Proceedings of the

American Association of

Veterinary Laboratory Diagnosticians

57th Annual Conference

Sheraton Crown Center
Westin Crown Center
Kansas City, MO
October 16-22, 2014

AWARDS

2014 Trainee Travel Awardees

Karan Agrawal	University of California, Davis
Lorelei Clarke	University of Georgia
Federico Giannitti	University of California, Davis
	Pathology Committee Travel Awardee
Heather Herd	Oklahoma State University
Yuekun Lang	Kansas State University
Tessa LeCuyer	Washington State University
Claire Miller	Washington State University/ North Dakota State University
Kelly Ray	Purdue University
Jamie Rothenburger	SWCVM—Saskatchewan
Kanako Sakaguchi	Louisiana State University
Dahai Shao	Iowa State University
Shankar Thangamani	Purdue University

2014 Staff Travel Awardees

Kristin Lenoir	Oklahoma State University
Mary Mengel	Purdue University
Dwayne Schrunk	Iowa State University
Karen Sverlow	University of California, Davis
Charlene Teitzel	Washington State University
Leyi Wang	Ohio Department of Agriculture

2014 ACVP/AAVLD Award

Jamie Rothenburger
SWCVM—Saskatchewan

AAVLD Strategic Plan
Adopted July 31, 2014

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

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

Motto: Advancing veterinary diagnostic laboratory science

Core values
The AAVLD is committed to these core values:
- Continuous improvement
- Engagement of members
- Effective communication
- Collaboration
- Support of One Health

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

American Association of Veterinary Laboratory Diagnosticians

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

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

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Acknowledgments

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

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

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2014 AAVLD

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

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Crown Center Exhibit Hall B

Moderators: Bruce King, François Elvinger

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Saturday, October 18, 2014

New York A

Moderators: Santiago Diab, Kevin Lahmers

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Toxicology

Saturday, October 18, 2014

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Moderators: Wilson Rumbeiha, Patricia Talcott

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Epidemiology 1

Saturday, October 18, 2014

Chicago C

Moderators: Bruce L. Akey, Belinda Thompson

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Virology 1

Saturday, October 18, 2014
Chicago A

Sponsor: ECL2

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Pathology 2

Sunday, October 19, 2014

New York A

Moderators: Kelli Almes, Arthur (Bill) Layton

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Bacteriology

Sunday, October 19, 2014

Atlanta A

Moderators: Timothy Frana, Deepanker Tewari

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Sunday, October 19, 2014
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Moderators: Ashley Hill, Marie Culhane

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Virology 2

Sunday, October 19, 2014

Chicago A

Sponsor: VMRD

Moderators: Ben Hause, Kristy Pabilonia

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Virology 3

Sunday, October 19, 2014
Chicago B

Sponsor: Biovet

Moderators: Susan Schommer, Jianqiang Zhang

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Posters

Saturday, 18 October – Sunday, 19 October
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AAVLD Plenary

Saturday, October 18, 2014

Chicago ABC

Moderators: François Elvinger, Thomas J. Baldwin

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Veterinary Molecular Pathology
Bridging the Gap Between Clinical Disease and Molecular Pathogenesis

Matti Kiupel

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

The term "pathology" originates from the Greek words "pathos" and "logos" and is therefore best translated as the "study of disease". Pathology was established as an essential part of modern medicine that studies the etiology, pathogenesis and outcome of diseases by the Italian physician Giovanni Battista Morgagni in 1761. Utilizing postmortem examinations, he based his studies on empiric data that identified and localized causes of diseases in different organ systems rather than explaining disease processes by an imbalance of bodily fluids (humoral pathology) as proposed by the ancient Greeks. Rudolf Virchow revolutionized pathology and created the foundation of modern scientific medicine by introducing the concept of cellular pathology, which evaluates cell and tissue architecture to identify and classify morphologic alterations that form the basis of disease. These principles of cellular pathology are still relevant today. By identifying morphologic alterations, both gross and microscopically, pathologists have been able to determine disease processes, their cause and also predict the clinical outcome, thereby establishing pathology as the central science in modern medicine and especially disease investigations, with modern microbiology underscoring its importance. By being able to associate an infectious agent with a specific microscopic alteration, pathology became essential in both diagnostics and pathogenesis research of infectious diseases. Classical gross and microscopic evaluations of tissues, however, are insufficient to provide a precise prognosis for an individual patient or determine the proper therapeutic approach, and also fail to show causality of lesions with infectious agents that are not microscopically identifiable. This has led to a shift in the paradigm of how pathologists analyze tissues for diagnostic purposes or pathogenesis research, now also using molecular methods to visualize targets of interest within microscopic lesions or extract target cells for further molecular analysis. The evolution of cellular pathology into molecular pathology has already allowed us to link tissue alterations more precisely to the clinical course of disease and to molecular mechanisms of disease processes. Modern molecular veterinary pathology has altered the way we perform daily diagnostic testing and contribute to our understanding of pathogenesis. Embracing molecular pathology as the future of our profession requires a shift in our training methods of young pathologists, a close collaboration with scientists across medical disciplines, and education of the public on the benefits of integrating such new technologies into routine diagnostics, despite the higher costs.

Speaker Biography: Dr. med. vet. habil. Matti Kiupel, BS, MS, PhD, DACVP, Fachtierarzt für Veterinär Pathologie, is a Professor in the Department of Pathobiology and Diagnostic Investigation at Michigan State University in the College of Veterinary Medicine where he serves as the Section Chief of Anatomic Pathology in the Diagnostic Center for Population and Animal Health. He is also the head of the histology and immunohistochemistry laboratory, the largest veterinary diagnostic molecular pathology laboratory in the country. Dr. Kiupel received his veterinary degree from the Freie University of Berlin, Germany in 1996. He completed his doctoral thesis research in 1999 on canine malignant lymphomas in a collaborative study between the University of Cambridge, UK, the University of Utrecht, Netherlands and the Freie University, Berlin, Germany. He completed a residency in anatomic pathology from 1996 until 1999 and a PhD on the pathogenesis on porcine circovirus in 2001 at Purdue University. Dr. Kiupel has received many honors and awards in Germany, The United Kingdom, and the United States. He has served on 30 PhD and MS graduate student committees (11 as chair), and been the primary advisor of 7 residents in anatomic pathology. His work focuses on incorporating education in veterinary medicine and pathology with training in human biomedical research designed to address translation of research findings from animal models to the clinical setting. He has published more than 200 peer-reviewed scientific manuscripts and numerous book chapters in the field of veterinary and comparative pathology and specifically tumorpathology, and has been the lead author on the WHO fascicle of neuroendocrine tumors of domestic animals. He also has maintained a strong interest in infectious, especially viral, diseases and has discovered numerous novel infectious diseases. He has an interest in ferret diseases and just finished an update of the chapter on viral diseases of ferrets for the next edition of "Ferret Biology and Diseases." Recently Dr. Kiupel has been invited to lead research into coral diseases for the Khalid Bin Sultan Living Oceans Foundation, exploring new frontiers in pathology and disease mechanisms.

Veterinary Laboratory Knowledge Translation and Transfer

Grant Maxie, Hugh Cai, Davor Ojkic, Durda Slavic, Josepha DeLay

University of Guelph, Guelph, ON, Canada

Entry into the molecular age has presented exciting challenges and opportunities for veterinary laboratory medicine. Based on their incoming caseload and their ability to solicit submissions, diagnostic labs serve as resources in identifying emerging issues, and then also able to utilize the new knowledge for test development. To be useful to veterinary laboratories and their clients, test results must of course be relevant, credible, accurate, unambiguous, and timely, and available at minimal cost to users. A tall order! In order to translate new knowledge into diagnostic applications offered to clients, the diagnostic lab must validate the test in-house, often based on a published peer-reviewed publication, and following the guidelines of the AAVLD Requirements for an Accredited Veterinary Medical Diagnostic Laboratory and the OIE (World Organization for Animal Health). When validating a new test for a common pathogen, diagnostic labs typically have a distinct advantage of incoming/archived case materials that can be used for test validation. When dealing with a new condition or agent – be it new strains of *Brachyspira*, new immunohistochemistry targets, white nose syndrome in bats, or porcine epidemic diarrhea virus - a strength of the AAVLD is the network of accredited sister labs willing to share material. Primers and probes can be designed, ordered, and delivered promptly to facilitate rapid test development. Synthetic control material may also be available for purchase. Given the availability of partial and whole genome sequencing, variant microbes can be identified speedily, trends in disease spread tracked, and disease control implemented. The desired outcome of this test development process is to fill a need and have a beneficial impact on client service. If sufficiently unique, the new test and its supporting documentation could be publishable in the Journal of Veterinary Diagnostic Investigation to be quickly and widely shared with the laboratory community.

Speaker Biography: After completing his DVM at WCVM, Saskatoon, in 1969, and PhD in Veterinary Clinical Pathology at the Ontario Veterinary College in Guelph in 1973, Dr. Maxie worked in Kenya investigating the pathology of trypanosomiasis and theileriosis (1974-77). He worked on faculty at the OVC (1977-82, tenured associate professor) and as a Veterinary Pathologist (1982-94), and then as Guelph Laboratory Head (1994-97), for Veterinary Laboratory Services of the Ontario Ministry of Agriculture, Food and Rural Affairs. He was board certified as an anatomic pathologist by the American College of Veterinary Pathologists in 1984. He is currently the Director of the Animal Health Laboratory (1997 -) and co-Executive Director (2007 -) of the Laboratory Services Division at the University of Guelph. Dr. Maxie was the editor-in-chief of the Canadian Veterinary Journal (1986 - 1991), and chair of the editorial committee of the CVMA until 1998. His scientific publications include 10 book chapters and >50 peer-reviewed articles. He edited the 3 volumes, and co-authored 3 chapters, in the 5th edition of "Jubb, Kennedy and Palmer's, Pathology of Domestic Animals", published in 2007, and is currently editing the 6th edition of this work. He is a past-president of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) (2007-08), a long-time member of the AAVLD Accreditation Committee and past-chair of the Strategic Planning Committee. He was the 2011-12 president of the Canadian Animal Health Laboratorians Network (CAHLN). Effective November 1, 2014, he will succeed Dr. Jerry Saliki as editor-in-chief of JVDI.

Leveraging Laboratory Data for Animal Health Surveillance

Brian McCluskey

USDA, APHIS, Fort Collins, CO

Building comprehensive and integrated animal health surveillance systems requires identifying and then leveraging appropriate surveillance streams to meet the specific purpose of the surveillance. Those purposes or objectives may include: 1) claims of disease freedom, 2) emerging disease detection, documentation and characterization, 3) continuity of business, 4) identifying changes in disease prevalence, temporal or geographic distribution and, 5) identifying areas requiring more intensive surveillance. The flow of laboratory test data into surveillance systems has proven invaluable for many surveillance purposes and objectives. Surveillance for classical swine fever, whose objective is to support the U.S. claim of disease freedom, includes a laboratory component. Similarly, current laboratory testing efforts to support the Swine Enteric Coronavirus reporting requirements are critical for emerging disease detection, documentation and characterization. Both of these laboratory streams have well-defined data requirements. Questions arise, however, about what surveillance data elements are best collected through the laboratory submission and testing process and which elements may be more appropriately collected outside of those processes. A minimum set of 15 animal health laboratory data elements to support surveillance was suggested by Kloeze et al. (Kloeze, J., Transboundary and Emerging Diseases, 2011). This data set was designed to support domestic and international disease reporting requirements, effective analysis and syndromic surveillance efforts. It was suggested that these 15 elements all be included on a laboratory submission form. The completeness and accuracy of the data on a submission form and subsequent accuracy of the data moved through the laboratory to the surveillance system thus relies primarily on the diligence and thoroughness of the submitter of the samples to the laboratory and secondarily on the laboratory's ability to capture that submitted data accurately. Is the laboratory sample submission and testing process the most appropriate place to collect surveillance data? Other potential options will be presented. In addition to questions about the appropriateness of surveillance data collection at the time of sample submission are questions about the information technology structures necessary to make this surveillance stream efficient and effective. Examples will be presented of current data management and transfer processes, including the laboratory messaging service, Veterinary Services Processing Streamlining and what is needed to move these and other IT systems forward.

Speaker Biography: Dr. Brian McCluskey received a Doctorate in Veterinary Medicine from Washington State University in 1987, and, following graduation, was in large animal practice, primarily dairy, in western Washington state. He joined APHIS in 1990 and was stationed in Charleston, West Virginia as a section veterinary medical officer. He was then sponsored by APHIS Cattle Diseases Staff in a graduate program at the University of Florida and after receiving his Masters degree in epidemiology, was stationed at the Colorado Area Office as the Area Epidemiology Officer. During this time he became a Diplomate in the American College of Veterinary Preventive Medicine. Brian then moved to the Center for Animal Health Monitoring in Fort Collins, CO as the Dairy Commodity Support Analyst. He received a PhD in Epidemiology at Colorado State University in 2003 and from 2003 to 2007 was the Director of the USDA's National Surveillance Unit. He joined the Senior Executive Service in 2007 as the Director of Veterinary Services Western Region. Brian was named APHIS, Veterinary Services Chief Epidemiologist, a senior scientist level position, in 2011.

Veterinary Pharmacovigilance and the Use of Diagnostic Laboratory Data and Services by the Animal Health Industry

Susan M. Stehman

Veterinary Medical Information and Product Support, Zoetis, Exton, PA

Animal health companies (AHC) utilize laboratory services during development, licensure and post licensing marketing of veterinary pharmaceutical, biological and diagnostic products. This discussion will focus on the use of laboratory data and services in monitoring the safety and efficacy of marketed veterinary products. The documentation, investigation, and assessment of reports of suspected adverse events associated with use of marketed veterinary medical products are collectively referred to as veterinary pharmacovigilance. Pharmacovigilance surveillance depends on spontaneous reporting from end users including pet owners, producers, distributors and veterinarians. Adverse events may include an unexpected effect, including adverse reactions, drug residue, or ecotoxicity, and/or a lack of efficacy relative to label indications. Human exposures, physical product defects and extra label use are also documented. The regulatory frameworks that define reporting requirements and timelines vary by type of product and licensing agency. Animal Health Companies typically use a pharmacovigilance database that allows recording of product incidents, has query capabilities for retrieving data to report trends and allows electronic submission to regulatory authorities. Adverse event investigations often involve utilization of diagnostic testing by an attending veterinarian in consultation with a technical veterinarian from the AHC to investigate a reported product incident. The breed, sex, age, concomitant medications and concurrent or pre-existing health conditions are all recorded. In herds, the number of patients exposed, morbidity and mortality data is collected. The attending veterinarian's clinical findings are entered using a standardized clinical sign dictionary (VEDDRA system). Pathology, microbiology, molecular biology, serology and toxicology tests may be used to investigate reports in an attempt to determine the cause of the event reported; to assess if the experience is related to an adverse product event; and to trend such reports over time. Beyond individual case investigations, examples of use of diagnostic laboratory services and data in pharmacovigilance include examination of bacterial isolates to monitor antimicrobial resistance patterns. Monitoring of vaccine efficacy and characterization of bacterial and viral isolates from ill but appropriately vaccinated animals allows identification of newly emerging strains or pathogens should efficacy fail. The goal of pharmacovigilance is to incorporate these findings in product profiles to provide safe and effective products for practical use, to extend claims and to quickly identify and resolve product issues not identified in pre-licensing clinical trials.

Speaker Biography: Dr. Susan Stehman is a Senior Livestock Veterinarian with Veterinary Medical Information and Product Support for Zoetis, Inc. where she provides technical service and conducts pharmacovigilance investigations of product adverse events involving pharmaceuticals, vaccines and diagnostic kits for ruminants, and poultry. Prior to joining Zoetis, Dr. Stehman worked as a livestock extension veterinarian at the Animal Health Diagnostic Center at Cornell from 1991 to 2007 in Veterinary Support Services where she supported disease investigations and helped to validate testing for ruminant herd health programs including the Johne's program and the New York State Cattle Health Assurance Program. Dr. Stehman worked in a private dairy practice in Lowville, NY and completed an internship and residency in Large Animal Ambulatory Medicine at Cornell University School of Veterinary Medicine. Dr. Stehman received her BS from the Pennsylvania State University, an MS in Veterinary Science at University of Massachusetts, Amherst and her VMD from the University of Pennsylvania.

USAHA / AAVLD Joint Plenary
Achieving Perspective to Feed the World
Monday, October 20, 2014
Crown Center Exhibit Hall B

Moderators: Bruce King, François Elvinger

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What Consumers Expect

Charlie Arnot

Center for Food Integrity, Gladstone, MO

Every organization operates with some level of "social license" — the privilege of operating with minimal formalized restrictions based on maintaining public trust. Social license is granted when you operate in a way that is consistent with the ethics, values and expectations of customers, employees, the local community, regulators, legislators and the media. Once lost, through a single event or a series of events that erode public trust, social license is replaced with social control — regulation, legislation, litigation or restrictive market action. Operating with social license is flexible and low cost. Social control increases costs, reduces operational flexibility and increases bureaucratic compliance. What can be done to maintain public trust that grants social license? You begin by recognizing that transparency is no longer optional. Anyone with a cell phone is an on-the-scene reporter. Research in recent years clearly indicates that consumers increasingly go online to look for information to answer their questions about food. Growing skepticism about food safety and the use of technology fuel online communities that are raising issues and making their voices heard with increasing volume and frequency. When CFI asked consumers what it takes for them to be more trusting of food, they said they don't believe that today's food system is transparent. They also believe that large companies are likely to put profit ahead of public interest. To overcome this bias, the food system must dramatically increase the commitment to transparency. CFI's consumer trust research has identified seven elements of transparency that can make a significant contribution to building trust. The research shows these elements had the most positive impact on those who tend to be most skeptical about the food system – women and early adopters. All of our research has shown that early adopters, those who are better educated, have higher incomes and broader social circles, and women tend to be significantly more skeptical than men and later adopters when it comes to food issues. As we increase both the distance most consumers have from farming, food processing and the level of technology we implement in food production, we must dramatically improve our ability and commitment to build trust with stakeholders who grant social license. To be successful we have to build and communicate an ethical foundation for our activity and demonstrate our commitment to practices that are ethically grounded, scientifically verified, and economically viable.

Speaker Biography: Charlie Arnot is recognized as a thought leader in food and agriculture. He is highly regarded as both a writer and sought-after speaker who engages audiences across the globe. Charlie has more than 25 years of experience working in communications, public relations and issues management within the food system. He is the founder and president of CMA, an employee-owned consulting firm with offices in Missouri, Iowa and Ohio. He also serves as CEO of the Center for Food Integrity, a national non-profit organization dedicated to building consumer trust and confidence in today's food system.

Meaningful Conversations

Randy Krotz

US Farmers and Ranchers Alliance, Chesterfield, MO

Abstract available in the Meeting App.

Speaker Biography: available in the Meeting App.

Animal Feed vs. Human Food

Jude Capper

Livestock Sustainability Consultant, Bozeman, MT

The global population is predicted to rise to over nine billion by the year 2050. As resources for food production will decline over this time, how we should ensure that our children and grandchildren have the same access to food that we currently enjoy? Groups opposed to animal agriculture contend that we should adopt a vegetarian or vegan diet in order to "save the planet", however, continuous improvements in efficiency have allowed U.S. livestock producers to considerably reduce environmental impact. Compared to 1944, U.S. dairy producers use 77% less feed, 90% less land, 65% less water and have achieved a 63% reduction in the carbon footprint per gallon of milk. Similarly, the modern U.S. beef industry uses 19% less feed, 12% less water, 33% less land and has a 16% lower carbon footprint than production systems characteristic of the 1970's. Moreover, the U.S. EPA reports that meat production contributes 2.1% of national GHG emissions. If all of the USA's 314 million inhabitants removed meat from their diet for one day per week, the annual reduction in national GHG emissions would only be equal to 0.30%. Reduced meat consumption would also necessitate new sources for the many by-products from animal agriculture, including leather, fertilizer, fats, fibers and pharmaceuticals. Another popular argument for reducing meat consumption is that human nutrient requirements could be met by shifting grain use from livestock feed to human food. Corn only accounts for 7% of the total feed used to produce a unit of U.S. beef, and globally, over 7 billion acres of pastureland are used to raise livestock. Only a small fraction of these are suitable for food crop production due to terrain, water or nutrient restrictions, and they also maintain habitats for many bird, animal and insect species that would be lost if converted to cropland. By-products from the food and fiber industries also play significant roles in feeding livestock. Approximately 37 lb of livestock feed is produced from every 100 lb of plants grown for human food – what would be the environmental consequences of instead diverting these human-inedible by-products to landfill? Furthermore, as 30% of all food purchased in the USA is discarded by the consumer, making a concerted effort to reduce food waste could significantly reduce environmental impacts. To maintain food availability for future generations, it is essential to continue the tradition of continuous improvement within animal agriculture that has reduced environmental impact over time, and to consider the additional areas where considerable reductions can be made.

Speaker Biography: JUDE L. CAPPER, Ph.D. undertook her BSc in Agriculture with Animal Science and her PhD in Ruminant Nutrition and Behavior at Harper Adams University College in Shropshire, United Kingdom. She held a postdoctoral position in Ruminant Nutrition and Environmental Impact in the Department of Animal Science at Cornell University, followed by an Assistant Professor position in the Department of Animal Sciences at Washington State University. Jude is currently an independent Livestock Sustainability Consultant based in Bozeman, MT; and holds adjunct professor and affiliate positions at Washington State University and Montana State University, respectively. Jude's current research focuses on modeling the environmental impact of livestock production systems, specifically dairy and beef. Current research projects include the effect of specific management practices and technology use upon environmental impact. Her principal professional goal is to communicate the importance of livestock industry sustainability and the factors affecting sustainability to enhance the knowledge and understanding of stakeholders within food production from the rancher and farmer through to the retailer, policy-maker and consumer. She has an active social media presence and spends a considerable amount of time de-bunking some of the more commonly-heard myths relating to resource use and the environmental impact of livestock production. Jude maintains websites relating to her work at: http://wsu.academia.edu/JudeCapper/Talks and http://bovidiva.com/ and has the Twitter handle of @Bovidiva. She can be contacted via email at jude@livestocksustainability.com.

Antibiotic Stewardship as a Driver of Legislative, Regulatory, and Consumer Agendas that will Shape the Future of Antibiotic Use in Agriculture

Mike Apley

College of Veterinary Medicine, Kansas State University, Manhattan, KS

The interface of antimicrobial use in food animals and the potential for selection of resistant organisms which could affect human health lies within a wide variety of food animal production systems. These systems are comprised of unique combinations of scale, physiological and disease challenges, technological inputs, and management intensity. Innovations in efficiency bring rewards in an economic system where commodity prices tend to approach the cost of production; early adapters of new efficiency technology obtain a competitive advantage in the period prior to the uniform adoption across the industry.

Antimicrobial use in livestock production will continue to evolve due to pressures from regulatory, legislative, and supply chain entities. The supply chain perspective may include evaluation of available data, but also involves marketing pressures driven by the latest trends on Twitter, Facebook, and the blogosphere; all of which we might agree pull us away from rational assessment of issues at least to some extent. Come to think of it, maybe the same argument could be made for legislative and regulatory pressures also. Regardless, the supply chain is the most likely to drive immediate and substantial changes in food animal antimicrobial use.

If we attempt to use data to drive decisions about antibiotic use in agriculture, our challenge becomes that of defining risks and benefits of antimicrobial use in different production scenarios and then evaluating these outcomes based on our collective values. As these values will seldom reach consensus, it is reasonable to assume that views of risks and benefits (and in fact the morality) of the use of antibiotics in food animals will seldom reach consensus.

The issues of food chain transfer, or direct transfer, of resistant bacteria such as Salmonella, E. coli, and Campylobacter from food animals to humans at least lend themselves to metrics which can help us evaluate the risk of certain practices within a food system. We may disagree about the probabilities associated with each node along a quantitative risk assessment, or about the acceptability of the overall calculated risk distribution, but at least we can find some points on which to focus. In contrast, the concept of the "reservoir of resistance" defies assessment of the system as a whole due to the nebulous nature of the concept, and paints us in the corner of deciding whether or not to invoke the precautionary principle.

Speaker Biography: Dr. Mike Apley is a veterinary clinical pharmacologist who works with food animal producers and veterinarians in the areas of drug use in food animals, antimicrobial resistance, and drug residues. His practice background includes general practice in central Kansas and a feedlot consulting/contract research practice based out of Greeley, CO. Prior to joining Kansas State University, he was on the faculty at Iowa State University.

Animal Welfare Landscape: Current Scientific and Consumer Challenges

Candace Croney

Center for Animal Welfare Science, Purdue University, West Lafayette, IN

Farm animal welfare remains a highly contentious topic in the US. Continuous confinement housing and behavioral restriction of animals continue to be primary areas of concern. However, a number of issues exist that are at least as significant in regard to potential infringement on animal well-being, but which have received comparatively less public attention. These include inappropriate animal handling and other poor quality human-animal interactions on farms. Handling of non-ambulatory animals continues to present a challenge for many farms, and painful practices, performed without analgesia, such as castration, tail docking and dehorning remain problematic. On-farm euthanasia methods and the timeliness of euthanasia decisions also warrant attention, along with the distress, injury and mortality that can occur during loading and transport of animals. While scientists, veterinarians, farmers and food animal industry organizations have invested significantly in addressing farm animal welfare, and consequently perceive themselves to be the go-to experts on the subject, a recent Purdue University study suggests that consumers do not necessarily look to these particular groups for information on animal welfare. An online survey of 798 US households examined relationships between key household characteristics (demographics, geographic location and experiences), reported levels of concern about animal welfare, and sources of information people use to inform themselves on the topic. Because of the level of media attention dedicated to recent undercover videos of swine care practices on farms, specific questions pertaining to modern pork production were posed. Over half of those surveyed (56%) could not identify a specific source for animal welfare information. Those who did have a source most commonly reported using information provided by the Humane Society of the United States (HSUS) and People for the Ethical Treatment of Animals (PETA). Respondents were most concerned about confinement housing of sows, identifying gestation and farrowing stalls as even more troubling than castration, teeth clipping or tail docking of piglets. Additionally, respondents reported acting on these concerns, with 14% subsequently decreasing their pork consumption by as much as 56%. It is increasingly critical for the scientific and veterinary communities to be well versed in current scientific advancements and challenges relative to farm animal welfare as well as the nature and reasons for public concerns. The latter is particularly important to facilitate improved communication, trust and perceived competence relative to current and emerging farm animal welfare issues.

Speaker Biography: Dr. Candace Croney is Director of Purdue University's Center for Animal Welfare Science and associate professor of animal behavior and well-being in the departments of Comparative Pathobiology and Animal Sciences. Her research focuses on understanding the relationship between animal cognition and well-being, the effects of rearing environments and enrichment on animal behavior and welfare, bioethical implications of animal care and use decisions, and public perceptions of animal agriculture. She serves as scientific advisor on animal welfare to several groups, including American Humane Association, Bob Evans Farms, McDonald's, the National Pork Board, P & G Inc., and Target.

Trends in Food Safety: Public Perception vs. Reality

Richard Raymond

Food Safety/Public Health Consultant, Windsor, CO

This talk will address recent trends in food safety and media coverage of outbreaks, such as the recent Foster Farms associated Salmonella outbreak, that tend to cause mistrust with the food industry in consumers' minds. The issue of the use of antibiotics in animals raised for food, and what, if any, risk this practice poses to human health thru the development of antibiotic use will also be discussed. Also discussed will be the use of technologies to increase production output and efficiency in an effort to feed a growing population with an increasing income. Finally, a discussion of where consumers are getting their information about agriculture and food production, and why that needs to change.

Speaker Biography: Dr. Raymond was a rural Family Physician in O'Neill, NE, for 17 years and then established and Directed Clarkson Hospital's Family Practice Residency Program in Omaha for 10 years. During this time he also served as the President of the Nebraska Medical Association. In January, 1999, Dr. Raymond was appointed by Governor Mike Johanns to be Nebraska's Chief Medical Officer. Dr. Raymond directed a large number of public health programs including investigations of food borne illness outbreaks and building public health preparedness. He also served as President of the Association of State and Territorial Health Officials. In July, 2005, Dr. Richard Raymond moved to Washington, D.C., when President George Bush appointed him Undersecretary for Food Safety at the U.S. Department of Agriculture. In this position, Dr. Raymond was responsible for overseeing the policies and programs of the Food Safety and Inspection Service (FSIS) which regulated the meat and poultry food industry and once again was a direct report to Mike Johanns, at that time the Secretary of the USDA. Dr. Raymond now consults and writes on food safety and public health issues from his home in Windsor, Colorado, and speaks on the same subjects both domestically and internationally. Dr. Raymond is a member of the Board of Trustees of the Co-WY Chapter of the National Multiple Sclerosis Society, the Business Advisory Board of Identigen, the Food Safety Solutions Advisory Board for Elanco/Eli Lilly, the Christie Club Property Owners Board of Directors and Tyson Food's Animal Well Being Advisory Board. He writes two food safety blogs monthly for Meatingplace.com and Feedstuffs Food Link Dr. Raymond has an ongoing working relationship with Eli Lilly/Elanco, consulting on food safety and public health issues, and an ongoing working relationship with Merck Animal Health regarding messaging as it relates to antibiotic use in animals raised for food.

The New Consumer Value Proposition

David Fikes

Food Marketing Institute, Arlington, VA

Strongly held beliefs about environmental issues, the ethical treatment of food animals, and the way a food item is produced are becoming more pronounced variables in the consciousness of the American consumer. These emotionally charged concerns - along with food safety considerations - are increasingly factoring into U.S. shopper's decisions about where they shop, the products they purchase and the brands they support. Additionally, consumers are expecting their food retailer to be engaged in these value considerations and in some instances, active advocates acting on behalf of the customer's views for improvements. Sharing research about what builds customer trust, trends regarding shopper values and emerging consumer attitudes about food safety and animal welfare considerations, we will explore the expanding role of the food retailer in addressing customer values. The new value proposition of consumers extends beyond economics and encompasses more esoteric concerns and belief systems. It is making exploration of these value-driven issues up and down the value chain a necessary conversation, requiring deeper dialogue, better information exchange and more intimate engagement between retailers and producers.

Speaker Biography: David Fikes is Vice President, Consumer/Community Affairs and Communications for Food Marketing Institute, the trade association for supermarkets, grocery stores and all venues of food retail. His areas of responsibility - which embrace consumer research, animal welfare issues, the challenges of communication and the many ways food retailers interact with their local community - all feed his fascination with people. Prior to his position with FMI, Fikes served as the Director of Communication for the American Frozen Food Institute. Before entering the world of food trade associations, Fikes worked for 20 years as an Episcopal priest, serving parishes in Georgia, Tennessee and Texas. He and his wife Lisa are the proud parents of three and half year old Harper Fikes, who maintains an active consumer interest in Dragon movies, sidewalk art and soap bubbles.

