MAGFAST, 20 min).

The performance of the rapid and ultra-rapid amplification programs were compared by the Friedrich-Loeffler-Institut (FLI, Germany) on 67 characterized field samples, 69 experimental infection samples and 35 ASF strains of different genotypes.

The IDASFTRI kit successfully detected all isolates and all genotypes tested without cross-reactions with other pathogens, showing 100% inclusivity (97/97) and 100% exclusivity (22/22).

The LDPCR (95%) was established around 5 copies/PCR. The MDL obtained with IDvet's extraction methods for spiked swine oropharyngeal fluids samples was 102.8 HAU/ml, and 103.8 HAU/mL for spiked swine blood and bone marrow samples.

The ultra-rapid and rapid amplification programs show excellent agreement (Kappa coefficient: 1 and correlation coefficient: 1,75 %) (mean difference $\Delta Cq = 1$).

Conclusion: The ID GeneTM African Swine Fever Triplex kit offers:

- the fastest amplification protocol on the market (35 minutes)

- excellent performance: high inclusivity on all tested ASFV strains; LDPCR = 5 copies / PCR

- Optimal test reliability thanks to endogenous and exogenous internal controls included in the kit

- Ready-to-use liquid format with economical ambient temperature shipping worldwide

The kit is currently under evaluation and validation by the EURL and FLI.

## Poster 130

### Insights into cellular tropism of porcine circovirus type 3 using an infectious clone with and without the expression of the antigenic GP5 epitope IV of porcine reproductive and respiratory syndrome virus * †

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Porcine circovirus type 3 (PCV3) was first identified in the United States in 2016. Since then, PCV3 has been reported globally and has been associated with reproductive failure and multisystemic inflammation. However, experimental reports of successful viral isolation have been limited and cellular tropism remains unknown in porcine and non-porcine cell lines. PCV3 is a circular, single-stranded, ambisense, DNA virus with an approximate genome size of 2 kb. The two major open reading frames are ORF1 encoding for the Replicase (Rep) and ORF2 encoding for the viral capsid protein (Cap). It is unknown if the PCV3 ORF2 can tolerate the addition of foreign epitopes. Therefore, objectives of this study were (1) to construct a full length PCV3 infectious clone; (2) to determine if the ORF2 can express additional epitopes by the insertion of PRRSV-GP5 epitope IV (14 amino acid, linear, B-cell, antigenic epitope); and (3) to determine the infectivity of PCV3 in porcine and non-porcine cell lines. The PCV3 genome (PCV3) and the PCV3 genome with the insertion of PRRS-GP5 IV epitope into the ORF2 C-terminus (PCV3-GP5IV) were cloned into the pBluescript expression vector flanked by *BamHI* restriction sites. Porcine cell lines (PK-15, primary porcine kidney, ST, 3D4/21) and non-porcine cell lines (HEK293T, Vero) were analyzed by IFA to determine if each cell line could be (1) transfected with concatemerized DNA, (2) able to recover infectious virus from transfection, and (3) be infected with rescued PCV3 or PCV3-GP5IV chimeric virus. PCV3 Cap and PRRSV-GP5 IV epitope expression were visualized in IFA by utilizing dual staining with anti-PCV3 Cap mAb and anti-PRRSV GP5 IV pAb followed by anti-rabbit IgG-DyLight 550 and anti-mouse FITC IgG. PCV3 and PCV3-GP5IV were transfected and rescued from PK-15, primary porcine kidney, and HEK293T cells. Additionally, PCV3 was transfected into 3D4/21 and ST cells, but infectious virus was not recovered. Viral infection demonstrated that PK-15, primary porcine kidney, and HEK293T cells are permissive to PCV3 and PCV3-GP5IV. Recircularization of a linear PCV3 genome with flanking *BamHI* restriction sites resulted in a fully self-replicative virus particle. Specific PRRSV-GP5 epitope IV pAb stain demonstrated the PRRSV-GP5 epitope IV can be fully expressed into PCV3 ORF2 C-terminus, showing potential for use as a live-vectored vaccine. The rescued PCV3 viruses were able to infect porcine kidney cell lines but not porcine macrophages (3D4/21) or testicular (ST) cell lines. Finally, PCV3 demonstrate to be capable to infect a human cell line, which warrants further research of PCV3 as a potential human pathogen.

* Graduate Student Poster Presentation Award Applicant
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## Poster 131

### Improving African swine fever surveillance using fluorescent rapid tests.

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African Swine Fever (ASF) is a highly infectious disease of swine, caused by the ASF virus (ASFV). Infection with ASFV correlates with a wide range of clinical syndromes from almost unapparent disease to hemorrhagic fever with high fatality rates. To date, there are no commercially available vaccines worldwide and therefore, ASF control is based on early diagnosis and the enforcement of strict sanitary measures. Lateral flow assays are user-friendly, low cost, provide rapid results, and have long-term stability, what makes them one of the most widely used techniques for point-of-care testing, accelerating the final diagnosis.

In this work, we described the optimization of two fluorescent lateral flow assays based on the use of europium-labelled nanoparticles. Using specific anti-p72 monoclonal antibodies, a fluorescent lateral flow assay was developed for the direct detection of ASFV antigen (Ag-assay). For the development of the rapid test for the detection of specific ASFV antibodies, p72 ASFV antigen was also bounded to europium-labelled nanoparticles (Ab-assay). Analytical sensitivity was determined using a negative blood sample spiked with p72 and with a highly positive serum, respectively. Finally, a group of samples was analyzed to determine diagnostic performance. For Ag-assay, a total of 15 experimental positive blood samples were evaluated for sensitivity determination, and 34 field negative blood samples were evaluated for specificity determination. For antibody detection, a total of 48 experimental positive serum samples were used to determine sensitivity, and 84 negative field samples (34 blood samples and 50 serum samples) were evaluated to determine specificity. For reading the results, a rapid test reader (Pacific Image Electronics) or UV-lamp was used. The optimized assays exhibited a significant increase in their sensitivity compared to the colorimetric assays, reaching up to a 10-fold increase employing recombinant protein or reference sera, respectively. Finally, assay performance was determined using the samples described above. For Ag-assay, an increase in assay sensitivity was obtained, especially for samples with Ct values above 20. Ab-assay showed the same sensitivity as IPT, increasing ELISA and colorimetric LFA's sensitivities.

In conclusion, the assays described in this work exhibited a marked increase in sensitivity while conserving the advantages of rapid tests. Therefore, they are a useful tool for improving ASFV surveillance through a fast and highly sensitive identification of infected animals, and an active surveillance of serological status.

## Poster 132

### Retrospective laboratory-based surveillance of rabies from July of 2010 to June of 2021 in South Georgia and nearby counties

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A retrospective study was performed between July 2010 and June 2021 on all case submissions for rabies testing requested at the Tifton Veterinary Diagnostic and Investigational Laboratory (TVDIL), College of Veterinary Medicine, University of Georgia (UGA). Rabies status was confirmed in brain samples using the Direct Fluorescent Antibody Test (DFAT) in a total of 792 samples. DFAT was performed by 4 different laboratories: UGA Athens Diagnostic Laboratory (39 cases), and Georgia Public Health Laboratories in the cities of Albany (291 cases), Decatur (121 cases), and Waycross (341 cases).

The animal samples for rabies DFAT originated from 23 species: 228 cats, 188 dogs, 86 raccoons, 66 bovine, 55 bats, 55 equine, 33 goats, 26 foxes, 10 opossums, 9 skunks, 9 swine, 7 squirrels, 6 bobcats, 2 beavers, 2 otters, 2 sheep, 2 rats, 1 alpaca, 1 antelope, 1 cheetah, 1 coyote, 1 deer, and 1 wallaby. Animals tested originated from 89 counties in Georgia, and 4 neighboring counties in Florida, 1 in South Carolina, and 1 in Alabama. There were 79 (9.97%) rabies positive cases across 10 species. In 13 (1.64%) cases, the test result was inconclusive (sample was unsuitable/unsatisfactory for diagnosis). The remaining 700 (88.38%) cases were negative.

Positive cases included 8 out of 9 skunks (88.89%), 5 out of 6 bobcats (83.33%), 51 out of 86 raccoons (59.30%), 8 out of 26 foxes (30.77%), 2 out of 55 bats (3.64%), 1 out of 33 goats (3.03%), 1 out of 55 equines (1.82%), 1 out of 66 bovine (1.52%), 1 out of 188 dogs (0.53%), and 1 out of 228 cats (0.44%) tested. The lowest percentage of positive cases was observed in the month of June (3.9%), followed by January (4.84%), August (7.02%), July (9.72%), and March (9.86%), with the remaining months having 10% or more positive cases each, with the highest percentage seen in May (13.85%).

Reason for submission for rabies test was human exposure in 414 cases (52.27%), animal exposure in 143 cases (18.06%), and both human and animal exposure in 55 cases (6.94%). No exposure was recorded in 75 cases (9.47%). History of exposure was unknown in 105 cases (13.26%), out of which 73 were necropsy cases in which the reason for testing was prompted by neurologic clinical signs, histopathologic findings, or other. A necropsy was performed in a total of 276 cases (34.85%), and included necropsies performed by TVDIL pathologists (208 cases) or by the submitting veterinarian (68 cases), in which either the head or brain was received for testing. Necropsy was performed in only 3 (3.8%) of the positive cases including a cow, a raccoon, and a dog.

To our knowledge this is the first study that presents prevalence of rabies using laboratory-based surveillance amongst multiple animal species in South Georgia and nearby counties, and it could be useful in reviewing and monitoring active local rabies control programs.

## Poster 133

### *Mycobacterium cookii* infection in Amazon milky tree frogs (*Trachycephalus resinifictrix*) of a captive collection

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Between 2014 and 2021, seven Amazon milk frogs (*Trachycephalus resinifictrix*) from a captive collection were found dead (5/7) or were euthanized (2/7). The euthanasia of the 2 frogs was performed due to weight loss, cloacal prolapse and discoloration of the skin. All frogs were in a good nutritional state and had granulomatous and necrotizing inflammation in multiple organs. The most consistent gross findings were splenomegaly (7/7) followed by hepatomegaly (4/7), hydrocoelom (2/7), renomegaly (2/7) and subcutaneous edema (1/7). Sheets of histiocytes with rare multinucleated giant cells, were present in the grossly affected tissues and some animals in bone marrow, lung, skin, striated muscle, small intestine, colon, and adipose tissue. A modified Ziehl-Neelsen acid fast stain and Fite's acid fast stains were negative for organisms in all sections examined. Acid fast positive bacteria were isolated from the liver (6/7) or spleen (1/7) from all animals. The isolates were identified as *Mycobacterium cookii* based on 16s gene and rpoB gene sequencing. Liver tissue from an unaffected milky frog that died due colonic carcinoma was negative for mycobacteria by culture. This report presents the first cases of natural infection of hylid frogs with *M. cookii.* The infection appears to be rapidly progressive and is considered the cause of death in the seven affected animals despite the failure to detect acid fast positive bacteria in tissue sections.

## Poster 134

### Characterization of disease-associated and non disease-associated *Streptococcus suis* isolates from pen-matched diseased and control pigs * †

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*Streptococcus suis* is an important pathogen in the US swine industry. The organism can be both disease-associated and commensal in swine. This can lead to misidentification of *S. suis* disease-associated strains. The objective of this study was to characterize *S. suis* isolates from pigs with *S. suis* meningitis and healthy pen-matched controls using coagglutination (aggl), PCR-based serotyping (mPCR) and whole-genome sequencing (WGS) to inform diagnostic and preventative measures.

A total of 329 *S. suis* isolates originating from various sample types of 35 nursery pigs from 8 different farms in Iowa and Minnesota were collected. The majority of the isolates were recovered from the upper-respiratory tract (URT; 75%), followed by bronchoalveolar lavage fluid (BALF), meninges, ileum, joint, cerebrospinal fluid (CSF), colon, pericardial fluid (PCF), sternal abscess and lung. A large number of the isolates (24%) were untypeable by aggl and mPCR; the most common typeable serotypes were serotypes 10 and 21 (8% each), and serotypes 1 and 7 (7% each). Thirteen pigs were diagnosed with bacterial meningitis caused by serotypes 1, 2, 4, 5, 10, 11 and 14. These serotypes were also isolated from the URT, BALF, joint, CSF, PCF, abscess, ileum, colon and lung of diseased pigs as well as from the URT of control pigs. A total of 34 isolates belonging to disease-associated serotypes recovered from different sample types of diseased (30) and control (4) pigs and 22 isolates of non-disease-associated serotypes recovered from control (15) and diseased (7) pigs as well as 13 isolates of disease-associated serotypes derived from routine submissions to Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) were selected for WGS. The traditional virulence-associated genes (VAGs) *sly*, *mrp* and *epf* were present in 53% of the disease-associated isolates only. However, the recently proposed VAGs (*ofs* and *srtF*) were present in the vast majority (93%) of the disease-associated isolates and only in 31% of the non-disease associated isolates. The most common sequence types (ST) were ST1 (29%) and ST119 (10%), with ST1 containing serotypes 1 and 14, and ST119 containing serotypes 5 and 11.

The results from this study showed that the disease-associated isolates were recovered from various samples from both diseased and control animals. Hence, this finding suggests that once a disease-causing *S. suis* is identified through histopathology, culture and serotyping, healthy cohorts could be screened for colonization by potentially virulent serotypes using nasal and/or tonsil swabs. In addition, one of the farms had two serotypes (5 and 11) causing disease, indicating that more than one serotype can be involved during a *S. suis* outbreak. Selecting a higher number of pigs for sampling could improve the isolate selection for autogenous vaccine production, therefore improving disease prevention.