Pathology 1

Saturday, October 18, 2014

New York A

Moderators: Santiago Diab, Kevin Lahmers

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Use of Iba1 Immunohistochemistry in the Diagnosis of Hepatic Histiocytic Sarcoma in a Dog: A Case for Kupffer Cell Neoplasia? * † +

Kenneth Kim[21], Rudy Bauer[12]

[1]Louisiana Animal Disease Diagnostic Laboratory, Vanderbilt University, Baton Rouge, LA; [2]Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, LA

Common sites of origin of histiocytic sarcoma complex in dogs include the skin, subcutis, joints, lymph node, spleen, lung, and bone marrow. Iba1 expression has been reported in subdural (Ide 2011) and ocular histiocytic sarcoma but not in other sites. A 7-year-old male neutered border collie presented with chronic weight loss and a 2 day history of vomiting and anorexia. CBC and chemistry demonstrated an albumin of 1.7 (2.6-4.2) and mild signs of cholestasis. Coagulation times were mildly elevated. Following diagnostic imaging and a short hospitalization the dog developed severe hemoabdomen and was euthanized. At postmortem examination approximately 90% of the left lateral liver lobe was expanded and effaced by coalescing, tan to yellow, firm, irregularly defined nodules. Similar nodules, ranging in diameter from 1 to 3cm, were found throughout all lobes. Additional findings included multifocal dark red nodules up to 2.5cm in diameter expanding the spleen, and enlargement of hepatic lymph nodes up to 3cm in diameter. Cytology of the hepatic nodules demonstrated pyogranulomatous inflammation with atypical histiocytes. Histopathologically, neoplastic cells filled hepatic sinusoids and formed unencapsulated, poorly demarcated, infiltrative, moderately cellular nodules composed of ill-defined packets. Cell morphology varied from spindle to polygonal to round with indistinct borders and moderate to abundant, pale, eosinophilic, often foamy cytoplasm. Nuclei were pleomorphic and ranged from spindle-shaped with clumped to marginated chromatin to round/oval nuclei with coarse chromatin and one to two distinct nucleoli. Mitoses averaged 20 per 10 HPF and were occasionally bizarre. Scattered neoplastic cells were multinucleate and no erythrophagocytosis was seen. Similar neoplastic cells effaced the hepatic lymph nodes and were seen in only 2 of the splenic nodules. Micrometastases were seen in the lung and tracheobronchial lymph node. Morphologically the neoplasm was preliminarily diagnosed as a poorly differentiated sarcoma. Histiocytic sarcoma (HS) was diagnosed based on neoplastic cells' cytoplasmic expression of Iba1. Results were supported with follow-up immunohistochemistry (IHC) using CD3, CD18, and CD20. Experimental IHC was attempted using 2 clones of anti-mouse CLEC4F antibody but staining was nonspecific. Investigation to rule in or rule out a dendritic cell origin is ongoing. The findings in this case illustrate the value of Iba1 in the diagnosis of HS in the canine liver. The large hepatic tumor burden, combined with the absence of a clear extrahepatic primary neoplasm, raise the possibility of primary hepatic HS.

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

Sudden Death Associated with Pulmonary Hemorrhage in Racehorses in New Mexico: 2010-2014

John Ragsdale, Norbert Takacs, R. Flint Taylor

Veterinary Diagnostic Services, New Mexico Department of Agriculture, Albuquerque, NM

New Mexico has a thriving horse racing industry that has an important role in the state's economy. The majority of racing and wagering involves thoroughbred horses, but quarter horses are raced as well. The industry in New Mexico has had a history of being dangerous for horses and riders. New Mexico racetracks averaged 3.5 horse fatalities per 1,000 starts from 2007 to 2011, versus a United States average of 2.0 horse fatalities per 1,000 starts from 2008 to 2010. To investigate why there were high numbers of race horse fatalities in New Mexico, the New Mexico Racing Commission (NMRC), the agency with oversight of the racing industry in New Mexico, began requesting necropsies of horses that had inexplicably died during training or racing. Horses with musculoskeletal breakdowns or other known causes of death were not submitted for necropsy. From 2010 to 2014, 17 postmortem examinations on racehorses seized by the NMRC were performed. The death of 12 of these horses was attributed to severe pulmonary hemorrhage (PH). The horses had a history of weakness, incoordination, and collapse followed by rapid death. There were 8 quarter horses and 4 thoroughbred horses. The vast majority of horses (11) were male. Five quarter horses were 5 years of age or younger and 3 were six years of age or older. All 4 of the thoroughbred horses were older than 6 years of age. The gross lesions included diffusely dark red, congested and hemorrhagic lungs that did not collapse when the thorax was opened. The lungs often had variable numbers of pleural hemorrhages, hemorrhage in the subpleural septa, and rib impressions on the parietal surface of both lungs. The trachea contained varying amounts of hemorrhage, and there were often large volumes of blood that exuded from the nares on the transport trailer and on the cooler floor. The microscopic lesions were severe congestion of the alveolar capillaries with extensive acute hemorrhage and edema within almost all of the alveoli, the airways, and the subpleural septae. There were multifocal pleural hemorrhages microscopically. There was almost no evidence of previous episodes of pulmonary hemorrhage in these horses. However, the marked acute hemorrhage in the lungs of the affected horses could have obscured any subtle evidence of pre-existing subacute or chronic hemorrhage. Toxicology testing of all of the horses was performed on urine and blood collected by representatives of the NMRC. Although the authors were not privy to the toxicology results of the individual horses due to the confidentiality of the NMRC's investigations, abnormal or illegal drugs were not reported to be present in these horses. No gross or microscopic lesions were found in the heart of these horses. The cause of the fatal PH in these horses has not been determined.

Abortion in a Miniature Donkey Associated with a Gammaherpesvirus Similar to *Equid Herpesvirus-7* # †

Tessa LeCuyer[12], Dan Bradway[2], James Evermann[12], Anette Rink[3], Tim Baszler[12], Gary J. Haldorson[12]

[1]Department of Veterinary Microbiology & Pathology, Washington State University, Pullman, WA; [2]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA; [3]Nevada Animal Disease and Food Safety Laboratory, Sparks, NV

Fetal tissues and placenta from a third trimester miniature donkey abortion were submitted to the Washington Animal Disease Diagnostic Laboratory for abortion diagnosis. Microscopic examination of formalin-fixed tissues revealed multifocal necrotizing placentitis. Several cells within the necrotic foci contained large, eosinophilic, intranuclear inclusions. Virus isolation from fresh frozen placenta identified a cytopathic, syncytia-forming virus. Polymerase chain reaction from the cultured virus using degenerate universal herpesvirus primers amplified a 699 base pair portion of the DNA polymerase gene. The PCR amplicon had 97% nucleotide identity with the DNA polymerase gene of *equid herpesvirus-7* (EHV-7), also known as *asinine herpesvirus-2*, a gammaherpesvirus. Additionally, the amplicon had complete identity with short sequences of asinine herpesviruses that have been associated with interstitial pneumonia in donkeys. EHV-7 has previously been isolated from nasal secretions of normal donkeys and mules, but to the authors' knowledge, this is the first report of abortion associated with EHV-7.

AAVLD Trainee Travel Awardee (Virology, Pathology)
† Graduate Student Oral Presentation Award Applicant

Investigation of Serum Amyloid A Levels in Fetal Heart Blood in Aborted Equine Fetuses

Erdal Erol[1], Carney Jackson[1], Steve Locke[1], Naomi Kelly[1], David Horohov[2], Craig N. Carter[1]

[1]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY; [2]Department of Veterinary Science, University of Kentucky, Lexington, KY

Serum amyloid A (SAA) is a highly conserved-acute phase protein synthesized predominantly by the liver and triggered by infection, inflammation, stress, neoplasia, trauma and toxins. SAA is well recognized and used in human medicine for diagnosis, prognosis and assessment of health. Increased levels of SAA are seen with inflammation, infections, surgical trauma in horses and, bacterial infections, arthritis, and septicemia in foals. A recent study showed that SAA levels are elevated in mares with ascending placentitis. However, to the authors' knowledge, SAA levels in equine fetal heart blood have not been reported. In this pilot study, SAA levels were measured in aborted equine fetuses. Blood from 65 aborted equine fetuses was obtained and, serum was separated and stored in -80C until use. A commercial SAA ELISA kit was used in total 65 serum samples in which 25 represented the abortion cases where an infectious disease process was identified (1st group) and, 40 cases represented the abortion cases where no infectious disease process was identified (2nd group) by the pathologists. SAA was elevated (ranging from 4 to 40 μg/ml) in 19 cases (including 3 EHV-1 and 1 leptospirosis cases) out of the first group. In the remaining 6 cases (which 5 cases were reported as focal placentitis), SAA levels were found to be very low (between 0 and 1 μg/ml). In 34 cases of the second group, SAA levels were near zero, whereas in 6 cases SAA levels were elevated (ranging 5,8 to 40 μg/ml). Because small molecules (oxygen, glucose, lactose, fatty acids and amino acids), but not large molecules, have been shown to cross placental barriers and, the equine fetus can produce large molecules such as immunoglobulin (e.g., fetal antibodies against leptospira), our results suggested that SAA was fetal origin and produced by the fetus as a response to infection. Preliminary data suggested that SAA testing can be used as a marker for further investigation of cases where a definitive diagnosis has not been made and learn more about fetal-pathophysiology and immunology.

Lesions Associated with *Eucoleus* sp. in the Non-Glandular Stomach of Wild Urban Rats (*Rattus norvegicus)*

Jamie L. Rothenburger[1], Chelsea G. Himsworth[23], Manigandan Lejeune[4], Piper M. Treuting[5], Frederick A. Leighton[16]

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This study describes the histological lesions in the non-glandular stomach associated with *Eucoleus* sp. infection in a wild, urban population of Norway (*Rattus norvegicus*) and black rats (*R. rattus*). Over a 1-year period, 725 rats were trapped in Vancouver, Canada and autopsied. A subset of 183 Norway rats and 15 black rats was examined for *Eucoleus* sp. infection in the mucosa of the upper gastrointestinal tract including ventral tongue, oropharynx, esophagus and non-glandular stomach. Additionally, non-glandular stomachs were examined for six distinct categories of histological lesions. The apparent prevalence of *Eucoleus* sp. in the upper gastrointestinal tract of Norway rats was 43% (79/183). Only one black rat was infected (1/15; 7%). Detailed statistical analysis was applied to Norway rats only. Among Norway rats, infection with *Eucoleus* sp. was significantly associated with hyperkeratosis, mucosal hyperplasia, keratin pustules and submucosal inflammation in the non-glandular stomach ($P<0.05$). *Eucoleus* sp. infection and/or related stomach pathology was present in 135/183 (74%) of rats. The odds of being affected by *Eucoleus* sp. or associated stomach pathology were greater in heavier (OR = 1.06, 95% CI = 1.00 – 1.12) and sexually mature rats (OR = 4.64, 95% CI = 1.23 – 17.10). These findings suggest that gastrointestinal *Eucoleus* sp. infection is common in wild urban rats and induces a substantial host response. The impact of these lesions and infection on individual rats and the population as a whole requires further investigation.

Identifying Vaccinal-Type Strains of BoHV-1 in Bovine Abortion Using Single Nucleotide Polymorphisms: 10 Herd Episodes ◊

Donal O'Toole[1], Myrna M. Miller[1], Christopher C. Chase[2]

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Three vaccine manufacturers in the United States currently sell multivalent vaccines containing modified live *bovine herpesvirus 1* (BoHV-1) for use in pregnant cattle. Their use has become popular since they can be used year-round. One disadvantage is that they can be abortifacient unless vaccination is done within the previous 12 months using specific vaccine products and in accordance with label directions. Diagnostically it is impossible to distinguish iatrogenic from natural abortion on the basis of herpetic-type lesions and virus isolation alone. Use of single nucleotide polymorphisms (SNPs) in BoHV-1 was proposed as a method to resolve whether outbreaks were likely to be iatrogenic (1). We selected 10 abortion episodes (2010 – 2014) where an apparent association existed between use of modified live BoHV-1 and abortion in the subsequent 1 – 3 months. In individual episodes the products were either used on or off label, according to the producer. All 10 episodes had SNP patterns consistent with those of commonly used modified live BoHV-1 strains. Use of SNP patterns is helpful in resolving whether abortion was likely due to vaccinal virus, particularly when disagreement existed between a producer and representatives of the vaccine manufacturer. 1: Fulton et al.: 2013. Vaccine 31(11):1471-1479.

◊ USAHA Paper

The Effect of Zinc Oxide Nanoparticles (Zno-NPs) and Diet Form (Dry and Moisture) on the Indices of Immune System, Blood Parameters and Gut Morphological of Broilers During Starter Period * †

Farhad Ahmadi, F. Mohammadi, M. Esfahani, V. Pahlavan, S. Pazhavand

Department of Animal Science, Azad University of Kurdistan, Sanandaj, Islamic Republic of Iran

This research was conducted to investigate the effect of different levels of zinc oxide nanoparticles (100 or 200 mg/kg diet) and diet form (dry or wet diet with 1:3 water to diet ratio) on the indices of immune system, blood parameters and gut morphological of broilers during starter period (from hatch to 21 days). A total of 240 one-day old male broiler chickens (Ross-308) were randomly allocated into 2×2 factorial experiment consisted of four groups with four replicates (15 birds / pen) for a 21-day trial. Experimental diet was: T1) dry diet+100 mg of Zno-NPs/ kg diet; T2) dry diet+200 mg of Zno-NPs/kg diet; T3) wet diet+100 mg of Zno-NPs/kg diet and T4) wet diet+200 mg of Zno-NPs/kg diet. The results of the research indicated that there were no significant ($P>0.05$) differences in heamatological parameters such as Hb, Hct and RBC although, the highest titer of mention parameters were observed in T3 (wet diet plus 100 mg of Zno-NPs/kg) and T4 (wet diet plus 200 mg of Zno-NPs/kg). Furthermore, the addition of Zno-NPs in wet diet significantly increased ($P<0.05$) the number of WBC, lymphocytes, and decreased ($P<0.05$) heterophil to lymphocyte ratio compared to the birds fed dry diet plus Zno-NPs. The total immunoglobulin and IgG concentration in the birds fed wet diet inclusion of 200 mg Zno-NPs was higher than other groups ($P<0.05$). As well, relative weight of bursa Fabricius and thymus in birds fed wet diet inclusion Zno-NPs was higher ($P<0.05$) than other group during the 21-day period. Duodenum and jejunum traits (villi height, depth of crypt and ratio those) was not affected by diet ($P> 0.05$). In conclusion, the present findings suggest that supplementation wet diet with Zno-NPs improved indices of immune response and the best response observed in the birds that fed with wet diet plus 200 mg of Zno-NPs per kg diet in comparison with other experimental diet during starter stage.

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

Pathology and Diagnosis of Necrotic Enteritis of Chickens ◊

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Diagnosis of necrotic enteritis produced by *Clostridium perfringens* (NE) in poultry can be challenging, mostly because this organism is usually found as a normal inhabitant of the gut, making it difficult to determine its role in pathogenesis. We reviewed the diagnostic features of 65 cases of necrotic enteritis in chickens, that were submitted to the Turlock, Tulare and San Bernardino branches of the California Animal Health and Food Safety Laboratory, between 2004 and 2013. Of these, 70% of the cases had focal or diffuse gross lesions in at least one portion of the intestine. Microscopic lesions consisted of mucosal intestinal necrosis, and in some cases necrotic changes reached the submucosa, with a few cases in which the necrosis extended into the muscularis. Heterophils were the dominant inflammatory cells in the initial stages of the disease, but mononuclear cells are also present in more chronic lesions. Large numbers of Gram positive rods, usually grouped in clusters, were seen associated with the necrotic lesions. Immunohistochemistry for *C. perfringens* performed in small intestine of 10 of the birds with NE revealed the presence of strongly positive intralesional rods in all the birds tested by this technique. Microscopic intestinal lesions were observed most frequently in the jejunum-ileum (61%), duodenum (43%) and the ceca (17%). *C. perfringens* type A was isolated from the 24 (100%) cases in which anaerobic culture of the intestine was attempted. Seven (29%) of these 24 isolates carried the gene encoding for beta 2 toxin, while 2 (8%) each of those isolates were positive for the genes encoding enterotoxin and Net B toxin, respectively. Coccidiosis was diagnosed by fecal floatation and/or histopathology in 50% of the cases and it was the most frequent predisposing factor, but it was not always present. The number of cases NE received in these three laboratories increased ~ 100% in 2009 and ~ 200% in 2013 when compared with the average annual submission over the previous 9 years. Diagnosis of NE cannot be based on gross examination alone and an acceptable level of certainty should be achieved by combining several diagnostic tests. Although NetB has been recently been associated with many cases of NE around the world, our results suggest that this toxin is not necessary for NE to occur. The dramatic increase in the number of cases of NE to our lab over the past few years can be related to the significant reduction in the use of antimicrobials.

◊ USAHA Paper

Toxicology

Saturday, October 18, 2014

Chicago B

Moderators: Wilson Rumbeiha, Patricia Talcott

Symbols at the end of titles indicate the following designations:

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

A Novel Nephrotoxic Mycotoxin: Pathology and Etiological Diagnosis

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We hereby describe a novel mycotoxin which should be considered in differential diagnosis of acute and chronic nephrotoxic insults in animals. Orellanine is a bipyridyl toxin which bears striking structural resemblance to the herbicide Paraquat. It is produced by mushrooms of the genus *Cortinarius* which grow throughout North America and Europe. Although clinical cases of orellanine poisoning in animals are yet to be reported, numerous human cases have been reported. In human cases, orellanine causes a slowly-developing severe oliguric renal failure in the course of 1-2 weeks post ingestion. Survivors develop chronic renal failure requiring hemodialysis for life. We believe that lack of awareness of this novel toxin as a danger to animals, coupled with lack of diagnostic tests, are reasons why intoxication cases in animals are yet to be identified. Using a mouse model, our lab has characterized renal lesions associated with orellanine toxicosis. In mice dosed with 10 mg/kg bw 2 X subcutaneously 2 hours apart, renal lesions consisted of acute tubular necrosis. For etiological confirmation, we have developed an analytical procedure utilizing LC/MS/MS method for detection and quantitation of orellanine in kidneys. Extraction of 0.1 gram of kidney in 0.4ml methanol:3M HCl, (10:1) LOQ is 5ppm LOD is 0.5ppm. The method can detect orellanine in suspect mushrooms or in fresh renal tissue such as a renal biopsy. Additional work is ongoing to develop tests based on less invasive specimens such as serum and urine. In conclusion, procedures based on pathology and analytical chemistry for diagnosis of a potent novel nephrotoxic mycotoxin orellanine have been developed. The LC/MS/MS-based procedure is available for etiological confirmation of suspected cases of orellanine-induced renal failure.

Phosphorus Toxicosis Following Fireworks Ingestion

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[2]Department of Veterinary & Biomedical Sciences Department, South Dakota State University, Brookings, SD

A four month old male Yorkshire terrier consumed Slider fireworks on July 1, 2012. The dog vomited a large amount of stomach contents the following day (July 2), was weak and also had diarrhea. It was taken to an emergency clinic and initial blood chemistry results demonstrated hypoglycemia while the alkaline phosphatase and alanine aminotransferase levels were within the normal range. Fluid therapy was initiated and the dog remained recumbent. An abdominal radiograph on July 3 revealed a small area of gas distended small intestine and ascites. No unusual masses were observed. An abdominocentesis procedure was unable to obtain a peritoneal fluid sample. The condition of the dog worsened. Exploratory abdominal surgery was attempted, but the dog died. The veterinary practitioner described a yellowish tan liver with specks of brown to red coloring. There was mild hepatomegaly and the liver was friable. Early gastric mucosal necrosis was suspected. The stomach contents were dark, brown fluid. The gastric mucosa near the greater curvature was purple and hemorrhagic. Formalin fixed liver and a fireworks sample were submitted to the Animal Disease Research and Diagnostic Laboratory at South Dakota State University, Brookings, SD. Microscopic examination of the liver revealed severe acute diffuse hepatocyte degeneration and necrosis. Centrilobular veins were surrounded by necrotic hepatocytes and hemorrhage. Hepatocytes near portal triads were swollen and vacuolated. The fireworks (Sliders) were analyzed using ICP-OES and contained 46, 154 ppm (4.6%) phosphorus, 62, 710 ppm (6.2%) potassium and 19,429 ppm (1.9%) magnesium on a dry basis. Fireworks typically contain potassium nitrate, potassium chloride, sodium nitrate and potassium perchlorate. Phosphorus, in the form of white phosphorus has been used in explosives. Clinical signs following ingestion of excess phosphorus include gastroenteritis with vomiting and diarrhea. After several days and animal can develop a secondary phase of severe liver damage with renal damage also. This case is compatible with phosphorus toxicosis caused by consumption of the Slider fireworks.

Hemolytic Crisis in Horses from Exposure to *Pistacia* spp

Leslie W. Woods[1], Kyla Walter[2], Carly Moore[2], Rana Bozorgmanesh[3], Gary Magdesian[3], Federico Giannitti[1], Mark L. Anderson[1], Birgit Puschner[21]

[1]California Animal Health and Food Safety Laboratory, School of Veterinary Medicine, Davis, CA; [2]Department of Molecular Biosciences, School of Veterinary Medicine, Davis, CA; [3]William Pritchard Veterinary Teaching Hospital, School of Veterinary Medicine, Davis, CA

Three adult horses were submitted to the California Animal Health and Food Safety Laboratory System for necropsy in October 2013. The first horse had anorexia, fever and pale mucus membranes 24 hours prior to death. Another horse on the premises had similar symptoms with mild ataxia and died the next day. A third horse died two days later. Microscopically, all three horses had hemoglobinuric nephrosis; one of which had severe, diffuse, centrilobular to midzonal, bridging hepatocellular necrosis with bile stasis. The horses had been on dry pasture with a pistachio orchard, supplemented with *Panicum* and alfalfa hay. Trimmings from the pistachio trees (small leaf and large leaf pistachio; *Pistacia atlantica*, *P. terebinthus*, respectively) were piled in the enclosure and clinical signs began after horses were seen consuming the clippings. Other plants identified within the enclosure included: coffee berry, coyote brush, penny royal, *Pistacia chinesis*, grape, oak and toyon. Fluorescent antibody test results for *Leptospira* spp. on the kidneys and in the urine were negative in all three horses and PCR test results were negative for *Leptospira* spp. in one horse. Silver stain on the kidneys from the third horse demonstrated spirochetes, however. Antibody titers in this horse to *Leptospira interrogans* serovars Pomona, Bratislava and Icterohaemorrhagiae were 1:6400, 1:6400 and 1:3200, respectively. Antibody titers to *Leptospira interrogans* in the first horse were 1:400 for serovars Canicola and Bratislava and 1:800 for Icterohaemorrhagiae, and in the second horse were 1:400 for Bratislava, Hardjo, Icterohemorrhagiae and Pomona. Pyrogallol was identified in the kidneys of 2/3 horses; *Pistacia terebinthus* and *P. atlanticus* contained 2.3 % and 6.6% gallic acid, respectively; all considered toxic levels. Extract from the pistachio inoculated on washed equine erythrocytes produced hemolysis whereas extractions of hay (leaves and seeds) did not demonstrating that the pistachio trees have oxidative properties (pyrogallol) which cause hemolysis and hemoglobinuria. The cause of the intravascular hemolysis seen in these horses was likely due to a newly recognized plant toxicosis (*Pistachia* sp.). This case also demonstrates the importance of evaluating more than one animal in an outbreak with multiple mortalities in a herd.

Intralaboratory Development and Validation of an Analytical Method for Determination of Aflatoxin M1 and B1 in Liver #

Dahai Shao, Paula M. Imerman, Dwayne E. Schrunk, Steve M. Ensley, Chong Wang, Wilson K. Rumbeiha

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

Aflatoxins are potent mycotoxins which negatively impact animal and human health. Among other effects, aflatoxins are especially hepatotoxic and immunosuppressive. The etiological diagnosis of aflatoxicosis which is based on analysis of contaminated feed matrices has disadvantages and is not quite confirmatory. Currently there are no tissue based methods for etiological diagnosis and confirmation of aflatoxicosis. A high performance liquid chromatographic method with fluorimetric detection and pre-column derivatization for the determination of aflatoxin B1 and M1 in bovine liver has been developed and validated. The selectivity, recovery, precision, matrix effect, limit of detection, limit of quantification, and linearity have been validated. Current method demonstrates good selectivity for both aflatoxins against bovine liver matrix. The limit of detection is 0.01 and 0.02 ng g-1 and limit of quantification is 0.03 and 0.07 ng g-1 for aflatoxin M1 and B1, respectively. The calibration curves show good linearity (R2 is 0.9999 and 0.9991 for aflatoxin M1 and B1, respectively) from 0.2 ~ 10.3 ng g-1. The mean recoveries calculated at three levels of fortification (0.2, 2 and 10 ng g-1) is 55% and 59% for aflatoxin M1 and B1, respectively, and the maximum relative standard deviation value for the intra-lab repeatability is 18% and 15% for aflatoxin M1 and B1, respectively. The comparison of post-extraction fortified liver at 2.0 ng g-1 with aflatoxin standard solution indicates the absence of liver matrix effect. These results indicate that the proposed method is suitable for the determination of aflatoxin B1 and M1 in bovine liver. The next step is interlaboratory validation of this test before it can be implemented for the routine analysis.

AAVLD Trainee Travel Awardee (Toxicology)

A LC-MS Method for the Analysis of Nitrate and Nitrite in Serum #

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[1]Department of Nutrition, University of California, Davis, Davis, CA; [2]Obesity and Metabolism Research Unit, Western Human Nutrition Research Center, USDA-ARS, Davis, CA

Current methods for nitrate and nitrite quantification in biological samples generally rely on either direct analysis by specialized equipment such as ion chromatographs with electrochemical detectors or by reduction (in the case of nitrate) followed by derivatization and quantification by relatively non-selective techniques such as fluorescence or ultraviolet-visible light spectroscopy. Here, we propose a novel method for the analysis of nitrate and nitrite in serum obtained from livestock using reduction (in the case of nitrate) followed by derivatization and analysis by liquid chromatography-mass spectrometry (LC-MS). Samples are enriched with 15N-nitrite, treated with a 20% sulfosalicylic acid solution to precipitate proteins, and reacted with an acidified solution of sulfanilamide and N-(1-napthyl)ethyelenediamine (i.e. Griess Reagent). Derivatized nitrite and 15N-nitrite are resolved from interferences on a 2.0 x 50 mm, 4 μm Polar-RP column (Phenomenex) with a 10 minute gradient of water and acetonitrile, both modified with 0.1% formic acid. Analytes are detected on a Quattro Micro (Waters) triple-quadrupole with positive mode electrospray ionization and multi reaction monitoring. On this system the method has a linear range of 1.0–50μM (0.07–3.5 ppm) nitrite in water and standard additions demonstrated 85-100% recovery in serum. These results indicate that the method is a highly selective screen for nitrite in serum using equipment commonly available in veterinary diagnostic laboratories across the country, and is comparable in sensitivity with ion chromatographic methods. We are currently evaluating the application of the method to other matrices such as aqueous humor. For nitrate quantification, the inclusion of 15N-nitrate will further allow correction for nitrate reduction efficiency. Additionally, as artifacts created during derivatization with the Griess reagent are separated from the nitrite adduct by chromatography, there is no risk of interferences affecting the quantification of nitrite in the sample, as is common with spectroscopic quantification. Additionally, the method is very amenable to batch processing and the total cost of reagents used during the assay are approximately $1/sample. Thus, the assay offers laboratories that do not possess specialized equipment a cheap, fast and sensitive method for the quantification of nitrate and nitrite in serum with possible application to other biological fluids using equipment already in their possession.

AAVLD Trainee Travel Awardee (Toxicology)

A New Approach to the Rapid Analysis of Toxins and Toxicants Using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry ◊

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[1]Department of Toxicology, Purdue University, West Lafayette, IN; [2]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been a commanding tool for the high-throughput analysis of biomolecules such as proteins, polymers, nucleic acids and bacterial cells/cultures. However, using MALDI mass spectrometry to analyze low-molecular weight analytes has historically been challenging. This is due to several factors which include the abundant presence of several matrix ions which can interfere with detection of analytes < 500 Da and limitations in instrument sensitivity. However, exploiting advances in the sensitivity of new MALDI instrumentation, and through matrix manipulations, a procedure has been adapted for the detection of toxins and toxicants that are of interest in diagnostic veterinary toxicology. Using a Bruker microflex™ LRF high performance bench-top MALDI-TOF-MS, which is equipped with an additional gridless reflectron, provides the superior resolution and mass accuracy needed to accommodate detection of low molecular weight analytes. Several toxins and toxicants, including microcystins, brodifacoum, strychnine, and ractopamine, have been analyzed using this MALDI-TOF-MS. Methods adapted for the analysis of these compounds included: external calibration of the instrument using α-cyanohydroxycinnamic acid matrix ions in both positive and negative ion mode, a reflector voltage set at 19.99 kV, a detector scan range of 0 to 1,000 Da, and approximately 1,000 laser shots of data summed per sample. Prior to analysis, the samples are co-crystallized with α-cyanohydroxycinnamic acid matrix in a ratio of 1:1. Using this method, microcystin-LR (995 m/z), microcystin-LA (910 m/z), microcystin-RR (1038 m/z), and microcystin-YR (1045 m/z) can be detected at concentrations as low as 0.01 ppm. Additionally, rodenticides such as brodifacoum (523/525 m/z), strychnine (334 m/z), and ractopamine (301 m/z) are also detectable with estimated detection limits ranging from 0.1 ppm to 1 ppm. Although some sample preparation is involved in using this method, the MALDI-TOF-MS is a high-throughput format technique that can prove to be a rapid reliable tool for detection and confirmation of a variety of toxins and toxicants for diagnostic veterinary toxicology.

◊ USAHA Paper

Comparison of Two Extraction mMethods for Monensin in Feed by LCMSMS ◊

Paula M. Imerman, Dwayne Schrunck, Ray Grover, Wilson K. Rumbeiha, Steve M. Ensley

Veterinary Diagnostic Lab, Iowa State University, Ames, IA

Monensin, Lasalocid, Salinomycin, and Narasin also known as polyether antibiotics are mainly used as coccidiostats in animal feed. These compounds possess unique structural properties which allow them to act as cation carriers across biological membranes. Thus they have microbiological activity against gram positive bacteria. Also ionophores can act as growth promoters in animals. Due to the potential of these compounds to be toxic above recommended levels or their interaction with certain drugs to potentiate their effects monitoring levels in feed is essential. This study compares two extraction methods for the determination of Monensin using LCMSMS as the output. AAFCO feeds at various concentrations (ppm) ie., low,(20), medium (300), and high (1200) were used in the study. Method 1 uses hexane:ethyl acetate (90:10) 5g/40ml and Method 2 uses methanol:water 5g/20ml (90:10) for extraction of the feeds. It was found that both extraction levels worked well for the low and medium levels falling within the range of standard deviation values given by the AAFCO. However at the high level (1200 ppm) it was found that extraction Method 2 with standard addition method of 500 and 1000 ppm worked best for accurate results. The LCMSMS method has an LOQ of 1ppm and an LOD of 0.1ppm for Monensin. In future for high samples gram to solvent ratio will be investigated to see if this can improve performance.