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

## Poster 135

### *Rhodococcus equi* isolate from an implant site swab in a dog * †

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An implant site swab from a post orthopedic surgery to repair a comminuted diaphyseal right radius and ulna fracture in a 2-year-old male intact Bernese Mountain dog was submitted to the VMDL. Bacterial culture was done due to stress shielding noticed in the radiographs. Aerobic culture of the implant swab site with enrichment broth revealed the presence of *Rhodococcus equi* and *Micrococcus luteus*. Bacteria commonly recovered from canine implants are *Staphylococcus pseudintermedius, Pseudomonas aueruginosa,* and *E. coli*, which are also known, biofilm producers. In this case, we recovered *R. equi,* which was identified by MALDI-TOF and was further confirmed by biochemical tests including CAMP, catalase, nitrate reduction and urease tests. On antimicrobial susceptibility testing the *R. equi* isolate was susceptible to chloramphenicol, erythromycin, doxycycline, gentamicin, rifampin and trimethoprim/sulfonamide. *R. equ*i is a Gram-positive bacteria found in soil, commonly causing pneumonia in foals. *VapA* plasmid is usually associated with isolates that cause clinical disease in foals. There are few reports of *R. equi* isolates recovered from immunocompromised dogs. We wanted to confirm the presence of virulence-associated *VapA* plasmid in the *R. equi* isolate recovered from the implant site swab by real-time PCR targeting a 75bp region of the *VapA* gene; the isolate was positive. Further, sequencing of the *Vap A* gene and phylogenetic analysis may provide insight into the virulent plasmid.

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

## Poster 136

### Prevalence of extended spectrum beta lactamase-producing Enterobacterales in clinical infections and fecal specimens in dogs and cats in southwestern Virginia

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Extended-spectrum beta-lactamase producing Enterobacterales (ESBL-E) are considered to be a serious threat to public health by the CDC and contribute to mortality, prolonged hospital stays, and dramatically increased healthcare costs. ESBL-E infections also occur in companion animals, and the incidence of these infections has been increasing over the past decade. Because people and pets are both affected by genetically-similar strains of ESBL-E, there is also concern for zoonotic and/or nosocomial spread of these pathogens in veterinary hospitals, so contact precautions are often put into place, adding to the expense for caring for infected patients. ESBL-E are typically opportunistic pathogens that may be carried subclinically in the gastrointestinal tract, but then may induce disease at other sites under the right circumstances. We hypothesized that the prevalence of fecal carriage of ESBL-E in companion animal fecal samples submitted to our laboratory would be approximately 10%, which is similar to reported ESBL-E carriage rates in dogs and cats in other locations.

We screened 112 canine or feline-source fecal samples that were submitted for routine fecal float or bacterial culture for ESBL-E using chromogenic, selective ESBL agar (CHROMagar ESBL). The prevalence of ESBL-E carriage was 8% (n=9), and most of the ESBL-E were *E. coli* (n=8). We then compared the fecal ESBL-E prevalence to the prevalence of ESBL-E clinical infections isolated at our diagnostic laboratory. The prevalence of ESBL-E clinical infections diagnosed in our laboratory has significantly increased over the past five years (Chi-square test for trend, p=0.003). Over the period of fecal sampling in 2022, 13.9% of clinical Enterobacterale infections were caused by ESBL-E; this indicates that the prevalence of ESBL-E fecal carriage is not significantly different from the prevalence of ESBL-E clinical infections in animals with infections caused by Enterobacterales (test of two proportions, p=0.13).

Our ESBL-E prevalence data will allow us to compare ESBL-E carriage across different veterinary populations, and will serve as a baseline for ESBL-E carriage in our patient population, which will allow for future assessment of changes in ESBL-E prevalence over time and of correlations between with ESBL-E carriage and clinical infections.

## Poster 137

### Biochemical characterization of *Clostridium novyi* isolated from dogs

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*Clostridium novyi* is a gram-positive obligate anaerobe that consists of diverse strains or "types", each capable of inducing unique pathology. C. *novyi* type B causes infectious necrotic hepatitis (INH), or black disease, in ruminants and rarely in other species, such as horses and pigs. Closely related to *C. novyi* is *C. haemolyticum*, previously known as *C. novyi* type D, which causes bacillary hemoglobinuria (BH) in ruminants. Rarely, *C. novyi* strains have been isolated from canine samples. Little is known about the role of *C. novyi* in canine disease, and canine isolates of *C. novyi* have not been fully characterized in the literature. Differentiation between *C. novyi* subtypes and *C. haemolyticum* is a diagnostic challenge with laboratory identification methods such as MALDI-TOF and 16s ribosomal RNA sequencing due to the high degree of similarity between all of the organisms in the group. This study evaluated the biochemical profiles of canine isolates of *C. novyi* in order to better characterize isolates that caused disease in dogs.

We isolated *Clostridium novyi/haemolyticum* from anaerobic culture of abdominal fluid specimens of two different dogs with bacterial peritonitis and hepatitis between 2020 and 2022. Both organisms were identified as *C. novyi/haemolyticum* by 16S rRNA sequencing. The isolates could not be identified to the species level using MALDI-TOF (Bruker Biotyper), with all scores <1.720. A panel of biochemical tests was performed, including urease, indole, nitrate reduction, gelatin hydrolysis, esculin hydrolysis, milk digestion, sugar fermentation, lipase, and lecithinase. Biochemical profiles were consistent with *C. novyi* and the major difference noted between the isolates was indole production. *C. novyi* type B and *C. haemolyticum* are typically indole-positive, while *C. novyi* type A is indole-negative. Canine-origin *C. novyi* isolates do not have a distinct, consistent biochemical profile, and therefore additional molecular testing will be required in order to more fully identify the organisms.

Importantly for veterinary bacteriology laboratories, routine methods of bacterial identification, such as biochemical testing, MALDI-TOF and 16s rRNA sequencing are inadequate to differentiate *C. novyi* and *C. haemolyticum*. In ruminants, non-culture methods are often used for diagnosis, and when cultivation is attempted, species and subspecies-level differentiation is often based on the clinical disease observed. However, in the rare cases of clinical canine cultures, the diagnostic challenges described here can lead to a final laboratory diagnosis of "*Clostridium* species" and further identification is often not pursued. As *Clostridium* spp. are reported to be some of the most common causes of anaerobic bacterial hepatitis in dogs, *C. novyi/haemolyticum* may be overlooked in canine cases due to difficulties in isolating and identifying these organisms.

## Poster 138

### A five-year retrospective analysis of serosurveillance data on cervid diseases ◊

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Members of the Cervidae family can harbor a number of pathogens that could potentially transmit to domestic animals and humans. Disease surveillance in cervid population is therefore important to protect the health and wellbeing of animals and humans. In the present study, we analyzed the serosurveillance testing data of free-ranging white-tailed deer (WTD) samples submitted to the Athens Veterinary Diagnostic Laboratory (AVDL) from 2017 to 2021. Sera were tested for the presence of antibodies to epizootic hemorrhagic disease virus (EHDV), bluetongue virus (BTV), bovine viral diarrhea virus (BVDV), Infectious bovine rhinotracheitis virus (IBRV) parainfluenza type 3 (PI-3) and *Brucella abortus/Brucella suis*. Seroprevalence of EHD and BT remained high over the last five years with an exception in 2020 where it showed a drastic decrease in prevalence. In 2017, 65.59% of samples tested were positive for EHDV antibodies and 62.04% samples were positive for BTV antibodies. In 2018, the numbers went slightly down to 55.70% for EHDV and 53.16% for BTV. The numbers remained relatively steady in 2019 with 59.38% samples showing positive results for EHDV and 57.81% samples positive for BTV antibodies. 2020 saw a drop in numbers with 20.27% positive for EHDV antibodies and 8.11% positive for BTV antibodies. In 2021, the positive numbers went back up again with 68.07% samples showing positive results for EHDV and 68.67% samples for BTV antibodies. The PI-3 antibody test results showed a range of 22.78-60.81% of tested samples giving a positive antibody response over the five-year period. The seroprevalence of BVD was low with a positivity range of 0-4.6.9%, and that of IBR was 1.27-7.81%. Overall, the data showed a relatively high seroprevalence of EHDV, BTV and PI-3 in the free-ranging WTD population whereas the data showed a very low seroprevalence of BVDV and IBRV in WTD over the last five-year period. None of the samples tested during the study period showed any evidence of Brucellosis in WTD. The clinical significance of this data in disease transmission and epidemiology in domestic animals need to be further investigated. Continuous monitoring of diseases in cervid populations in proximity to livestock and human habitats is critical in understanding the epidemiology and taking control measures to protect livestock and public health from transmissible infectious diseases.

◊ USAHA Paper

## Poster 139

### Isolation and molecular characterization of fowl adenovirus and avian reoviruses at TVDML, 2020-2022

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Aviadenoviruses, commonly known as fowl adenoviruses (FAdV), and avian reoviruses (ARV) are considered economically important viral pathogens for the poultry industry. Fowl adenoviruses are separated into five species (A–E). Historically considered incidental or opportunistic, recent data strengthen the role of some FAdV strains as primary pathogens in chickens. This has been demonstrated for some FAdV–1 strains (species A) causing adenoviral gizzard erosion (AGE) and some FAdV–4 strains (species C) which play a major role in the etiology of hepatitis hydropericardium syndrome (HHS). Other strains, primarily those belonging to species D and E, are responsible for severe liver damage leading to the condition known as Inclusion Body Hepatitis (IBH). Avian reoviruses (ARV) are classified in the genus Orthoreovirus and are known to cause arthritis/tenosynovitis in broilers and turkeys, which is characterized by swelling of the hock joints and lesions in the gastrocnemius tendons leading to lameness. Avian reoviruses are also associated with other avian diseases, such as stunting-malabsorption syndrome, myocarditis, hepatitis, and respiratory and enteric diseases. While presumptive diagnosis of both FAdV and ARV infections is possible based on gross lesions, confirmation is needed using viral isolation and/or RT-PCR. Additionally, the high genetic diversity and constant evolution of these viral species necessitates continuous monitoring of genetic changes and viral types/species.

Here we present the isolation and characterization of 81 FAdV and 20 ARV at the Texas A&M Veterinary Medical Diagnostic Laboratory between July 2020 and June 2022. The 81 FAdV were isolated in primary chicken embryo liver cells from 121 submitted samples. The isolates were confirmed by PCR targeting the *hexon* gene and the resulting products were submitted for sequencing. Phylogenetic and sequence identity analyses showed that 89% of the isolates (n = 72) belonged to species E serotype 8b, 6% (n = 5) of the isolates belonged to species D serotype 11, and the remaining 5% of the isolates (n = 4) were classified as species E serotype 8a. The 20 ARV were isolated via drop CAM (SPF eggs) from 55 samples. The isolates were confirmed by three-step RT-PCR targeting the *sigma C* gene and the resulting products were submitted for sequencing. Despite the low number of isolates, phylogenetic and sequence identity analyses showed high diversity among the studied ARV. The isolated viruses belonged to genetic clusters 1.1 (n = 3, 15%), 2.1 (n = 4, 20%), 2.3 (n = 4, 20%), 3 (n = 1, 5%), 4 (n = 1, 5%), 5.1 (n = 3, 15%), and 6 (n = 4, 20%).

The results and genetic data generated herein facilitate studying the diversity of circulating FAdV and ARV as well as provide valuable information for the poultry industry on the temporal and spatial distribution of the species/types of these important viruses.

## Poster 140

### Detection and disease diagnosis trends (2010 – 2021) of five swine systemic bacterial pathogens at Iowa State University Veterinary Diagnostic Laboratory

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*S. suis, G. parasuis, M. hyorhinis, A. suis,* and *M. hyosynoviae* are the most significant bacterial pathogens in swine production. This study investigated temporal trends in detection and disease diagnosis for these five pathogens using 12 years of data from the ISU VDL.

Submissions with bacterial culture for *S. suis* and *G. parasuis* increased significantly by 1% and 3% on average yearly, respectively, with the largest increase occurring between 2018 to 2019 and 2020 to 2021. In contrast, *A. suis* culture submissions decreased by 2% on average each year. No statistical change was observed in the positive PCR rate for *G. parasuis* and *M. hyosynoviae* over the studied period, but a 4% increase was observed in the positive PCR rate of *M. hyorhinis*.

Over the 12 years, *S. suis* disease was the predominant agent of neurological disease (73% of all cases), while *G. parasuis* disease contributed to at least 47% of all polyserositis cases. A significant increase was frequently observed in the etiological diagnosis of arthritis [e.g., arthritis due to *S. suis* increased by 15% average year, *G. parasuis* (8%), *M. hyorhinis* (5%), and *A. suis* (19%)]; however, *M. hyosynoviae* disease diagnosis decreased by 13% average year. Bronchopneumonia due to *S. suis* and *G. parasuis* increased by 4% and 9% on average each year, respectively, over this period, while the same lesions associated with *M. hyorhinis* increased by 52% in the most recent couple of years. Meningitis associated with *S. suis* and *G. parasuis* disease increased by 4% and 22% in 2020 and 2021. While *M. hyorhinis* and *S. suis* disease associated with polyserositis increased yearly by 4% and 12%, respectively, *G. parasuis* decreased by 2%. In contrast, no significant trend was observed in *A. suis* polyserositis. Within disease diagnosis cases, *S. suis* and PRRSV were the most common observed polymicrobial interaction (104 cases on average each year), followed by *G. parasuis* and PRRSV (70 cases on average each year).

This study showed that detection and disease associated with four of five systemic bacterial agents have increased over 12 years. The increase can be manifold, e.g., improvements in diagnostic data systems, pig industry growth, increased awareness of disease investigation, advancements in diagnostic techniques, and changes in the prevalence of the agent or the dissemination of more pathogenic variants.

## Poster 141

### Surveillance of companion and exotic animals for SARS-CoV-2 and evaluating transmission potential within veterinary medicine *

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SARS-CoV-2 is a highly pathogenic, novel coronavirus, which is the cause of the ongoing COVID-19 pandemic. With a total of 85.4 million human cases of COVID-19 in the United States and 70% of households owning a pet, millions of companion animals have potentially been exposed to the virus through their owners, a process called reverse zoonosis. SARS-CoV-2 has been reported to infect both wild and domestic animal species after contact with infected humans. Uncertainty currently exists on SARS-CoV-2 transmission from domestic animals to humans, whether directly or as a mechanical vector. Veterinarians and veterinary personnel work at the forefront of emerging zoonotic diseases and have an increased likelihood of exposure to these diseases. Improved understanding of the transmission risk of SARS-CoV-2 in animals and the veterinary community can direct appropriate resource allocation and data-driven protocols that protect the health of veterinary personnel. Our goal is to measure the prevalence of SARS-CoV-2 in companion animals, including exotic species presenting to a veterinary teaching hospital to assess transmission risk of this virus.

With informed owner consent, we collected oral swabs, nasal swabs, and opportunistic blood samples from domestic and exotic mammal species seen at the CSU Veterinary Teaching Hospital (VTH) and those owned by students, staff, and faculty associated with the VTH. Oral and nasal samples were tested using quantitative PCR to detect SARS-CoV-2 nucleic acid, while serum was tested for neutralizing antibodies against SARS-CoV-2 using the plaque reduction neutralization test (PRNT). Study enrollment is ongoing with the goal to obtain a minimum of 340 individual animal samples with a representative range of household pet species.

Preliminary qPCR tests have detected SARS-CoV-2 definitively in 1 nasal swab of 88 canine oral/nasal samples and 1 feline oral swab in 74 feline oral/nasal samples. This data supports existing studies indicating that SARS-CoV-2 is present in our companion animals.

Data derived from this study will provide infection and seroconversion rates of domestic and exotic pets with SARS-CoV-2 and contribute to a better understanding of transmission risk of the virus in a veterinary setting. Study results will support informed decision making by veterinary professionals regarding hospital biosafety protocols and use of personal protection equipment (PPE) during animal handling, as well as provide more comprehensive information to pet owners regarding zoonotic risks of SARS-CoV-2.

* Graduate Student Poster Presentation Award Applicant

## Poster 142

### Validation of ASFV real-time PCR in a 384 well plate format using the QuantStudio 6 Pro

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African Swine Fever (ASF) is a foreign animal disease that is highly contagious and can be deadly in both domestic and feral swine populations. ASF can be found in countries around the world and has recently spread to the Dominican Republic and Haiti in the western hemisphere. The U of MN Molecular Diagnostic Lab (MVDL) is a Level 1 high throughput NAHLN testing laboratory and is responsible for accurate and timely surveillance testing for the presence of African Swine Fever virus, the causative agent of ASF. Real-time PCR detection of this virus is a well-established method for diagnosing outbreaks of ASF, so ways to increase testing capacity in a lab of limited space need to be explored. Validating the NAHLN ASFV real time assay in a 384 well plate format using the QuantStudio 6 Pro (QS6) quadruples the testing capacity per plate while using one quarter of the space when compared to a 96 well real time instrument like the ABI 7500 Fast. A methods comparison was conducted between the 7500 Fast 25 µl real-time NAHLN protocol and a 25 µl QS6 384 well and 20 µl 384 well protocol designed at the MVDL. The lower reaction volume of 20 µl is the optimal reaction volume to use in plates with a smaller well diameter. Parameters compared in this study include analytical sensitivity (ASe), inter- and intra-plate repeatability, diagnostic sensitivity (DSe), and diagnostic specificity (DSp). ASe was assessed by diluting the positive amplification control down to 10-5 and comparing the Ct values from these dilutions in triplicate on the 7500 Fast 96 well assay, the 384 well QS6 25 µl assay, and the 384 well QS6 20 µl assay. The limit of detection for all assays was found to be equivalent between all platforms and reaction volumes. Intra-plate repeatability was determined by running 12 replicates of the 10-2 positive amplification control on a single 384 well plate using the QS6 20 µl reaction volume protocol. The mean of this data set is Ct 34.86 with a standard deviation of 0.229 and a coefficient of variation (CV) of 0.66%. Inter-plate repeatability was assessed by examining this same dilution run in triplicate across three different real time PCR sessions. The mean for this data is Ct 34.97 with a standard deviation of 0.48 and a CV of 1.37%. DSe and DSp were determined by testing a sample set of 61 negative porcine tonsil and spleen homogenates that were negative on the 7500 Fast protocol using both 384 well QS6 methods. Thirty of these negative samples were spiked with ASFV training panel material to simulate positive field samples. All samples were extracted using the MagMAX Core kit and tested using the 7500 Fast and QS6 protocols. The 384 well QS6 20 µl assay was found to provide equivalent results to the 7500 Fast 96 well assay with a DSe and DSp of 100%. These parameters indicate that a higher throughput 384 well real time PCR for the detection of ASFV would be acceptable for clinical use and will aid in increasing testing capacity in the lab.

## Poster 143

### Genetic characterization of *Streptococcus equi* subspecies *zooepidemicus* isolates from a 2021 outbreak in Indiana with increased sow mortality

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High mortality events due to *Streptococcus equi* subspecies *zooepidemicus* (*Streptococcus zooepidemicus*) in swine in the United States were first reported in Ohio and Tennessee in September and October 2019 and later in Pennsylvania in December 2019. In February 2021, another *S. zooepidemicus* outbreak occurred in two-year-old adult sows from a 2400 sow herd production system in Indiana, resulting in 66 deaths within a six-week period. To investigate if the Indiana outbreak isolates were related to those isolates from Ohio and Tennessee outbreaks, whole genome sequencing (WGS) using Illumina MiSeq platform and subsequent analysis was performed.

WGS analysis of four outbreak isolates from Indiana revealed that while these isolates were closely related to a *S. zooepidemicus* isolate from a horse in Iowa (ISU38408), they were genetically distant to the Ohio and Tennessee outbreak isolates, the ATCC 35246 strain responsible for 1974 high mortality outbreaks in China, and an isolate from one pig with septicemia from Arizona (AZ-45470). These data suggest variation in pathogenicity between strains and that more than one strain of *S. zooepidemicus* may cause high mortality events.

The genome sequences of one Indiana outbreak isolate (IN-6992) and the *S. zooepidemicus* horse isolate (ISU38408) were further closed using Nanopore sequencing. Comparative genomic analyses were performed and genomic islands and putative virulence genes were identified. Comparative genomic analyses revealed that IN-6992 and ISU38408 had an average nucleotide identity of 99.9%. 96.1% of nucleotides of IN-6992 aligned with ISU38408, and 99.9% of nucleotides of ISU38408 aligned to IN-6992. Compared with ISU38408 genome, 1135 SNPs, 17 insertions, and 8 deletions were found in the genome of IN-6992. When IN-6992 was compared with the Ohio and Tennessee outbreak isolates, three genomic islands (GI-1, GI-3, and GI-10) were present only in IN-6992. One genomic island (GI-13) was identified specifically in the genome of IN-6992 but was absent from the genomes of all isolates included in this study. Thus, the genes encoded by this genomic island could serve as a diagnostic biomarker for this *S. zooepidemicus* strain. In addition, recently reported *S. zooepidemicus* virulence factors M-like protein gene (*szM*) and the newly identified Fic domain-containing protein gene (*bifA*) were absent in the Indiana outbreak isolates but were present in all tested Ohio and Tennessee outbreak isolates. These findings provide significant and timely insights into understanding, tracking, and possible prevention of severe outbreaks caused by *S. zooepidemicus.* In addition, identification of bacterial virulence biomarkers will greatly improve our response to future outbreaks caused by *S. zooepidemicus.*

## Poster 144

### Development and evaluation of MG2Vec: a transformer neural network for metagenomic shotgun sequencing based BRD pathogen detection # * ◊

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Metagenome shotgun sequencing is gaining recognition as a reliable method for infectious disease diagnostics. One of its major improvements over traditional diagnostic methods is the potential for unlimited multiplexing making it possible to simultaneously test for multiple pathogens in a clinical sample. However, the complexity of metagenomic data and the computational requirements for analyzing and interpreting them have been major hurdles to the widespread adoption of metagenome-based diagnostics. We developed and evaluated a machine learning and transformer neural network algorithm for identification of pathogen sequences in the metagenome data. The entire metagenome is broken down as *k-mers* and graphically represented, similar to *de-bruijn* graphs for genome assembly. Based on the co-occurrence of *k-mers* identified from these graphs, the neighborhood structures for each k-mer are recognized to retrieve relevant information. We used a transformer neural network made of multi-headed attention and feed-forward mechanisms to learn features associated with the *k-mers* for pathogen detection. Bovine respiratory disease clinical samples with varying pathogen availability were used to assess the efficacy of the algorithm. The average F1 scores which represent both precision and recall for our machine learning algorithm were estimated to be 0.985 and 0.981 using multilayer perceptron classifier and deep learning classifier respectively. Average F1 scores for pathogens were 0.534 and 0.631 using the Multilayer Perceptron classifier and Deep Learning classifier respectively.

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

## Poster 145

### One Health: veterinary laboratories uniquely prepared to perform human diagnostic testing during SARS-CoV-2 pandemic

*Natasha Hodges[1], Izabela Ragan[2], Tracy Webb[3], Treana Mayer[1], McKenzie Sparrer[1], Molly J. Carpenter[2], Christie Mayo[1]*

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The ongoing SARS-CoV-2 pandemic has highlighted gaps in the efficiency and implementation of timely diagnostic testing. Early in the pandemic, most health institutions were overwhelmed with diagnosing and treating COVID-19 patients; however, many biotechnology companies and academic institutions rapidly mobilized resources to help aid in these efforts, with veterinary diagnostic laboratories (VDLs) serving as opportune sites for processing high volume human testing during the SARS-CoV-2 pandemic. When effectively mobilized, VDL facilities, equipped with the necessary instruments and trained personnel, represent an invaluable resource for emergency disease testing in pandemic situations. VDLs routinely process and test large numbers of samples for pathogens, including outbreak, surveillance, and regulatory testing in animal populations. In order to optimize the integration of VDLs into future pandemic response efforts, additional information is needed about the process and impact of VDLs on the current SARS-CoV-2 pandemic. This includes how many VDLs performed human SARS-CoV-2 testing, implementation protocols and challenges, length of testing, volume of samples tested, management and methods of sample collection, financial implications and potential One Health connections that arose from human sample testing.

Understanding how VDLs supported human SARS-CoV-2 testing provides a useful template for improved, coordinated disease surveillance efforts across multiple health disciplines. Within the veterinary diagnostic community, we share general principles and expertise that includes test development, quality assurance, supply chain troubleshooting, and data security and transfer. Our aim is to collect data from accredited VDLs to measure the impact of Emergency Use Authorization (EUA) SARS-CoV-2 testing for humans conducted by veterinary diagnosticians. A 17-question survey has been approved by the CSU Institutional Review Board for distribution and data collection.

Study results will help inform and optimize pandemic response efforts and identify benefits from additional cross-disciplinary collaborations between human and veterinary diagnosticians. Optimizing One Health approaches to disease surveillance and testing can lead to early identification and improved mitigation of future zoonotic events. This comprehensive One Health perspective will aid in future pandemic preparedness.

## Poster 146

### Logistic regression for automated assigning taxonomical classification to sequences

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Phylogenetic classification is being performed with increasing frequency in veterinary diagnostic laboratories for pathogens where sufficent sequencing data are available. With the reduced cost of genetic sequencing, unprecedented volumes of sequencing data are being generated globally. It has become routine to obtain a genetic sequence of a pathogen of interest to identify genetic variations of diagnostic significance. The Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) has implemented a machine learning approach using logistic regression as a supplemental method for phylogenetic analysis to assign a classification to genetic sequences.

The ISU-VDL hosts a repository of prior classified swine influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV) sequences, which serve as training data for machine learning tasks. Classifiers for IAV and PRRSV are retrained on a weekly basis, and are used to classify all newly generated sequences on a real-time basis. Measures of accuracy, precision, and recall are checked for each new classifier and are regularly above 90% for each. The machine learning classifier benefits from being computationally fast as it runs with linear time complexity, and does not require a specifically curated reference set for classification as all prior classified data can be used for training. The output of the classifier is a probability score for inclusion in a designated phylogenetic clade. The method at the ISU VDL has been integrated into a pipeline that sends out nightly reports of new detections, and also doubles as an early detection system for irregular events. New classifications that fall below a minimum threshold for classification probability are flagged for further phylogenetic follow-up outside of the classification pipeline.

To date, the classifier has flagged a number of human-to-swine reverse-zoonotic events within the H3 subtype influenza and identifies IAV sequences outside of the United States that are submitted to the ISU VDL. The use of machine learning for the classification of sequences has proven to be an accurate supplementation to traditional phylogenetic methods, which is easy to implement in automated pipelines and decreases workload within diagnostic laboratories. In addition, this automated machine learning approach to phylogenetic classification provides an ‘early warning’ system that detects unusual sequences of diagnostic significance and potential emerging pathogens.

## Poster 147

### Development of a direct reverse transcription real-time polymerase chain reaction assay for the detection of porcine reproductive and respiratory syndrome virus (PRRSV) in clinical samples * †

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Direct RT-rtPCR (dRT-rtPCR) is emerging as a time-efficient and cost-effective alternative to traditional extraction-based RT-rtPCR. A dRT-rtPCR assay would be invaluable to high throughput diagnostic laboratories, increasing testing capacities and lowering the cost of consumables. As such, the Veterinary Diagnostic Laboratory at Iowa State University conducted an exploratory study to determine if a model virus, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), could be tested via dRT-rtPCR.

To this end, processing fluids, oral fluids, and serum previously confirmed positive for PRRSV were used in this study. The effect of heat treatment (HT) on the utility of dRT-rtPCR, i.e., 65°, 80°, 95° C for 5 minutes, vs. no HT, was determined using PRRSV isolates (cell culture, n=8) and clinical specimens (n=15). To address congealing of clinical samples when heated, samples were tested undiluted, 1:2, 1:3, or 1:4 in phosphate-buffered saline (PBS) with HT alone or HT supplemented with PCR enhancing additives proteinase K (PK, 2.0mg/mL) or N-Acetyl Cysteine (NAC, 1.0mM). Subsequently, PK (0.5mg/mL, 1.0mg/mL, or 2.0mg/mL) in conjunction with HT as well as sample input volume (3uL, 5uL, or 8uL) were evaluated to establish optimum reagent concentration and template volume. Five PCR enhancing additives combined with 1:2 dilution, PK (0.5mg/mL), and 95°C HT were investigated: Triton X-100 (1.0%), Sodium dodecyl sulfate (SDS, 0.05%)/Tween-20 (2.0%), Tween-20 (2.0%), Bovine Serum Albumin (BSA, 0.4mg/mL), or PK added to HT or PCR reaction mix. All treatments were compared to magnetic bead-based extraction and evaluated for assay sensitivity.

In viral isolates, 65°C and 80°C HT significantly reduced PRRSV detection in dRT-rtPCR compared to standard RT-rtPCR ($p<0.0001$ and $p<0.05$, respectively), while no difference was observed for 95°C. Congealing of processing fluids and serum was significantly reduced after a 1:2 dilution and treatment with PK (2.0mg/mL). Thus, 1:2 dilution of the sample in PBS heated for 95°C along with PK were selected for subsequent experiments. As no significant difference in Ct was noticed across all the clinical samples at different doses of PK, a 0.5mg/mL was employed. Interestingly, PRRSV detection was significantly compromised with increased sample input volumes ($p<0.001$). Using 3uL, the lowest volume incorporated in this study, all PCR enhancing additives listed above were tested, and the performance of PK was consistent across clinical samples, with Ct values close to standard magnetic bead-based extraction.