◊ USAHA Paper

The Effect of *Foot-and-Mouth Disease Virus* Inactivation on the Status of Serum Mineral and Vitamin Concentrations §

Dwayne E. Schrunk

Department of Toxicology and Nutrition, College of Veterinary Medicine, Iowa State University, Ames, IA

The effect on serum trace mineral and vitamin concentration of three USDA APHIS approved treatments for the inactivation of *Foot-and-Mouth Disease virus* (FMDV) from animal specimens were compared. In order to ship animal blood serum from some foreign countries into the United State the samples must be treated to inactivate the FMDV. Several treatment options for inactivation of FMDV are approved by the FDA, of which three seem the most appropriate for serum. The three options are to expose the specimen to heat (72 C for at least 30 minutes), to make the specimen acidic (pH <= 5.5 for at least 30 minutes), and/or to make the specimen basic (pH >= 10 for at least two hours). Previously analyzed serums were pooled to produce a sample sufficiently large enough to compare the various treatment options. Analysis was performed to determine the concentration of Vitamins A and E, as well as trace minerals Ca, Cu, Fe, K, Mg, Mn, Mo, P, Se, and Zn. When compared to regularly used methods for analysis of trace minerals and vitamins in serum the preliminary data indicates that making samples acidic works best for trace mineral analysis and making samples basic works best for analysis of vitamins. The average recovery of the trace minerals was 91% for the acid treatment as compared to our routine analysis procedure. Additionally, analysis of two reference serums using the acid treatment produced results in the acceptable range. For Vitamins A and E the average recovery was 100% and 74%. Further investigation into the effect of the treatments on the stability of the vitamins and minerals on storage is ongoing.

§ AAVLD Staff Travel Awardee

Epidemiology 1

Saturday, October 18, 2014
Chicago C

Moderators: Bruce L. Akey, Belinda Thompson

1:00 PM **Outbreak and Elimination of PRRSV from Switzerland in 2012/2013 - Collaboration of Swiss Animal Health Authorities and QIAGEN Leipzig ◊**
Nevena Djuranovic, Carsten Schroeder, Christine Gaunitz, Guido Fritsch, Patrica Scheer, Barbara Thur. 59

1:15 PM **Genotype Prevalence and Mixed-Genotype Infections Revealed by Group A *Rotavirus* Full-Genome Sequencing in Cattle and Swine**
Elizabeth G. Poulsen, Haixia Li, Barbara Breazeale, Joe Anderson, Qing Sun, Ben Hause, Chester McDowell, Juergen Richt, Gary A. Anderson, Richard Hesse, Jianfa Bai 60

1:30 PM **Behavioral Aspects of Swine Oral Fluid Sample Collection**
Ashley Holmes, Apisit Kittawornrat, Yaowalak Panyasing, Christa Goodell, Karthik Subramanya, Steve Hoff, Chong Wang, Jeff Zimmerman 61

1:45 PM **Studies of the Emerging Pestivirus Species *Pronghorn Virus*; Antigenic Cross Reactivity with other Pestiviruses, Recent Detection in Wildlife and Clinical Presentation in Goats and Deer**
Julia F. Ridpath, Fernando Bauermann, Shollie M. Falkenberg, John D. Neill, Peregrine L. Wolff. 62

2:00 PM **The Prevalence and Epidemiology of *Equine Rhinitis A Virus* and *Equine Rhinitis B Virus* Urine Shedding in Horses**
Steven Grubbs, Robert Keene, Andres Diaz-Mendez, Laurent Viel, John Tuttle, Christian M. Leutenegger 63

2:15 PM **Dual Infections with *Canine Parvovirus* Type 2 (CPV-2) and *Dog Circovirus* (DCV) in Michigan Dogs**
Annabel G. Wise, Roger K. Maes, Thomas P. Mullaney, Tuddow Thaiwong, Matti Kiupel 64

2:30 PM **Point of Need Detection of Canine Respiratory Disease Pathogens on POCKIT, a Portable Molecular Detection System ◊**
Jessie D. Trujillo, Uri Donnett, Chuan Fu Tsai, Yun-Long Tsai, Pei-Yu Lee, Fu-Chun Lee, Hsiu-Hui Chang, Pin-Hsing Chou, Bor-Huah Chen, Li-Juan Ma, Yu-Han Shen, Hsiao Fen Grace Chang, Thomas Wang 65

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

Outbreak and Elimination of PRRSV from Switzerland in 2012/2013 - Collaboration of Swiss Animal Health Authorities and QIAGEN Leipzig ◊

Nevena Djuranovic[1], Carsten Schroeder[2], Christine Gaunitz[2], Guido Fritsch[2], Patrica Scheer[3], Barbara Thur[4]

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We describe an outbreak of Porcine Reproductive and Respiratory Syndrome (PRRS) in Switzerland, introduced into a Swiss PRRSV negative population by boar semen and how a good collaboration of the Swiss authorities together with a reliable supplier of high quality veterinary diagnostics helped to eradicate the disease in only seven weeks. The Swiss pig population is 1.5 million and free of PRRS. Import of live pigs into Switzerland is only permitted after quarantine. Previous to 2013 up to 32,000 doses of boar semen per year were imported without restrictions. Boar facilities tested approximately 10% of boars every 4 weeks using ELISA and PCR from serum. In addition, boars delivering semen for Switzerland were tested every 2 weeks in between by PCR from semen samples. On November 27th 2012, PRRSV was detected in a German boar facility delivering boar semen to Switzerland. One blood sample and semen sample from two boars were PRRSV positive. The outbreak was confirmed by the detection of PRRSV in up to 90% of the boars in one boar-stable. The Swiss authorities were informed by the German boar station on November 28th. By that time 26 Swiss farms had received boar semen from the infected boar facility in the last 2 weeks, 5 of those farms received semen from the two boars which were initially tested as PRRSV positive. All 26 farms and all 61 contact farms were put on quarantine on November 29th. The sudden, overnight requirement of PRRSV PCR for 87 farms was handled by the Swiss Institute of Virology and Immunology (IVI) in collaboration with QIAGEN Leipzig. In total, over 15,000 tests (ELISA and PCR) were conducted in a seven week time frame, where in over 7200 PCR. Samples were collected by veterinarians, vet students, and employees of the Swiss Pig Health Service. The testing was conducted in 3 Swiss laboratories. The costs for sample collection, diagnostic reagents, and testing service were estimated at € 1 Mill. On January 11th, the PRRS free status of Switzerland was confirmed and restrictions lifted. The following new regulations, for importing boar semen into Switzerland, are now implemented by the Swiss authorities: The foreign boar station must have EU approval and be free of Aujeszky. Testing for PRRSV must be performed on blood and semen samples by PRRSV PCR and ELISA. Swiss farms using fresh semen are not permitted to sell pigs for 4 weeks. This ban is lifted if blood samples from such farms are tested PRRSV negative 4 weeks after using such semen samples. Frozen semen can be used on Swiss Farms only three months after collection. During this monthly examination for PRRSV must be done on the original boar station. Virotype PRRSV PCR kit is officially approved by the German and Swiss authorities for PRRS control. The PCR assay allowed to eradicate the 2012 PRRS outbreak and will be used to reliably prevent PRRSV from entering Switzerland.

◊ USAHA Paper

Genotype Prevalence and Mixed-Genotype Infections Revealed by Group A *Rotavirus* Full-Genome Sequencing in Cattle and Swine

Elizabeth G. Poulsen[1], Haixia Li[1], Barbara Breazeale[1], Joe Anderson[1], Qing Sun[1], Ben Hause[13], Chester McDowell[2], Juergen Richt[2], Gary A. Anderson[1], Richard Hesse[1], Jianfa Bai[1]

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Rotavirus infections cause severe gastroenteritis in both humans and animals and are responsible for approximately 600,000 human deaths per year worldwide. *Rotavirus* groups A, B, and C are known to infect mammals and cause losses in production animals. Rotaviruses are members of the Reovirus family and have a double-stranded RNA genome comprising 11 segments approximately 680-3,500 bp in length. Kansas State University Veterinary Diagnostic Laboratory (KSVDL) processed more than 300 porcine and bovine fecal samples of suspected rotavirus infection for whole genome sequencing. By employing the single primer amplification technique (SPAT) combined with next-generation sequencing on the Illumina MiSeq, we obtained 240 full-length or near full-length genome sequences. In cattle, 100% (n=85) of the fecal samples sequenced were identified as group A rotavirus, while in swine, 37% (n=57) of the samples yielded group A sequences, 59% (n=91) were group C and 18% (n=28) were group B strains, with some mixed-group infections. In cattle, six VP4 and six VP7 genotypes were identified, with P[5]-G6 being most prevalent (present in 75% [n=63] of samples). In swine, six VP4 and seven VP7 genotypes were identified, with P[7]-G9 and P[23]-G4 being the most common genotypes (present in 26% [n=15] and 25% [n=14] of group A samples, respectively). Two alleles were identified for each of the other nine segments, with one exception in cattle (3 alleles for NSP1) and two exceptions in swine (3 alleles each for NSP1 and NSP3). Genotypes were considerably species-specific. However, a small number of porcine samples with bovine genotypes were identified, strongly suggesting cross-species transmission. Fourteen percent (n=21) of the porcine samples yielded full or near-full genome sequences of more than one rotavirus (8% [n=13] groups A and C, 4% [6] groups A and B, 1% [1] groups B and C, and 1% [1] groups A, B, and C). Several bovine and porcine samples also contained sequences for more than one genotype for a particular gene segment. The VP7 segment was the most common mixed genotype, with 9 porcine and 9 bovine samples yielding multiple VP7 genotypes. Similar to other studies, our results confirmed the co-circulation of diverse rotaviruses in farm animals and probable frequent interspecies infection. Whole genome sequencing is a valuable tool for determining the genome diversity and epidemiology of *Rotavirus* infections in pigs and cattle.

Behavioral Aspects of Swine Oral Fluid Sample Collection

Ashley Holmes[1], Apisit Kittawornrat[1], Yaowalak Panyasing[1], Christa Goodell[3], Karthik Subramanya[2], Steve Hoff[2], Chong Wang[1], Jeff Zimmerman[1]

[1]Department of Veterinary Diagnostics and Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Agricultural & Biosystems Engineering, Iowa State University, Ames, IA; [3]IDEXX Laboratories, Westbrook, ME

The use of swine oral fluid specimens in research, diagnostics, and surveillance has been the focus of recent investigations. The majority of this work has focused on the detection of pathogen-specific antibody or nucleic acid in the oral fluid matrix (1, 2). In contrast, the behavioral aspects of oral fluid collection have been under-researched and many questions remain. The general focus of the present study was on the process of oral fluid sample collection, with the specific aim of evaluating the effect of the location and number of ropes provided in the pen on pig oral fluid sampling behavior. The ultimate goal of this line of research is to determine the number of ropes to be placed as a function of the number of pigs in the pen. The specific questions addressed in this study were: (1) The effect of the number of ropes in the pen on oral fluid sampling behavior; (2) the effect of the location of the rope(s) within the pen on sampling behavior. Sixty 5-week-old pigs were divided into two groups of 30 (15 gilts, 15 barrows) and placed in two pens of identical size and structure. For 9 days pigs were acclimated to the pen and familiarized to oral fluid collection using 2 cm (3/4 inch) 3-strand 100% cotton rope. During acclimatization and throughout the study, oral fluids were collected at approximately 7:00 in the morning. The process of harvesting oral fluids and quantifying the sample is described in detail elsewhere (1). Ropes for collecting oral fluids were hung at the corners of the pens. Four cameras synchronously took pictures at 2 second intervals throughout the 20 minute sampling period. Pictures were used to quantify pig behavior. “Rope contact” was defined as a picture showing a pig’s mouth closed around the rope. Observations were taken at both the group and individual level. That is, the whole pen (30 pigs per pen), and a subset of pigs (10 ear-tagged pigs in each pen). The tagged pigs were chosen at random and marked with colored ear tags. Analysis of data showed that the total volume of oral fluid, the number of pigs contacting rope(s), and the total time that pigs chewed rope(s) increased as more ropes were provided. Pigs showed a clear preference rope placed in certain locations, but the basis for this preference was undeterminable. Overall, these data imply that the collection of oral fluid samples can be improved by understanding the behavioral aspects of the process. References: 1. Kittawornrat A, et al.: 2014, Vet Micro 168:331-339. 2. Mur L, et al.: 2013, Vet Micro 165:135-139.

Studies of the Emerging Pestivirus Species *Pronghorn Virus*; Antigenic Cross Reactivity with other Pestiviruses, Recent Detection in Wildlife and Clinical Presentation in Goats and Deer

Julia F. Ridpath[1], Fernando Bauermann[1], Shollie M. Falkenberg[2], John D. Neill[1], Peregrine L. Wolff[3]

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The Pestivirus genus of the family *Flaviviridae*, is composed of a group of antigenically related positive strand RNA viruses. Recognized species of the genus include *bovine viral diarrhea virus* types 1 and 2, *classical swine fever virus* and *border disease virus*. Putative species include *Bungowannah virus*, *giraffe virus*, *HoBi-like virus and pronghorn virus* (PHV). PHV was first isolated from an immature, blind pronghorn antelope. Phylogenetic analysis shows that PHV is more distant from the recognized species of pestivirus than the recognized species are to each other. In the study reported here, antigenic comparison, using hyperimmune goat serum, demonstrated that the antigenic cross reactivity between PHV and the recognized pestivirus species is low. For over a decade, no other detection of PHV was reported. However, in a PCR-based survey of samples collected in the state of Nevada from wildlife in the last three years, PHV was detected in mule deer, big horn sheep and mountain goat samples. Clinical presentation following infection of goats with PHV included mild, short-term pyrexia and a decrease in circulating lymphocytes. Infection of white tailed deer resulted in death in 2 out of 6 animals inoculated. Pre-existing antibodies, against BVDV in deer, did not prevent replication of PHV or decreases in circulating lymphocytes. PHV was not transmitted to penmates following infection of either goats or white tailed deer.

The Prevalence and Epidemiology of *Equine Rhinitis A Virus* and *Equine Rhinitis B Virus* Urine Shedding in Horses

Steven Grubbs[1], Robert Keene[1], Andres Diaz-Mendez[2], Laurent Viel[2], John Tuttle[1], Christian M. Leutenegger[3]

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Equine Rhinitis A Virus (ERAV) has been considered to be a common respiratory viral infection in horses worldwide. Primary viral replication takes place in the pharyngeal region and spread from horse to horse through nasal secretions. The purpose of this study was to determine the prevalence of *Equine Rhinitis A Virus* and *Equine Rhinitis B Virus* (ERBV) in post-race urine samples from horses in three distinct geographic regions in the US using specific RT-PCR. Two-hundred sixty-eight post-race urine samples were collected from normal Thoroughbred and Quarter Horses from California, Pennsylvania, and Florida and submitted to IDEXX laboratories for detection of ERAV and ERBV by specific RT-PCR. Urine samples were stored at 40C for less than 3 months. The mean particles/mL in urine was determined for all *Equine Rhinitis Virus* positive samples. One-hundred eighteen urine samples were submitted from California. All 118 urine samples were ERBV negative, whereas 33 of 118 (27.9%) were ERAV positive. The mean ERAV particles/mL in urine was 8,866,620. One-hundred urine samples were submitted from Pennsylvania horses. There were 23 of 100 (23%) RT-PCR ERAV positive compared to 0 of 100 urine samples ERBV positive. The mean ERAV particles/mL in urine was 403,811. Fifty urine samples were submitted from Florida horses. Eleven of 50 (22%) were ERAV positive, whereas 0 of 50 were ERBV positive. The mean ERAV particles/mL in urine was 2,549,642. To confirm the presence of live-virus urine shedding, rabbit-kidney-13 (RK-13) cells were exposed to four separate filtered RT-PCR ERAV positive urine samples. Samples were incubated and examined every 24 hours for cytopathic effect (CPE). Cells were considered negative if CPE was not observed after 3 passages. Two of 4 samples had evidence of CPE and further investigated by electron microscopy. Briefly, the two isolates were concentrated by ultracentrifugation, supernatants were discarded, and the pellets fixed in 200uL of 0.1% gluteraldehyde in PBS. Conventional electron microscopy was performed on the preparation. Virus particles consistent with the Apthovirus genus of the *Picornaviridae* family were observed and electron micrographs were taken. Overall, 67 of 268 (25%) urine samples were positive by RT-PCR for ERAV, whereas all 268 urine samples were ERBV negative. Horses from California (27.9%) had the highest percent ERAV positive, followed by horses from Pennsylvania (23%) and Florida (22%). Urine shedding of live ERAV was established in 2 horses by propagation in RK-13 cells and confirmed by electron microscopy. Natural infection by ERAV in this population of horses resulted in viral RNA (mean ERAV particles/mL - 3,940,024) shed in urine. The mechanism of ERAV urine viral shedding requires further investigation along with the impact of urine viral shedding on ERAV transmission in horses.

Dual Infections with *Canine Parvovirus* Type 2 (CPV-2) and *Dog Circovirus* (DCV) in Michigan Dogs

Annabel G. Wise[1], Roger K. Maes[12], Thomas P. Mullaney[12], Tuddow Thaiwong[12], Matti Kiupel[12]

[1]Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI; [2]Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI

A Michigan dog breeding facility experienced outbreaks of bloody diarrhea in March 2013 and February 2014. Despite a history of vaccination, fatalities occurred in a number of both adult dogs and puppies. One dog from the 2013 outbreak and two dogs from the 2014 outbreak were submitted to the Diagnostic Center for Population and Animal Health (DCPAH) for necropsy. Both gross and histologic findings were consistent with CPV-2 infection. Histologically, the affected segments of the small intestine of the dogs submitted had a marked loss and necrosis of mucosal crypts. There was marked lymphoid follicle depletion in the spleen and Peyer's patches. Numerous mesenteric lymph nodes had marked sinusoidal histiocytosis and small focal areas of granulomatous inflammation. Real-time PCR testing confirmed the presence of very high levels of CPV-2 DNA in affected tissues. Immunohistochemistry demonstrated the presence of abundant amounts of CPV-2 antigen within crypt epithelial cells in the affected segments of the small intestine, but only few positive cells in lymphoid follicles in spleen, lymph nodes and Peyer's patches. In September 2013, archived tissue DNA from the CPV-2 infected dog submitted from the March 2013 outbreak was retrospectively tested for DCV. A previously described real-time PCR for DCV detected very high levels of the virus, revealing a case of dual infections with DCV and CPV-2. Subsequently, the two dogs from the 2014 outbreak were also determined to be dually infected with both viruses. An in situ hybridization assay for DCV, newly developed at DCPAH, detected abundant amounts of viral nucleic acid in all lymphoid tissues, most commonly in the cytoplasm of histiocytic cells. Large amounts of DCV were also detected within the cytoplasm of macrophages in areas of granulomatous inflammation. Nuclei of regenerating crypt epithelial cells were rarely positive. Kupffer cells in the liver were positive for both CPV-2 and DCV. The presence of DCV viremia in surviving dogs was also evaluated, based upon its significance in *porcine circovirus* pathogenesis. Significantly, two surviving dogs, one from each outbreak, were viremic for DCV when tested 2 months to 1 year post-recovery. Overall, the marked lymphoid depletion and the abundance of DCV in all lymphoid tissues suggest an immunosuppressive role of DCV, potentiating a concurrent CPV-2 infection. Alternatively, CPV-2 infection could induce the target cells for subsequent DCV replication.

Point of Need Detection of Canine Respiratory Disease Pathogens on POCKIT, a Portable Molecular Detection System ◊

Jessie D. Trujillo[1], Uri Donnett[1], Chuan Fu Tsai[2], Yun-Long Tsai[2], Pei-Yu Lee[2], Fu-Chun Lee[2], Hsiu-Hui Chang[2], Pin-Hsing Chou[2], Bor-Huah Chen[2], Li-Juan Ma[2], Yu-Han Shen[2], Hsiao Fen Grace Chang[2], Thomas Wang[2]

[1]Center for Advanced Host Defenses, Immunobiotics and Translational Comparative Medicine, Iowa State University, Ames, IA; [2]GeneReach USA, Boston, MA

Canine Distemper virus (CDV), *Canine Herpes virus-1* (CHV-1), *Canine Parainfluenza virus* (CPIV), *Canine Respiratory Corona virus* (CRCoV), *Canine Adenovirus-2* (CAV-2), Canine Influenza virus (CIV), and *Bordetella bronchiseptica* are pathogens resulting in Canine Respiratory Disease (CRD). Proper diagnosis of CRD pathogens is paramount for patient care, population medicine, and biosecurity. Time to diagnosis is critical due to their highly infectious nature and ability to cause sometimes life threatening disease. When tested, samples are shipped to reference laboratories, delaying diagnosis and thus hindering infectious disease control. Here we evaluate pathogen specific insulated isothermal PCR (iiPCR) assays in the field deployable device, POCKIT, for the detection of important pathogens in the dog. Published or de novo, real time PCR (qPCR) assays were validated as reference assays on the BioRad CFX96. Limits of detection (LOD) were determined via pathogen standards and were performed side by side for both platforms. Clinical samples (30 positive/30 negative) were tested side by side, in triplicate. When sufficient clinical samples were not available, various dilutions of the pathogen standard or vaccine were tested as surrogate positives. Reference assay LOD for all canine pathogens fell one to 3 logs below one infectious unit except *B. bronchiseptica* with an LOD of one infectious unit. LOD for iiPCR assays on POCKIT are equivalent or within on log of the reference assays. Acceptable sensitivities for iiPCR assays on POCKIT are 98.5%, 100%, 93.3% and 96.7% for CIV, CDV, CAV-2 and CPIV assays. Specificity for all assays was 96-100%.For *B. bronchiseptica*, CHV, and CRCoV Sensitivity were 53.3%, 76.7% and 71%. These three reagent sets have been redesigned accordingly and evaluation of their sensitivity and specificity are underway. POCKIT portable molecular detection system has exceptional performance in detection of several relevant pathogens such as Canine Distemper and Canine Influenza viruses and can have profound impact on infectious disease control in canine populations such as kennels, shelters and urban areas.

◊ USAHA Paper

Virology 1

Saturday, October 18, 2014
Chicago A

Sponsor: ECL2

Moderators: Christie Mayo, Lalitha Peddireddi

1:00 PM **Rapid Isothermal Detection of *Porcine Epidemic Diarrhea Virus* (PEDV) RNA**
Yogesh Chander, Thomas W. Schoenfeld, Kyoung-Jin Yoon, Sagar M. Goyal, David A. Mead *69*

1:15 PM **Development and Evaluation of a Colloidal Gold Particles-Based Immunochromatographic Strip for Specific Detection of *Porcine Epidemic Diarrhea Virus* #**
Yuekun Lang, Xi Li, Dianlei Guo, Fang Fu, Xiangling Wang, Xueyun Zhang, Kunpeng Li, Li Feng, Wenjun Ma *70*

1:30 PM **Development and Evaluation of a Duplex Real-Time RT-PCR for Detection and Differentiation of Classical and Variant Strains of *Porcine Epidemic Diarrhea Viruses* from the United States** §
Leyi Wang, Yan Zhang, Beverly Byrum *71*

1:45 PM **Real-Time RT-PCR comparison to Ensure Accurate Detection of PEDV and TGEV** ◊
Douglas Marthaler, Marie G. Culhane, Kurt D. Rossow, Yin Jiang *72*

2:00 PM **Kinetics of the *Porcine Epidemic Diarrhea Virus* (PEDV) Humoral Immune Response (IgM, IgA, and IgG) in Serum and Oral Fluid Specimens from Pigs Infected Under Experimental Conditions**
Luis G. Gimenez-Lirola, Hai T. Hoang, Qi Chen, Dong Sun, Darin Madson, Drew Magstadt, Leslie Bower, Paulo Arruda, Mahesh Bhandari, Jeff Zimmerman, Jianqiang Zhang, Kyoung-Jin Yoon *73*

2:15 PM **Disease Investigation and Virus Isolation of *Porcine Deltacoronavirus***
Melinda Jenkins-Moore, Leo Koster, Colleen Bruning-Fann, Montserrat Torremorell, Mary Lea Killian, Steve Tousignant *74*

2:30 PM **Detection and Characterization of a *Porcine Deltacoronavirus* from Pigs with Diarrhea** ◊
Yan Zhang, Leyi Wang, Beverly Byrum *75*

2:45 PM **Characterization of Genotypically Distinct Enteric and Respiratory *Bovine Coronaviruses*** ◊
Alexa Ukena, Joe Anderson, Ben Hause, Elizabeth G. Poulsen, Richard Hesse *76*

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Rapid Isothermal Detection of *Porcine Epidemic Diarrhea Virus* (PEDV) RNA

Yogesh Chander[1], Thomas W. Schoenfeld[1], Kyoung-Jin Yoon[2], Sagar M. Goyal[3], David A. Mead[1]

[1]Lucigen Corporation, Middleton, WI; [2]Iowa State University, Ames, IA; [3]Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN

Recent emergence of *porcine epidemic diarrhea virus* (PEDV) in the United States has caused significant economic concern to the swine industry. Since its first detection in April 2013, this virus has rapidly spread across the United States. While many diagnostic tests are available for PEDV detection, they cannot be used at penside, highlighting the need of such a rapid diagnostic test. This project is intended to address the need for a sensitive, specific, affordable, and fast solution for penside detection of PEDV. As a first step in providing a penside test for PEDV, we have developed an isothermal molecular detection assay. This test is based on reverse transcription loop-mediated isothermal amplification (RT-LAMP) using OmniAmp DNA polymerase. Detection is based on signal generation by a fluorescent intercalating dye that binds to the double stranded RT-LAMP DNA product. This chemistry is intended to be used eventually with a low-cost, portable, and easy to operate instrument, which is being developed concurrently to facilitate penside detection of PEDV and other agricultural pathogens. LAMP primers were designed to target nucleocapsid (N) gene of PEDV and reaction conditions were optimized for specific detection of PEDV. The time to result was under 30 minutes with no false positives in the negative control. Analytical sensitivity (50 copies of RNA/μl) of the assay was found to be comparable to real time RT-PCR. Analytical specificity of LAMP assay was evaluated using nucleic acid extracts from other swine pathogens such as TGEV, PCV-2, PRRSV, and SIV that are commonly found in swine production systems. We also developed a simple heat lysis protocol without the use of any expensive equipment as a method of sample preparation for low resource settings. This sample preparation method and RT-LAMP assay were used to test a cohort of residual clinical samples previously tested positive by RT-PCR, resulting in clinical sensitivity of 80% and 100% specificity. The combination of performance, time to result, ease of operation and interpretation, low cost and compatibility with less complex instrumentation suggest that this test platform can be used in testing laboratories or penside along with other diagnostic assays to provide timely results to swine producers.

Development and Evaluation of a Colloidal Gold Particles-Based Immunochromatographic Strip for Specific Detection of *Porcine Epidemic Diarrhea Virus* #

Yuekun Lang[2], Xi Li[1], Dianlei Guo[1], Fang Fu[1], Xiangling Wang[1], Xueyun Zhang[1], Kunpeng Li[1], Li Feng[1], Wenjun Ma[2]

[1]Division of Swine Infectious Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Harbin, China; [2]Department of Diagnostic Medicine/Pathobiology, Kansas State University, College of Veterinary Medicine, Manhattan, KS

Porcine epidemic diarrhea virus (PEDV), a member of the *Coronaviridae* family, causes acute diarrhea and dehydration with significant morbidity and mortality in pigs. The economic impact of the PEDV has substantially increased, particularly in Korea, China, Japan, and the United States. It is difficult to diagnose PEDV simply by clinical signs and histopathological lesions. Although many techniques can be used for the detection of PEDV including enzyme-linked immunosorbent assays (ELISA), immunofluorescence (IF) tests and RT-PCR, they are time consuming and must be tested in laboratory. In the present study, a rapid immunochromatographic (ICG) strip based on a conjugate of colloidal gold and monoclonal antibody (mAb) was developed for the rapid, sensitive detection of PEDV in samples from the field. The two specific monoclonal antibodies (mAbs) against PEDV were produced and used as the capture and the detection mAbs, respectively. The detection limit of the ICG strip for PEDV is 105 TCID50/ml, and the assay can be completed in 10 minutes. A cross-reactivity test indicated that the ICG strip was highly specific to PEDV showing no cross-reactivity with *porcine transmissible gastroenteritis coronavirus* (TGEV), *rotavirus* (RV), *porcine reproductive and respiratory syndrome virus* (PRRSV), *classic swine fever virus* (CSFV), and *porcine pseudorabies virus* (PRV) The results of the recovery test from the fecal samples in the field were in good agreement with those obtained by ELISA. The correlation between the two methods was K=0.93, 95% and CI: 0.90-1.00 when testing the fecal samples. Accordingly, the use of ICG technology provided an efficient, effective, and rapid means of detecting the presence of coronavirus PED antigen in field samples and indicates that it is a very useful tool for diagnosis in the field.

AAVLD Trainee Travel Awardee (Virology)

Development and Evaluation of a Duplex Real-Time RT-PCR for Detection and Differentiation of Classical and Variant Strains of *Porcine Epidemic Diarrhea Viruses* from the United States §

Leyi Wang, Yan Zhang, Beverly Byrum

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

Porcine epidemic diarrhea virus (PEDV) was identified in May of 2013 in the United States (US). Since then, PEDV has been detected in 29 US states, causing significant economic losses to US swine industry. A new variant strain of PEDV was identified in late January 2014. Compared with classical PEDV, the variant PEDV strain contains three deletions, one insertion, and several nucleotide variations in first 1170 nt of the S1 domain in the spike gene. Variant PEDV has been detected in samples from multiple states by our laboratory as well as other laboratories in the US. It is critical to detect and differentiate variant PEDV from the classical PEDV during outbreaks to enhance control and prevent PED associated disease. Here, we report the development and validation of a duplex real-time RT-PCR assay for detection and differentiation of the variant and the classical strains of PEDV. The primers and probes were designed by targeting the conserved and deletion regions of the first 1170 nt of S1 region, respectively. The developed duplex real-time RT PCR has a high sensitivity (1 genome copy detection limit for both variant and classical PEDV) and specificity (no cross reaction with other porcine viruses). In addition, the positive field samples detected by this new assay were further confirmed by subsequent DNA sequencing. The duplex real-time RT-PCR offers a rapid and sensitive method to detect both classical and variant PEDV from clinical samples, and will allow differentiation of variant strains from classical PEDV strains.