These results suggest that dRT-rtPCR could be a promising alternative to traditional RT-rtPCR. However, further validation using additional specimen types and pathogens is required.

This study further supports that sample type, sample dilution and volume, HT, and PCR enhancing additives are crucial for a successful dRT-rtPCR, and PK along with HT are important factors for detecting PRRSV through dRT-rtPCR.

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

## Poster 148

### Effect of freeze-thaw on the detection of PRRSV RNA by RT-qPCR * †

*Berenice Munguia-Ramirez[1], Betsy Armenta-Leyva[1], Alexandra Henao-Díaz[2], Fangshu Ye[3], Kent Doolittle[4], Silvia Zimmerman[4], Luis G. Gimenez-Lirola[1], Jeffrey Zimmerman[1]*

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Re-testing samples is common both in diagnostic and research settings, but the effect of freeze-thaw cycles on nucleic acid detection by PCR is largely unexplored. The objective of this project was to quantify the effect of multiple freeze-thaw cycles on the detection of PRRSV RNA and a porcine endogenous reference gene (internal sample control) in serum and oral fluids.

Serum samples (n = 10) used in the study were from pigs experimentally inoculated with wild-type PRRSV (n = 5) and corresponding negative control pigs (n = 5). Oral fluid samples (n = 10; 6 PRRSV positive and 4 negative) were from individually housed 14-week-old pigs vaccinated with a PRRSV modified-live vaccine (MLV) (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica, Inc., Duluth, Georgia) and sampled from -7 to 42 days post vaccination (DPV).

The experiment consisted of exposing samples to 2, 5, 10, or 15 complete freeze-thaw cycles, testing for PRRSV RNA and a porcine internal sample control, and then quantifying the freeze-thaw effect by linear regression on SAS 9.4 (SAS Institute, Cary NC). To conduct the experiment, samples were aliquoted (1 ml) into 2 ml tubes to create 4 complete sample sets (one per "treatment"). Samples were submitted to freeze-thaw cycles by freezing at -80°C and then thawing at 4°C overnight. After all freeze-thaw "treatments" were completed, samples were tested using a commercial RT-qPCR mix that detects both PRRSV RNA and a porcine internal sample control simultaneously (RealPCR*NA PRRS Types1-2 RNA Mix, IDEXX Laboratories, Inc., Westbrook, Maine, USA).

In serum, regression analysis showed that freeze-thaw cycles had little impact on the detection of PRRSV RNA and the internal sample control. That is, all samples were positive for both targets and the slope of the regression was -0.135 (95% CI: -0.37, 0.34) and 0.018 (95% CI: -0.06, 0.09) for PRRSV and the internal sample control, respectively. In oral fluids, the intended targets were detected in all samples, but the freeze-thaw effect was more discernable. That is, the slope of the regression was 0.208 (95% CI: -0.02, 0.43) and 0.193 (95% CI: 0.06, 0.32) for PRRSV RNA and the internal sample control, respectively.

Samples in diagnostic laboratories commonly undergo freeze-thaw cycles in the course of testing and retesting, but the effect of this process on the results is largely unquantified. This study demonstrated that PRRSV RNA and a porcine internal sample control was relatively resistant to multiple freeze-thaw cycles, albeit more so in serum *vs* oral fluids. These results suggest that further studies are needed to address the effect of freeze-thaw on other pathogens.

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## Poster 149

### Novel virulence-associated gene frequency across capsular serotypes and sequence types in *Streptococcus suis*

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*Streptococcus suis* is a significant burden to the swine industry and an animal welfare concern[1]. Both commensal and pathogenic strains subsist in pigs, and identifying clinically relevant strains is important in autogenous vaccine development and disease control programs. Recently discovered Virulence-Associated Genes (VAGs), known as SSU_RS03100, SSU_RS09155, and SSU_RS09525, have been reported to be indicators of pathogenicity[2]. Our group aims to explore relationships between these VAGs among serotypes and sequence types (STs) that have previously been associated with pathogenic pathotypes[1]. 127 *Streptococcus suis* isolates were subjected to WGS (Whole Genome Sequencing) during routine diagnostics at Newport Laboratories between late 2021 to April 2022. Frequencies of VAGs SSU_RS03100, SSU_RS09155, and SSU_RS09525 across STs previously reported to be associated with pathogenic pathotype[1] were observed to be ST1(1,.96,1), ST25(1,1,1), ST28(.93,1,1), ST29(1,.67,1), ST94(.67,.67,.67), ST373(1,1,1), and ST977(1,1,1). Frequencies of VAGs SSU_RS03100, SSU_RS09155, and SSU_RS09525 across serotypes previously reported to be associated with pathogenic pathotype[1] were observed to be 1(1,1,1), ½(.9,1,1), 2(1,1,1), 7(1,.86,1), 14(1,1,1), and 23(1,1,1). Our results suggest that serotypes and STs reported to be pathogenic align well with the presence of the novel VAGs. However, many serotypes and STs exhibited 100% prevalence of the novel VAGs though these were not associated with pathogenicity[1]. These isolates represented a small proportion of our sample set. Additional investigation of these less common serotypes and STs could further reveal trends with regards to the novel VAGs. We believe that each predictive approach has merit and their use in tandem can increase confidence in differentiating between commensal and pathogenic strains for autogenous vaccine development.

1. Estrada AA, Gottschalk M, Rossow S, Rendahl A, Gebhart C, Marthaler DG (2019) Serotype and genotype (multilocus sequence type) of *Streptococcus suis* isolates from the United States serve as predictors of pathotype. J Clin Microbiol 57:e00377-e419. https://doi.org/10.1128/JCM.00377-19

2. Estrada AA, Gottschalk M, Gebhart CJ, Marthaler DG (2022) Comparative analysis of *Streptococcus suis* genomes identifies novel candidate virulence-associated genes in North American isolates. Veterinary Research 53:23 https://doi.org/10.1186/s13567-022-01039-8

## Poster 150

### Whole genome sequencing of *Leptospira* isolates from animals: a One Health approach to improve the biobank for future genomic research

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Leptospirosis is an important neglected zoonotic disease. The genus *Leptospira* is currently divided into 68 species classified into four phylogenetic clusters. Leptospires are maintained by chronic carrier hosts in their renal tubules and excreted into the environment through their urine. Infection in humans or animals results from direct contact with infected reservoir animals or indirect exposure to contaminated environments. Integrating the knowledge of human, animal, and environmental health is essential for controlling and predicting zoonotic diseases. Though the culture of pathogenic *Leptospira* from animals is inherently difficult due to fastidious growth, a recovered isolate is essential for accurate epidemiology and the ability to perform comprehensive genome analysis. In this study, we sequenced the genomes of *Leptospira* isolated from animals and identified species that improve our biobank for future genomic research in diagnosis and control strategies.

*Leptospira* strains used in this study were isolated using Hornsby-Alt-Nally (HAN). Genomic DNA was prepared by centrifugation of exponential-phase cultures and extracted with the Maxwell RSC Purefood Purification Pathogen kit. DNA from 105 *Leptospira* isolates was whole genome sequenced using Illumina Nextera XT DNA Library Kit. Comparing these 105 isolates, along with publicly available reference genomes, species identity was confirmed by inspecting the 16S rRNA gene, calculating the average nucleotide identity, and comparing assemblies using kSNP (a reference-free phylogenetic analysis program). Genomes have been deposited in the NCBI Sequence Read Archive and GenBank. These genomes can be used to compare local isolates for epidemiological studies. For example, an isolate that is *L. borgpetersenii* can be a vary greatly genetically from the *L. borgpetersenii* in the biobank. It is important to have a diverse database for comparison.

This work highlights the importance of culture and encourages the ability to isolate *Leptospira*, a notoriously fastidious gram-negative bacteria, in HAN medium from animal samples. Whole genome sequencing indicated that at least four different species were isolated: 12 *L. interrogans*, 76 *L. borgpetersenii*, 1 *L. santarosai*, 6 *L. kirschneri* and 10 cultures that contained mixed species. The present study shows the diversity of the *Leptospira* species isolated from animals. The increasing availability of *Leptospira* genomes in our biobank has created new opportunities for future genomic studies. With these genomes there can be improvement of molecular epidemiological studies and the identification of relevant target genes by comparative genomics. This will help improve the relevance of future diagnostic tools and discovery of vaccine candidates.

# Poster 151

## Performance of RT-qPCR for the detection of *Mycoplasma spp.* in milk samples and results in the evaluation of diagnostic accuracy on pooled samples

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Mastitis is a common and costly disease among dairy cows. Mastitis pathogen identification is critical in defining effective herd management strategies.

Many molecular tests have been developed, but the bacteriological culture still is the gold standard for mastitis diagnostics. *Mycoplasma* spp., being a highly contagious and slow-growing organism, is the pathogen with high interest and diagnostic demand.

This study aimed to evaluate the performance of an RT-qPCR assay for the detection of *Mycoplasma* spp. and identification of *M. bovis* in the field and spiked milk samples. Additionally, the pooling capability of the assay was evaluated. RT-qPCR results were compared to standard mycoplasma culture.

Field milk samples (n = 88) were collected from mycoplasma culture submissions; 48% were positive (n = 42), and 52% were negative (n = 46). A portion of the mycoplasma positive samples (n = 11) represented cases where individual positive cows were identified by pre-culture pooling procedure that combined milk from 5 cows. All pools from the field represented samples with milk from 1 positive cow mixed in approximately equal proportions with 4 negative cows. Information about mycoplasma's load and, identification at species level was not provided by routine mycoplasma culture. Therefore, spiked milk samples (n =147) were prepared, inoculating known concentrations of *M. bovis* and other *Mycoplasma* spp. in milk known to be negative by culture and qPCR. Bacterial concentrations artificially inoculated ranged from 10^4 to 10^2 cfu/ml. Performances of RT-qPCR vs culture were evaluated for the entire set of dilutions, either if tested as individual cows or as pools of 5 samples (1 positive / 4 negatives).

Detection by culture was conducted on modified Hayflick agar and incubation at 37°C with 5% CO2 for 3 -7 days (NMC 2017). Molecular testing was performed using kits and instruments supplied by ThermoFisher Mastitis Diagnostics. DNA from milk was extracted by MagMAX™ CORE Mastitis & Panbacteria Module and processed by KingFisher. The RT-qPCR chosen for the evaluation was VetMAX MastiType Myco8 Primer Mix 1.

Overall analysis of this study found that culture and RT-qPCR performed similarly.

On-field samples, it was obtained a 98% agreement; 2% of disagreement was associated with samples resulting in *Mycoplasma* spp. positive by culture but inconclusive by RT-qPCR. Samples in disagreement represented a pooled sample and the respective individual cow, both highly contaminated with other organisms.

For spiked samples, it was obtained a 100% agreement at the lowest level of 10^3 cfu/ml; the same samples if pooled with 1 to 4 negative samples, still got 100% detection by RT-qPCR at genus and species levels.

Data of this study will be confirmed by testing a higher number of field samples.

For Research Use Only. Not for use in diagnostic procedures.

## Poster 152

### Molecular characterization of *Mycoplasma* spp. in red-tailed hawks (*Buteo jamaicans*) by PCR-RFLP

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Red-tailed hawks (*Buteo jamaicans*) submitted to the Alabama Veterinary Diagnostic Laboratory (AVDL) were tested for the presence of mycoplasmas. Fifty-four birds were tested by polymerase chain reaction (PCR) and 28 (51.8%) were found to be positive for mycoplasmas. The DNA amplicons from the positive birds were then analyzed by restriction fragment length polymorphism (RFLP) using four different restriction enzymes. According to the RFLP patterns generated, eight different species of mycoplasmas were identified among the 28 positives. The RFLP pattern of one mycoplasma species matched with *Mycoplasma gypis*. Three species matched reference field strains at the AVDL and the other four species patterns did not match any reference pattern available. These eight mycoplasma species were then examined for host specificity, pathogenicity, and infection frequency. The results of this study show that, in red-tailed hawks, mycoplasmas are commonly detected and multiple mycoplasma species can be identified. The mycoplasmas detected do not display high host specificity and show no apparent pathogenicity. The results of this study show that PCR-RFLP assays are effective at characterizing different mycoplasma species detected in raptors.

## Poster 153

### Rapid strain typing of viral hemorrhagic septicemia virus by metagenomic sequencing of Pacific herring (*Culpea pallasii*) tissue on the GridION long-read sequencing platform

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Viral Hemorrhagic Septicemia Virus (VHSV) was detected by real-time PCR, following NVSL-SOP-0331, on a tissue pool of brain, liver, and heart from herring collected by a regional zoo. To test the deployability of our unbiased metagenomic sequencing workflow for viral strain typing, an aliquot of the same extracted RNA was converted to second-strand cDNA by sequence-independent single primer amplification (SISPA) and used as template for Nanopore library construction.

During sequencing, data were analyzed in real-time on the web-based analysis suite Epi2Me, by Oxford Nanopore. Metagenomic classification was performed using the WIMP workflow and reads were aligned to VHSV strain DK-9895174 (Accession no. MK829413.1, genotype I-a) on the Fastq Custom Alignment module.

Within 90 minutes of sequencing initiation, we had captured 220 reads aligning to our reference (a total of 174.7Kbp with an average of 86.9% nucleotide identity to the reference). After 4.5h, we downloaded the cumulative data and mapped all raw reads to MK829413.1. Mapped reads (n=716) were I assembled into two contigs using SPAdes. Our total assembly length was 11,547bp and these two contigs covered 85.2% (9505 bases) of the reference length 11,159bp when aligned.

Depth of sequencing at the N and G protein coding regions allowed us to determine that our strain was genotype IVa, and when searched against the NCBI non-redundant nucleotide database using megaBlast, the contigs of our assembly matched most closely to VHSV isolate Coho salmon/Washington/20-036527/2020 complete genome accession number MW574348.1.

By performing unbiased metagenomic sequencing on SISPA-amplified nucleic acids extracted from pooled tissues of a single animal we were able to obtain 99.8% raw read coverage of the entire VHSV genome, and this was in our sample with the highest Ct value by real time PCR. Even more unexpectedly, the sample with the lowest Ct value yielded the fewest VHSV reads from our metagenomic workflow, covering just 61.4% of the VHSV genome. We don't yet understand the correlation (if any) between qPCR results and the probability of detecting viral signatures in metagenomic sequencing, but we have shown that the GridION third-generation sequencing platform is capable of confidently detecting viruses in clinical samples within a few short hours. This reduced turnaround promises to become a boon to the utilization of genomic data for tracking and responding to emerging infectious diseases.

## Poster 154

### Efficiency standardized PRRSV serum RT-qPCR results * †

*Betsy Armenta-Leyva[1], Berenice Munguia-Ramirez[1], Ting-Yu Cheng[2], Alexandra Henao-Díaz[3], Kent Doolittle[4], Silvia Zimmerman[4], Luis G. Gimenez-Lirola[1], Jeffrey Zimmerman[1]*

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Over time, the repeatability and reproducibility of PCRs has been improved by the widespread use of commercial extraction and amplification kits. However, further improvement may be achieved by expressing results relative to a reference standard (RS), as is done in basic research when PCR results are expressed as "efficiency standardized quantification cycles" (ECqs):

$ECq = E^{-\Delta Cq}$ where E = amplification efficiency and ΔCq = (sample Cq - RS Cq).

The objective of this study was to explore the application of the ECq methodology to routine diagnostic testing.

Samples of known status were created by vaccinating pigs (n = 4) with a PRRSV MLV (Ingelvac® PRRSV MLV) and collecting serum samples (n = 44) on days post vaccination (DPV) 0, 5, 8, 11, 14, 17, 21 and 28. Samples were tested using commercial reagents (RealPCR* DNA/RNA Spin Column Kit, RealPCR*RNA Master Mix and RealPCR*NA PRRS Types 1-2 RNA Mix, IDEXX Laboratories, Inc.) and the MIC PCR™ Cycler (Bio Molecular Systems, Australia).

Target specific (PRRSV RNA) and matrix-specific (serum) reference standards (RS) were created by rehydrating a PRRSV MLV vaccine (Ingelvac® PRRS MLV) with PRRSV-free serum and then diluting ($1 \times 10^4$) with the same matrix. Testing was performed in 48-well plates with RSs (n = 4) included on each plate. Sample results and plate RS efficiencies were calculated by commercial software (MIC PCR™, v2.10.4). The "E" for each plate was then calculated as the mean of the 4 RSs on the plate and the ΔCq was for each sample calculated as (sample Cq – mean RS Cq).

Across 8 plates, the mean plate RS Cq and E responses were 30.6 and 90%, respectively. All serum samples were negative on DPV 0 (ECq 0, Cq ≥ 40) and all were positive on DPVs 5 - 28 (mean ECq = 8.4, mean Cq = 31.1). In this study, ECq represents the PRRSV RNA fold change in a sample relative to the plate reference standards. For example, an ECq of 8.4 indicated that the concentration of PRRSV RNA was 8.4 times the concentration in the RS.

Accounting for amplification efficiency (E) improves test consistency because sample Cqs are directly related to E and assuming 100% E will lead to the over estimation of target concentration. In addition, all results have an ECq numeric value, i.e., "indeterminate" Cq results become zeros. Therefore, it is possible to calculate cutoffs and evaluate diagnostic performance using receiver operating characteristic analysis.

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

## Poster 155

**Development of a standard virome panel for the detection of animal viruses using next-generation sequencing**

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Next-generation sequencing (NGS) give us tremendous ability to detect and characterize emerging, re-emerging, zoonotic, and foreign animal disease (FAD) viruses in unbiased manner. In public health laboratories, reference standards and guidelines are available for implementation and validation of NGS, quality control, and testing of vaccines and other biologicals. However, Veterinary Diagnostic Laboratories (VDLs) don't have such reference standards and guidelines for optimization and validation of NGS protocols. To fill this gap, we developed animal virome panels of selected RNA (n=9) and DNA (n=5) viruses, as a reference material for NGS method optimization and validation from nucleic acid extraction to library preparation. We quantified and pooled RNA and DNA viruses based on $TCID_{50}$/mL at high and low virus titers and determined their Ct values with PCR. We spiked clinical samples with these pooled viruses and optimized the virus enriched NGS library preparation methodology and bioinformatics pipeline. We detected all viruses in the reference standard successfully. We believe these quantified RNA and DNA viral panels will be very helpful for the VDLs to use as a reference material for optimization and validation of their NGS assays for viral disease investigation and whole genome sequencing.

## Poster 156

### Whole genome sequencing to characterize and solve identification conflicts in the diagnosis of *Edwardsiella piscicida* and *Edwardsiella ictaluri*

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Bacteria included in the genus *Edwardsiella (E.)* are gram-negative, glucose fermentative, and catalase positive. *E. tarda*, *E. hoshinae*, *E. ictaluri*, *E. piscicida,* and *E. anguillarum* are the species currently recognized in this genus. Differentiation of *Edwardsiella spp* based on their phenotypic characteristics is challenging. Ten isolates from fish samples previously examined by phenotypical characterization, 16S rDNA sequencing, and VITEK MS system presented conflicting results among these 3 identification methods. All ten isolates were identified as *E. hoshinae* by Vitek. Five were identified as *E. ictaluri* and five were identified as *E. piscicida* by phenotypical characterization and 16S rDNA sequencing. To help solve the conflicts between these results and further characterize these isolates, whole-genome sequencing was performed on iSeq 100. Frozen *Edwarsiella spp* (-80°C) strains were inoculated on blood agar plates and incubated at 30°C for 48 hours. DNA from characteristic colonies was extracted using DNeasy blood and tissue kits (Qiagen), measuring its concentration using Qubit4. The library was prepared using Nextera DNA Flex library prep kit, and sequencing was performed on iSeq 100. Data were analyzed using MicroRunQC and NARMS AMRFinder workflow shared by FDA. Five isolates shared identity of over 99.96% with E. ictaluri_93_146, and five isolates shared identity of over 99.2% with E. piscicida reference strain 18EpOKYJ. Sequencing coverage for each isolate was 30x or higher. The identification of these bacterial isolates was consistent with phenotypic characteristics and 16S rDNA. Antimicrobial resistance genes were identified from three *E. ictaluri* and all five *E. piscicida* isolates. These results will be discussed during the presentation.

## Poster 157

### Nanopore sequencing: the promising tool for closing the gap in avian bacterial pathogens and generating high-quality closed genome sequences

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Genomic characterization of different bacterial pathogens allows for a better understanding of disease dynamics and subsequently improves the formulation of effective prevention and eradication practices. The low cost and high accuracy of most short-read DNA sequencing platforms make them well suited to high-throughput bacterial genomics. However, due to the nature of the generated short reads, they cannot resolve all genomic repeats and only generate incomplete bacterial genome drafts. Oxford Nanopore Technology (ONT), a platform that can generate ultra-long reads of million base-pair lengths, enables resolution of genomic complexities and allows the generation of closed complete bacterial genomes, achieving the ultimate genomic characterization of bacterial agents. ONT presents a low-cost and versatile tool for generating these high-quality genomes for severely understudied pathogens. In this project, a total of seventeen bacterial genomes of three important avian bacterial pathogens [*Pasteurella multocida* (n=13)*, Ornithobacterium rhinotracheale* (n=3) and *Avibacterium paragallinarum* (n=1)] from clinical isolates were sequenced using Illumina and ONT sequencing platforms. The hybrid assembly approach with the Unicycler assembler was used to harness the high accuracy of the short-read sequencing platform (Illumina) with the long reads generated by ONT to close the gaps, complete and finish these genomes. The availability of these complete genomes will pave the way for a better understanding of bacterial populations, pathogenicity factors and antimicrobial resistance of these pathogens, which offers new insight into the prevention and control strategies of these diseases in various poultry species.

## Poster 158

### Detection and subtyping of epizootic hemorrhagic disease virus with sensitive and specific real-time PCR assays

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Epizootic hemorrhagic disease (EHD) is an infectious viral disease transmitted by biting midges, the genus of *Culicoides*. The causative agent, Epizootic hemorrhagic disease virus (EHDV) is a double-stranded RNA non-enveloped virus, in the family of *Reoviridae* and the genus of *Orbivirus*. There are 10 double-stranded RNA genomic segments coding for seven structural proteins and five nonstructural proteins in the virus core. Currently, 7 different serotypes are identified worldwide. Among them, EHDV1, 2, and 6 have been reported in the United States. Previously, the EHDV screening PCR assay in our laboratory was adapted from the method published in the OIE Manual of diagnostic tests and vaccines for Terrestrial animals 2018. In the current study, a real-time PCR assay for screening of EHDV and a multiplex PCR assay for subtyping of EHDV are verified in our laboratory. These assays were based on the newly released OIE Manual of diagnostic tests and vaccines for Terrestrial animals 2021. In this report, we compared and verified the current EHDV screen PCR assay with the previous 2018 EHDV screening PCR assay. The results showed that the current EHDV PCR screen assays are approximately 100-fold more sensitive than the 2018 methods and detected more positive samples from the clinical samples submitted to our laboratory during the EHD outbreak in white-tailed deer in Ohio in 2021. Besides, the multiplex PCR assay for subtyping EHDV is sensitive and specific with successful subtyping of EHDV viruses from all the screened EHDV positive samples.

## Poster 159

### Success and challenges of testing for influenza A virus in swine samples collected in 2021

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Influenza A virus (IAV) is one of the most important respiratory pathogens of swine. It is also of public health importance and the USDA has a surveillance program to track the trends of this infection over time. Therefore, it is one of the pathogens most commonly tested at the Minnesota Veterinary Diagnostic Laboratory. During 2021, a total of 11,061 swine samples were tested for IAV by RT-PCR. Of these samples, 23% were lung, 38% were nasal swab/wipe, 34% were oral fluids and the remaining 5% were other specimen types. The overall positivity rate was 16.8% using Matrix gene PCR and considering the specimen type, it was 16% for lung, 9% for nasal swab/wipe and 23% for oral fluids. Of the positives, 735 samples were further tested by PCR to determine the subtype. Of these, 29% were H1N1, 26% were H1N2, 29% were H3N2 and the remainder 16% were other combinations (including mixed infections). Virus isolation was attempted in 714 of the positive samples with an overall success rate of 61%. The success rate for virus isolation was found to be dependent of the Ct value and the specimen type. For example, virus isolation was successful in all samples with a Ct value lower than 20, regardless of the specimen type. In contrast, the virus isolation success rate was 83% for lung, 70% for nasal swabs/wipes and 47% for oral fluids, in samples with a Ct≤30. Sequencing of the hemagglutinin gene (H) and the neuraminidase gene (N) was successful in 264 and 160 samples, respectively. Similar to what was observed for virus isolation, the sequencing success rate was dependent of the Ct value and the specimen type. H1 sequences were classified in 10 different clades while H3 sequences were classified in 4 different clades. Due to the high genetic variability of IAV, several cases were identified where the tests did not perform as expected. Examples of this are: 1) cases where the subtyping PCR was unable to detect a subtype, while Matrix PCR and sequencing were successful; 2) cases where the Matrix and subtyping PCR worked well but the H or N gene could not be sequenced; 3) cases where the PCR tests performed well but a rapid pen-side test performed during virus isolation failed to detect the growing virus. In Summary, influenza virus is an important pathogen of swine and is commonly found in diagnostic samples. Due to its high genetic and antigenic variability, IAV will continue to create challenges for swine veterinarians and diagnosticians. Therefore, the availability of multiple, robust, affordable and up to date diagnostic methods is critical for the accurate diagnosis and monitoring of influenza infection.

## Poster 160

### Evaluation of IDEXX's RealPCR Infectious Bronchitis RNA Mix PCR for the detection of infectious bronchitis virus RNA

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Infectious Bronchitis Virus (IBV) is an economically significant avian coronavirus in the meat and egg-laying poultry industry. Early detection and quantification of IBV is essential for preventing potential outbreaks in flocks and production of vaccines. Newport Laboratories, recently acquired by Vaxxinova, is an autogenous vaccination company that characterizes and quantifies pathogens using real-time polymerase chain reaction (PCR) for vaccine production.

The aim of this study is to evaluate the detection of IBV RNA using the IDEXX PCR reagents. The IDEXX PCR can be used to extrapolate the bio-concentration of IBV RNA in a sample. Using a standard curve, made from a dilution series of Synthetic IBV RNA, we can achieve a 3-copy sensitivity. These results indicate that the IDEXX RealPCR IBV assay is suitable for signaling the presence of IBV RNA over a 6-log range of unknown samples without assay inhibition. Following the detection of IBV RNA in a sample, we evaluated multiple IBV strains at various concentrations relative to one another within the same sample using different IBV strain specific PCRs.

## Poster 161

### Detection of porcine parainfluenza virus 1 from metagenomic whole genome sequencing of swine samples

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Porcine parainfluenza virus 1 (PPIV1) is a single-stranded, segmented RNA virus, belonging to the family of *Paramyxoviridae*. Since first detected in 2013 in slaughterhouse pig samples in Hong Kong, China, PPIV1 has been reported in multiple countries in Asia, Europe, and the Americas. Retrospective studies with RT-PCR test and serological test suggest PPIV1 is widespread in swine in the United States. PPIV1 is believed to be transmitted via the respiratory route and causes mild respiratory clinic signs such as coughing and sneezing. In this study, samples from four pigs were subjected to metagenomic sequencing for other research purposes, and a nearly full-long genome of PPIV1 was accidentally identified in one out of the four pigs. The methods used for the study and the results are summarized here.

RNA extracted from lung homogenate, and nasal swab were subjected to sequencing library preparation using Zymo-Seq RiboFree Total RNA Library Kit. The libraries were pooled and then sequenced with a MiSeq V2 (2 x 250 bp) reagent kit. Sequencing data were analyzed using CLC Genomics Workbench 22.0 following steps of 1) quality analysis, 2) adapter and low-quality trimming, 3) taxonomic profiling against the Reference Viral DataBase (RVDB) and the swine genome, 4) de novo assembling, 5) BLAST against the ref_viruses_rep_genomes database, 6) coverage analysis and 7) phylogenetic tree construction.