§ AAVLD Staff Travel Awardee

Real-Time RT-PCR comparison to Ensure Accurate Detection of PEDV and TGEV ◊

Douglas Marthaler, Marie G. Culhane, Kurt D. Rossow, Yin Jiang

University of Minnesota, St. Paul, MN

Porcine epidemic diarrhea virus (PEDV) is major cause of severe diarrhea and dehydration in pigs. Belonging to the *Coronaviridae* family, PEDV is an enveloped, positive-sense, single-stranded RNA virus with a genome size of approximately 28kb. The first detection of PEDV was reported in 1971 from England while Japan, China, South Korea, and Thailand also have reported PEDV infections. The United States first detected PEDV in May 2013. The veterinary diagnostic laboratories quickly developed sensitive and specific real time RT-PCR (RRT-PCR) assays to detect PEDV in a variety of porcine and environmental samples. In this study, we compared the PEDV-TGEV multiplex RRT-PCR assay developed at the University of Minnesota (UMN) to a commercial TGEV-PEDV multiplex RRT-PCR assay. Porcine intestinal samples, fecal samples, fecal swabs, oral fluid samples, and environmental samples are routinely submitted to UMN Veterinary Diagnostic Laboratory for enteric pathogen testing. Sample homogenates were extracted with the MagMax 96 Viral RNA Isolation Kit (Thermo Scientific), according to manufacturer's instructions. The commercial TGEV-PEDV multiplex RRT-PCR assay was preformed, according to manufacturer's instructions, while the UM RRT-PCR assay utilized the Path-ID Multiplex One-Step RT-PCR kit (Thermo Scientific, according to manufacture's instructions. A total of 396 samples, consisting of porcine oral fluids (n=39), intestinal homogenates (n=107), fecal (n=136), fecal swabs (n=47), feedback (n=12) and environmental samples (n=55), were compared with the UMN TGEV-PEDV multiplex RRT-PCR and the commercial TGEV-PEDV multiplex RT-PCR assays. The UMN TGEV-PEDV multiplex RRT-PCR assay had lower Ct values compared to the commercial TGEV-PEDV multiplex RRT-PCR assay. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 53 more positive PEDV samples (oral fluids (n=6), intestinal homogenates (n=9), fecal (n=6), fecal swabs (n=13), and environmental samples (n=19)) compared to the commercial TGEV-PEDV multiplex assay. The additional positive PEDV samples as indicated by the UMN TGEV-PEDV multiplex assay, but negative by the commercial TGEV-PEDV multiplex assay, were confirmed positive by a secondary UMN PEDV RRT-PCR assay, which targeted the N gene. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 11 more positive TGEV samples (intestinal (n=4) and fecal (n=7)) compared to the commercial TGEV-PEDV multiplex RRT-PCR. The UMN TGEV-PEDV RRT-PCR assay had superior performance over the commercial TGEV-PEDV multiplex RRT-PCR assay. Accurate detection of PEDV and TGEV in clinical samples is important to minimize the spread of these two viruses. The role of the clinical diagnostic laboratories is to provide high sensitivity and specificity assay to help prevent and control pathogens and many assays must be evaluated before choosing the best assay to support the swine industry.

◊ USAHA Paper

Kinetics of the *Porcine Epidemic Diarrhea Virus* (PEDV) Humoral Immune Response (IgM, IgA, and IgG) in Serum and Oral Fluid Specimens from Pigs Infected Under Experimental Conditions

Luis G. Gimenez-Lirola, Hai T. Hoang, Qi Chen, Dong Sun, Darin Madson, Drew Magstadt, Leslie Bower, Paulo Arruda, Mahesh Bhandari, Jeff Zimmerman, Jianqiang Zhang, Kyoung-Jin Yoon

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

Since first isolation in April 2013, *porcine epidemic diarrhea virus* (PEDV) has becomes more endemic in U.S. herds causing tremendous economic losses to the swine industry. The key to preventing the spread of PEDV is to control the movement of animals and fomites contaminated with the virus combined with surveillance. Therefore, serology is a vital tool used to control and to further understand this disease. Anti-PEDV antibodies have been detected in sera from swine with naturally occurring or experimentally induced PED by indirect ELISA2, IFA test3, and serum-virus neutralization test. However, the kinetics of the antibody response against PEDV has not been described to date. We described the ontogeny of the whole virus-specific IgM, IgA, and IgG responses in serum and oral fluid from pigs experimentally inoculated with a 2013 U.S. PEDV isolate. Fifty-six 3-week old pigs were inoculated with a PEDV isolate (USA/Iowa/18984/2013) via gastric gavage. Serum samples were collected on day 0 post inoculation (DPI) and every 7 days thereafter for 56 DPI, while pen-based oral fluid were collected daily for the first week, and twice a week thereafter for 56 DPI using methods previously described4. The same PEDV isolate was propagated in Vero cells, pelleted, processed and used as whole virus antigen for ELISA plate preparation. ELISA conditions such as coating conditions, reagent concentrations, incubation time and buffer compositions were optimized for simultaneous detection of anti-PEDV IgM, IgA, and IgM antibodies in serum and oral fluid specimens. Anti-PEDV IgM, IgA, and IgG responses over time in serum and oral fluid after experimental inoculation showed that, in a short-term, low-level IgM response was detected first (between 7 to 10 DPI) which was followed by a strong IgA and a moderate IgG response. Both IgA and IgG antibodies started to gradually decline after 4 weeks post inoculation. In this study, we addressed the significance of whole virus-based IgM, IgA, and IgG responses at different stages of post-infection. In conclusion, whole virus-based IgM, IgA, and IgG responses were detectable in both serum and oral fluid over time post inoculation with a strong IgA response and a moderate IgG response that could correlate with immune protection against PEDV infection.

Disease Investigation and Virus Isolation of *Porcine Deltacoronavirus*

Melinda Jenkins-Moore[1], Leo Koster[1], Colleen Bruning-Fann[2], Montserrat Torremorell[3], Mary Lea Killian[4], Steve Tousignant[5]

[1]BPA, USDA-NVSL, Ames, IA; [2]VS, USDA-APHIS, East Lansing, MI; [3]Department of Animal Science/Veterinary Medicine, University of Minnesota, St. Paul, MN; [4]Avian, USDA-NVSL, Ames, IA; [5]Department of Computer Science, University of Minnesota, St. Paul, MN

Recently viruses closely related to deltacoronaviruses in China have been identified in multiple locations in the United States by PCR and sequence analysis. In early March, a breed to wean multiplier facility located in Illinois with approximately 2500 sows and 4000 suckling pigs started to experience inappetence and diarrhea in the gestating sows. Diarrhea and high mortality was subsequently observed in nursing piglets. An investigation was initiated to identify the source of the deltacoronavirus introduction. Samples were collected and submitted to the National Veterinary Services Laboratories and the University of Minnesota for diagnostic testing. Intestinal samples were found to be positive by PCR for *porcine deltacoronavirus* (PDCoV) and *rotavirus C*. PDCoV virus was isolated from one of the intestinal samples. Whole genome sequencing was performed on the isolate and was 99% identical to PDCoV HKU15 strain IN2847 from Ohio and PDCoV 8734/USA-IA/2014.

Detection and Characterization of a *Porcine Deltacoronavirus* from Pigs with Diarrhea ◊

Yan Zhang, Leyi Wang, Beverly Byrum

Ohio Department of Agriculture, Animal Disease Diagnostic Laboratory (ADDL), Reynoldsburg, OH

During the late January and early February in 2014, a diarrheal disease occurred in several pig farms in Ohio. The clinical signs were similar to those caused by *porcine epidemic diarrhea virus* (PEDV), including watery diarrhea in sows and death in piglets. However, the mortality in piglets was lower (30%–50%) than that typically observed with PEDV infection. Fecal and intestinal samples from one farm were negative for PEDV, *transmissible gastroenteritis virus*, rotaviruses, and *Salmonella*. Examination of the samples by electron microscopy showed *coronavirus*-like virus particles. However, all samples were negative by a pan-*coronavirus* PCR. A *deltacoronavirus* was detected in all samples. Histological lesions were moderate comparing with that caused by PEDV currently circulating in the US. The virus is closely related (99% identity) to the *porcine coronavirus* HKU15 reported in Hong Kong in 2012. This is the first time that the virus has been detected in diarrheal disease. To date, this virus has been detected in at least 10 states in US.

◊ USAHA Paper

Characterization of Genotypically Distinct Enteric and Respiratory *Bovine Coronaviruses* ◊

Alexa Ukena, Joe Anderson, Ben Hause, Elizabeth G. Poulsen, Richard Hesse

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

Bovine Coronavirus (BCoV) causes enteric and respiratory diseases in cattle and is a component of the bovine respiratory disease complex (BRD). The Kansas State Veterinary Diagnostic Laboratory (KSVDL) routinely performs quantitative PCR surveillance on the respiratory viruses associated with BRD. Surveillance over a 3 year period demonstrated that 13% of 1,135 tissue samples and 29% of the 776 swab samples submitted were positive for BCoV. BCoV was the sole pathogen detected in 34% of tissue samples and 56% of swab samples. The spike gene on a subset of positive samples was sequenced. The Spike glycoprotein is believed to be responsible for host range and is the leading mediator of viral entry. Along with the data provided by KSVDL, genomic information of the spike gene was compared with data publicly available in Genbank. Looking at the phylogenic tree, the spike genomes fell into two distinct clades; Clad one being the vaccine strains and Clade two being the current respiratory disease. The virus isolates from KSVDL were primarily respiratory samples but there was one enteric sample and all samples align in Clade 2 in sub Clade B. The enteric also fell in this range along with the respiratory samples. Future work will investigate the antigenic relationship between enteric and respiratory viruses however our results suggest that BCoV is one of the major contributors to BRD. Further studies of complete BCoV genomes are needed to elucidate the genetic basis for host tropism and the underlying mechanisms of pathogenesis as well as provide information to assess the serological differences between the respiratory and enteric viruses.

◊ USAHA Paper

Pathology 2

Sunday, October 19, 2014

New York A

Moderators: Kelli Almes, Arthur (Bill) Layton

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Variant Strain of *Porcine Epidemic Diarrhea Virus* Caused Mild Histological Lesions in the Small Intestines of Piglets ◊

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

Ohio Department of Agriculture, Animal Disease and Diagnostic Laboratory (ADDL), Reynoldsburg, OH

In January 2014, we detected a variant strain (OH851) of PEDV in samples from a swine farm in Ohio. Genome sequence comparison showed that there was a high nucleotide similarity in either the complete genome (99%) or the full-length spike (S) gene (97%) between variant PEDV and currently circulating PEDV strains from US, whereas a low nucleotide identify (<= 89%) was observed in the first 1,170 nt of the S1 region between them. Importantly, the S1 domain of the OH851 strain is closely related (99% identical) to another PEDV strain (CH/HBQX/10) reported in China, indicating at least two genotypes of PEDV circulate in the US. Histological changes of piglets infected with the variant strain of PEDV were observed in the small intestines (jejunum, ileum), including mild segmental to multifocal villous atrophy, villous fusion, and superficial enterocyte attenuation. Mild lymphoid depletion in 1/5 pigs was observed in colonic lymph nodes. These microscopic changes were much milder than anticipated for PEDV associated infection. No evidence of infection of virulent PEDV, rotaviruses, and TGE virus. No colibacillosis, clostridial enteritis, coccidiosis or cryptosporidiosis was observed microscopically in any of the 44 intestinal sections examined. Further research is needed to monitor the evolution of the variant PEDV as well as virulent PEDV in US swine populations.

◊ USAHA Paper

Histological Lesions in Piglets Associated With a *Swine Deltacoronavirus* ◊

Yan Zhang, Leyi Wang, Jeffrey R. Hayes, Craig Sarver, Beverly Byrum

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

Porcine deltacoronavirus is a member in the genus Deltacoronavirus of the family *Coronaviridae*. The virus was first recognized in pigs in Hong Kong in 2012. Between the end of January and the beginning of February 2014, samples from piglets and sows were received from outbreaks of diarrheal disease resembling porcine epidemic diarrhea (PED) or transmissible gastroenteritis (TGE) from several pig farms in Ohio. Mortality ranged from 30 – 50% in piglets. All samples were negative for PED and TGE viruses. Subsequently, we detected *deltacoronavirus* from all samples. Phylogenetic study indicated that the newly detected virus was closely related to the *porcine deltacoronavirus* reported in Hong Kong. Further study showed this virus was detected in samples from nine out of ten states, demonstrating wide distribution of this virus in the US. Sequence analysis of all isolates from the nine states suggested that a single genotype is circulating in the US. Histopathologic alterations, such as attenuation and cytoplasmic vacuolation of superficial enterocytes, villus atrophy and villus fusion in small intestinal sections were similar to, but less severe, than those observed in piglets affected with PED virus infection.

◊ USAHA Paper

Treatment-Associated Pathologic Findings in Wisconsin Cattle

Melissa J. Behr

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Most pathologies of cattle are due to infectious disease. One our most common pathologic submission in Wisconsin (>90% of cases) is the 7-14-day-old dairy calf with hypotonic dehydration, metabolic acidosis and electrolyte imbalance secondary to diarrhea caused by *Cryptosporidium parvum, rotavirus* and/or *coronavirus*. Intestinal lesions may be subtle due to rapid autolysis, though villous atrophy or mild inflammation (tips of villi) may be found. In addition, lymphoid depletion (stress, acidosis, utilization), abomasitis (dehydration, improper lubrication of mucosa), and mild, culture-negative cholangitis (possible ascending infection) are often seen. Most calves do not have failure of passive transfer (FPT). Treatment-associated lesions include ruminitis, which in some cases is associated with tubing with milk, aspiration pneumonia due to improper tubing, nephrosis (dehydration; gentamicin toxicity), renal papillary necrosis (rare, non-steroidal anti-inflammatory drugs, NSAIDs), antibiotic-induced yeast or clostridial overgrowth, and iron, selenium or sodium toxicity. Recently, a notable trend identified older calves with shipping fever bronchopneumonia being treated with dexamethasone, a long-acting corticosteroid. Most calves with respiratory disease are 6 weeks of age or older—perhaps early detection is difficult because sick calves continue to drink milk. Thus, some calves thought to be in the acute stage clinically, actually have advanced disease. Furthermore, treatment with dexamethasone may alter pulmonary defense mechanisms, decreasing lung clearance and eventually, worsening their already advanced state of disease. Adult cattle are submitted less often to our laboratory. Undesirable outcomes have included a few vaccine reactions (anaphylaxis due to bacterins; abortion due to modified-live IBR vaccines), enteritis due to *C. difficile* following multiple antibiotic courses, and post-surgical peritonitis.

Descriptive Epidemiology, Gross and Histologic Lesions of Toe-Tip Necrosis in Western Canadian Feedlot Cattle

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Toe-tip necrosis (TTN) is a disease of feedlot cattle involving any combination of inflammation and necrosis of the corium; inflammation, necrosis and lysis of P3; or both. TTN typically affects hind feet and more commonly lateral claws. Toe-tip necrosis syndrome (TTNS) refers to sequela associated with TTN that includes lameness and unthriftiness due to arthritis, tenosynovitis, cellulitis and embolic pneumonia. Therefore, TTNS is of concern to feedlot operators and veterinarians for financial and humane reasons. Studies reported herein include a computerized health record search; analysis of health records of animals dead or euthanized because of TTNS; and the post-mortem examination of hind feet of feedlot cattle. A search of health records for 501,438 feedlot cattle, under the care of a beef industry consultancy, compiled over the 6 year period 2007 to 2012, inclusive, found that about 2/10,000 animals died or were euthanized due to TTNS, but that incidence varied between 1 and 24/10,000 at different times and feedlots. These figures are conservative as only animals that died or were euthanized because of TTNS were included. Some animals with TTNS likely survived without being diagnosed or survived following treatment. Retrospective analysis of 702, veterinarian diagnosed, cases of TTNS found 55% were yearlings and 45% were calves <1 year; and 71% were steers and 29% were heifers. Of those animals with a post-mortem diagnosis of TTNS, the mean and median interval between arrival and death was 42.7 and 27.0 days, respectively. Deaths due to TTNS occurred in all months, but were most common between September and November. The post-mortem study was initiated in the fall of 2012. Three veterinary feedlot practices collected 1 or both hind feet from cattle dead or euthanized because of TTNS. The same practices also submitted hind feet from other cattle in the same feedlots, but that had died or been euthanized because of other disease, e.g., bronchopneumonia and myocarditis. In total, 1 or more feet were submitted from 79 animals believed to be affected with TTNS and 67 animals that died or were euthanized with other diseases and believed to be free of TTN. During the 'blind' examination of 39 feet, the presence of apical white line separation (AWLS) correctly predicted the presence of TTN in 100% of claws; and the absence of AWLS correctly predicted the absence of TTN in 97% of claws. A gross exam of other claws with TTN also found AWLS; that lesions progressed proximally from the tip; and no displacement of the P3 relative to the hoof wall. The histologic exam of 6 affected claws revealed no evidence of vasculitis, which might be consistent with BVD virus infection; no evidence of laminitis, which might be consistent with a metabolic disturbance related to feeding a high grain ration and ruminal acidosis; and no evidence of embolic disease. Studies to identify risk factors for the development of AWLS of the hind feet of feedlot cattle are ongoing.

Oak Poisoning in Yearling Cattle

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A herd of approximately 200 yearling cattle were driven from high mountain pasture in the early autumn. The animals were allowed to overnight in an area containing a mixture of Juniper trees, Sagebrush and oak brush. A small pond was also present to provide a water source for the night. The following morning, six yearling heifers were found dead along with numerous sick animals, many of which had moderate to severe dyspnea, blood in the feces and straining. The animals were then moved further down the mountain onto winter pasture. Several more animals died during the next few days, then the deaths resided. The significant renal lesion was characterized by renal tubular necrosis of the convoluted tubules with the formation of granular and hyaline casts. In some tubules, necrosis of the epithelium was severe, leaving only a few tubular cells and bare basement membranes. In less severely affected tubules, fewer numbers of tubular cells were necrotic or missing. Intratubular hemorrhage was also observed along with necrotic epithelial cells within some of the tubules. Some tubular cells were less severely affected and contained only degenerative changes with no necrosis. The completeness of necrosis in groups of tubules with intratubular hemorrhage distinguishes the nephrosis of acute oak poisoning from that of most other causes. The clinical history of exposure to oak (*Quercus* sp.) and the presence of acute renal nephrosis is diagnostic for oak poisoning. Oak poisoning in ruminants, and occasionally horses can be caused by the ingestion of oak leaves, stems and acorns. The toxic substances are gallotannins which are hydrolyzed to tannic acid, gallic acid and pyrogallol which appeared to be the active toxic metabolites. In Western Colorado cattle graze areas abundant with oak brush each year without incident. However, if the feed is limited and they graze heavily on the oak leaves or acorns oak toxicity can occur. Interestingly, the black bear (*Ursus americana*) graze heavily on oak brush in the late fall consuming large numbers of acorn with little or no effect References: 1. Maxie MG, Newman SJ: 2007, UrinarySystem. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG. pp. 473. Saunders Elsevier, Philadelphia, PA.

Pathology of Select Organs after Zilpaterol Supplementation and Variation in Select Hematologic and Anatomic Parameters

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Supplementation of zilpaterol hydrochloride (ZH; Zilmax®) to cattle has been unscientifically implicated as having a negative impact on cattle well-being. Currently, there is no scientific evidence to support or refute these claims. This study was designed to determine the effects of supplementation on hematologic profiles, select organ weights, organ histopathology and cardiac anatomic features at slaughter. Heifers (n=20; 556±7 kg BW) were separated into two groups: Control (CON): no ZH, or 2) Zilpaterol treated (ZIL): supplemented with ZH at 7.56 g/ton (DM basis). The trial was 25 d (-2 to 22d) in duration, with 3 blood-collection periods [-2 to 3d (ZH supplementation started on d 0); 12 to 15d, and 20 to 22d (withdrawal period)]. For each collection period, blood was collected via indwelling jugular catheters (inserted on d -3, 11, and 19) into EDTA tubes and submitted for a complete blood count. At slaughter animal, carcass, liver, kidney, lung, adrenal and heart weights were recorded. Heart necropsy included right ventricular weight, combined left ventricle and septal weights. The right AV, pulmonic, left AV, and aortic circumferences were recorded. Thickness of right and left ventricular free walls and interventricular septum were measured. Cardiac weight ratios and valve diameter ratios were compared. Organ to body carcass weight ratios were determined. No differences were found between groups in any of the standard leukogram parameters excepting hemoglobin ($p=0.01$) which was slightly higher in the ZIL group but within normal reference range and was this difference was present prior to treatment and remained constant. Liver ($p=0.02$) and kidney ($p=0.02$) weights as percent of carcass weight were reduced in treat cattle. Raw liver weight ($p=0.08$) and kidney ($p=0.11$) weights tended to be less and carcasses tended to be heavier in the ZH group. The data demonstrates a metabolic effect evidenced by reduction in hepatic and renal mass as a percentage of carcass weight. Other organ (heart, lung, adrenals) to carcass weight ratios remained similar suggesting this was not a pure carcass weight impact. No treatment associated histopathologic lesions were seen in the organs sampled. No pathologic effects of treatment were found. As cattle may be presented for necropsy late in the feeding period it is important to know the effects if any that beta agonist might have. The absence of any cardiac anatomic differences suggests that beta agonist is not likely a direct contributor to increased reports of cardiac failure in feedlot cattle.

Sodium Toxicity in Neonatal Dairy Calves

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Ten dairy herds from 5 states submitted tissues from calves <14 days of age to the lab for work-up; all calves were fed milk replacer (MR); some calves had a history of abnormal gait, stiffness, muscle twitching, or seizures, but most had no neurologic signs other than lethargy and depression; morbidity and mortality rates ranged from 25% to 100%; most calves had diarrhea, but many were negative for our most common agents of diarrhea (*Cryptosporidium, rotavirus* and *coronavirus*). No consistent gross or histologic lesions were found; brains were almost always non-lesional; acute neuronal necrosis ("dead reds") was found rarely; no eosinophilic meningoencephalitis was seen; a few calves had pneumonia. When available, serum sodium level was found to be >155 mmol/L: calves are at risk of neurologic disease when serum sodium values exceed 160 mmol/L. Brain sodium levels were 1800 ppm or higher (wet matter basis); normal brain sodium value is <1400 ppm, and levels >1800 ppm are confirmatory of sodium toxicosis. In 6/10 herds, milk replacer or electrolytes were the source of excessive dietary sodium, often because softened water was used with a high sodium concentration (>500 ppm); mixing errors were also found. Water that has passed through a softener can have very high levels of sodium, particularly when the water is very hard; it should not be used to mix up commercial MR or used as a source of potable water unless it has been tested and verified to have low levels of sodium <100 ppm. In 4/10 herds, heat stress and feeding MR were thought to be the cause of sodium toxicosis. Since neonatal calves don't drink much water during the first week of life, mild dehydration and feeding MR may be sufficient to cause sodium toxicity in some herds. Submission of fresh brain to the laboratory is essential; serum, plasma, ocular fluid and cerebrospinal fluid (CSF) are also appropriate samples. MR can be submitted to the lab for percent total solids, Na concentration, and osmolality. Sodium toxicosis should be considered if a calf dies within 4-24 hours of onset of signs, with no evidence of neonatal calf diarrhea or other diseases (abomasal tympany or ulcer, omphalophlebitis, pneumonia, sepsis, meningoencephalitis). The likelihood of sodium toxicity increases if the calf has neurologic signs. Histologic cerebrocortical lesions of salt poisoning of neonatal calves may differ from those seen in pigs.

Hepatogenous Chronic Copper Toxicity in a Charolais Heifer ◊

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A six-month old Charolais heifer presented for a two day history of lethargy and inappetence. The calf had been orphaned and was currently unweaned, received 6 L of commercial milk replacer twice daily and was kept in a 1-acre paddock of unimproved "weedy" pasture. On presentation, the calf exhibited severe icterus and was estimated to be 10% dehydrated with hard, pelleted feces. Blood collected for an assessment of the packed cell volume (PCV; 64%) and total solids concentration (6.0 mg/dL) was dark brown in color. Nitrate toxicity is the most common cause of methemoglobinemia in cattle but does not commonly cause icterus. Furthermore, clinical signs of nitrate toxicity were not evident. The owner was unaware of exposure to other exogenous oxidants that may cause methemoglobinemia. A complete blood count and serum biochemical profile revealed a mature neutrophilia and lymphocytosis and elevated concentrations of GGT, CK, AST, and bilirubin consistent with a hepatopathy. The heifer developed hemoglobinuria the following day; potential causes include leptospirosis, copper toxicity, water intoxication, alloimmune or autoimmune hemolytic anemia (IMHA), bacillary hemoglobinuria, postparturient hemoglobinuria, eperythrozoonosis, babesiosis and ingestion of toxic plants. Antibiotic therapy was initiated due to the possibility of bacterial infection; abdominal ultrasound was unremarkable and a liver biopsy was delayed due to the ongoing hemolysis. The calf was subsequently found to be seronegative for leptospirosis and anaplasmosis and the calf was treated with dexamethasone due to the possibility of IMHA. However, hemolysis continued over the next 72 hours and the PCV fell to 12%; a blood transfusion was performed using 3.5L of whole blood. The following day the patient appeared to stabilize and a liver biopsy was performed. Histopathologic analysis of the biopsy sample revealed extensive bridging fibrosis with lymphoplasmacytic pericholangitis suggestive of toxic hepatopathy. A plant-based etiology for the observed changes was suggested by pigmentary change from chlorophyll pigments. The copper concentration was found to be significantly elevated (210 ppm). Analyzed copper levels in the milk replacer were not elevated and local soils are not deficient in molybdenum or sulfate, which moderate copper levels by binding copper molecules. Hepatogenous chronic copper toxicity results after ingestion of hepatotoxic plants (e.g., *Senecio*, *Heliotropium* spp.) leads to liver damage and an increase in hepatocyte affinity for copper. This diagnosis was made by the presence of *Senecio* spp. in the pasture, elevated liver copper, histopathologic changes indicative of a plant based toxic hepatopathy, and absence of other copper sources. This case demonstrates the disease can be a diagnostic challenge and should be included on the differential list for patients exhibiting methemoglobinemia and signs of liver disease.

◊ USAHA Paper

Bacteriology

Sunday, October 19, 2014

Atlanta A

Moderators: Timothy Frana, Deepanker Tewari

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Isolation of a Novel *Campylobacter* Species from Bovine Genital Sample

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Campylobacter fetus comprises two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*. *C. fetus* subsp. *venerealis* causes bovine venereal campylobacteriosis (BVC), a sexually transmitted disease of cattle that if not properly controlled leads to severe economic loss particularly to industries with focus on breeding programs. Therefore, accurate identification of the organism is essential. *C. fetus* subsp. *venerealis* can be differentiated from closely related *Campylobacter* spp. by their growth behavior in 1% glycine and susceptibility pattern on cephalothin followed by confirmation with molecular method. At the Wisconsin Veterinary Diagnostic Laboratory we use a real-time polymerase chain reaction (rt-PCR) assay that simultaneously targets a gene that is present in *C. fetus* and a gene that is reportedly unique to *C. fetus* subsp. *venerealis*. Recently, a slow growing, gram-negative curved bacterium was recovered from bovine vaginal wash and swab. The organism exhibited *C. fetus* subsp. *venerealis* characteristics by biochemical and susceptibility patterns and was negative by PCR for *Campylobacter fetus* but positive for *C. fetus* subsp. *venerealis*. Comparative 16S sequence based identification analysis indicated that the isolate belongs to the genus *Campylobacter*. Cluster analysis derived from the 16S rDNA sequences revealed that the isolate is more closely related to other campylobacters than to *C. fetus* subsp. *venerealis*. Genotyping by Pulsed Field Gel electrophoresis (PFGE) did not produce meaningful results. Further, the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and cellular fatty acid methyl ester analysis yielded no identification. These findings suggest that the isolate may be novel and deserves further study as potential veterinary pathogen.

Isolation and Identification of *Helcococcus ovis* from Bovine Joint Fluid

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Lung tissue and joint fluid were submitted to the University of Missouri College of Veterinary Medicine Veterinary Medical Diagnostic Laboratory for Bacteriology and Molecular Diagnostic examination. The samples were obtained from a Holstein calf with clinical signs of possible pneumonia and swollen joints. As per standard operational procedure the submitted samples were cultured aerobically on Blood and MacConkey agars as well as under microaerophilic conditions (5% CO2, 95% air) on Chocolate and Eugon Chocolate Agars. Both submitted samples were found to contain mixed microbial growth. The lung contained light growth of isolates subsequently identified as *Corynebacterium, Staphylococcus* and *Streptococcus* species. The joint fluid contained light growth of a hemolytic *Bacillus* on aerobic culture and heavy growth of minute grey colonies on Chocolate and Eugon Chocolate agars cultured in the presence of CO2. This latter isolate was found to be KOH and catalase negative and appeared microscopically as paired Gram-positive cocci resembling *Micrococcus*. Automated bacterial identification systems (Thermo Fisher Scientific Sensititre AP90 Gram-positive Identification system) failed to identify the isolate even to the level of genus. Ribosomal RNA (16S) sequencing was employed as a diagnostic tool to identify this unusual microbe. Results from sequencing showed one nucleotide difference out of 1423 base pairs between this isolate and the *Helcococcus ovis* type strain s840-96-2. *Helcococcus ovis*, a facultative anaerobic Gram positive catalase negative cocci was first recovered together with *Trueperella pyogenes* from multiple tissues during post mortem examination of two adult sheep in Scotland. Like that report we have demonstrated an aerobic growth dependency on *Staphylococcus aureus* and are unaware of the clinical significance of this isolate in the joint fluid of the bovine. Independent molecular evaluation of the submitted samples demonstrated that the lung was PCR positive for *Mycoplasma*, but not *M. bovis*; the joint fluid was *Mycoplasma* negative by PCR. Microbiological and molecular testing is still ongoing.