Six to nine million reads were obtained for each sample in the sequencing. Taxonomic profiling found that host sequences account for over 90% of the total reads in the three lung samples, and only less than 0.1% of the reads are classified as viral sequences. In contrast, 3.4% of the reads in the nasal swab sample belong to viral sequences, and only 38.1% of the reads are from the host genome, while the other 58.5% may be from bacteria and fungi. De novo assembling of the viral reads and then BLAST searching in the ref_viruses_rep_genomes database identified a near full-long genome of PPIV1 in the nasal swab sample. Taxonomic profiling was conducted against all PPIV1 sequences downloaded from the NCBI Nucleotide database to the reads from the four lung samples, and no PPIV1 sequence was identified in the samples. The nearly full-long genome identified in the nasal swab is 15,340 bp. BLAST searching demonstrated that it has 95.67% sequence identity to the full-long 15,396 bp reference strain (RefSeq: NC_025402.1). A maximum-likelihood phylogenetic tree was constructed using the newly identified PPIV1 genome and all available full-long and nearly full-long genomes in the NCBI Nucleotide database. These genomes are clustered into two distinct clades. One clade consists of this genome, all other genomes obtained in the United States (n=4), and two from China. The other clade includes the isolates obtained from Asia, Europe, and South America.

## Poster 162

### Development of two novel INgene® PRRSV qPCR assays of broad inclusivity intended for screening and typing.

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Porcine reproductive and respiratory syndrome (PRRS) is probably the most relevant viral disease affecting swine production. One hallmark of PRRSV is the extreme genetic diversity of its genomic RNA due to mutation and recombination. The two major genotypes, the European (type 1) and the North American (type 2) diverge by approximately 40% at their sequence, and intra-type variation can be up to 30%. Molecular tools are key for PRRSV diagnostics, but qPCR assays need to be redesigned frequently to account for emerging variants.

In this work, two updated assays for PRRSV detection are presented. INgene® PRRSV NA-EU assay is a triplex qPCR assay based on hydrolysis probes that differentiates the NA and EU types, together with a workflow control. Assay inclusivity was tested with synthetic RNA and with a panel of 13 reference strains, covering main lineages/subtypes including highly pathogenic strains. Analytical sensitivity $LOD_{RT\text{-}PCR\ 95\%}$ was of 5 copies/ul in the fast mode. INgene® PRRSV Universal is a multiplex real time PCR assay based on hydrolysis probes that detects both PRRSV types and a workflow control. The assay showed wide inclusivity and higher sensitivity than the differential assay with most of the reference strains and field samples tested, including serum, oral fluids and semen. Both assays share the same FAST cycling protocol.

Two new qPCR assays for PRRSV detection were developed, the PRRSV NA-EU is broadly inclusive for typing and the Universal is highly inclusive and sensitive assay for surveillance.

## Poster 163

### Use of *lipL32* rt-PCR to identify animal carriers of *Leptospira* spp.

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Leptospirosis is a neglected zoonotic disease of worldwide importance caused by pathogenic spirochetes belonging to the genus *Leptospira* and causes significant morbidity and mortality in both humans and domestic animals. Animals excrete these bacteria in their urine, resulting in environmental contamination and potentially leading to zoonotic transmission. Leptospires are also transmitted via the genital tract in domestic livestock. This study showed the importance of using *lipL32* rt-PCR to identify animal carriers of *Leptospira* spp.

A total of 747 kidney (471 rodents and 276 mongooses) and 354 urine (21 goat, 33 sheep, 6 horses, 15 dog and 279 cattle) samples were tested by *lipL32* rt-PCR during May 2020- December 2021.

Positive carrier status was identified by *lipl32* rt-PCR in 186/747 (24.9%) kidney samples (150 rodents and 36 mongooses) and 45/354 (12.7%) urine samples (1 goat, 2 sheep, 3 horses, 5 dogs and 34 cattle). These findings indicate that those animals are reservoir hosts of this zoonotic disease, showing how various species of animals might play a role in the epidemiologic cycle of leptospirosis and the potential risk for exposure and infection to humans, domestic animals, and other species at risk. The rt-PCR has high sensitivity and specificity, being useful for screening to identify animals infected with *Leptospira* spp. Our findings suggest that additional studies to isolate leptospires from naturally infected animals is essential to better understand the epidemiology of leptospirosis and to update diagnostics and bacterin-based vaccines used for control.

## Poster 164

### A qualitative impact evaluation of the Ohio Animal Disease Diagnostic Laboratory * ◊

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The Ohio Animal Disease Diagnostic Laboratory (ADDL) services various animal agriculture sectors in the state of Ohio that have identified committed goals to increase their production systems exponentially over the next 20 years. Will the ADDL be able to meet the demands of its clients over the next 20 years, as animal agricultural production systems expand? What impact does the ADDL's performance have on its clients, animal agriculture, and the nation? A qualitative evaluation was conducted to identify issues that might hinder the laboratory's ability to keep up with the predicted growth of its clientele, to assess what impact this might have on the animal agriculture industry, and its correlation to supporting public health measures in Ohio.

Since 2015, genetics and regulatory industries have significantly increased their spending on ADDL services, with genetics making up a majority of client spending. If these trends continue over the next 20 years, genetics will contribute approximately 56.8% of ADDL's revenue generated by testing services. Private practitioners, researchers, and swine industries have decreased their client spending which may be a result of some clients choosing to outsource samples to other veterinary diagnostic laboratories (VDLs) that may be more competitive in price and turnaround time. The following laboratory sections: avian serology, bacteriology, molecular, and pathology are expected to see increases in laboratory testing. The remaining laboratory sections are expected to see a decline in testing each year. Lastly, average turnaround time ratios for each laboratory section revealed that the virology and serology sections are experiencing difficulties in minimizing turnaround times which is a result of understaffing in those sections of the laboratory.

Furthermore, according to the opinions gathered during stakeholder interviews, the Ohio ADDL has made a significant impact in the lives of its clients and the animal agricultural industries in Ohio. The clients agreed that having the ADDL in Ohio was essential for the success of their businesses despite drawbacks in performance. Stakeholders also agreed that services provided by the ADDL allowed them to better monitor animal health and prevent the spread of disease, ultimately supporting public health initiatives and maximizing profitability for those clients.

* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

## Poster 165

### NAHLN avian disease PCR assays: alternative reagents ◊

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A limited number of equipment and reagents are approved for NAHLN Influenza A viruses (IAV) and Avian paramyxovirus Type-1 (APMV-1) testing, and alternative reagents and equipment are essential to assuring the continuity of testing supplies during an outbreak situation. Aside from outbreak testing, NAHLN laboratories regularly conduct active or passive surveillance testing. The sample number for routine diagnostics and surveillance testing tends to be smaller due to the ability to pool samples. Thus, both low and medium-throughput platforms for IAV and APMV-1 testing should be encouraged, especially equipment and reagents already available in NAHLN labs for routine diagnostic testing. Low and medium-throughput platforms also require fewer plastic consumables, which is especially important as supply chain deficiencies have become a significant challenge for diagnostic laboratories during the current COVID-19 pandemic.

This project evaluated alternative equipment and reagents from Indical Bioscience to supplement the repertoire of approved supplies for IAV and APMV-1 testing. The IndiMag 48s allows for rapid nucleic acid purification of up to 48 samples per extraction using only two plastic consumable cartridges. The IndiMag 48s allows for 1, 8, or 24 sample plastic formats for increasing flexibility, additional plastics savings, and further environmentally friendly. Thus, the IndiMag 48s is a viable medium-throughput option for the IAV and APMV-1 assays. The IndiMag Pathogen Kit is an approved reagent for Foot-and-mouth disease virus, Classical swine fever virus and African swine fever virus PCR assays and was proven as an excellent alternative extraction kit for IAV and APMV-1 assays. The IndiMag Pathogen Kit is packaged in 2 different kit formats, the standard manual fill kit and the prefilled cartridges. Both formats are available for IndiMag 48S and KingFisher family extractors. The prefilled design streamlined the extraction process and is especially valuable when rapid testing results were needed during an investigation or outbreak situation. The IndiMix JOE is a premix master mix that includes the primer/probe for Intype IC internal control for a multiplex PCR. The Intype IC RNA can be used as an extraction control when included during the extraction process, which is very important for extracting nucleic acid from sample types with high inhibitory, such as tissues or environmental samples for post-cleaning and disinfection testing in an outbreak. Overall, this study provides data to support the IndiMag 48S, IndiMag Pathogen manual and prefilled formats, IndiMix JOE, and Intype IC RNA as an alternative for NAHLN IAV and APMV-1 testing.

◊ USAHA Paper

## Poster 166

### Correlation of avian reovirus tenosynovitis- and viral RNA and development of *in situ* diagnostic assay for avian reovirus antigens # + * †

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Avian reovirus (ARV) is a non-enveloped RNA virus causing tenosynovitis in chickens and turkeys. Etiological diagnosis is based on virus isolation and/or reverse-transcriptase real-time polymerase chain reaction (RT-qPCR) from tissues with characteristic gross lesions. However, due to the ubiquity of the virus, virus isolation and qPCR may yield false-positive results. Therefore, developing specific direct detection methods such as immunohistochemistry or *in-situ* hybridization is necessary for accurate disease diagnosis. The aim of this study is to investigate 1) the correlation between the severity of histological lesions and viral RNA (PCR Ct values), and 2) to develop an *in-situ* diagnostic methods to confirm the presence of ARV antigens in lesions of tenosynovitis. Twenty-eight cases from 2016 to 2022 were retrieved from the archive of Veterinary Diagnostic Laboratory, Iowa State University. Case selection criteria included: 1) reported clinical signs of lameness, 2) histopathologic features of tenosynovitis and synovial hyperplasia, and 3) ARV nucleic acid detection by qPCR. A subset of cases with histological lesions of tenosynovitis but negative qPCR results for ARV were used as negative controls. Histologic features of tenosynovitis were scored using a published scoring system (Sharafeldin et al., 2014) and based on 1) severity of inflammation, 2) synovial proliferation, and 3) presence of lymphoid nodule(s), neovascularization, and/or fibrosis. qPCR protocol was based on a previous study (Tang and Lu, 2016). A mouse polyclonal antibody (Pab) was developed with chicken reovirus (ARV type-1 Fahey-Crawley strain) and turkey reovirus (field isolate ISU21-18681) and validated with indirect immunofluorescence assay. The mouse Pab was used for *in situ* viral antigen detection by immunohistochemistry (IHC). Our results showed that qPCR-positive cases (68%, 19/28) had more severe inflammation and synovium proliferation ($p < 0.05$), and the sum of histological scores was significantly higher in qPCR-positive cases. However, there was no correlation between the severity of lesions and the amount of nucleic acid detected by qPCR. The lack of correlation may be due to confounding factors such as co-infections with other pathogens or the lack of specificity of the histopathology for ARV diagnosis. This observation indicates the importance of reliable *in situ* viral detection tests that can support ARV diagnosis and pathogenicity studies to elucidate the role of ARV in cases of tenosynovitis. Our newly-developed Pab was able to stain macrophages in the tenosynovitis of ARV experimentally infected turkeys. Further studies will evaluate the performance of IHC and its complementary role with histopathology and qPCR for ARV-related tenosynovitis diagnosis.

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

## Poster 167

### A retrospective analysis of bovine respiratory syncytial virus in bovine diagnostic cases submitted to the Iowa State University Veterinary Diagnostic Laboratory

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Bovine respiratory syncytial virus (BRSV) continues to represent a significant component of respiratory diagnostic cases received at the Iowa State Veterinary Diagnostic Laboratory (ISU-VDL), representing the 5$^{th}$ most diagnosed respiratory pathogen and accounting for approximately 4% (66) of all respiratory diagnoses (1593) from 9/1/2020 to 9/1/2021. In the same time interval, 55 case accessions with histopathology, representing 64 individual animals were diagnosed with BRSV and positive by real-time RT-PCR or immunohistochemistry (IHC) in lung tissue received at the ISU-VDL. Lung tissue from all 64 animals was retrospectively evaluated by routine histopathology for morphologic changes in airways (i.e., bronchi and bronchioles), alveolar spaces, and the pulmonary interstitium. Bronchopneumonia was the most frequent histological change observed (61 animals, 95.3%), followed by bronchitis and necrotizing bronchitis or bronchiolitis (58 animals, 90.6%). Syncytial cells were observed in bronchi and bronchioles of 18 animals (28.1%) with eosinophilic, intracytoplasmic inclusion bodies observed in 8 animals (12.5%). Interestingly, varying degrees of diffuse alveolar damage were evident in 20 animals (31.3%), characterized by the presence of hyaline membranes. There were bacterial culture results from 60 individual lungs (52 out of 55 cases accessions) with *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni*, and *Trueperella pyogenes* isolated in 25, 14, 13, and 4 animals, respectively. PCRs for bovine herpesvirus 1 (BoHV-1), bovine coronavirus (BCoV), and bovine viral diarrhea virus (BVDV) were performed on 51 individual and 6 pooled lung cases. BCoV and BVDV nucleic acid was detected individually in five and three animals, respectively. A high amount of BRSV nucleic acid was detected in all diagnosed BRSV cases, with an average cycle threshold value of 25.4 (16.4-34.9) across individual and pooled cases. A bovine respiratory bacterial multiplex PCR panel detecting *P. multocida*, *H. somni, M. haemolytica*, and *M. bovis* was performed individually on 42 lungs with 4 pooled PCR reactions. *P. multocida*, *H. somni*, *M. bovis*, and *M. haemolytica* nucleic acid were detected in 30, 22, 20, and 13 cases, respectively. Clinical cases of BRSV in cattle are often complicated by co-infection by secondary bacteria and less commonly respiratory viruses. Interestingly, approximately 30% of the animals diagnosed with BRSV demonstrated lesions of diffuse alveolar damage that can result in acute respiratory failure and death. These findings indicate that BRSV continues to represent a diagnostic challenge, requiring careful interpretation of histologic changes and ancillary diagnostics to formulate accurate diagnoses in clinical cases of bovine respiratory disease.

## Poster 168

**Avian cholera outbreak in wild waterfowl and greater sandhill cranes in a wetland in New Mexico ◊**

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In late December, 2021, two lesser snow geese and two greater sandhill cranes were submitted for postmortem examination to investigate the death of 30 birds that included a variety of duck species, lesser snow geese and greater sandhill cranes at a waterfowl management area. At postmortem examination, the birds were in good body condition. There were no significant gross lesions in the snow geese. The two greater sandhill cranes had gross lesions of multifocal necrotizing hepatitis and splenitis. The histopathology of all four birds was similar with random multifocal necrotizing heterophilic hepatitis and splenitis with intralesional bacteria. *Pasteurella multocida* was isolated from a pooled liver and spleen sample from each species. PCR testing was negative for avian influenza virus and avian paramyxovirus. The diagnosis of avian cholera (fowl cholera) was made. The staff at the waterfowl area began removing carcasses of dead birds as soon as they were found limiting the outbreak to only 100 dead birds.