A Taqman Multiplex Real-Time PCR for Rapid Detection of Viral and Bacterial Pathogens Associated with Bovine Respiratory Disease Complex

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Bovine Respiratory Disease Complex (BRDC) is the most significant infectious disease in cattle. The disease is multifactorial, with either stress or reduced immunity allowing primary and/or secondary infections by viral and bacterial pathogens to occur. *Bovine viral diarrhea virus* (BVDV), *Bovine respiratory syncytial virus* (BRSV), *Bovine coronavirus* (BCoV) and *Bovine herpesvirus* type 1 (BHV-1/IBR), *Mannheimia haemolytica* (Mh), *Pasteurella multocida* (Pm), *Histophilus somni* (Hs), *Bibersteinia trehalosi* (Bt) and *Mycoplasma bovis* (Mb) are 9 pathogens that are commonly associated with BRDC. A Taqman multiplex real-time PCR assay consisting of three triplex reactions was developed and validated for simultaneous detection of these 9 pathogens involved in BRDC. The complete panel includes a triplex reaction for detection of the BRD viral RNA pathogens (BVD, BRSV and BCoV), a triplex reaction for DNA pathogens (*M. bovis*, BHV1/IBR and *Pasteurella multocida*) and the third triplex assay for the detection of the BRD bacterial pathogens: *M. haemolytica*, *H. somni* and *B. trehalosi*. Each individual singleplex reaction was optimized separately and subsequently multiplexed, with PCR amplification efficiencies of 92.1-105.4% and correlation coefficients of 0.99-0.999. Assay specificity was assessed by testing non-target and closely related species and there was no cross-reactivity observed. Comparison of detection limit and reaction efficiency between singleplex and multiplex assays indicated that multiplexing does not inhibit the detection sensitivity of the assay. Testing on 70 clinical samples using the assay showed good sensitivity and specificity. This study provides the basis for further evaluating the assay's diagnostic performance in clinical service for rapid detection of viral and bacterial pathogens associated with Bovine Respiratory Disease Complex (BRDC).

Development and Evaluation of a Real-Time PCR Assay for Detection of *Salmonella* Heidelberg from Clinical and Environmental Samples

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Salmonella Heidelberg represents an emerging health concern both for veterinary patients and humans due to zoonotic transmission. Recent food-borne outbreaks related to this serotype have also demonstrated the potential hazard it presents to the safety of our human food supply. While there are currently no demands for regulatory testing related to S. Heidelberg, management and testing requirements may be forthcoming in an attempt to reduce its impact from livestock and poultry food product sources. Twenty-one S. Heidelberg isolates along with 21 non-S. Heidelberg isolates recovered from environmental and clinical submissions to the California Animal Health and Food Safety Lab System (CAHFS) were submitted for whole-genome sequencing and analysis was performed to determine if a suitable gene target could be identified for PCR detection. After preliminary evaluation in silico, and a putative Type II restriction enzyme sequence was selected for further evaluation. A real-time PCR assay was developed that identified a 62 base-pair target in this region conserved in S. Heidelberg but not present in other serotypes. PCR testing on a panel of 26 different S. Heidelberg isolates demonstrated 100% detection utilizing this target. When tested against 22 non-Heidelberg *Salmonella* sp. organisms, 21 of the strains generated negative results to this target. The single discrepant result yielded a PCR product from a S. Newport isolate with a Ct of 36.8 in which a large concentration of DNA (403 ng/µl) was tested; dilution of this sample to a concentration closer to that obtained for other isolates tested in this assay (140 ng/µl) did not result in a detectable PCR product on repeat testing. Consistent detection was obtained with concentrations of 1.5 X 103 cfu/ml in broth samples without enrichment. No interference was seen when samples were co-inoculated with S. Heidleberg and non-Heidelberg *Salmonella* serotypes. Side by side comparisons between this real-time PCR assay and traditional culture with serotyping performed on tetrathionate broths from twenty field samples incubated for 18-24 hours at 42 °C yielded 100% agreement between the two methods. This assay may prove to be useful for sensitive and specific detection of S. Heidelberg in clinical and environmental samples.

Prevalence, isolation and characterization of *E. coli* O104 in cattle feces †

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The hybrid serotype of *E. coli* O104:H4, reported in 2011 outbreak in Germany, possessed characteristics of two pathotypes, Shiga toxin-producing and enteroaggregative *E. coli*. Although O104:H4 has not been detected in cattle, other serotypes (O104:H21, O104:H2, O104:H7, O104:H12) have been detected in cattle and other animals. The objectives of our study were to determine the prevalence of *E. coli* O104 in feedlot cattle feces, isolate and characterize the strains. A total of 757 rectal content samples from cattle, representing 29 different feedyards, were collected at a slaughter plant in July, 2013. Fecal samples were enriched in *E. coli* broth for 6 h at 40 C and then subjected to PCR- and culture-based methods of detection. DNA extracted from pre and post enriched fecal samples were tested by a multiplex PCR to detect serogroup O104 and associated virulence genes (*terD*, *ehxA*, *stx*1, *stx*2, *bfpA*, *aggA*, *eae* and *flicH4*) of the hybrid pathotype. Culture-based method of detection involved immunomagnetic separation with O104 beads, plating on selective chromogenic medium, followed by serogroup confirmation by PCR. Isolates of O104 were tested by PCR assays to determine capsular antigen of O8/O9 serogroups, virulence genes and flagellar types. Of the 757 samples, 38(5%) and 349 (46%) samples were positive before and after enrichment respectively for O104 serogroup-specific gene. A total of 143 O104 isolates were obtained and 92 of them were positive for O8/O9 capsular antigen genes. Of the 51 O104 isolates, only 16 (31.4%) carried *stx*1, none of them carried *eae*. Subtyping of *stx*1 was performed based on amino acid sequences and all 16 isolates carried *stx*1c. *In silico* restriction fragment length polymorphism (RFLP) was performed to compare with the subtyping based on amino acid sequences. *In silico* RFLP was also compared with conventional PCR-RFLP. The results from PCR/RFLP and *in silico* RFLP were in agreement with those obtained by subtyping based on amino acid sequences. The O104 isolates harbored diverse flagellar (H) antigens with 36 isolates containing H7, 4 H2, 1 each of H21 and H1. Pulsed-field gel electrophoresis (PFGE) was performed to assess the genetic relatedness of the *stx*-positive O104 isolates. The 16 *stx*1-positive isolates formed three PFGE clusters and the 13 isolates from one feedyard were of the same PFGE subtype (100% similarity). Cattle shed serogroup O104 in feces, but only a few strains (11.2 %) carried *stx*1 gene and none of the isolated strains carried genes characteristic of the hybrid pathotype.

† Graduate Student Oral Presentation Award Applicant

An Improved Primer Set for the Genotyping of *Clostridium perfringens* * †

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Clostridium perfringens is a widely distributed Gram-positive bacterium responsible for a wide range of diseases in humans and animals. *C. perfringens* strains are categorized into five toxinotypes (A-E) according to their complement of four major toxin genes. There is an important variation within the cpa gene, which encodes the alpha toxin present in all toxinotypes of *C. perfringens*. In addition, the gene encoding Beta2 toxin is present as two alleles with ~70% sequence homology and substantial sequence variation in each allele. Collectively, this presents a diagnostic challenge for accurately genotyping isolates for the cpa and cpb2 genes. This study focused on strain variation between isolates from calves and the effect of this variation on genotyping accuracy. New primer sets for amplifying cpa and cpb2 were designed based on all known sequence variants of the cpa and cpb2 genes. The new primer set identified isolates carrying cpb2 and cpa in samples that did not show bands corresponding to these genes using previously published and widely used primer sets. This primer set represents an important improvement in the diagnostic genotyping of *C. perfringens* field isolates.

* Graduate Student Poster Presentation Award Applicant
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Survey of Respiratory Pathology in Wild Urban Rats (*Rattus norvegicus* and *Rattus rattus*) # * †

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Norway (*Rattus norvegicus*) and black rats (*R. rattus*) are common commensal pests that live in association with human habitats and carry a number of zoonotic pathogens, yet we know little about wild rat ecology, including their natural diseases. We describe the gross and histological lesions of the respiratory tract in a sample of 711 wild urban rats. Over a 1-year period, rats were trapped from an inner city neighborhood in Vancouver, Canada and autopsied. A subset was examined for 19 distinct categories of histological lesions in the respiratory tract. Samples were tested, using serology and PCR, for known rat respiratory pathogens. Grossly-evident lesions were rare (8/711; 1%). Upper respiratory tract (URT) inflammation (rhinitis, submucosal and periglandular lymphoplasmacytic tracheitis, and/or tracheal intraluminal necrotic debris) was present in 93/107 (87%) rats and was associated with Cilia Associated Respiratory Bacillus (CARB) and *Mycoplasma pulmonis* ($P \leq .05$), and heavier rats (OR = 1.09, 95% CI = 1.05 – 1.14 per 10 g). Lower respiratory tract (LRT) inflammation (peribronchiolar and/or perivascular lymphoplasmacytic cuffing) was present in 152/199 rats (76%) and inflammation was associated with CARB and *M. pulmonis* ($P \leq .02$), and heavier rats (OR = 1.20, 95% CI = 1.14 – 1.27 per 10 g). *Pneumocystis carinii* was detected in 48/102 (47%) rats using PCR, but was not significantly associated with lesions. This is the first detailed description of pathology in the respiratory system of wild rats and demonstrates that respiratory disease is common. Although the impact of these lesions on individual and population health remains to be investigated, respiratory disease may be an important contributor to wild rat morbidity and mortality.

AAVLD Trainee Travel Awardee (Pathology, Bacteriology/Mycology)
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Minimal Inhibitory Concentration Antimicrobial Susceptibilities of Coagulase Positive *Staphylococcus sp.* Associated with Canine Pyoderma (2011-2013)

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Skin swabs or skin biopsies from dogs with pyoderma were submitted to the University of Illinois Veterinary Diagnostic Laboratory for bacterial culture and susceptibility testing as part of a study investigating potential for exchange of antimicrobial resistance and/or virulence factors between *Staphylococcus species* during mixed infections. Dominant among the isolates recovered were coagulase positive *Staphylococcus species*, predominantly *Staphylococcus pseudintermedius* (N=327), *Staphylococcus schleiferi* (N=38) and *Staphylococcus aureus* (N=17). Isolates were identified by Gram stain, catalase, coagulase, ONPG broth, and/or Sensititre™ GPID or Biolog™ GP2 systems as needed. Only 12 dogs had significant co-infections with multiple *Staphylococcus species*, *S. pseudintermedius* with either *S. aureus* or *S. schleiferi*. Susceptibilities were performed using Sensititre™ Companion Animal 1F or 2F MIC panels, following Clinical Laboratory Standards Institute M31-A3 and Vet01-A4/S2 guidelines and interpretations. Penicillin resistance was common among *S. pseudintermedius* isolates (MIC50=1.0 μg/ml, MIC90=16.0 μg/ml) while *S. schleiferi* were more susceptible (MIC50≤ 0.06 μg/ml, MIC90=1.0 μg/ml). A similar trend was observed for ampicillin with a *S. schleiferi* MIC90 = 1.0 μg/ml and MIC90 ≥ 4.0 μg/ml for both *S. pseudintermedius* and *S. schleiferi*. Based upon oxacillin breakpoints of 4.0 μg/ml for *S. aureus* and 0.5 μg/ml for *S. pseudintermedius* and *S. schleiferi*, 35.3%, 30.4% and 34.2% of these isolates exhibited methicillin resistance, respectively. While the MIC50 values were all 0.25 μg/ml, MIC90 values were 8.0, 4.0 and 2.0, respectively. There was good correlation between the oxacillin breakpoints and the presence of the *mecA* gene detected by PCR. Fluoroquinolone resistance was more common among *S. schleiferi* isolates (MIC50=1.0 μg/ml, MIC90=2.0 μg/ml), and both *S. aureus* and *S. pseudintermedius* had MIC50 values of ≤0.25 μg/ml but MIC90 values of ≥ 4.0 μg/ml for both enrofloxacin and marbofloxacin. The prevalence of methicillin resistance among coagulase positive Staphylococci is much higher for dogs with pyoderma (~30%) than the prevalence reported for healthy dogs (~6% for methicillin resistance) possibly as a result of selection pressure associated with antibiotic treatment.

Virulence Factors Genes (siet, lukI and lukF) in *Staphylococcus* Species Isolated from Canine Clinical Specimens

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The epidemiology and study of virulence factors in methicillin-resistance (MR) *S. pseudintermedius* and *S. schleiferi* subsp. *coagulans* isolated from dogs is sparse. The aim of this study was to investigated the presence of the genes siet, lukI and lukF, which encode for important virulence factors in *S. schleiferi* subsp. *coagulans*, *S. pseudintermedius* and *S. aureus* isolated from canine clinical specimens. In addition, related epidemiological information related with previous antimicrobial therapy, breed, gender, age and prevalence of methicillin-resistant isolates by year was analyzed. A total of 577 coagulase positive *Staphylococcus* species were collected from canine clinical specimens, and epidemiological information was collected from the clinical history submitted to the lab. The incidence of the genes siet, lukI and lukF were determined by PCR. A significant difference ($p \leq 0.05$) indicated that isolates from dogs with a history of antimicrobial therapy have a higher risk of being MR positive, than from dogs without therapy. Within the *S. aureus* group, the panton-valentine leukocidin genes were tested in the MRSA group with four testing positive, 12.9% (4/31). LUK-I and siet genes were found mainly in *S. pseudintermedius*, but also in *S. aureus* and *S. schleiferi* subsp. *coagulans*. An important finding in this study was the discovery of the genes siet and lukI in *S. aureus* and *S. schleiferi* subsp.*coagulans*. Collectively, these findings support the need for further epidemiological and clinical studies concerning virulence factors in *Staphylococcus* species of canine origin, which may be a potential hazard for public health.

Fatalities Caused By *Streptococcus dysgalactiae* subsp. *equisimilis* in Horses

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Streptococcus dysgalactiae subsp. *equisimilis* (*S. equisimilis*) causes severe clinical syndromes in humans with high mortality rates including arthritis, pleuropulmonary infections, peritonitis, intra-abdominal and epidural abscesses, meningitis, endocarditis, puerperal septicemia, neonatal infections, necrotizing fasciitis, myositis, and streptococcal toxic-like syndrome. *S. equisimilis* has been reported in horses from aborted placenta, less frequently from abscessed lymph nodes, and the upper respiratory system infections in horses. However, fatalities caused by *S. equisimilis* in horses are poorly described and, comprehensive and exhaustive studies are limited. The goal of this study is to describe fatalities of horses caused by *S. equisimilis* and examine the genotypes of equine isolates of *S. equisimilis* by using multilocus sequence analysis, and M virulence protein gene (emm) typing combined with phenotypic characterization. 105 equine necropsies where *S. equisimilis* were isolated were retrospectively analyzed. The necropsies were performed at the University of Kentucky Veterinary Diagnostic Laboratory between January 1, 2010 and December 31, 2013. In 22 cases, *S. equisimilis* was reported as only causative microorganism leading to death. In 15 of these cases, *S. equisimilis* caused abortion due to placentitis with or without fetal septicemia. *S. equisimilis* also caused osteoarthritis/ osteomyelitis/arthritis (5 cases), and septicemia (2 cases). In 29 other cases, *S. equisimilis* was diagnosed as contributing or associated agent to the fatality (or euthanasia) causing placentitis/ abortion (9 cases), pleuritis (4 cases), pneumonia (4 cases), septicemia (4 cases), dermatitis/ cellulitis/ myelitis (3 cases), osteomyelitis/arthritis (2 cases), cystitis (1 case), omphalitis (1 case), and mesenteric abscess (1 case). Other contributing microorganisms identified in these cases were *S. aureus*, *S.equi* subsp. *zooepidemicus*, *P. caballi*, *Neorickettsia risticii*, *R. equi*, *A. equi* subsp. *equi*, *K. pneumonia*, *E. coli*, *Enterococcus* spp and *Streptococcus* spp. In 54 necropsy cases, *S. equisimilis* was recovered from tissues but not associated with any disease/fatality. 12 *S. equisimilis* isolates representing these groups were confirmed to be *S. equisimilis* by PCR using streptokinase gene specific primers. In addition, multilocus sequence types (MLSTs) and sequence types of the emm gene were obtained and compared to human disease case *S. equisimilis* isolates. These preliminary results shown that these *S. equisimilis* isolates recovered from equine necropsy cases have new MLSTs and emm types suggestive of a genetic lineage distinct from human isolate clonal complexes described to date.

A Novel *Avibacterium* sp. Causes Mortality in Laying Hens ◊

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In January 2014, a disease outbreak characterized by elevated mortality and reduced egg production occurred in a commercial in-line high-rise laying hen operation. White leghorn chickens in 3 of 9 houses on this farm were affected. Each house had an average capacity of 111,000 birds and affected flocks ranged in age from 39 to 48 weeks. The flock manager reported mildly swollen heads and conjunctivitis in a few hens housed in each of these three flocks. Macroscopic lesions consisted primarily of hepatosplenomegaly, air sacculitis, and pneumonia. Abundant caseous exudate was present in anterior thoracic air sacs and lungs were consolidated with exudate in bronchi. Livers, lungs, air sacs, and spleens were collected using aseptic technique and pooled. All samples were cultured using routine procedures on blood agar plates under aerobic and anaerobic conditions. *Avibacterium* sp. was isolated from lungs and air sacs and *E. coli* was isolated from livers. Biochemical tests suggested that *Avibacterium* isolated from these chickens was not *Avibacterium paragallinarum*, the etiologic agent associated with infectious coryza. To identify the species of *Avibacterium*, the 16S ribosomal gene of selected isolates was amplified by PCR and sequenced. The 16S rRNA phylogenetic tree of veterinary *Pasteurellaceae* species confirmed that this isolate is not *A. paragallinarum* and is different from other *Avibacterium* sp. previously isolated from chickens in the United States.

◊ USAHA Paper

Development of a Multiplex PCR Assay for the Identification and Speciation of Pathogenic Vibrionaceae

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There are currently several emerging pathogens within the family Vibrionaceae that are causative agents of disease in both humans and fish. Identification and speciation of these organisms using classical culture and biochemical methods yield results that are frequently inconsistent, necessitating diagnostic techniques that are accurate, rapid, and cost effective for proper Vibrionaceae identification. Multiplex PCR has proven useful for *Vibrio* spp. in previous studies, although these tests have typically been limited to human pathogens such as *Vibrio cholerae*. We have designed a multiplex PCR assay for the detection of six fish and human pathogens within the Vibrionaceae family: *Vibrio alginolyticus, Vibrio (Listonella) anguillarum, Vibrio ordalii, Vibrio parahaemolyticus, Vibrio vulnificus*, and *Photobacterium damselae* subsp. *damselae*. Each of these species was correctly and consistently identified when tested against their corresponding American Type Culture Collection (ATCC) strains and known clinical isolates, and no reaction was observed when tested against a variety of non-*Vibrio* isolates. Fifty-five presumptive *Vibrio* spp. clinical isolates were additionally tested by multiplex PCR, MALDI-TOF mass spectrometry, and sequencing of multiple gene targets. There was a high degree of concordance between mass spectrometry and multiplex PCR results, though PCR showed a greater ability to differentiate between *V. anguillarum* and *V. ordalii* isolates. The PCR assay demonstrated high accuracy compared to other available techniques and is also highly modular, as it can be successfully run as triplexes or a single hexaplex reaction and retains the potential of having additional primer sets incorporated into it for the detection of other *Vibrio* spp. This multiplex PCR assay represents a useful diagnostic tool for the proper identification of multiple Vibrionaceae species that will be beneficial for aquaculture and in both veterinary and human medicine.

Use of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) for the Speciation of Pathogenic *Vibrio* in Fish # †

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Many *Vibrio* spp. cause disease resulting in significant morbidity and mortality in farmed fish and significant economic losses to fish producers around the world. Speciation of *Vibrio* isolates is time consuming, difficult, and costly due to species diversity and inconsistency of phenotypic and biochemical traits within species. Therefore, improved methods of *Vibrio* speciation would aid identification accuracy and improve disease diagnosis. MALDI-TOF MS has been shown to be a rapid and cost effective method for speciation of numerous bacteria, but its accuracy for *Vibrio* spp. identification has not been reported. Fifty-five presumptive *Vibrio* spp. isolates were subjected to three diagnostic approaches: MALDI-TOF MS (Bruker Biosciences Corp.), conventional aerobic bacteriology with biochemical phenotypic testing, and DNA sequence analyses using three conserved genes, *rpoB*, *rpoD* and *toxR*. DNA sequencing was presumed to be the most accurate and speciation by this method was completed on all 55 isolates. There was moderate agreement between the sequencing data and MALDI-TOF MS; however, certain species such as *Vibrio ordalii*, were inaccurately identified by MALDI-TOF MS. Thirty-four isolates (61.8%) had MALDI-TOF MS scores >2.0, suggestive of accurate speciation and these included American Type Culture Collection (ATCC) strains of *Listonella (Vibrio) anguillarum*, *Vibrio alginolyticus*, *Vibrio vulnificus*, *Vibrio ordalii*, and *Photobacterium damselae* subsp. *damselae*. An additional five isolates (9.1%) were identified to the genus level with scores between 1.8 and 2.0. Two isolates (3.6%) produced scores <1.8 indicating inability to confidently assign genus or species. Fourteen isolates (25%) produced no score and thus no identification. It was also noted that the tube extraction method produced more consistent and higher quality scores compared to the direct transfer method. Conventional aerobic bacteriologic methods were applied to the same isolate set, including consideration of the effects of added salt (with or without 2% NaCl incorporated in the media), temperature (15° vs 20°C), and incubation time (24 vs 48 hours). Speciation using conventional aerobic bacteriology was inaccurate and inconsistent, and dependent upon salt concentration, incubation temperature and time. However, ATCC strains were correctly identified provided the media included 2% NaCl, an incubation temperature of 15°C and 24 hours of growth. Only one clinical isolate, *P. damselae* subsp. *damselae*, was concordantly identified by both MALDI-TOF MS and conventional aerobic bacteriology. These findings indicate the MALDI-TOF MS database may benefit from data collected from additional *Vibrio* isolates in order to improve accuracy and permit confident use in *Vibrio* speciation of fish-origin.

AAVLD Trainee Travel Awardee (Bacteriology/Mycology)
† Graduate Student Oral Presentation Award Applicant

Epidemiology 2 / Parasitology

Sunday, October 19, 2014
Chicago C

Moderators: Ashley Hill, Marie Culhane

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Application of MIQE Guidelines for Use in a Veterinary Diagnostic Laboratory PCV2 qPCR

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There is widespread lack of transparency, standardization and assay quality control found in publications that report qPCR assays. The Minimum Information for publication of Quantitative real-time PCR Experiments guidelines (MIQE) provides researchers a set of recommendations for properly reporting qPCR results. The Boehringer Ingelheim Vetmedica, Inc Health Management Center (HMC) recently implemented several MIQE enhancements to the TaqMan real-time PCV2 qPCR to further define limit of quantification (LOQ) and accuracy. Enhancements included linearizing the supercoiled plasmid in the standard curve that can lead to DNA quantity overestimation (1,2) and testing samples in triplicate. The objective of this paper was to evaluate the impact MIQE changes had on a known quantity PCV2 virus. Both the crossing quantity (Cq) and qPCR copy numbers generated for PCV2 controls were compared. All controls were tested with TaqMan real-time PCR reagents using a supercoiled PCV2 10x10 DNA plasmid both provided by Life Technologies Corp., Grand Island, NY. The plasmid was linearized by a Hind III digest kit (Promega corp., Madison, WI) to create qPCR standards. All samples were extracted followed by PCR in triplicate. A LOQ of 3.5 copies was established per published guidelines although they may still be deemed positive. Individual positive extraction control aliquots (n= 162) have been tested since June of 2012. Eighty-sevenwere quantified using the traditional method, prior to implementation of MIQE changes, and 75 following implementation of the changes. The average Cq's and qPCR copies/reaction ± the SE number generated were 24.07±0.80 and 7.58x106 ± 8.08x107, respectively, for the traditional; and 23.84±0.23 and 1.06x104 ± 2.62x103 respectively, for the MIQE implemented method. The actual quantity for the PCV2 virus extraction control was 5.0x103 TCID50 as determined by culture. On average the quantity generated for the PCV2 virus extraction control was 3 logs higher for the traditional method as compared to the improved method. This improved method determined a more accurate estimate of the PCV2 extraction control compared to the traditional method. Practitioners need to be aware of the methods of detection and quantification and limits of detection in their chosen laboratory and take caution when directly comparing qPCR results from various laboratories. References 1. Hou Y, et al. 2010, Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: microalgal pcna as the model gene. Plos One 5(3):e9545. 2. Lin C, et al. 2011, Quantification bias caused by plasmid DNA conformation in quantitative real-time PCR assay. Plos One 6(12):e29101.

Initial Evaluation and Validation of High-Throughput (384-well) Real-Time PCR for Testing Bulk Milk Samples

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Surveillance and preparedness for disease outbreaks is one of the most important missions of veterinary diagnostic laboratories nationally. In particular, PCR-based testing has key advantages for surveillance and outbreak response, including the ability to rapidly scale-up for high-volume, rapid turn-around testing. A California-Wisconsin veterinary diagnostic laboratory partnership was formed to evaluate a 384-sample PCR platform using an endemic virus, *Bovine Viral Diarrhea virus*, and milk as the sample matrix. The approach presents proof-of principle data for "outbreak scalability," with the additional intent to evaluate and validate a test that could be used as a component of routine dairy health management testing. Validation was performed as recommended by the National Animal Health Laboratory Network (NAHLN) Methods Technical Working Group MTWG). Eight BVDV detection methods (four extraction methods and two PCR amplification procedures) were compared. Extraction method evaluations were chosen for equivalency to the method considered by the NAHLN for testing *Foot-and-Mouth Disease virus* in milk, and from approaches used for milk testing by accredited AAVLD laboratories. Analytical sensitivity was quantified using a synthetic plasmid for each combination of extraction, amplification, and detection method. All assays evaluated included an internal control used for detecting PCR inhibition. A panel of BVDV reference strains were used to confirm detection of all BVDV genotypes known to be present in the U.S. Comparison between 96-well and the 384-well platforms was performed for all steps included in the assay validation. An important enhancement was the addition of a visible dye to aid technicians in confirming that assay wells were accurately loaded with both reagent and sample. Milk from a persistently infected (PI) BVDV cow serially-diluted into bulk tank milk showed that detection of a single PI animal in a bulk tank sample to which 1,000 cows contributed. Alternatively, sensitivity was calculated using the number of pounds a PI cow contributed to a bulk milk tank and data suggests that detection for a single animal persistently infected with BVDV is possible up to a 1:10,000 dilution. Individual milk samples and bulk tank milk samples (n=462) of various sizes and origin were used in the field validation testing. Assay performance was statistically similar between the 96-well and the 384-well platform (p=0.01). Overall, the 384-well approach shows promise for not only high-throughput endemic disease testing, but may also provide a feasible candidate for further evaluation by the NAHLN for enhancing national foreign animal disease response testing capacity and capability.

Rapid Detection of Pathogens from Swine Clinical Samples Using a Broad Spectrum Microbial Detection Array ◊

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To best safeguard human and animal health requires early detection and characterization of disease events. This must include effective surveillance for emerging infectious diseases. Both deliberate and natural outbreaks have enormous economic and public health impacts, and can present serious threats to national security. To evaluate the initial utility of a novel and comprehensive microbial detection technology, the Lawrence Livermore Microbial Detection Array (LLMDA) to expedite faster and better detection of emerging and foreign animal disease pathogens, we analyzed a series of swine clinical samples from past disease events. The LLMDA (1) contains probes to detect >8000 species of microbes including 3,856 viral, 3,855 bacterial, 254 archaeal, 100 fungal, and 36 protozoan species that were sequenced through June, 2013. This microarray targets both conserved and unique genomic regions of sequenced microbial strains. The automated data analysis algorithm, Composite Likelihood Maximization, is integrated with a web interface that enables LLMDA data analysis within 30 minutes. Clinical (serum, oral fluids, tissues and fecal) samples from past disease outbreaks were collected by or submitted to Kansas State University. The samples were shipped to Lawrence Livermore National Laboratory and nucleic acid samples were extracted using Trizol. The samples were amplified using random amplification, fluorescently labeled and hybridized to the LLMDA. *Porcine circovirus 2* (PCV2) and *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) were the most dominant pathogens detected. We found that oral fluids were a good substitute for serum for pathogen detection. In addition to PCV2 and PRRSV, the LLMDA also detected other previously undetected viral co-infections including *porcine parainfluenza*, *astrovirus*, and *bocavirus* from oral fluid samples. Common bacterial co-infections detected by the LLMDA were *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, *Staphylococcus* sp. and *Enterococcus* sp. We then compared array results with PCR results in the detection of PCV2 and PRRSV. Initial results showed that the LLMDA detected PCV2 and PRRSV from pig serum samples at Ct of 30 or less. Additional sensitivity testing is under way. In summary, we have demonstrated that the broad spectrum microbial detection technology, the LLMDA, is sensitive in the detection of known and emerging swine pathogens. It can be used to identify viral and bacterial co-infections, discover unknown pathogen outbreaks, and correlate the effects of microbiome to the health of animals. Its most appropriate, cost-effective application presently is as a secondary diagnostic test to assist further in evaluation of situations where primary syndromic testing does not identify a causative agent. References 1. Gardner S, Jaing C, McLoughlin K, Slezak T: 2010, A microbial detection array (MDA) for viral and bacterial detection. BMC Genomics 11:668.

◊ USAHA Paper

Bioinformatics for Improved Pathogen Detection: Maintenance of the Virotype® PRRSV RT-PCR Reagents for Improved Accuracy ◊

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Accurate pathogen detection is essential in many fields, ranging from infectious diseases diagnostics in humans and animal medicine to pathogen screening for biosecurity. However, the development of tools for specific pathogen detection can be very complex due to the existence of many pathogenic strains, with varying mutations, alongside the ever-present threat of new emerging strains. Typical examples include the *Influenza A virus* and *Porcine Respiratory and Reproductive Syndrome virus* (PRRSV), which can have catastrophic economic consequences for the swine industry. Both *Influenza A virus* and PRRSV have high mutation rates and regional strain variations exist. Virotype PRRSV NA/EU real-time PCR reagents are designed to detect North American and European PRRSV strains in a multiplex format with an internal positive control. Bioinformatics is used for surveillance of QIAGEN assays. Through the routine use of pathogen genomic characterization, the bioinformatics team can access the success rates of current assay oligonucleotide design, and when necessary identify critical sequences that may require assay is adaptation. In 2013, diligent bioinformatics alerted critical sequence changes, which might impact the accuracy of the virotype PRRSV NA/EU Reagent. After notification from the bioinformatics team that the reagents were missing strains of the Midwestern region of US (Iowa), in silico PCR was applied to compare virotype PRRSV primer and probe design with PRRSV strains in the database. This analysis allowed for design modification for assay oligonucleotides, which was implemented to maintain accurate detection of the regional PRRSV strains, and maintain detection accuracy of other known strains. The analytical sensitivity of the modified virotype PRRSV NA/EU oligonucleotides was performed in a translational research lab at Iowa State University (Trujillo). Utilizing purified RNA, from select PRRSV strains, we evaluated several modified oligonucleotides for the virotype PRRSV NA/EU Reagent alongside another commercially available PRRSV detection reagent. Results show that by utilization of bioinformatics data to aid in assay evaluation and redesign, the modified virotype PRRSV NA/EU Reagent could accurately detect the regional strain and conventional strains. Furthermore, the modified virotype PRRSV NA/EU Reagent demonstrated improved sensitivity of detection as compared to the other commercially available PRRSV detection reagents for the regional isolate (strain Iowa 21). Academic collaboration and attainment of sequence information for atypical virus isolates coupled with diligent deployment of bioinformatics aided in assessment and successful redesign of oligonucleotides utilized in the QIAGEN virotype PRRSV EU/NA Reagent. Diligent deployment of bioinformatic analysis on a regular basis or in response to a reported outbreak, with new sequences continually being added to the internal database through collaboration will insure assay performance.