*Pasteurella multocida* is a gram-negative coccobacillus bacterium that is the causative agent of avian cholera (fowl cholera). Avian cholera is known to occur in at least 180 species of birds. Among wild birds, it most frequently occurs in North American waterfowl within wetlands and nesting areas. Avian cholera is typically an acute respiratory and septicemic disease with death occurring within 24-48 hours. *P. multocida* is most likely to be transmitted between birds by ingestion of the bacterium from the contaminated environment as nasal secretions of sick birds and carcasses of dead birds contain large numbers of bacteria that contaminate the environment. Immediate removal of carcasses from the outbreak area is important for the management of avian cholera in wild birds as the bird carcasses will continually contaminant the environment with *P. multocida* if left to naturally decompose or be consumed by scavengers. How *P. multocida* is transmitted between outbreak areas is not known, but it is believed that some lesser snow geese and Ross's geese can be carriers of *P. multocida*. In some wetlands, an increase in the population of lesser snow geese has been associated with an increase in the incidence and mortality numbers of avian cholera. It has been shown that *P. multocida* cannot be isolated from the wetland environment after 7 weeks of an outbreak. Thus, long term persistence of the bacterium in the environment is not likely the source of multiple outbreaks in the same area that occur years apart.

In acute avian cholera, gross lesions may or may not be present. Typical gross lesions include generalized congestion; hemorrhages in the heart, epicardial fat, abdominal fat, mucus membranes and gizzard; multifocal necrotizing hepatitis; and multifocal necrotizing splenitis. The microscopic lesions in the liver and spleen are coagulative necrosis and heterophilic inflammation. Heterophilic inflammation can also occur in the lungs and other organs.

◊ USAHA Paper

## Poster 169

### Transmission electron microscopy detection of viruses in goats with enteritis in California *

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Diarrhea is the most common and costly syndrome affecting newborn small ruminants. Despite recent advancements in molecular methods, the role of viruses in this enteric disease remains largely elusive. We investigated the spectrum of viruses detected in caprine enteritis cases from 2020 to present using our database of intestinal contents and fecal samples submitted to our transmission electron microscopy (TEM) laboratory for suspected viral enteritis in goats, with all but one ranging in age from one day to two weeks. Direct TEM provides a quick method of viral screening that presents a high specificity at the family level of virus classification, despite its lower sensitivity compared to molecular diagnostic methods. The technique utilizes ultracentrifugation to cleanse fresh enteric samples of unwanted debris and isolate viral particles via concentration gradients. Optimal usage of direct TEM allows for the reliable and unbiased detection of novel viruses that might remain otherwise unidentified by diagnostic methods due to their high specificities. From January 2020 to June 2022, we confirmed the presence of enteric viruses in 12 out of 60 suspected caprine cases using direct TEM. This includes viruses from the reoviridae, adenoviridae, parvoviridae, and picornaviridae families, as well as small round virus-like particles ranging from 18-30 nm. Of these 12, only 2 were confirmed with molecular or immunological methods on the available enteric virus panel (e.g., sequencing, PCR, and ELISA). Without TEM screening, 83% of these caprine enteric viruses would not have been detected. This not only demonstrates the need for the expansion of the existing enteric panel to include more tests for epidemiologically-relevant pathogens, but also the necessity for TEM in the detection of novel viruses and known pathogens emerging as significant epidemiological threats. The value of synergism cannot be overstated in diagnostic systems. TEM should be run in parallel with these highly-specific molecular methods to act as a safety net for the identification and characterization of viruses new and old, guiding and accelerating the sequencing pipeline to enable subsequent diagnosis using molecular methods.

* Graduate Student Poster Presentation Award Applicant

## Poster 170

**Alteration of colonic mucin glycosylation in acute swine dysentery + * † ◊**

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Infection with strongly β-hemolytic strains of *Brachyspira hyodysenteriae* (Bhyo) leads to swine dysentery (SD), a production-limiting disease characterized by mucohemorrhagic diarrhea and typhlocolitis in grower-finisher pigs. Previous *in vitro* studies have shown that Bhyo growth is increased in the presence of free sialic acid and N-acetylglucosamine (GlcNAc), but not in the presence of other monosaccharides, and specific sialic acid may serve as an adhesion epitope for Bhyo. It has also been shown that fucose induces a chemotactic response in Bhyo; however, a later study showed that the overall fucosylation of mucin collected from pigs with SD was decreased when analyzed by tandem mass spectrometry. Herein we describe the local expression of four different mucin glycans in colonic tissues of pigs with and without acute SD to determine if the disease is associated with the same changes in overall mucin glycosylation observed in previous studies that were conducted *in vivo* or by tandem mass spectrometry.

Four different lectins targeting sialic acid in an α-2,6 linkage, sialic acid in an α-2,3 linkage, GlcNAc, and α-linked L-fucose were used. Formalin-fixed spiral colon samples were obtained from a total of 36 gilts (12 controls, 12 inoculated with Bhyo, 12 inoculated with Bhyo and fed a highly fermentable fiber diet). Pigs were euthanized within 72 hours after clinical SD was observed, or at the end of the study on DPI 16 (between DPI 10 and 16). Standardized images were captured and quantification of staining specific to the above targets was performed using a commercial software program.

GlcNAc expression in pigs infected with SD was significantly lower in the lower half of the colonic glands but significantly greater in the upper half of the glands ($P<0.05$) compared to controls. The difference in distribution may indicate a continuous secreting activity into the colonic lumen. The increased expression of GlcNAc in the upper glands in the pigs with SD was in line with a previous study, which showed that GlcNAc significantly promoted the growth of Bhyo. Fucose expression in pigs infected with SD was significantly higher throughout the full thickness of the colon ($P<0.05$), which supports its chemotactic effect on the spirochetes. Pigs fed a higher fermentable fiber diet had a lower increase of fucose ($P<0.05$), suggesting the diet lessened fucosylation. Pigs with SD had significantly lower expression of the two lectins targeting different sialic acid linkages in the bottom half of the glands, and a lower or not different expression of sialic acids in α-2,6 linkage and in α-2,3 linkage in the upper half of the glands, respectively. These findings indicate that these two linkages of sialic acids may not be major adhesion epitopes for Bhyo or they have been utilized or degraded by local bacteria. Overall, there was a significant alteration of mucin glycosylation in acute swine dysentery.

+ AAVLD/ACVP Pathology Award Applicant
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◊ USAHA Paper

## Poster 171

### Natural outbreak of avian influenza in a group of striped skunks in the Northwest

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During May-June 2022, two groups of striped skunk (*Mephitis mephitis*) kits encompassing a total of seven total kits, housed at a wildlife conservatory in northwestern Idaho presented for inappetence, lethargy, tremors and terminal seizure like activity. The clinical progression occurred over a period of <24 hours and ultimately culminated in death. All seven skunk kits were submitted dead to the Washington Animal Disease Diagnostic Laboratory (WADDL) for postmortem examination with canine distemper being the primary differential diagnosis. Six of the seven kits were examined and the seventh was saved fresh and frozen. Gross findings for all examined skunk kits were unremarkable. Histologically, the most consistent histologic finding was multifocal and random hepatic necrosis (6/6 kits) with interstitial pneumonia and lymphoid necrosis in multiple lymphoid organs including the spleen and lymph nodes (4/6 kits). Two kits, one from each group, were negative by PCR for canine distemper. Due to the current avian influenza (AI) outbreak in the US and the reported susceptibility of skunks to influenza A infection, two kits were tested and were positive for H5 avian influenza by PCR. Follow up immunohistochemistry revealed strong and specific immunoreactivity in multiple affected organ systems within the kits to AI antigen.

Highly pathogenic avian influenza (HPAI) is a lineage of influenza A defined by the presence of H5 or H7 hemagglutinins in combination with the virus' ability to cause disease and mortality in chickens. HPAI infection are potentially zoonotic and have significant public health implications. HPAI infection has been reported in multiple species including birds, humans, pigs, felids, mice, and mustelids such as ferrets and martens. Skunks are part of the *Mephitidae* family and are closely related to mustelids. Influenza H1 infection in skunks in Canada has been previously reported.

Here we present, to the authors' knowledge, the first description of HPAI infection in the striped skunk within the US. This case series aims to outline the clinical presentation, gross findings and histologic lesions associated with natural infection of HPAI H5 in skunk kits. This report highlights the importance of avian influenza as a differential in cases of rapidly progressive and fatal disease in US skunks.

## Poster 172

### Vaccine induced bovine herpesvirus-1 encephalitis in three calves

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Three 2 to 4-month-old Simmental calves were submitted to the University of Kentucky Veterinary Diagnostic Laboratory from a local farm with a 10-day to 6-week history of lethargy and depression. The farm reported clinical signs which were initially responsive and then refractory to treatment with Zactran (gamithromycin), Resflor (florfenicol), dexamethasone, Exceed (ceftiofur crystalline free acid), and Banamine (flunixin meglumine). The calves had been recently vaccinated with commercially available bovine rhinotracheitis-parainfluenza 3-respiratory syncytial virus-*Mannheimia haemolytica-Pasteurella multocida* modified live virus intranasal vaccine prior to development of clinical signs. At autopsy, all three calves had a severe non-suppurative meningoencephalitis, ventriculitis, and vasculitis most severely affecting the periventricular white matter, hippocampus, and fourth ventricle. Upon presentation, two of the three calves had pulmonary cranioventral consolidation with only one of the three calves exhibiting fibrinonecrotic tracheitis. Real time PCR analysis of representative samples of brain and lung were positive for bovine herpesvirus-1 at Ct Values of 19.09, 31.81 & 31.59 (brain), 31.95 & 31.81 (lung). Cell culture using bovine turbinate cells (BT cells CRL-1390) isolated bovine herpesvirus-1 from all PCR positive tissues. Virus isolates of lung and brain were submitted to Kansas State University and South Dakota State University for next generation sequencing for vaccine differential. Samples submitted had viral sequences that were 99.8-100% similar to vaccine sequences. These findings support vaccine-induced bovine herpesvirus-1 encephalitis, tracheitis, and interstitial pneumonia.

## Poster 173

### Alveolar echinococcosis in a series of dogs in Washington state

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*Echinococcus multilocularis* is an important and emerging clinically relevant zoonosis which has been increasingly reported in expanding regions of North America over the past decade. *E. multilocularis* primarily exists in a sylvatic cycle involving wild canids and small rodents. Wild canids are the definitive host and harbor the adult tapeworms in the intestinal tract with subsequent release of eggs into the feces. The life cycle is completed with small rodents acting as intermediate hosts for the development of the larval stage in the form of a visceral hydatid cyst. Canids infected with the adult stage are asymptomatic. Dogs rarely develop the hydatid stage of the parasite, a condition known as alveolar echinococcosis (AE). Following an insidious course, AE manifests as a severely debilitating and often fatal disease which most commonly affects the liver. Timely diagnosis can be difficult and is often hampered by a general unawareness of the disease. In this report, we describe a series of cases of AE in dogs in Washington State.

In all four cases, radiographic findings revealed moderate to marked hepatomegaly. Abdominal ultrasound identified multiple, variably sized, smoothly marginated, hyperechoic nodules/masses with anechoic contents and irregular intraluminal margins diffusely throughout the hepatic parenchyma. Cystic fluid was collected via ultrasound-guided needle aspirate, and PCR from this fluid confirmed *Echinococcus* sp. infection in three of the four cases. Cytologic findings of the cyst fluid in all dogs included membranous structures and calcareous corpuscles.

Treatment included oral benzimidazole and praziquantel administration, and surgical resection when possible. In cases in which surgical resection was not feasible due to the extent of disease, ultrasound-guided drainage with or without ethanol ablation of the hepatic cysts was attempted as a therapeutic technique.

In one case, euthanasia was selected and gross necropsy revealed a markedly enlarged liver with nearly complete obliteration of the hepatic parenchyma by multilocular cystic masses. Histologically, the hepatic masses were consistent with markedly inflamed and degenerate hydatid cysts with PAS-positive membranes and calcareous corpuscles. This series of cases aims to outline diagnostic findings of AE in dogs and raise awareness of hepatic AE as a differential diagnosis for liver disease in dogs in the United States.

## Poster 174

### Gingival squamous cell carcinoma in lions: report of two cases

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Oral neoplasia is an important cause of illness in captive felids and can represent up to 51% of lesions in the oral cavity. These neoplasms include tumors arising from the mouth, pharynx, gums, teeth, tongue, tonsils, salivary glands, mandibular and maxilla bones, and have highly variable morphologic characteristics and clinical behavior, which poses a diagnostic challenge.

The clinic and pathologic findings of well-differentiated gingival squamous cell carcinoma (SCC) associated with bone lysis in 14 and 21-year-old lions, respectively, that were euthanized due to poor long-term prognosis are described. In both cases, clinical signs consisted of episodes of mild oral bleeding mixed with drooling, lesion-associated destruction of the rostral mandible and symphysis by neoplasms measuring 5.0 x 3.5 x 2.0 cm, and 2.5 x 1.1 x 0.3 cm, respectively. Both lions were euthanized 14 days and 3 months after diagnosis. Microscopically, the neoplasms consisted of markedly pleomorphic keratinocytes arranged in solid nests, and supported by moderate to abundant fibrovascular stroma. Centrally, some nests contained keratin pearls and, occasionally, individual keratinocytes exhibited dyskeratosis. The mitotic index was high. Histopathologic evidence of metastasis of the SCC to a local lymph node and lung was observed in one case.

SCC is the most common oral malignant neoplasm diagnosed in domestic cats and some non-domestic felids, but it has been rarely reported in lions. The incidence of SCC in lions appears to be sporadic when compared with domestic cats and other non-domestic felids. Oral SCCs are considered locally invasive and poorly responsive to chemotherapy and euthanasia is usually elected due to local clinical complications inherent to the location of the mass. The differential diagnosis of oral SCCs in lions include oral papillomas, fibromatous epulis of periodontal ligament origin (FEPLO) and ameloblastomas.