◊ USAHA Paper

An Empirical Approach to Confidence Intervals of Positive and Negative Predictive Values Extrapolated from Case Control Studies

Stephane Guillossou

Global Diagnostic Marketing and Medicine, Zoetis, Florham Park, NJ

The fit of purpose of a diagnostic test can be assessed with a cross-sectional study. Estimates of sensitivity, specificity, prevalence, and positive and negative predictive values (PPV/NPV) can be assessed, as well as the respective 95% confidence intervals (95CI). Unfortunately, cross-sectional study can be difficult to implement and case-control studies are often preferred when assessing the performance of a diagnostic test. Then, the sensitivity and specificity observed are extrapolated to calculate predictive values given different prevalence. This approach provides good point estimates of PPV/NPV but does not allow an easy calculation of the 95CI. A simulation strategy is proposed to estimate the confidence intervals of PPV/NPV. From the sensitivity and specificity estimates, the bayesian 95CI are calculated. These confidence intervals present the advantage of distributions and therefore can be used to sample from. The sampling outputs are computed with a given prevalence value in order to establish a posterior distribution of the predictive values. The respective 95CI are established. The simulation is repeated for all prevalence values between 0 and 1 with increments of 0.01. All computations are realized in R 3.0.1. The simulation is illustrated using two hypothetical tests (Test 1: Se=99%, Sp=99%; Test 2: Se=95%, Sp=100%) and is evaluated in two populations of different size (Pop. a: 100 infected, 100 healthy; Pop. b: 100 infected, 1000 healthy). Graphical outputs illustrating PPV/NPV and their respective 95CIs in function of the prevalence, sensitivity, and specificity. Test 1 and test 2 are compared and despite the difference between the positive predictive values at low prevalence, large overlapping of their confidence intervals illustrate the importance of considering both the value and the precision of the value before inferring decision upon fit for purpose of the test. Additional comparison illustrates the impact of sample size on the predictive values' confidence intervals. The empirical method hereby described presents a convenient alternative to obtain 95% confidence intervals of predictive values extrapolated from sensitivity and specificity estimates from case-control studies. This method can be use to compare predictive value before selecting a test over another in a particular population. Alternatively, the simulation model could be reversed and used to infer ideal sample size for obtaining the precision targeted for the PPV/NPV.

Oklahoma Equine Cases Presenting with Clinical Signs of Central Nervous System Disease– 2012-2013 § ◊

Kristin M. Lenoir, Janisue C. Jones, Perla Encarnacion-Astudillo, Grant Rezabek

Serology, Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, OK

The identification of a pathogen underlying equine neurologic symptoms plays an important role in equine and public health. A probable diagnosis can lay the groundwork for risk analysis and suspected diagnosis for equines and other mammals in the geographic vicinity, including humans. This can also facilitate preventive and control measures relevant to the disease. Testing for common agents that underlie equine central nervous system (CNS) disease, however, remained low from 2008-2011 at The Oklahoma Animal Disease Diagnostic Laboratory (OADDL). In an effort to increase testing, the Oklahoma Department of Agriculture, Food and Forestry (ODAFF) subsidized a diagnostic profile for horses presenting CNS symptoms. This profile included *West Nile Virus* (WNV) and *Eastern Equine Encephalitis Virus* (EEE) by IgM capture ELISA on serum as well as *Equine Herpes Virus 1* (EHV-1) by PCR on whole blood and nasal swab. Supplemental diagnostics for Equine Protozoal Myelitis (EPM), Western Equine Encephalitis (WEE), Venezuelan Equine Encephalitis (VEE), Rabies, or plant toxins was available as fee-for-service to the client. OADDL solicited submissions from Oklahoma veterinarians and attempted to collect demographics, vaccination history, and clinical signs on all cases. A total of 269 Oklahoma-resident CNS cases were submitted from January 2012 through December 2013, with 147 cases meeting the full sample requirements for the subsidized program. The data are presented as a percentage, with positive results over the total number of cases submitted for each test. Twenty-nine percent (69/237) were positive for WNV. All EHV-1 submissions (167 nasal swabs, 190 whole blood samples) were negative and all EEE submissions (213) were negative. Equine Protozoal Myeloencephalitis (EPM) combined IFAT emerged as a common supplemental test request for which 68% (38/56) had a positive titer. Two out of 10 brain specimens tested for rabies virus were positive. An inherent challenge to serological testing of horses with CNS disease is that it only identifies a probable agent, but does not confer a definitive diagnosis. Results may indicate exposure to more than one pathogen. For example, of 5 WNV-positive cases also tested for EPM, 4 were positive for EPM. A low percentage of cases reported current vaccination status at time of submission: 29% WNV, 31% EEE, and 25% EHV-1. This data contributes to surveillance for both common and zoonotic CNS pathogens and promotes awareness among equine veterinarians and owners.

§ AAVLD Staff Travel Awardee
◊ USAHA Paper

Salmonella spp. Serotypes Isolated from Drag Swabs at a Veterinary Diagnostic Laboratory and Public Health Implications

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Salmonella spp. is an important zoonotic cause of gastroenteritis in humans and animals. Most cases in humans are due to bacterial contamination of food (foodborne illness) and less commonly due to direct contact with affected animals or contaminated water. Many serotypes of *Salmonella* spp. have been documented to cause foodborne disease, with *Salmonella enterica* serotype Enteritidis (SE) from contaminated eggs most commonly reported. Testing of environmental drag swabs from poultry housing facilities is a validated screening tool for SE. As specified by the FDA under the Egg Safety Rule, the identification of SE from an environmental drag swab results in the holding of eggs until further negative testing is obtained. The identification of other *Salmonella* spp. serotypes may not result in regulatory implication even though other serotypes have been associated with foodborne illness from the consumption of poultry products. The objective of this study was to determine the prevalence of different Salmonella serotypes isolated from drag swabs submitted to the Utah Veterinary Diagnostic Laboratory (UVDL) during a 3-year period. A total of 868 drag swabs were tested from 2009 through 2011. Of those, *Salmonella* spp. was isolated from 126 swabs (14.5%) using tetrathionate enrichment followed by plating onto Hektoen enteric agar and XLT-4 (Xylose-Lysine-Tergitol 4) agar or MSRV (Modified Semi-Solid Rappaport Vassiliadis) agar. Suspect *Salmonella* spp. colonies were presumptively identified using an LIA (Lysine Iron Agar) slant and a TSI (Triple Sugar Iron) slant and then were identified to genus using API 20E strips. The *Salmonella* spp. isolates were submitted to the National Veterinary Diagnostic Laboratories (NVSL) for serotyping. The most common (27/126 = 21%) serotype identified was *Salmonella* Typhimurium. *Salmonella* Kentucky was the second most commonly (23/126 = 18.2%) isolated serotype. *Salmonella* Enteritidis was isolated from 7 (5.6%) drag swabs. *Salmonella* Typhimurium is the second most common serotype associated with foodborne illness nationally and was the serotype identified the most commonly at the UVDL from 2009 through 2011. However, its detection in drag swabs does not currently result in federal regulatory action.

Retrospective Testing for the Emergence of *Porcine Deltacoronavirus* in US Swine at Iowa State University Veterinary Diagnostic Laboratory

Avanti Sinha, Phillip Gauger, Jianqiang Zhang, Kyoung-Jin Yoon, Karen Harmon

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Porcine deltacoronavirus (PDCoV) belongs to the order Nidovirales, family *Coronaviridae*, and genus Deltacoronavirus. PDCoV is a single-stranded, positive-sense, enveloped RNA virus containing a genome of approximately 25 kb. PDCoV was first identified in Hong Kong in a surveillance study published in 2012. According to the study, PDCoV HKU 15-44 and HKU 15-155 strains have been present in pigs in Hong Kong since 2009 and 2010 respectively. PDCoV in U.S. swine was identified for the first time in February 2014 and its presence has been reported in several research studies thereafter. Up to May 15, 2014, PDCoV has been detected in 14 U.S. states according to the National Animal Health Laboratory Network (NAHLN) laboratory testing summary from USDA. However, it remains unknown if PDCoV had been introduced to the U.S. earlier than February 2014. The present retrospective study is being conducted to produce evidence of the possible presence of PDCoV in samples submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) between 2012 and 2013. A total of 1,073 samples (fecal samples, environmental samples, and oral fluids) submitted between November 2012 and December 2013 were tested using a PDCoV-specific real-time reverse transcription PCR (rRT-PCR) assay targeting the membrane (M) gene segment. PDCoV M gene was first detected from a fecal sample acquired on 19th August 2013 from a pig in Minnesota with a history of diarrhea but negative for *Porcine Epidemic Diarrhea Virus* (PEDV) by rRT-PCR. Subsequently, PDCoV was detected in samples collected on 29th August 2013 in two additional pigs from Illinois which were also negative by rRT-PCR for PEDV and *Transmissible Gastroenteritis Virus* (TGEV) and PCR for *Lawsonia intracellularis*, and negative by immunohistochemistry for PEDV, group A *rotavirus* and TGEV. Therefore, at this time and with available samples submitted to the ISU-VDL, it can be inferred that PDCoV has been present in US swine at least since August 2013, suggesting it did not emerge in the US at the same time period as PEDV.

Molecular Epidemiology of *Porcine Epidemic Diarrhea Virus* in US Swine †

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Porcine epidemic diarrhea virus (PEDV) was detected for the first time in US swine in April 2013 and has spread to 30 US states as of May 2014. However, little is known about the molecular epidemiology of PEDVs in US swine temporally and spatially. In this study, we characterized the genetic profile and diversity of PEDVs circulating in US swine since its emergence. Sequencing for PEDV S1 region was chosen, since this region harbors the postulated neutralizing epitopes, and performed on 167 cases among which full-length genomic sequences were determined on 7 cases. Sequences were compared to 11 additional US PEDV strains whose whole genomic sequences were previously reported as well as 216 non-US PEDV strains with sequences available in GenBank. Among the 178 US PEDVs collected from 23 states, S1 sequences of 156 cases from 22 states had 99.0-100% nucleotide (nt) identity to each other, including the PEDVs initially sequenced after the outbreak in April 2013 (hereafter designated as original US strain). In contrast, S1 sequences of the remaining 22 cases from 10 states had only 92.4-93.8% nt identity to the original US strains, while they shared 99.6-100% nt identity to each other (hereafter designated as variant US strain). Sequence alignment showed that all US PEDV variants had the same patterns of nucleotide changes including some deletions and insertions, when compared to original US PEDVs. Whole genome sequencing demonstrated that the variants had 99.6-100% nt identity to each other and 98.8-99.2% nt identity to the original US strains. Phylogenetic analyses using the S1 nucleotide sequences showed that the variant US strains clustered with some PEDV strains reported from China, which were distantly related to the original US strains. When using the whole genomic sequences, the variant strains still formed a cluster distinct from the original US strains, but its relatedness to the cluster of the original strains was not as distant as that observed in S1-gene based dendrograms. We further developed a S1-gene based differential real-time RT-PCR to quickly distinguish the original strain from the variant strain of PEDV. Retrospective testing of over 1,000 archived fecal samples from December 2012 at Iowa State University Veterinary Diagnostic Laboratory revealed that the original US strain was present from April 15, 2013 whereas the variant US strain was present from May 16, 2013. Our study indicates that there are at least two genotypes of PEDV co-circulating in US swine and the two genotypes of PEDV may be derived from different ancestors but would have been introduced into the US concurrently. The data from this study suggest that within each genotype, PEDVs in the US have not undergone significant genetic changes.

† Graduate Student Oral Presentation Award Applicant

Improved Diagnostic Performance of a Commercial *Anaplasma* Antibody Competitive Enzyme-Linked Immunosorbent Assay Using Recombinant Major Surface Protein 5–Glutathione S-Transferase Fusion Protein as Antigen ◊

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The current study tested the hypothesis that removal of maltose binding protein (MBP) from recombinant antigen used for plate coating would improve the specificity of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay (cELISA). The number of 358 sera with significant MBP antibody binding (≥30%I) in *Anaplasma*-negative herds was 139 (38.8%) when tested using the recombinant major surface protein 5 (rMSP5)-MBP cELISA without MBP adsorption. All but 8 of the MBP binders were negative (<30%I) using the commercial rMSP5-MBP cELISA with MBP adsorption, resulting in 97.8% specificity. This specificity was higher than some previous reports, so to improve the specificity of the commercial cELISA, a new recombinant antigen designated rMSP5–glutathione S-transferase (GST) was developed, eliminating MBP from the antigen and obviating the need for MBP adsorption. Using the rMSP5-GST cELISA, only 1 of 358 *Anaplasma*-negative sera, which included the 139 sera with significant (≥30%I) MBP binding in the rMSP5-MBP cELISA without MBP adsorption, was positive. This resulted in an improved diagnostic specificity of 99.7%. The rMSP5-GST cELISA without MBP adsorption had comparable analytical sensitivity to the rMSP5-MBP cELISA with MBP adsorption and had 100% diagnostic sensitivity when tested with 135 positive sera defined by nested polymerase chain reaction. Further, the rMSP5-GST cELISA resolved 103 false-positive reactions from selected sera with possible false-positive reactions obtained using the rMSP5-MBP cELISA with MBP adsorption and improved the resolution of 29 of 31 other sera. In summary, the rMSP5-GST cELISA was a faster and simpler assay with higher specificity, comparable sensitivity, and improved resolution in comparison with the rMSP5-MBP cELISA with MBP adsorption.

◊ USAHA Paper

Possible Direct Transmission of *Histomonas meleagridis* in Peafowl # * † +

Lorelei L. Clarke[1], Horacio Shivaprasad[2], Silvia Carnaccini[2], Robert B. Beckstead[3], Daniel R. Rissi[1]

[1]Department of Pathology, University of Georgia, Athens, GA; [2]California Animal Health and Food Safety, University of California, Davis, Tulare and Turlock, CA; [3]Department of Poultry Science, University of Georgia, Athens, GA

Histomonas meleagridis is a flagellate protozoan organism that causes severe necrotizing typhlitis in chickens and turkeys as part of the condition known as "blackhead disease". It typically uses the cecal nematode *Heterakis gallinarum* as a vector and reservoir for infection, but direct transmission has been both naturally observed and experimentally induced in turkey poults. While it is commonly recognized that other gallinaceous birds are susceptible to *H. meleagridis* infection, there is only a single report in the veterinary literature describing the disease and its transmission by *H. gallinarum* in peafowl. In that report, transmission of *H. meleagridis* was accomplished by feeding young peafowl embryonated eggs of *H. gallinarum*, and resulted in high morbidity and mortality rates, indicating that peafowl are likely more susceptible to infection than chickens or pheasants and that it poses a significant concern for peafowl producers. A review of the archived cases at the University of Georgia Athens Veterinary Diagnostic Laboratory and the California Animal Health and Food Safety Laboratory System yielded 4 cases (2 from each institution) of young (1 week old to 19 weeks old) peafowl with gross and histological findings characteristic of *H. meleagridis* infestation, including a bilateral, transmural fibrinonecrotic typhlitis and multifocal necrotizing hepatitis with intralesional trophozoites morphologically consistent with *H. meleagridis*. The 2 cases diagnosed at UGA also had concomitant necrotizing air sacculitis with intralesional trophozoites, and one of these 2 cases had pulmonary granulomas with intralesional *Aspergillus* spp. hyphae. Immunohistochemistry for *Trichomonas* spp., which cross-reacts with *H. meleagridis*, and genotyping for *H. meleagridis* was done to confirm the diagnosis. There was no evidence of *H. gallinarum* infestation in all 4 cases. Infection in these cases may have occurred by 1) ingestion of embryonated *H. gallinarum* eggs; or 2) direct ingestion of *H. meleagridis* from the environment. Direct infection has not been previously documented in peafowl and further tests will be developed in these cases to determine whether lateral infection could have occurred.

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

Efficacy of BioMed TF-Transit Tubes in Comparison to Gold Standard BioMed InPouch TF during Transit ◊

Julia Boehler[1], Alicia L. Alexis[1], John Ragsdale[1], Pascale Leonard[2], Kim Reiten[1], Dustin Cox[1], Suzanne Townsend[1], Tim J. Hanosh[1]

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Tritrichomonas foetus (*T. foetus*) is a flagellate protozoan parasite that is the etiologic agent responsible for the severe reproductive disease in cattle known as bovine trichomonosis. New Mexico Department of Agriculture Veterinary Diagnostic Services (NMDA-VDS) has validated a Real-Time PCR assay that was recently discussed in the Journal of Veterinary Diagnostic Investigations by Effinger and Colleagues as the only assay performed by a laboratory (Laboratory F) in their study that had perfect agreement (kappa = 1.0) with the nPCR and subsequent sequencing results they acquired. To maintain this detection level of the *T. foetus* organism for the clients the laboratory serves, NMDA-VDS performed a study to determine the efficacy of the newly offered BioMed TF-Transit tubes in comparison to the gold standard BioMed InPouch transport system while in transport. This study also served to determine the impact on samples of variable transport times with regard to the detection of *T. foetus* by Real-Time PCR. The transport study was performed by using a pure strain of *T. foetus* (sequenced) to prepare a stock 10-fold serial dilution (neat through 10-6). The BioMed TF-Transit tubes and BioMed InPouch transport system were then inoculated with each dilution series in triplicate with a negative sample included per group (22 samples per collection system per group; 176 total samples). Four groups of side-by-side comparison collection methods were produced including a laboratory control group, a 48 hour transport group, a 72 hour transport group, and a 96 hour transport group. Once inoculated, samples were prepared for transport with logged temperatures throughout transport time. Upon return, each group was processed through specimen receiving for molecular processing following standard diagnostic procedures. The Molecular Biology department performed the NMDA-VDS validated chemical lysis extraction and Real-Time PCR method and added a standard *T. foetus* reference dilution series on each plate for development of a standard curve and limit of detection. The results of this study provided data toward the acceptance of the BioMed TF-Transit tube as an efficacious transport system and evidence revealed comparable analytical sensitivity when compared to the BioMed InPouch transport system. Additionally, this study yielded valuable information on acceptable transport times from collection date to date receipt in laboratory, with 96 hours being a permissible and evidence-based transport time when utilizing the validated Real-Time PCR method at NMDA-VDS.

◊ USAHA Paper

Improvements in *Tritrichomonas foetus* Molecular Diagnostics

Carly Ginter Summarell, Thomas Hairgrove, Megan Schroeder, Mangkey A. Bounpheng

Texas A&M Veterinary Medical Diagnostic Lab, College Station, TX

Bovine trichomoniasis is a venereal disease with worldwide distribution caused by *Tritrichomonas foetus* (*T. foetus*). *T. foetus* is transmitted during coitus and results in infertility, abortion and diminished calf crops. Cows are often able to clear infection but bulls become lifelong carriers. *T. foetus* infection is of significant economic concern to the cattle industry due to economic losses associated with reduced breeding efficiency. There is no effective treatment, and management strategies are currently limited to testing prior to breeding and culling of infected bulls. Two diagnostic testing methods available are microscopic identification in preputial smegma cultures and quantitative polymerase chain reaction (qPCR). Both methods require incubation in InPouch media after collection. Notable challenges associated with *T. foetus* diagnostic testing include: cost, associated with sample collection in InPouch media, inconsistencies in shipment conditions, incubation time and temperature, incubation time burden, and labor burden of InPouch sample processing. To overcome these challenges, an innovative qPCR that utilizes direct smegma testing was developed, eliminating cost of the InPouch, need for incubation, and decreasing time and labor burden for submitter and laboratory. Smegma is collected into a sample tube and used directly for nucleic acid purification. Thus, without incubation in InPouch media, faster results are enabled, allowing faster movement of animals. Assay performance was evaluated using 166 bulls, 56 positives and 110 negatives. T.foetus positive or negative status was determined using InPouch culture microscopic identification. Using microscopic identification as the reference test, the TVMDL currently employed qPCR, which utilizes samples cultured in InPouch media, exhibited 95% diagnostic sensitivity and 100% specificity, McNemar's P-value of 0.25. The new, direct qPCR without prior culture exhibited 100% diagnostic sensitivity and 99% specificity, McNemar's P-value of 1.00. No significant difference was observed between each qPCR compared with the microscopic identification. The agreement between the current and new direct qPCR was 98% (kappa=0.95), P-value of 0.125, indicating no significant difference between the two tests. However, the new direct qPCR identified four additional positive animals and Cq values were lower for all positives: 13.6-33.5 for new direct qPCR vs. 18.7-37.4 for current, post-culture qPCR. The new direct qPCR results enabled better data interpretation since all Cq values were outside of the inconclusive and suspect range. In summary, the new direct qPCR assay offers significant improvements: easier sample collection process, omission of the InPouch cost and incubation time, easier sample processing and nucleic acid purification in the diagnostic lab, faster results and overall lower cost. These improvements benefit producers, veterinarians, and diagnostic labs in their efforts to control *T. foetus*.

Virology 2

Sunday, October 19, 2014

Chicago A

Sponsor: VMRD

Moderators: Ben Hause, Kristy Pabilonia

8:00 AM **Enhanced Sensitivity of an Antibody Enzyme-linked Immunosorbent Assay using *Equine Arteritis Virus* Purified by Anion Exchange Membrane Chromatography ◊**
Chungwon Chung, Amanda L. Grimm, Carey L. Wilson, Udeni BR Balasuriya, Peter Timoney, Chandima-Bandara Bandaranayake-Mudiyanselage, Stephen Lee, Travis McGuire *121*

8:15 AM **Evaluation of a Commercially Available Competitive ELISA (cELISA) for the Detection of Antibodies to *Equine Arteritis Virus* (EAV)**
Kristin Pfahl, Chungwon Chung, Jianqiang Zhang, Yun Young Go, Juliana Campos, Kathleen Shuck, Amanda L. Grimm, Ethan Adams, Peter Timoney, Udeni BR Balasuriya *122*

8:30 AM **Evaluation of a Newly Developed Insulated Isothermal RT-PCR Assay and a Real-Time RT-PCR Assay for the Detection of *Equine Arteritis Virus* Nucleic Acid in Equine Semen**
Udeni BR Balasuriya, Ashley Skillman, Kathleen Shuck, Bora Nam, Peter Timoney, Yun-Long Tsai, Li-Juan Ma, Pai-Chun Yang, Hsiu-Hui Chang, Pei-Yu Lee, Hsiao Fen Grace Chang, Hwa-Tang Wang *123*

8:45 AM **Development of a Multiplex Assay to Determine Antibodies to Different Glycoproteins of *Equine herpesvirus 1***
Laura B. Goodman, Heather Freer, Susanna Babasyan, Alicia Rollins, Gillian A. Perkins, Edward J. Dubovi, Bettina Wagner. *124*

9:00 AM **First Diagnosed Case of Vesicular Stomatitis in the United States in 2014**
Melinda Jenkins-Moore, Dawn Toms, Harry Vogt, Mary Lea Killian, Annette Olson, Katherine Mozingo, Angela M. Pelzel-McCluskey *125*

9:15 AM **Break (45 min)**

10:00 AM **SNP Analysis Used to Select Conserved Regions for an Improved *Newcastle Disease Virus* Real-time RT-PCR Test ◊**
David Suarez, Lauren Marbut. *126*

10:15 AM **Detection of H5 and H7 Highly Pathogenic Avian Influenza Virus with Lateral Flow Devices: Performance with Healthy, Sick and Dead Chickens**
Erica Spackman, J. T. Weaver, Sasidhar Malladi. *127*

10:30 AM **Characterization of H1N2 Variant Influenza Viruses in Pigs ◊**
Jinhwa Lee, Michael A. Duff, Jingjiao Ma, Qinfang Liu, Yuekun Lang, Bhupinder Bawa, Jianfa Bai, Juergen Richt, Richard Hesse, Wenjun Ma *128*

10:45 AM **Pathogenicity of Chimeric H17N10 Bat Influenza Virus in a Mouse Model**
Jinhwa Lee, Bin Zhou, Jingjiao Ma, Qinfang Liu, Wei Wang, Bhupinder Bawa, MIchael Duff, Yuekun Lang, David E. Wentworth, Juergen Richt, Wenjun Ma *129*

11:00 AM **Updating PCR Assays for Influenza Subtyping ◊**
Mia Kim Torchetti, Janice C. Pedersen, Mary Lea Killian, Nichole L. Hines, David Suarez *130*

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Enhanced Sensitivity of an Antibody Enzyme-linked Immunosorbent Assay using *Equine Arteritis Virus* Purified by Anion Exchange Membrane Chromatography ◊

Chungwon Chung[1], Amanda L. Grimm[1], Carey L. Wilson[1], Udeni BR Balasuriya[2], Peter Timoney[2], Chandima-Bandara Bandaranayake-Mudiyanselage[1], Stephen Lee[3], Travis McGuire[1]

[1]Research & Development, VMRD Inc., Pullman, WA; [2]University of Kentucky, Lexington, KY; [3]University of Idaho, Moscow, ID

In the present study, a rapid and easily-scalable method for purifying *Equine arteritis virus* (EAV) using an anion exchange membrane chromatography capsule (AEC) was developed. The relative advantage of AEC-purified EAV was evaluated based on the following parameters: 1) The presentation quality of the epitope defined by GP5-specific monoclonal antibody 17B7, and 2) The relative sensitivity of an antibody competitive enzyme-linked immunosorbent assay (cELISA) using AEC-purified antigen compared to an otherwise-identical commercial antibody cELISA using differential centrifugation-purified antigen. AEC-purified EAV antigen contained ~86.3% GP5 monomer while differential centrifugation-purified EAV contained less than 29.4% GP5 monomer. Improvement of cELISA analytical sensitivity without sacrifice of analytical specificity was clearly evident when cELISAs based on the two purification methods were evaluated using sensitivity check sets composed of borderline positive/negative sera from three horses vaccinated with a commercial modified live attenuated vaccine (MLV), and a time point serum set sequentially collected from an MLV-vaccinated horse. Furthermore, the AEC-purified antigen cELISA had 44.2% to 46.4% higher agreement with the virus neutralization (VN) test than the cELISA derived from differential centrifugation-purified EAV when tested with 43 borderline EAV-seropositive samples as defined by the VN test. In addition, the AEC-purified antigen cELISA had highly significant ($p = 0.001$) robustness indicated by intra-laboratory repeatability and inter-laboratory reproducibility when evaluated with the sensitivity check sets. The results suggest that the use of AEC-purified antigen in the cELISA may significantly contribute to further harmonization of the antibody cELISA with the OIE-prescribed VN test.

◊ USAHA Paper

Evaluation of a Commercially Available Competitive ELISA (cELISA) for the Detection of Antibodies to *Equine Arteritis Virus* (EAV)

Kristin Pfahl[1,2], Chungwon Chung[3], Jianqiang Zhang[1], Yun Young Go[1], Juliana Campos[1], Kathleen Shuck[1], Amanda L. Grimm[3], Ethan Adams[3], Peter Timoney[1], Udeni BR Balasuriya[1]

[1]Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY; [2]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY; [3]Veterinary Medical Research and Development, Pullman, WA

Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), an economically important respiratory and reproductive disease of horses. The virus neutralization test (VNT) is the principal serological assay used to detect evidence of EAV infection by most laboratories, and currently is the World Organization for Animal Health (OIE) prescribed test for EVA for international trade. The assay is used for diagnosis, epidemiological surveillance, trade, and pre and post vaccination screening of horses. Although the VNT is the most highly sensitive and specific serodiagnostic test for this infection, it has the disadvantage being expensive, labor-intensive, and time-consuming to perform. Furthermore, results can vary among laboratories when inadequate attention is paid to standardization of both test reagents and test protocol. Moreover, serum cytotoxicity caused by anti-cellular antibodies directed against RK-13 cells can present difficulties in test interpretation. To overcome these disadvantages, a commercial competitive enzyme-linked immunosorbent assay (cELISA) to detect antibodies to EAV has been developed. The objective of this study was to compare the cELISA to the VNT for detection of EAV antibodies. A total of 3300 archived equine sera were tested by the commercially available cELISA and by VNT as the OIE approved gold standard. These included 768 sequential samples from horses experimentally inoculated with various EAV strains, 22 sera from horses inoculated with selected archived strains of EAV, and 1510 field samples randomly selected from those submitted for serological testing to the OIE EVA reference laboratory at the Maxwell H. Gluck Equine Research Center. The positive cELISA cut-off of ≥35% inhibition (%I) was used as recommended by the manufacturer. The cELISA results and VNT results from the experimental inoculated horses were used to calculate the specificity and sensitivity of the cELISA. In general, the pattern of antibody development determined by cELISA correlated very well with the VNT. However, seven samples positive by ELISA were negative by VNT (false positive) and four samples positive by VNT were negative by ELISA (false negative). The sensitivity and specificity of the cELISA were calculated to be 99.1% and 97.8%, respectively, compared to the VNT. These results confirm that the cELISA is highly specific and sensitive and when fully validated, may be accepted by the OIE as an alternative test to the VNT for detecting EAV-specific antibodies in equine sera.

Evaluation of a Newly Developed Insulated Isothermal RT-PCR Assay and a Real-Time RT-PCR Assay for the Detection of *Equine Arteritis Virus* Nucleic Acid in Equine Semen

Udeni BR Balasuriya[1], Ashley Skillman[1], Kathleen Shuck[1], Bora Nam[1], Peter Timoney[1], Yun-Long Tsai[2], Li-Juan Ma[2], Pai-Chun Yang[2], Hsiu-Hui Chang[2], Pei-Yu Lee[2], Hsiao Fen Grace Chang[2], Hwa-Tang Wang[2]

[1]Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY; [2]GeneReach USA, Lexington, MA

Prevention of venereal transmission of *equine arteritis virus* (EAV) is critical in minimizing the spread of the virus through the movement of carrier stallions or virus infective semen. Identification of the carrier stallion is therefore of critical epidemiological importance in the prevention and control of EAV infection. The objective of this study was to develop a reverse transcription insulated isothermal polymerase chain reaction (RT-iiPCR [POCKIT™]) targeting open reading frame 7 (*ORF7*) and compare it to a previously described real-time RT-PCR (rRT-PCR). The field-deployable POCKIT™ system incorporates fluorescent probe-based iiPCR technology. It can provide test results from extracted nucleic acid within one hour. The limit of detection of RT-iiPCR was determined to be 10 copies of *in vitro* transcribed EAV *ORF7* RNA. Analysis of viral RNA extracted from 10-fold dilutions (10^{0} to 10^{-10}) of tissue culture fluid containing the Virulent EAV Bucyrus strain (VBS) and the KY 84 strain showed that RT-iiPCR could detect EAV nucleic acid up to 10^{-6} and 10^{-7} dilutions, respectively, which was 10-fold higher than the rRT-PCR. Both assays generated positive signals with nucleic acid extracted from twenty-six known laboratory, field, and modified live virus vaccine strains. Both assays were highly specific and there was no cross reactivity with thirteen other important equine pathogens. Accuracy of both assays was evaluated against virus isolation (gold standard) for the detection of EAV in 118 archived sequential semen samples from experimentally infected carrier stallions. The relative sensitivity, specificity and accuracy were 98.41% (62/63), 100.00% (55/55), and 99.15%, respectively, for the rRT-PCR (Cohen's kappa value 0.98), and 100.00% (63/63), 98.18% (54/55), and 99.15%, respectively for the RT-iiPCR (Cohen's kappa value 0.98). Finally, a spin column-based extraction method (PetNAD™ Nucleic Acid Co-prep kit) and a portable automatic extraction method (taco™ mini Automatic Nucleic Acid Extraction System) intended for point-of-need applications were shown capable of extracting EAV RNA comparable to the MagMAX™-96 Viral RNA Isolation Kit. The RNA extracted with taco™ mini had similar sensitivity to the other two extraction methods in the RT-iiPCR. These results suggest that both rRT-PCR and RT-iiPCR are equivalent for the detection of EAV in semen samples.

Development of a Multiplex Assay to Determine Antibodies to Different Glycoproteins of *Equine herpesvirus 1*

Laura B. Goodman[1], Heather Freer[1], Susanna Babasyan[1], Alicia Rollins[1], Gillian A. Perkins[2], Edward J. Dubovi[1], Bettina Wagner[1]

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY; [2]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

Development of rapid, sensitive and high-throughput assays for the assessment of immunity to *equine herpesvirus* type 1 (EHV-1) is important for vaccine development, clinical diagnostics and the evaluation of protective EHV-1 antibody levels in horses. The current gold standard is the serum neutralization (SN) test, which is time intensive and does not differentiate between EHV species. We have developed a new multiplex test based on two major EHV-1 antigens (glycoproteins C (gC) and gD). The antigens of interest were cloned and stably expressed in a mammalian expression system as IL-4 fusion proteins. Recombinant gC and gD antigens were then purified and coupled to fluorescent beads, which were used to quantify antigen-specific antibodies in horse serum on a Luminex platform. The assay was validated on serum samples from 58 horses. Validation samples included naturally EHV-1 infected, vaccinated or non-exposed horses with known SN titers ranging between <2 and 768. Multiplex-based antibody values were highly correlated with SN ($R=0.87$, $p<0.0001$). A group of 15 naïve horses were also monitored prior to and at monthly intervals following vaccination for one year to establish reference levels for protective immunity. In conclusion, the new multiplex EHV assay is an efficient alternative to SN testing.

First Diagnosed Case of Vesicular Stomatitis in the United States in 2014

Melinda Jenkins-Moore[1], Dawn Toms[1], Harry Vogt[3], Mary Lea Killian[2], Annette Olson[1], Katherine Mozingo[1], Angela M. Pelzel-McCluskey[3]

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Vesicular stomatitis virus (VSV) is a member of the *Rhabdoviridae* family mainly affecting horses, mules, donkeys, cattle, and swine. In the United States (US) the virus is usually seen in outbreaks in the southwestern part of the country. Typically oral vesicles, ulcers, and erosions are seen in and around the mouth, but animals can also manifest similar lesions around the feet, udder, or prepuce. There are 2 main serotypes seen in the US, New Jersey (VSV-NJ) and Indiana type 1 (VSV-IN1). The last outbreak of VSV-IN 1 occurred in 1998. The last outbreak of VSV-NJ was in the states of New Mexico and southern Colorado in 2012. On May 21, 2014 serum and swab samples from five horses located in Southern Texas were collected and submitted to the National Veterinary Services Laboratories (NVSL) for VSV Foreign Animal Disease (FAD) testing. Three of the five horses had crusty, swollen muzzles and vesicles of the oral mucosa on May 18. By May 21st two more horses had similar lesions while the lesions of the original three were resolving. NVSL tested the serum of all five by cELISA and compliment fixation (CF) tests and inoculated the swabs on cell culture for virus isolation (VI). Two of five horses had titers on the CF test to VSV-NJ. Three of the five horses were positive on the cELISA test for VSV-NJ. Two of the five horses (the two that were negative for cELISA and two of the three that were negative on the CF) were positive for VSV-NJ virus by isolation in less than 24 hours. Reisolation was performed to confirm the results. Whole genome sequencing indicated the virus was greater than 99% homologous to a strain from New Mexico associated with the 2012 outbreak.

SNP Analysis Used to Select Conserved Regions for an Improved *Newcastle Disease Virus* Real-time RT-PCR Test ◊

David Suarez, Lauren Marbut

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Newcastle disease virus is a RNA virus with high sequence diversity that may cause a severe disease in susceptible poultry. The severe form of the disease is considered a foreign animal disease in the United States and in many other countries, and rapid detection is critical for successful eradication when disease outbreaks occur. Real-time RT-PCR (rRT-PCR) has become the most commonly used test for screening clinical samples for the virus. Sequence mismatches of primers and probe to the circulating field strains has resulted in false negative tests or reduced sensitivity which can compromise our goal of rapid and early detection. With the rapid increase in the number of sequences of NDV available in public databases, it provides new opportunities for tests to be developed that are to the most highly conserved regions of the viral genome. A new approach for finding the best sites for primers and hydrolysis probes was developed using the single nucleotide polymorphism (SNP) analysis to calculate variability at every nucleotide of the genome and then use a boxcar average approach to identify the most conserved regions. A total of eight different regions that were highly conserved and were amenable to a rRT-PCR test were empirically tested to identify the most promising tests for additional study. Sensitivity, specificity, and end-point detection were considered after the most promising tests were optimized. Several promising tests were identified that could potentially replace or provide an alternative to the existing matrix rRT-PCR test used in the U.S. The SNP analysis approach can be used for any pathogen for molecular diagnostic testing.

◊ USAHA Paper

Detection of H5 and H7 Highly Pathogenic Avian Influenza Virus with Lateral Flow Devices: Performance with Healthy, Sick and Dead Chickens

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Rapid detection of highly pathogenic avian influenza virus (HPAIV) in the field is critical for effective disease control and to differentiate it from other diseases, such as Newcastle disease. Lateral flow devices (LFD) are commercially available and provide a fast, highly specific, on-site test for type A influenza. Because of the low analytic sensitivity of LFD tests at low virus concentrations, targeted sampling of sick and dead birds has been proposed in order to increase detection probability. In order to quantify how clinical condition correlates to the detection of HPAIV with LFDs and whether delayed testing of dead chickens affects detection we exposed 50 chickens to a low dose of an H5 HPAIV and 50 chickens to a low dose of H7 HPAIV. Low doses were used in an attempt to increase mean death times. Oro-pharyngeal swabs were collected from all birds at 12, 24, 36, 48, 60, 72, 84, 96 and 108 hrs post exposure. During sample collection each chicken was scored as healthy, sick or dead. Half of the dead birds were placed in an empty isolator and samples were not collected until the next sample time, so swab collection was delayed 12hr. All swab samples were tested at the time of collection with a commercially available US licensed LFD for avian influenza virus and were subsequently tested with quantitative real-time RT-PCR to quantify the virus in each swab sample. With the combined data of both experiments 9.1% of healthy chickens, 82.4% of the sick chickens, 90.3% of the dead birds tested immediately, and 91.3% of the dead birds with 12hr delayed sampling were positive with the LFD. There was a direct correlation between the titer in the sample and whether the LFD was positive; the lowest titer the LFD could detect was 10^4.6 50% egg infectious doses per ml (EID50/ml) and at the titer of 10^5.6 EID50/ml and above 100% of samples were positive. Delaying testing of dead birds by 12 hrs did not affect results and titers from swabs collected after a 12hr delay were significantly higher than those collected from freshly dead birds.

Characterization of H1N2 Variant Influenza Viruses in Pigs ◊

Jinhwa Lee, Michael A. Duff, Jingjiao Ma, Qinfang Liu, Yuekun Lang, Bhupinder Bawa, Jianfa Bai, Juergen Richt, Richard Hesse, Wenjun Ma

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Introduction of the 2009 pandemic H1N1 virus (pH1N1) into swine herds has led to reassortment between the pH1N1 and endemic swine influenza viruses (SIVs) worldwide. Recently, reassortant H3N2 and H1N2 variants that contain only the M gene from pH1N1 and the remaining seven genes from North American triple-reassortant (TR) SIVs have emerged. These variant viruses have caused more than 300 cases of human infections and one death in the USA, creating a major public health concern. To date, the pathogenicity and transmissibility of H1N2 variant viruses has not been investigated using an animal model. Through passive surveillance of Kansas swine herds, we isolated 25 H1N2 SIVs: 16 of these viruses are reassortant viruses with genes from pH1N1 and 12 of those are variant viruses with only the M gene from pH1N1. This suggests that H1N2 variants with only M gene from pH1N1 have become established in Kansas swine herds. To further determine the pathogenicity and transmissibility of novel reassortant H1N2 viruses, we selected two reassortant H1N2 SIVs from our isolate pool to infect pigs: one is a swine H1N2 variant virus (swH1N2v) with the M gene from pH1N1; the other is a reassortant H1N2 virus (2+6 rH1N2) with two surface genes from endemic North American TR H1N2 SIVs and six internal genes from pH1N1, using a human H1N2 variant (huH1N2v) and an endemic TR H1N2 SIV (eH1N2) isolated in 2011 as controls. All four viruses were able to infect pigs and replicate in the lungs. Both H1N2 variant viruses caused more severe lung lesions in infected pigs when compared to the eH1N2 and 2+6 rH1N2 viruses. Although all four viruses are transmissible in pigs and were detected in the lungs of contact animals, the swH1N2v replicated more efficiently than the other three viruses in the respective sentinel animals. Additionally, the huH1N2v displayed delayed and inefficient nasal shedding in sentinel animals. Taken together, the swine and human H1N2 variant viruses are more pathogenic and the swH1N2v more transmissible in pigs and could pose a threat to public and animal health.

◊ USAHA Paper

Pathogenicity of Chimeric H17N10 Bat Influenza Virus in a Mouse Model

Jinhwa Lee[1], Bin Zhou[2], Jingjiao Ma[1], Qinfang Liu[1], Wei Wang[2], Bhupinder Bawa[1], MIchael Duff[1], Yuekun Lang[1], David E. Wentworth[2], Juergen Richt[1], Wenjun Ma[1]

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The subtyping of *influenza A viruses* (IAV) is based on surface hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Until 2010, 16 HA and 9 NA were known and all were found in the aquatic birds. The recent discovery of novel H17N10 and H18N11 bat influenza virus (BIV) genomes has challenged this traditional notion and expanded the natural host spectrum for influenza viruses. No live BIVs so far are available because they are not cultivable in substrates for known IAV which has been a major obstacle in further characterizing these viruses. In this study, chimeric BIVs (cH1N1/H17 and cH3N2/H17) containing HA and NA coding regions from known IAVs (PR8/H1N1 and TX98/H3N2) in the genetic background of H17N10 BIV were generated using reverse genetics. The chimeric BIVs replicated efficiently in Madin-Darby canine kidney (MDCK) cells comparable to the parental PR8/H1N1 and TX98/H3N2 viruses. To further investigate the pathogenicity of chimeric BIVs in vivo, 6-7 week old BALB/c mice were intranasally challenged with 103 TCID50 of both chimeric and parental viruses. The PR8/H1N1 and cH1N1/H17 caused 100% and 75% mortality respectively, whereas the TX98/H3N2 and cH3N2/H17 viruses were not virulent in mice. All the viruses replicated efficiently in mouse lungs. Histopathologically, both wild type and chimeric viruses were able to cause typical influenza like lesions characterized by varying degree of broncho-alveolar epithelial degeneration and necrosis and interstitial pneumonia. Anti-influenza A nucleoprotein antibody showed moderate to strong immunoreactivity in all mouse lungs except cH3N2/H17 infected mouse which showed a rather weak immunoreaction. In summary, our experiments demonstrate that chimeric BIVs, which HAs and NAs from the IAVs but the packaging regions from the novel BIV replicate and are virulent in mammalian host. These chimeric viruses could be used as an invaluable tool to better understand the novel BIVs.

Updating PCR Assays for Influenza Subtyping ◊

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The recent event of a low pathogenic avian influenza A(H7N9) causing significant morbidity and mortality in humans from China prompted national veterinary laboratories worldwide to ensure the ability to detect this emergent strain. Diversity long recognized in the H7 lineage has resulted in the use of assays which target viruses circulating by geographic region, thus while the Type A assay detected the virus of concern, the regionally specific H7 subtype assay did not. Rapid evaluation of available molecular assays was made possible thanks to the timely sequence data shared by the Chinese Authorities to the public database at the Global Initiative on Sharing All Influenza Data (GISAID, www.gisaid.org), but complete validation of assays can be a long process, especially when needed for use by multiple labs. The current H5 assay has also been under revision to include detection of distinct viruses from Mexico identified as the result of an interlaboratory collaboration for the harmonization of IAV diagnostics between Mexico, Canada, and US. In these cases, it is important to review testing algorithms, identify and implement interim actions, and keep stakeholders informed of progress and next steps. The National Animal Health Laboratory Network (NAHLN) provides the framework needed for such communication and has served as a "proving" ground for harmonizing approaches to methods comparison and validation efforts. The approach to validation, progress to date, and lessons learned towards improved transparency and communication for the H5 and H7 assays will be reviewed.

◊ USAHA Paper

Characterization of a Novel Influenza Virus in Cattle with Bovine Respiratory Disease

Ben Hause[1], Emily Collin[2], Yuekun Lang[1], Wenjun Ma[1], Runxia Liu[3], Feng Li[3]

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We previously reported the isolation of a novel virus, provisionally designated C/swine/Oklahoma/1334/2011 (C/OK), with 50% overall homology to human *influenza C viruses* (ICV), from a pig in Oklahoma. The novelty of C/OK virus prompted us to investigate whether C/OK virus could exist in a non-swine species. Significantly, we found that the C/OK-like virus was widespread in U.S. bovine herds, as demonstrated by reverse transcription (RT)-PCR and serological assays. Genome sequencing of three bovine viruses isolated from two herds in different states further confirmed these findings and detailed studies on the biology of this virus suggested that it represents a new genus in the family *Orthomyxoviridae* (proposed influenza D virus, IDV). To further our understanding of the epidemiology of this virus in cattle, we screened 208 samples collected from cattle with bovine respiratory disease (BRD) that were submitted for diagnostic testing from multiple states. Approximately 5% of the samples were positive for IDV by RT-PCR. A similar percentage of samples (3.4-7.2%) were positive for established BRD pathogens *bovine viral diarrhea virus*, *bovine herpesvirus* 1 and *bovine respiratory syncytial virus* while *bovine coronavirus* was the most commonly identified virus (34% positive). Virus isolation for IDV was successful from 10 RT-PCR positive samples and full genome sequencing identified >96% identity to C/OK. Phylogenetic analysis of the hemagglutinin esterase protein identified two well supported clades. Hemagglutination inhibition assays using rabbit polyclonal antiserum generated against representatives from each clade further demonstrated significant (>4-fold) antigenic differences between viruses in the two clades. These results demonstrate that IDV is commonly present in animals with BRD and that genetic and antigenic variability is present in circulating viruses. Further work is necessary to investigate an etiological role for IDV in BRD. The finding of C/OK-like virus in swine and cattle suggests that this new virus may spread and establish infection in other mammals, including humans.

Virology 3
Sunday, October 19, 2014
Chicago B

Sponsor: Biovet

Moderators: Susan Schommer, Jianqiang Zhang

11:00 AM **Rapid and Sensitive Detection of *Canine Distemper Virus* by One-Tube Reverse Transcription-Insulated Isothermal Polymerase Chain Reaction**
Rebecca P. Wilkes, Fu-Chun Lee, Yun-Long Tsai, Ping-Hua Teng, Pei-Yu Lee, Hsiao Fen Grace Chang, Hwa-Tang Thomas Wang *144*

11:15 AM **The Effect of Coagulant Treatment on the Constituents of Swine Oral Fluid Specimens**
Kevin C. O'Neill, Korakrit G. Poonsuk, Drew Grosz, Dong Sun, Yaxuan Sun, Dwayne E. Schrunk, Timothy G. Ellis, Chong Wang, Timothy Frana, Kyoung-Jin Yoon, Jeff Zimmerman *145*

Symbols at the end of titles indicate the following designations:

§ AAVLD 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

Simultaneous Detection of *African Swine Fever Virus* Antibodies in Serum and Oral Fluid Using a Recombinant p30 Antibody ELISA

Luis G. Gimenez-Lirola[1], Lina Mur[2], Belen Rivera[2], Sergio Lizano[3], Christa Goodell[3], Raymond R. Rowland[4], Mark Mogler[5], DL Hank Harris[5], Carmina Gallardo[6], Marisa Arias[6], Jose Manuel Sanchez-Vizcaino[2], Jeff Zimmerman[1]

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African swine fever (ASF) is a devastating, highly contagious disease classified as a Foreign Animal Disease (FAD) in the U.S. Serology has been widely used in ASFV control programs in the Iberian Peninsula and Sardinia as a tool for the detection of ASFV carrier animals. Among ASFV proteins considered to be candidate antigens for serological tests, structural proteins p30, p54, and p72 are the best described, most highly studied, and most widely used in commercial ASFV serum antibody ELISAs1. Serum and oral fluid antibody-positive samples were generated by experimental inoculation of 17 pigs with an attenuated ASFV isolate (NHV) that produces chronic infection. Oral fluid and serum samples were sequentially collected over days post inoculation (DPI 0, 6, 12, 15, 19, 26, 33, 40, 47, 54, and 61) using methods previously described2. The performance of the optimized ELISA was also evaluated using serum (n = 200) and oral fluid (n = 200) samples from animals (n = 400) known to be free of ASFV infection. The antigen used in the ELISA was selected by evaluating the serum antibody response of ASFV-infected pigs against three recombinant antigens (rp30, rp54, rp72) using a multiplex fluorescent microbead-based immunoassay (FMIA; Luminex® Corporation). Antibody was detected at 6 DPI against p72 (11%) and at 12 DPI for both p30 (100%), and p54 (89%). All pigs (100%) were positive at DPI 12 for p30, at DPI 15 for p54, and at DPI 19 for p72. Recombinant p30 was selected as antigen target for subsequent development of an antibody ELISA. ASFV rp30 antibody ELISA was able to detect ASFV antibodies by DPI 12 in both serum and oral fluid specimens run on the same plate simultaneously. The evaluation of known ASFV negative field samples showed specificities of 99.5% and 100% for serum and oral fluid samples, respectively. Given the increased surveillance efficiency provided by oral fluid sampling and the ability to corroborate results using serum samples, the ASFV rp30 antibody would be a highly useful under conditions that warrant ASFV surveillance.

Development and Testing of a Multiplex Molecular Diagnostic Assay for Simultaneous Detection and Differentiation of Multiple Bacterial and Viral Causes of Respiratory Disease in Pigs ◊

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A multiplex molecular assay for the rapid and sensitive diagnosis of respiratory disease in pigs has been developed. The assay enables detection and differentiation of 4 bacterial (*M. hyponeumoniae, A.pleuropneumoniae, H.parasuis*, and *S.suis*) and 6 viral pathogens (PRRS, Flu, PCV2, Pseudorabies Virus, ASF, CSF) with no cross-reactivity to clinical and genetic near-neighbor organisms. In addition to endemic diseases, the assay is able to detect and differentiate between African Swine Fever and Classical Swine Fever. The 25-plex assay has sensitivity of 1000 infectious units or less per mL of porcine oral fluid. For Influenza detection, an LOD of 200 infectious units per mL has been demonstrated. The assay is expected to be of utility in clinical diagnosis of important endemic diseases as well as surveillance for FADs.

◊ USAHA Paper

African Swine Fever Virus, *Classical Swine Fever Virus*, and *Foot-and-Mouth Disease Virus* Detection By Multiplex Reverse Transcription Quantitative Polymerase Chain Reaction in Swine Oral Fluids

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African wine fever (ASF), Classical swine fever (CSF), and Foot-and-mouth disease (FMD) are highly contagious animal diseases of significant economic and social importance. Pigs infected with ASFV and CSFV develop clinical signs that may be indistinguishable from other diseases. Likewise various causes of vesicular disease can mimic clinical signs of FMD. Early detection is critical to limiting the impact and spread of these disease outbreaks, and the ability to perform surveillance for all three diseases rapidly and cost effectively using a single diagnostic sample is highly desirable. This study assessed the feasibility of simultaneous ASFV, CSFV, and FMDV detection by multiplex reverse-transcriptase quantitative polymerase chain reaction (mp RT-qPCR) in swine oral fluids simply collected through use of a chewing rope. Animal groups were experimentally infected independently with each virus, observed for clinical signs, and oral fluids collected and tested throughout the course of infection, in four independent studies conducted months apart. All animal groups chewed on the ropes readily before and after onset of clinical signs and before the onset of lameness or serious clinical signs. ASFV was detected as early as 3 days post infection (dpi), 2-3 days before onset of clinical disease; CSFV was detected at 5 dpi, coincident with onset of clinical disease; and FMDV was detected as early as 1 dpi, 1 day before the onset of clinical disease. Equivalent results were observed in four independent studies and demonstrate feasibility of oral fluids and mp RT-qPCR for surveillance of ASFV, CSFV, and FMDV in swine populations.

Early Post Natal CSFV Infection Can Result in Persistently Infected Piglets

Sara Muñoz[1], Rosa Rosell[12], Lester Josue Perez[3], José Alejandro Bohorquez[14], Maria Teresa Frias[3], Lorenzo Fraile[5], Maria Montoya[1], Lorena Cordoba[1], Mariano Domingo[16], Felix Ehrensperger[7], Nicolas Ruggli[8], Artur Summerfield[8], LLilianne Ganges[1]

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Classical swine fever (CSF) remains one of the leading threats to the pig industry worldwide. In recent years, CSFV has been prevalent essentially in Asia and in Central and South America, with sporadic occurrence in Europe also. The broad range of disease severity observed with CSFV infections in pigs depends on the virulence of the strain and on the age, breed, health and immune status of the pig. Interestingly, a trend towards milder, chronic clinical manifestations of CSF has been observed particularly in endemic countries such as Cuba and China. Pigs infected with low-virulent strains appear mostly healthy and shed virus continuously or intermittently for months, representing a major problem for disease control. The pathogenesis of such unapparent infections and their implications for virus progression in pig populations especially in endemic regions is poorly understood. The presence of low virulent CSFV strains in the field has mainly been related to the "pregnant carrier sow syndrome" involving trans-placental infection of the foetus leading to persistently infected piglets. In contrary, the role of post natal infection for viral persistence in the field is poorly documented and not well understood. Therefore, the aim of this study was to evaluate the ability of CSFV to induce viral persistence upon early postnatal infection. This was explored with two recent low virulent CSFV field isolates, an endemic virus from Cuba and a recent isolate from Spain. Two litters of 10 piglets were infected via the intranasal route at the day of birth with the Cuban and the Spanish isolate, respectively. During the observation period of 6 weeks post infection, most of the infected piglets remained clinically healthy despite persistent high virus titres in the serum. Importantly, these animals were unable to mount any detectable humoral immune response. No virus could be isolated from serum of the two sows and of the few piglets that had seroconverted. This study shows that early post natal infection can result in a high proportion of seronegative persistently infected piglets, which has important implications for disease control and eradication in countries where CSFV is currently endemic.

Genetic Variation Observed in BVDV Isolated from 34 Persistently Infected Cattle Generated in One Outbreak

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Bovine viral diarrhea viruses (BVDV) are single stranded RNA viruses that belong to two different species within the pestivirus genus of the Flavivirus family. Similar to other viruses with single stranded RNA genomes, BVDV isolates exist as quasispecies, or a swarm of individual viruses, each with small numbers of unique nucleotide differences. Exposure to bovine viral diarrhea viruses may result in acute and persistent infections. Persistent infections are the result of exposure in utero prior to 125 days gestation. Persistently infected (PI) cattle are immunotolerant to the viral strain with which they were infected in utero which prevents them from clearing the virus and results in viral replication in numerous tissues and lifelong shedding of the virus. Previously it has been shown that a greater number of nucleotide substitutions were introduced into the BVDV viral genome during acute infections of pregnant cattle than of non-pregnant cattle. In this study we looked at the stability of the BVDV genome from viruses isolated over time in 34 PI cattle generated during one outbreak of BVDV2. The outbreak occurred in a group of 136 bred heifers assembled by a cattle buyer and purchased in 2004 to increase a research herd. Between March and May of 2004, 128 live calves were born of which 8 died as neonates. A total of 41 out of the remaining 120 calves tested positive for persistent BVDV infection based on positive tests on 2 samples collected at least two weeks apart. Of this group, 34 survived long enough to have blood samples collected in December of 2004 and February and March of 2005. Virus was isolated from the buffy coat of each animal at each sampling point and the 5' UTR and E2 region sequences generated by PCR amplification followed by cycle sequencing. Phylogenetic analysis revealed that the viruses were highly similar but belonged to two distinct clades. The viruses isolated at the three different time point from 14 of the 34 cattle were identical in even the highly variable region of the E2. Minor differences were observed among viruses isolated from the other cattle at different time points. Segregation into the two clades was consistent for viruses across all the time points. To determine if viral variants from both clades were part of the viral swarm for each of the isolates sequencing of individual clones was performed. This was done by cloning the E2 PCR amplicon generated from the March buffy coat samples into a bacterial vector and then sequencing at least 100 of the recombinant vectors. Sequencing revealed that the viral swarms from isolates segregated to one clade did not include variants that matched the other clade. These results suggest that while variants are generated at a higher rate following infection of pregnant animals, infection of the fetus resulting in persistent infection is the result of infection with a single variant.

One-Step Triplex Real Time RT-PCR Assay for Simultaneous Detection and Differentiation of Three Vesicular Viruses in Swine ◊

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Vesicular Stomatitis (VS) and Swine Vesicular Disease (SVD) are two worldwide livestock diseases that are of economical importance. They cause vesicular lesions, ulcerations of the tongue and oral tissues, and coronary bands in infected animals. VS has two major serotypes that are *Vesicular stomatitis Indiana virus* (VSIV) and *Vesicular stomatitis New Jersey virus* (VSNJV). SVD only has one serotype, but animals are often co-infected with VS strains, making it difficult for accurate clinical diagnosis. Therefore, rapid detection and accurate differentiation of these viruses is critical for effective disease management. Here we describe a novel one step triplex real time RT-PCR for simultaneous detection and differentiation of VSIV, VSNJV and SVDV. The most reserved region of the L gene of VSV and the 5'UTR of SVDV were selected as detection targets. Our results showed that the multiplex assay generated similar sensitivity levels as compared to its corresponding single-target PCRs. The amplification efficiencies of multiplex real time PCRs were 96.1%, 98.6% and 98.1%, and the correlation coefficients of Ct values from the standard curves generated by the multiplex reaction and its corresponding singular reactions were 0.9994, 0.9953 and 0.9995, for VSIV, VSNJV and SVDV, respectively. The detection limits of triplex real time PCRs were about 10 copies per reaction for the three viruses, which are comparable to its corresponding singular real time PCR reactions. When primers and probes of the three viruses were used in the same reaction on individual virus template, only the corresponding channel generated signal, and there is no cross-reaction or interference observed. Similar specificity was also obtained from singular reactions. This assay may be a cost-effective alternative for rapid and accurate detection and differentiation of VSIV, VSNJ and SVDV strains. This assay may be especially useful when co-infections with two or three of these viruses occur in the same animal.

◊ USAHA Paper

Establishing Critical Diagnostic Capability for Foot-And-Mouth Disease in Red Deer

Reinhold Kittelberger[1], Charles Nfon[2], Kurtis Swekla[2], Zhidong Zhang[2], Kate Hole[2], Hilary Bittner[2], Tim Salo[2], Courtenay O'Sullivan[1], Michaela Hannah[1], Richard Swainsbury[1], Rudolfo Bueno[1], Richard Clough[1], Andrew mCfADDEN[1], Richard Spence[1], Soren Alexandersen[2]

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New Zealand has the largest population of farmed red deer in the world (1.1 million) along with a significant feral deer population. Deer are susceptible to foot-and-mouth disease (FMD) although clinical signs for FMD in deer are much less severe than in other ruminants. Consequently, during any FMD outbreak in New Zealand laboratory testing of large numbers of red deer samples would potentially be required. Currently the accuracy of available diagnostic tests for detecting FMD in red deer is unknown as these tests have been developed for other livestock species. The aim of this project was to determine which diagnostic tests are the most appropriate for use with red deer. This was a collaborative research project between the National Centre for Foreign Animal Disease (NCFAD), Winnipeg, Canada and the Animal Health Laboratory (AHL), Ministry for Primary Industries in New Zealand. At NCFAD 10 red deer were intra-nasally inoculated with the FMD serotype O virus, monitored for clinical signs, and samples taken regularly over a four week period. Samples were tested by RT-PCR, virus isolation, antigen detection using pen-side tests and ELISAs and antibody detection by the virus neutralization test (VNT), two O-serotype specific structural protein (SPO) ELISAs, and four serotype-independent non-structural protein (NSP) ELISAs and a NSP pen-side test. Only one animal developed clinical signs. It tested positive by RT-PCR in various swabs, lesion materials and serum. In an in-house NSP-ELISA, a commercial NSP-ELISA and an antibody NSP pen-side test, the same animal showed positive results from day post inoculation (dpi) 14 onwards. Two other commercial NSP ELISAs detected anti-NSP serum antibodies with lower sensitivity. The animal became positive in the VNT and an in-house SPO-ELISA at dpi 9 and in a commercial SPO-ELISA on dpi 11. Another three animals were RT-PCR positive only in nasal swabs. Six of the red deer that were RT-PCR negative were re-inoculated intramuscularly with the same O-serotype FMDV at dpi 14 after the first inoculation. None of these animals became RT-PCR or NSP-ELISA positive but all six animals became positive in the VNT, the in-house SPO-ELISA and the commercial SPO-ELISA. Currently at the AHL in New Zealand further evaluation of diagnostic specificity and sensitivity is being undertaken on the diagnostic tests that performed well at NCFAD using inactivated samples collected from the experimentally infected red deer and samples from FMDV free red deer from New Zealand. In summary, this study has provided evidence that red deer are not easily infected with the FMD serotype O virus. The experimental infection of 10 red deer has shown that RT-PCR, a commercial NSP-ELISA, SPO-ELISA and a NSP pen-side test demonstrate good specificity and sensitivity for detecting FMDV infection in red deer. On-going work is further evaluating the performance of these tests in New Zealand.