## Poster 175

### Massive hepatic trematodosis in juvenile bald eagles (*Haliaeetus leucocephalus*) * †

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Cases of hepatic trematodosis by opisthorchiid flukes have been reported in North American fish-eating raptors, particularly bald eagles. Infection with these flukes often causes varying degrees of granulomatous cholangitis, pericholangitis, and necrosis of adjacent hepatocytes, as well as subsequent hepatic fibrosis. Trematode identification in affected birds has been complicated by the inability to dissect intact specimens from tissues. Infections have been attributed to *Amphimerus elongatus* based on hosts, organs, morphology identified at histopathology and examination of adult worm fragments. Between 2007 and 2018, five juvenile bald eagles with massive hepatic trematodosis were identified at the University of Minnesota Veterinary Diagnostic Laboratory. The flukes were slender and extremely fragile making it impossible to extract them whole. On a microscopic level, numerous viable and fewer degenerate flukes were identified in the parenchyma. Degenerate flukes were associated with a granulomatous inflammation and hepatocyte necrosis in adjacent liver parenchyma. Based on histopathology, flukes were non-spinous, had ventral suckers measuring 80 – 93μm in diameter, and had uteri containing numerous golden, operculated eggs (about 25.0 x 12.0μm). These features were reminiscent of *Amphimerus elongatus*. The unfixed frozen liver sample of one eagle was analyzed by polymerase chain reaction and sequencing targeting the large subunit (LSU) gene of the parasite. The fluke DNA sequence shared 99.6% similarity to *Erschoviorchis anuiensis*, a newly described opisthorchiid species infecting the liver and pancreas of fish-eating birds in Europe and Asia. The clinical significance of the trematodosis is unknown despite its massive degree, since the cause of death of 4 of the eagles was attributed to West Nile virus infection (based on the presence of encephalitis and myocarditis). The fifth bird likely died of emaciation. An alternate cause of the emaciation other than the massive hepatic trematodosis was not apparent in this animal. Previous studies have found infection by *E. anuiensis* in several different piscivorous bird species to be highly pathogenic and an important cause of morbidity and mortality. The close resemblance of the gross and histological findings among the five eagles suggests that all 5 eagles were infected with the same fluke species. Additional testing is required to a. confirm that the opisthorchiid is *E. anuiensis* or a closely related new species, b. evaluate how widespread this specific trematodosis is among the juvenile bald eagle population in the upper midwest of the United States and c. determine the clinical effect of the trematodosis.

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## Poster 176

### Two cases of canine *Echinococcus multilocularis* in Missouri

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*Echinococcus multilocularis*, which is the causative agent of human alveolar echinococcosis, involves canids as definitive hosts. Dogs can be a source of human infection when they are definitive hosts (intestinal echinococcosis: IE) while dogs are considered sentinels of environmental contamination when they serve as aberrant intermediate hosts (alveolar echinococcosis: AE). In the United States, *E. multilocularis* is endemic in Alaskan wildlife (e.g., arctic fox) and has been detected in wild canids (e.g., red fox and coyote) in the 13 north-central states (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin and Wyoming); however, echinococcosis is not regarded as a major public health concern in the contiguous United States. In 2017 we diagnosed a juvenile canine case of IE in Missouri. It was the first case of IE in a pet dog in the contiguous US and the first detection of a European strain (E4 haplotype) of *E. multilocularis* in the US. In 2018 we encountered a canine case of AE in Missouri. Results from genotyping of this AE case were also consistent with the E4 haplotype. Because of increasing encroachment of fox and coyote populations into suburban and urban areas in the United States, there is growing concern of spillover from infected foxes and coyotes and potential human exposure from the infected dogs and/or the contaminated environment. The purpose of this study is to describe the clinicopathological findings and the molecular characterization of a canine case of IE and a canine case of AE in Missouri and to raise awareness of echinococcosis among veterinary diagnosticians.

## Poster 177

**Highly pathologic avian influenza in three red fox kits (*Vulpes vulpes*) # + * †**

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Three red fox kits (*Vulpes vulpes*) were submitted by a wildlife rehabilitation facility in southeastern Michigan to the Michigan Department of Natural Resources (DNR) for post-mortem examination. The foxes came from three separate dens in Lapeer, Macomb, and St. Clair counties between April 1 and April 14, 2022. Highly Pathologic Avian Influenza (HPAI) has been confirmed in avian species in all three counties, and the vixen from two of these dens were observed predating on waterfowl. At presentation, all kits were reported to have severe neurologic signs including circling, tremoring, blindness, and seizures. Two of the three kits died within the first six hours following presentation, while the third initially responded to supportive care but later died. All kits had similar gross post-mortem findings including multifocal patchy dark red regions throughout the lungs and similar reddened regions throughout the brain parenchyma. Histologically, all kits exhibited moderate to severe lymphoplasmacytic and neutrophilic meningoencephalitis that was predominately vasocentric with adjacent neuronal necrosis and satellitosis. Additionally, all kits had mild to moderate interstitial pneumonia and multifocal cardiomyocyte degeneration with mineralization. Brain tissue, oral/nasal swabs, tracheal swabs, and anal swabs from each fox kit were individually tested for avian influenza, avian influenza H5, and avian influenza virus A (H5N1) virus clade 2.3.44 by RT-qPCR. Cycle threshold (CT) values for avian influenza virus H5 2.3.44 RT-qPCR ranged from 12.28 – 17.32 in brain tissue, 20.88 – 37.32 in oral/nasal swabs, 28.76 – 37.33 in tracheal swabs, and all anal swabs tested negative. To rule out other common causes of respiratory and neurological disease in canids, canine distemper virus immunohistochemistry, rabies virus direct fluorescent antibody test, and immunohistochemistry and PCR for eastern equine encephalitis virus were performed and were negative. Transmission of HPAI from birds to mammals is of great concern and serves as a reminder that HPAI is a pathogen with zoonotic potential, and thus monitoring of virus evolution and adaptive mutation is imperative.

In the current outbreak of HPAI, H5N1 has been detected in birds throughout North America, with detections in domestic poultry and wild birds in 35 and 39 states to date, respectively. The first report of H5N1 in red foxes occurred in the Netherlands in May 2021 during an outbreak in wild birds. In addition to the cases in Michigan, in May 2022, several other neurologic red fox kits with HPAI were reported in Ontario, Minnesota, and Wisconsin. In the Ontario cases, virus was detected in brain tissue, and sequencing results indicated the same strain of HPAI found in the current North American outbreak and 2021 Netherlands outbreak (H5N1 a/goose/Guangdong/q99g (Gs/GD) lineage).

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## Poster 178

### The diagnostic value of rectal biopsies in horses with clinical signs of intestinal disease

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Diseases of the gastrointestinal tract of horses remain a challenge to diagnose and treat. Rectal biopsies (RBs) are frequently used in an attempt to diagnose some of these conditions. This study evaluated the diagnostic value of RBs in horses with clinical signs of intestinal disease. RBs of 79 horses with a history of one or more signs of malnutrition, weight loss, abnormal tolerance glucose test, hypoproteinemia, colic, chronic diarrhea, hematochezia, tenesmus, rectum neoplasm and rectum prolapse received between March 1, 1991 and July 31, 2020 were evaluated.

There were 18 cases (22.8%) of proctitis, 4 cases (5.0%) of colitis, 1 case (1.3%) of neoplasia and 56 cases (70.9%) with no microscopic abnormalities. Proctitis was minimal to mild in 12 (66.6%), and moderate to severe in 3 (16.7%) of the RBs analyzed. The severity of proctitis was not reported in 3 RBs (16.7%). In most cases (n=13; 72.2%) the proctitis was lymphoplasmacytic to pleocellular and chronic. In 4 cases (22.2%), the proctitis was mostly neutrophilic and in 1 case (5.6%), it was eosinophilic. All colitis (n = 4; 100%) were mild to moderate, and they were lymphoplasmacytic, focal to multifocal and chronic (n=3; 75%) or neutrophilic and multifocal (n=1; 25%). The case of neoplasia was a melanoma.

Using exclusively RBs as a diagnostic tool, it was not possible to determine the etiology of enteric diseases in any of the horses of this study.

Based on this limited number of samples, RBs have very limited accuracy to determine the etiology of enteric diseases of horses, but in a relatively small number of cases (29% of our cases) provided information about the lesions occurring in the colon or rectum.

## Poster 179

### Ferret systemic coronavirus - associated disease: a case report + * †

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Coronaviruses are enveloped, single-stranded RNA viruses characterized by "crown-like" radiating spikes on electron microscopy (EM). Two pathotypes of coronavirus disease have been described in ferrets: epizootic catarrhal enteritis (ECE) and ferret systemic coronavirus (FRSCV)-associated disease. ECE is characterized by lesions restricted to the gastrointestinal tract, whereas FRSCV is associated with lesions in several organs. Ferret coronavirus genotype 1 and 2 have been identified although not strictly associated with any particular syndrome.

A 2-year-old female ferret died following a 2-week history of progressive neurological signs. Necropsy findings included enlargement of both kidneys by multiple soft, tan, round, raised, up to 9 mm in diameter foci throughout the renal parenchyma. Lungs were congested and edematous, and with dozens, up 1 cm in diameter foci similar to those described in the kidneys. Histologically, there was severe, chronic, multifocal to coalescing pyogranulomatous nephritis with vasculitis, interstitial fibrosis, hyaline casts, tubular degeneration, necrosis, and regeneration. Similar pyogranulomatous inflammatory foci were also noted in the lungs, brain and diaphragm. Pan-coronavirus immunohistochemistry of the kidney revealed a positive signal associated with the renal lesions. RT-PCR of kidney was positive with ferret coronavirus genotype 2 primers and negative with ferret coronavirus genotype 1 primers. No virus particles were detected on negative-contrast EM or virus isolation on kidney samples.

Ancillary testing coupled with the gross and microscopic lesions confirmed FRSCV-associated disease. The route of transmission and pathogenesis for FRSCV remain unknown. Given the clinical and morphological similarities between feline infectious peritonitis and FRSCV-associated disease, a similar pathogenesis has been proposed. Four types of granulomas in FRSCV infections have been described based on the presence of necrosis, neutrophils, or diffuse granulomatous inflammation. More than one of these can coexist in the same animal, which could indicate multiple episodes of viremia. Vasculitis can be variable and is often not identified microscopically. It has been proposed that vasculitis is an acute lesion which can be obscured by the more chronic granulomatous reaction. A presumptive diagnosis of FRSCV-associated disease can be made based on clinical signs in conjunction with gross and histologic lesions. A final diagnosis, however, requires molecular testing.

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## Poster 180

### Nasal lymphoma in a free-ranging white-tailed deer (*Odocoileus virginianus*)

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A free-ranging, young adult, white-tailed deer buck from central Texas was observed to have bilateral, smooth, swollen masses on the head, located rostral and medial to the eyes. Four other deer across the state of Texas had also been observed with similar facial masses around the same time. This deer was monitored over a month-long period, during which time the masses were reported to have progressively grown, nearly doubling in size to the point they were obstructing the deer's vision. The deer was euthanized due to continued mass growth and loss of body condition. On postmortem gross examination, the masses were fluctuant and on cut section contained large cystic cavities filled with clear fluid and tan, fibrinous material. The underlying skull surface was slightly deformed and had several small cavities through which the fluid was draining. Upon cut section of the skull, the ethmoid turbinates contained a large, destructive, soft, yellow-tan mass beneath the area of the facial masses. Microscopic examination determined the nasal mass to be a lymphoma with a large cell morphology. Slides were submitted to a referral laboratory for CD3, CD20, and CD79 immunohistochemical staining. Fresh tissue samples were submitted for aerobic and anaerobic bacterial culture, virus isolation, and bovine leukemia virus PCR. This represents a unique case of nasal lymphoma in a white-tailed deer. Although lymphoma is well-described in this species, the disease is typically multicentric or nodal in distribution with solitary nasal lymphoma not being reported.

## Poster 181

### A one-year retrospective summary of first-week mortality in commercial broilers submitted to the Alabama State Veterinary Diagnostic Laboratory System

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In today's fast-growing broilers, the first week is the time when major morphophysiological changes occur in the digestive, immune, and thermoregulatory systems of the post-hatch chicks. A setback in these developments can result in consequences such as poor performance, condemnations, and high mortalities. As such, first-week mortality can be an indicator of a flock's performance during the rearing period. In this study, the records of the Alabama State Veterinary Diagnostic Laboratory System (ASVDLS) were analyzed to determine the causes of first-week mortality in commercial broilers.

In 2021, 106 cases containing a total of 1128, 1 – 7 days old, mostly live, broiler chicks, with a history of high mortality, were submitted to the ASVDLS-Auburn branch for necropsy. The birds were received from 45 grow-out farms from a commercial poultry complex in Alabama. In every case, a complete necropsy was performed and samples were collected for further laboratory analyses. Upon necropsy examinations, dehydration, yolk sacculitis, and omphalitis were observed in 93% of submissions. Of the 1128 birds necropsied, 610 had dehydration and 338 of those also had yolk sacculitis. The dehydrated chicks were small for their age with the skin of the tarsometatarsus being dry, shriveled, and dark. Many of these chicks also had pale kidneys with ureters distended with urate. Histologically, the kidney had multifocal tubular epithelial degeneration with intratubular deposition of urates and basophilic spheroids. From bacterial culture on yolk sacs, eighteen bacterial pathogens were isolated by aerobic culture. Of these, *Escherichia coli* was isolated from 33% of submissions as a pure culture and from 58% of cases as a mixed culture; and *Enterococcus* spp., mostly as a mixed culture with *E. coli*, was isolated from 53% of cases. *Salmonella* was isolated from the yolk sacs or chick box swabs from 23% of submissions and the predominant serotype was Kentucky (60% of the isolates). Intestinal virus isolation was attempted from 6 submissions by polymerase chain reaction, and Reovirus was isolated from 1 case.

This study clearly demonstrates that dehydration and yolk sac infection are major causes of first-week mortality in broilers. While dehydration can be secondary to yolk sac infections, it can also result from adverse environmental conditions without an infection. Moreover, baby chick nephropathy, most likely associated with a viral etiology, can also result in severe dehydration due to kidney damage as observed histologically. Although extensive virus isolation was not made on these submissions, reovirus was isolated from 1 of 6 cases as tested. In the cases of yolk sac infection, *E. coli* and *Enterococcus* spp. were found as the major insults. The potential sources of bacterial infection in yolk sacs include contaminated eggs, inadequate incubation conditions, and poor hatchery and brooder environments.

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