Detection of BVDV in Cattle Semen- How Common are Persistent Testicular Infections?

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The detection of BVDV in the semen of bulls is well known. Persistently infected (PI) bulls shed virus continuously and at a high titer and will result in the infection of all susceptible females that are inseminated. While not presenting as high a risk, semen from acutely infected bulls may also contain BVDV. Virus may be detected for a short time at and soon after the period in which the bull is viremic. Virus levels tend to be low but can occasionally infect a susceptible female, presenting a risk for the introduction of BVDV into a naïve herd or into a BVDV free population. Until recently, persistent testicular infections (PTI) were considered to be extremely rare. Prior to the last decade, only one case had been reported globally but in the last 3-4 years, several cases have been detected in Australia and the USA. Virus levels in the semen of bulls with PTIs can be variable but at times high and could present a significant threat to susceptible herds. Further, it is expected that semen from a bull infected with a BVDV2 virus would initiate infection in a herd where there is only immunity to BVDV1 (either naturally or by vaccination). Detection of PI bulls is straightforward and PI bulls are now unlikely to reach a semen collection center. Even if the serological status of a bull is not known prior to arrival at a collection center, it is usual that there will be a 3-4 period of isolation, during which semen is not eligible for dispersal. During this time, it is likely that an acute infection will have resolved. However, PTIs are more problematic. To ensure that an animal does not have a PTI, the semen of all seropositive animals must be screened. As a result of intermittent virus detection in semen, several collections must be tested over several weeks to ensure that a bull is free of BVDV. Virus isolation in cell culture has been the most common method to screen semen for freedom from BVDV. However, there are several disadvantages including toxicity of the semen, presence of bacteria, the need to culture relatively large quantities of valuable semen for optimal sensitivity and a comparatively long time to obtain results, with associated high cost. There can also be issues with differences in performance of virus isolation between laboratories. Transport of samples to the laboratory must use a reliable cold chain, ideally using LN, to avoid reduction in virus infectivity during transport or handling in the laboratory. Real time PCR offers many advantages for the screening of semen, including rapid turn around, lower cost, and high analytical sensitivity that is rarely compromised by suboptimal transport or storage. This presentation will compare virus isolation and real time PCR for BVDV using a standard magnetic bead based nucleic acid extraction on semen from bulls with PTI. A pan-pestivirus assay is recommended. In all situations, real time PCR gives results that are superior to and more consistent than those obtained by virus isolation.

Evaluation of Real-Time PCR Assays for the Detection of Viruses in Semen of Livestock

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Artificial breeding is widely used with livestock for the efficient transport and use of breeding animals with superior genetic characteristics. Semen and embryos are transported both nationally and internationally. While embryos can be washed and treated to minimize the likelihood of transfer of viruses, there are greater challenges with the supply of semen that is free of viruses of concern. While donor animals can be screened and be shown to be free of infection with some pathogens, there are many situations where this is not possible. There are many diseases where semen is collected from seropositive animals and although the donor is no longer viremic, virus may be present in the semen. Consequently, health protocols for semen collection centers often require semen to be screened for freedom from viral contamination. Examples included BVDV, BHV1, BTV & EHDV in cattle, EAV in horses and PCV2 and PRRS in swine. For some countries, other agents such as *Akabane* and *Schmallenberg viruses* can be a concern in cattle semen and *Bungowannah virus* from pig semen. Virus isolation in cell culture has been the most frequently used technique to screen semen for freedom from viral infections. However, there are several disadvantages with the use of cell cultures. These can include toxicity of the semen to the cells, presence of contaminating bacteria, the need to culture relatively large quantities of valuable semen for optimal sensitivity and a comparatively long time to obtain results, with associated high cost. There can also be issues with differences in performance of virus isolation between laboratories. Finally, transport of samples to the laboratory must use a reliable cold chain, ideally using liquid nitrogen, to avoid reduction in virus infectivity during transport or handling in the laboratory. With the availability of real time PCR protocols (qPCR or qRT-PCR for RNA viruses) for most commonly encountered viral infections, this technology offers many advantages over virus isolation for the screening of semen. These include rapid turn around, lower cost, and very high analytical sensitivity that is rarely compromised by suboptimal transport or storage conditions. This presentation will provide comparisons of virus isolation and real time PCR for a wide range of viruses using a standard magnetic bead based nucleic acid extraction protocol and common PCR reaction conditions for all viruses. Only the primers and probes vary for each individual virus. In all situations, real time PCR gives results that are superior to and more consistent than those obtained by virus isolation in cell culture and should be recommended as a preferred method for the screening of semen for freedom from virus contamination.

Rapid and Sensitive Detection of *Canine Distemper Virus* by One-Tube Reverse Transcription-Insulated Isothermal Polymerase Chain Reaction

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Canine distemper virus (CDV) has been associated with outbreaks of canine infectious respiratory disease in shelters and boarding kennel environments. Aiming to aid point-of-care diagnosis of canine distemper, a simple CDV detection method was developed on the basis of hydrolysis probe-based insulated isothermal polymerase chain reaction and POCKIT™ Nucleic Acid Analyzer. Analytical sensitivity (limit of detection) of the established CDV reverse transcription (RT)-iiPCR was about 11 copies of in vitro transcribed RNA per reaction. CDV RT-iiPCR generated positive signals from CDV, but not *Bordetella bronchiseptica*, *canine parvovirus*, *canine herpesvirus*, *canine adenovirus*, *canine influenza virus* (subtype H3N8), *canine parainfluenza virus*, and *canine respiratory coronavirus*. To evaluate accuracy of the established reaction in CD clinical diagnosis, 110 specimens from dogs suspected with CDV infection were tested simultaneously by CDV RT-iiPCR and real-time RT-PCR. CDV RT-iiPCR demonstrated excellent sensitivity (100%) and specificity (100%), compared to real-time RT-PCR. Generating reliable results from clinical samples within an hour using a portable device, the established method has great potential to be used for point-of-care diagnosis of CD.

The Effect of Coagulant Treatment on the Constituents of Swine Oral Fluid Specimens

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Swine oral fluid specimens are commonly used for monitoring bacterial and viral infections in commercial swine populations using either PCR- or antibody-based assays. The focus of the work presented herein is improvement in assay performance by "cleaning up" the sample itself. Coagulants are commercially available, inexpensive chemical agents used in wastewater cleanup. The aim of this study was to determine the effect of a variety of coagulants on the innate properties of porcine oral fluids. Oral fluid samples submitted to the ISU-VDL for routine testing were aggregated to a total volume of 5.75 L, stirred to assure uniformity, and aliquotted into 30 ml volumes. Nine commercially available coagulants were evaluated against a negative control at 9 concentration levels (0.01, 0.1, 1, 10, 100, 250, 500, 750, 1000 ppm) for their effects on pH (UB-5, Denver Instrument, Inc.), mineral content, total protein, and turbidity (2100 AN, Hach Industries). To perform the testing, all samples were stored at 4°C until treated with the coagulant, after which they were agitated, centrifuged, and the liquid fraction was immediately separated and analyzed. Total protein concentration was determined using a BCA protein assay (Pierce™), and the trace content of magnesium, phosphorous, potassium, calcium, manganese, iron, copper, zinc, selenium, and molybdenum was measured using inductively coupled plasma spectrometry (820, Varian Inc.). In general, as the concentration of coagulant increased, pH also increased between 6.5 and 6.8, although not when the coagulant contained a metal cation. The log nephelometric turbidity units (NTU) consistently decreased as the coagulant concentration increased, while total protein concentration was not affected (i.e., decreased) by any of the 9 nine coagulants except one. Coagulant treatment consistently and significantly decreased iron and copper concentrations; whereas zinc and phosphorous were variably affected, depending on the coagulant. The fact that coagulants achieve a marked decrease in suspended solids (i.e., decreased turbidity) with minimal effect on total protein and pH suggests that coagulant treatment could improve the handleability and diagnostic utility of swine oral fluids.

Posters

Saturday, 18 October – Sunday, 19 October
San Francisco

Symbols at the end of titles indicate the following designations:

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

Poster 01

Rapid and Sensitive Diagnosis of *Feline Immunodeficiency Virus* Using Reverse Transcription-Insulated Isothermal Polymerase Chain Reaction with POCKIT™ System, a Point-of-Need PCR Detection Platform

Rebecca P. Wilkes[1], Stephen A. Kania[1], Hsiu-Hui Chang[2], Li-Juan Ma[2], Yun-Long Tsai[2], Pei-Yu Lee[2], Hsiao Fen Grace Chang[2], Hwa-Tang Thomas Wang[2]

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Feline immunodeficiency virus (FIV) is an important infectious agent of cats. Clinical syndromes caused by FIV include immunodeficiency, opportunistic infections, and neoplasia. In this study, a 5' LTR/gag region-based reverse transcription-insulated isothermal PCR (RT-iiPCR) that amplified all known FIV strains was developed to facilitate point-of-need FIV diagnosis. The RT-iiPCR method works in the POCKIT™ Nucleic Acid Analyzer, which is a field-deployable device and capable of generating automatically interpreted iiPCR results from nucleic acids within one hour. Limit of detection 95% of the FIV iiPCR was calculated to be 95 copies of standard in vitro transcriped RNA per reaction. Endpoint dilution studies with serial dilution of a clinical sample showed sensitivity of lyophilized FIV RT-iiPCR reagent were comparable to that of a reference nested PCR. The established reaction had no cross reactivity with non-targeted feline pathogens, including *feline herpesvirusv*, feline infectious peritonitis type 2/*feline coronavirus* 2, *feline calicivirus*, *feline leukemia virus*, *Mycoplasma haemofelis*, and *Chlamydophila felis*. When evaluating 72 blood samples, test sensitivity, specificity, and accuracy of FIV RT-iiPCR were determined against a reference nested PCR assay to be 97.67 % (42/43), 100.00 % (29/29), and 98.61%, respectively. A kappa value of 0.97 was obtained, indicating an excellent correlation between these two methods. In conclusion, the lyophilized FIV RT-iiPCR reagent has potential utility for rapid and easy point-of-need detection of FIV in cats, especially in resource-limited facilities.

Poster 02

Canine Dysautonomia: Outbreak Investigation in a Litter of Puppies †

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Canine dysautonomia (CD) is a sporadic and generally fatal disorder of the autonomic nervous system of dogs in the United States and Western Europe. The first reported case in the US occurred in 1989 in Wyoming. Since that time, CD has been laboratory-confirmed in only the eastern half of the state.1 Most reported cases in the US are in Missouri, Kansas, Wyoming, Colorado, Nebraska, Southern Illinois, the Texas panhandle, and Western Kentucky. Typically, CD affects single individuals, with one published report involving multiple littermates. Cases are recurrent on some properties. The cause of CD is unknown. The disease is generally progressive with euthanasia or natural death in >92% of cases after a short clinical course. A commonly reported epidemiological feature is recent exposure to excavated soil, suggesting a role for a soil-associated agent or biotoxin. We report a CD outbreak affecting a litter of Havanese puppies near Kansas City, Missouri. Of five littermates, four were briefly exposed (<5 minutes) to small areas of the back and front yards with a history of excavation; this was the only time they were allowed outdoors. The fifth puppy was sold hours prior to the exposure event and no disease developed. The other four developed fatal disease 10 – 14 days after putative 'exposure' to disturbed soil. Histological lesions typical of CD were present after a clinical course of 3 – 9 days. The only unusual feature in two puppies whose spinal cords were examined was extensive degeneration of lower motor neurons in all levels examined. One 'exposed' puppy was sold and introduced to a household with an unrelated four-month-old golden retriever-cross, which developed clinical signs of CD 16 days later; disease was confirmed histologically in the unrelated dog. The dam and sire of this affected litter remain alive and healthy. This is the second reported episode of CD affecting multiple littermates. The occurrence of CD in an unrelated dog shortly after the introduction of one affected puppy may be an unusual coincidence or evidence of horizontal transmission. References- 1 Hull N et al.: Canine Dysautonomia in Wyoming: 24 cases (2004-2012). AAVLD Annual Meeting, Greensboro NC. Oct 2012.

† Graduate Student Oral Presentation Award Applicant

Poster 03

Development and Validation of a Taqman Multiplex Real-Time PCR for Simultaneous Detection of Major Viral and Bacterial Pathogens Causing Canine Diarrhea

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Diarrhea in dogs can be caused by an array of viral, bacterial and parasite pathogens. The most common canine diarrheagenic viral and bacterial pathogens are canine enteric coronavirus (CEcoV), canine parvovirus 2 (CPV2 including subtypes 2a, 2b and 2c) and canine distemper virus (CDV), and 6 bacterial pathogens including *Salmonella spp.*, *Lawsonia spp.*, *Cambylobactor jejuni*, *Clostridium perfringens enterotoxin A*, *Clostridium difficileToxA and Clostridium difficileTox B* strains. We describe here a Taqman-based multiplex real-time PCR for simultaneous detection of these viral and bacterial pathogens that are commonly seen in canine diarrhea cases. The complete panel consists of three separate multiplex real-time PCR reactions, each targets 3 viral or bacterial species or species groups. The assay was optimized and validated using synthetic targets and canine diarrhea clinical samples. The PCR amplification efficiencies of the assay are in the range of 91% to 99.5% and correlation coefficients are between 0.99 and 0.999. Analytical performance including specificity and sensitivity of the assay were assessed. No cross-reactivity was observed in non-target reactions. The sensitivity of the assay was evaluated by comparing each multiplex with individual singleplex assays, which demonstrated that detection sensitivity was not compromised by multiplexing the real-time PCR reactions. The assay was further validated using 130 clinical samples with known pathogen status, the pathogens can be detected by both singleplex and multiplex reactions with 100% sensitivity. The assay analytical and diagnostic performance is promising for its use in simultaneous detection of common viral and bacterial pathogens causing canine diarrhea.

Poster 04

Susceptibility of Multiple Drug Resistant Bacterial Pathogens to Fosfomycin

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Treatment options are limited for dogs infected with bacterial pathogens that are resistant to multiple classes of antibiotics. Recent evidence suggests that fosfomycin is being rediscovered as an effective treatment option in a broad range of clinical presentations, but is especially of interest in urinary tract infections. Due to an increase in isolation of resistant bacterial pathogens from clinical samples, the bacteriology section of the Indiana Animal Disease Diagnostic Laboratory (ADDL) has recently received requests from Purdue Veterinary Teaching Hospital clinicians for susceptibility testing beyond the standard commercially available MIC panels, specifically for the drug fosfomycin. This ongoing study includes pathogens with resistant MIC profiles on the standard companion animal panel isolated in the ADDL from clinical specimens of companion animals. The MIC profiles, fosfomycin E-test results, site of origin and clinical history are analyzed to identify circumstances in which fosfomycin may be an attractive option for primary antimicrobial treatment. Isolates of *E. coli* from urine and skin samples were consistently susceptible to fosfomycin while *Pseudomonas aeruginosa* isolates were often resistant. These data supplement the literature with further evidence that fosfomycin may be an effective option for treating otherwise resistant pathogens in veterinary patients, and support the use of the fosfomycin E-test in veterinary diagnostic laboratories.

Poster 05

Prevalence and Antibiotic Susceptibility Dynamics of Bacterial Isolates from Canine Skin Infections at a Purdue Veterinary Teaching Hospital (2004–2013) # * ◊

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Skin disorders are the most common clinical presentation in dogs varying from acute to chronic, and often require life-long treatment. Microorganisms including yeast and bacteria are considered to be both predisposing and perpetuating factors.The selection of appropriate antimicrobial therapy can be significantly aided by knowledge of the most common bacterial isolates in a geographical area and their antibiotic susceptibility profiles. We designed a ten year retrospective study to include culture and susceptibility data from skin samples (swabs and scrapings) submitted to the Indiana Animal Disease Diagnostic Laboratory in 2004 through 2013 from Purdue's Veterinary Teaching Hospital. The aims of this study are to identify and quantify the most frequently isolated bacteria from about 250 skin samples and their antibiotic susceptibility patterns in order to generate objective local data to aid veterinarians in their selection of an antibiotic for initial empirical therapy.Our data indicates that the most commonly isolated microorganisms are *Staphylococcus pseudintermedius*, *Corynebacterium* sp., *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Streptococcus* sp. (both alpha and beta-hemolytic).The most commonly isolated bacterium from each year of the study was *S. pseudintermedius* which was present in more than 50% of the samples included in this study. We analyzed the antibiotic susceptibility profiles of the five most common bacterial species to identify changes over time, such as increasing resistance to certain classes of drugs.This retrospective study identifies consistencies and trends of change in canine skin infections which may result in improved empirical treatment.

AAVLD Trainee Travel Awardee (Bacteriology/Mycology, Epidemiology)
* Graduate Student Poster Presentation Award Applicant
◊ USAHA Paper

Poster 06

Getting Inside the Identification of Coagulase Positive *Staphylococci* Frequently Isolated from Companion Animal Clinical Specimens §

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Correct identification of coagulase positive *Staphylococcus* species in companion animals is essential for antimicrobial drug selection and for the potential of zoonotic transmission. *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* subspecies *coagulans* are considered part of the normal flora in companion animals; differently *Staphylococcus aureus* is considered a transient microorganism. These three species of *Staphylococci* are commonly associated with mild or severe diseases in dogs and cats. *Staphylococcus pseudintermedius* belong to the *Staphylococcus intermedius* group (SIG). *Staphylococcus intermedius* and *Staphylococcus delphini* are also members of the of the SIG but are not frequently isolated from companion animals. Molecular techniques have been suggested as the ideal method to identify staphylococci found in dogs and cats, based on the difficulty to differentiate members of the SIG and *S. schleiferi* subspecies *coagulans* from *S. aureus*. The aim of this study was to evaluate the use of the matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) in combination with Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany) to identify coagulase positive staphylococci isolated from companion animal clinical specimens. In addition, an in-house method, based on conventional tests: coagulase, phenol-red-mannitol salt agar, mannitol, maltose, trehalose and Voges-Proskauer (VP) test was also assessed. The two methods were evaluated by comparison with species identification by sequencing of rpoB gene. In this study, a total of 50 *Staphylococci* were identified by the in-house method and MALDI-TOF. Sixteen isolates were tested by rpoB gene sequencing and of these, 12 (75%) were correctly identified by MALDI-TOF and the in-house method. Seven *S. schleiferi* subspecies *coagulans* and 6 *S. pseudintermedius* were tested by the three methods, 4 (57%) and 6 (100%) were identified to the level species by the three methods respectively. When MALDI-TOF results were compared with sequencing, 15 isolates were correctly identified to the genus and species level by MALDI-TOF, with a score higher than 2. MALDI-TOF scores were higher than 2 for 42 isolates, which indicates high confidence on the identification of the *Staphylococcus* species. Thirty-four isolates identified by the in-house method were identified as the same *Staphylococcus* sp. by MALDI-TOF. Further investigation, increasing the sample size and including members of the SIG will be performed.

§ AAVLD Staff Travel Awardee

Poster 07

Use of a Commercial Blocking ELISA Test Kit for Detecting Antibodies to Canine Influenza Virus

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Blocking ELISA test kits have been used to detect antibodies to the viral nucleoprotein (NP) of *influenza A viruses* in avian, equine, human, and porcine species. A commercial kit developed specifically for avian species has also been reported to successfully identify antibodies to viral NP in canine sera. Nucleoprotein of the influenza A viruses is thought to be highly conserved across virus subtypes affecting multiple species. Detection of antibodies to canine influenza virus (CIV) in canine sera was compared between Zoetis' Flu DETECT® BE, a test kit licensed for detecting antibodies to avian influenza virus, and hemagglutination inhibition (HI). Following the manufacturer's test kit instructions, each 96-well plate included three wells each for positive and negative controls. Duplicates of each canine serum sample were simultaneously evaluated on the same test plate. Samples originated from veterinary hospital, employee-owned dogs not vaccinated with a CIV vaccine and from research dogs involved in a CIV vaccine study. For the latter samples, testing was performed before and after vaccination and later after challenge with a heterologous CIV isolate. All research dogs were negative for CIV antibodies with HI prior to study entry. Sample optical density (OD) values were obtained and Sample/Negative (S/N) calculated as detailed within the test kit instructions. Samples with S/N ratio < 0.6 are positive and samples with S/N ≥ 0.6 are negative. To date, samples of veterinary hospital, employee-owned dogs not vaccinated for CIV are negative for CIV antibodies with HI and therefore sensitivity and specificity for Flu DETECT® BE cannot be calculated. Flu DETECT® BE yielded two positive results but S/N values are near the cut-off of 0.6. For the research dogs' serum samples (n=145), sensitivity is 57.5% and specificity is 96.9%. Flu DETECT® BE yielded false negative test results for this group of dogs compared to HI yet S/N values are also near 0.6 and HI CIV antibody titers are generally low. Flu DETECT® BE is unable to differentiate antibodies produced by the vaccine administered to the research dogs and those produced by experimental infection. Additional veterinary hospital, employee-owned dogs not vaccinated with a CIV vaccine will be tested to determine the ability of Flu DETECT® BE to identify dogs naturally infected with CIV.

Poster 08

Determination of Optimal *In vitro* Drug Ratios of Trimethoprim/Sulfamethoxazole and Trimethoprim/ Sulfadiazine Against Equine Pathogens ◊

Carmen Sadaka[123], Luca Guardabassi[3], Theofanis Kanellos[2], Michael T. Sweeney[1], Jeffrey L. Watts[1]

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Strangles is a highly contagious disease caused by *Streptococcus equi* that affects a horse's lymph nodes in the upper respiratory tract. Combinations of trimethoprim and sulfonamides are commonly used for the treatment of strangles in horses. Currently, the interpretive criterion used for antimicrobial susceptibility testing of trimethoprim/ sulfamethoxazole (TMP/SMX) with veterinary pathogens is based on human data. The purpose of this study was to develop MIC data for TMP/SMX that supports the establishment of veterinary-specific breakpoints against equine strains of *S. equi*. Data was generated using the common veterinary combination TMP/sulfadiazine (SDZ) to determine if TMP/SMX can be used as the class agent for susceptibility testing. *In vitro* susceptibility studies were conducted to evaluate activity of various TMP/SMX and TMP/SDZ drug ratios against 59 equine pathogens isolated from clinical cases of strangles. Minimal inhibitory concentration (MIC) broth microdilution assays and disk diffusion assays were conducted in compliance with CLSI methods. Fractional inhibitory concentration (FIC) assays using the checkerboard technique were also conducted to determine optimal drug ratios for synergy. Overall SMX, SDZ and TMP exhibited poor activity against 22 isolates (MIC>2048 μg/ml), 25 isolates (MIC>2048 μg/ml) and 3 isolates (MIC>4096 μg/ml), respectively. Additionally, individual sulfonamides and the combination antimicrobials exhibited poor activity in strains where TMP lacked activity. Despite evidence of poor *in vitro* activity to individual sulfonamides, FIC indices indicated that synergy (FIC index ≤ 0.5) occurred at TMP-to-sulfonamide combination ratios ranging from 1:1 to 1:256. These results imply that lack of activity of TMP may be conditional for the poor activity of the combination antimicrobials and that the last step of the pathway in which dihydrofolate reductase is inhibited by TMP may be the limiting step. More research will be conducted to validate these observations. Since the pharmacologic behavior of drugs may be different between species, the clinical utility of TMP/SMX and TMP/SDZ for equine strangles could be improved by performing pharmacokinetic and efficacy studies at the new proposed ratios: 1:40; 1:80; 1:160 and 1:256.

◊ USAHA Paper

Poster 09

Laboratory Diagnosis of *Theileria equi* Infection in Horses Imported into the Republic of Korea

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*Theileria equ*i (*T. equi*) is a tick-borne hemoprotozoan parasite that causes piroplasmosis in horses. The growth of international equine sports and horse trading has increased the possibility of introducing *T. equi*, which leads to a need in stringent serological screening of horses prior to entry into non-endemic areas, including the Republic of Korea. In this report, we describe our laboratory diagnosis cases of *T. equi* infection in horses imported into the Republic of Korea. Blood samples were collected from 1,127 imported equids undergoing post arrival quarantine from January, 2013 to April, 2014. Serological tests against *T. equi* were done by Competitive Enzyme-Linked ImmunoSorbent Assay (c-ELISA), Indirect Fluorescent Assay (IFA) and Complement Fixation Test (CFT). Detection of the agent is assessed by microscopy of blood smears and by the nested PCR assay for Equi Merozoite Antigen-1 (EMA-1) gene. Phylogenetic analysis was conducted with 18S rRNA and EMA-1 region. Of tested samples, three imported horses (US-42 from the USA, CH-2 and CH-10 from China) were shown to be positive by IFA (titer 1:640). The c-ELISA, specific to EMA-1, was positive in the Chinese horses even though the PI value of CH-10 was in borderline. CH-2 and CH-10 showed negative reaction in CFT. Although the level of EMA-1 specific antibodies of US-42 was negative in c-ELISA with a PI value of 38% (PI > 40% were considered positive), it showed positivity at dilution of 1:16 in CFT. The blood smear did not show any traces of parasites in RBCs. Amplified fragment of 218 bp was observed in the second PCR assay targeting EMA-1 gene. The phylogenetic analysis based on the 18S rRNA and EMA-1 gene showed that US-42 was closely related to the isolates from USA, Brazil and South Africa whereas CH-2 and CH-10 were closely related to the isolates from Korea, China and Mongolia. Therefore, it can be concluded that the infected horses were in the transitory phase, following acute infection by a *T. equi* isolate that was currently circulating in the exporting countries. Under the quarantine control, the pre-export period also assures this conclusion. The horses could have acted as reservoir, and contributed to the spread of *T. equi* during the quarantine period. However, the transmission mode and the seasonal disadvantage might have helped reducing in the risk of subsequent infection in the herd.

Poster 10

Sudden Death Due to Exercise-Related Hemoperitoneum Associated with Idiopathic Mesenteric Hemorrhages in 4 Horses

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Hemoperitoneum in horses can either be idiopathic or result from a variety of causes including splenic or hepatic rupture, rupture of the mesenteric arteries, rupture of the uterine artery during parturition, arteritis due to *Strongylus vulgaris* larval migration, vascular leakage from neoplasms or abscesses and coagulopathies. Although the true frequency of hemoperitoneum in horses is unknown, the condition has been reported rather infrequently in the scientific literature. The aim of this work is to describe unique pathological findings in 4 cases of hemoperitoneum associated with idiopathic mesenteric vessel hemorrhages in athlete horses. All 4 horses were male (3 geldings and a stallion), died suddenly during exercising, and were submitted to the CAHFS laboratory system in 2012-2013. Three cases occurred in racehorses, two Thoroughbreds 4 and 5 years of age, and one Standardbred 5 years of age, and one case occurred in a 14-year-old jumping Thoroughbred. Pathologic examination revealed moderate to severe hemoperitoneum associated with multifocal perivascular suffusive hemorrhages affecting multiple branches of the cranial mesenteric artery. The hemorrhages resulted in the formation of hematomas in the mesentery along and surrounding the affected vessels. Death in all cases was attributed to peracute hypovolemic shock. Major hepatic, splenic, and mesenteric vessel rupture, neoplasia, abscesses, trauma, and *Strongylus vulgaris* mesenteric arteritis were ruled out at necropsy, and *Salmonella* sp. infection was ruled out by PCR in all cases. Anticoagulant rodenticide screens for the detection of warfarin, diphacinone, chlorophacinone, coumachlor, brodifacoum, bromadiolone, and difethialone was negative in the liver in all 4 cases. Hepatic levels of lead, manganese, cadmium, copper, iron, zinc, molybdenum, arsenic, mercury, and selenium were all in acceptable concentrations, and no toxic organic compounds were detected in liver or urine by liquid- and gas- chromatography mass spectrometry in the only 2 horses analyzed by these techniques. Although a specific cause for the lesions has not been yet identified, the similarity in the clinical histories and consistency in the pathological findings in all 4 cases suggest a possible common etiology leading to increased vascular permeability concurrently in multiple mesenteric vessels. Bacterial endotoxemia (lipopolysaccharide) has been associated with similar but milder pathological findings in horses in both spontaneous and experimental cases, although the clinical picture of endotoxemia in horses is not typically characterized by hemoperitoneum and sudden death. We were unable to assess endotoxemia in these cases because of the lack of premortem blood needed for the analysis. Additional investigations are needed in order to better characterize this sporadic syndrome.

AAVLD Trainee Travel Awardee (Pathology)

Poster 11

Bovine Coronavirus Antisera Detects *Equine Coronavirus* in the Intestine of Equids with *Equine Coronavirus*-Associated Enteritis §

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Equine coronavirus (ECoV) has been associated with emerging outbreaks of pyrogenic, enteric and neurologic disease in horses. Based on pathologic examinations and specific detection of ECoV genome by qPCR, we recently confirmed 2 spontaneous cases of necrotizing enteritis associated with ECoV infection in a miniature horse and a miniature donkey. Because ECoV and bovine coronavirus (BCoV) both belong to the *Betacoronavirus* 1 species, we used antibodies (Ab) directed against BCoV by means of 2 validated immunological tests, a direct fluorescent Ab test (FAT) and an immunohistochemical test (IHC), to assess the presence of coronaviral antigen in the small intestine of these 2 cases. For the FAT, fresh jejunal tissue from the horse was frozen in optimum cutting medium compound (OCT, Sakura) for 24 hrs, cryosectioned at 8 μm, and fixed in cold acetone for 10 min. Bovine anti-BCoV polyclonal Ab (American Bioresearch Lab) directly labeled with fluorescein isothiocyanate was applied to the sections at a 1:40 dilution for 30 min at 37°C. Following a 15 min rinse in carbonate buffer pH 9.2, coverslips were applied and the slides evaluated using a fluorescence microscope. For the IHC, formalin-fixed small intestines from both ECoV-infected equids were routinely processed and embedded in paraffin. Formalin-fixed paraffin-embedded small intestine from a horse that was negative (on frozen samples) for ECoV and BCoV by validated PCR tests, was used as a negative control. Four μm sections were mounted on charged slides and dried overnight. After deparaffinizing, rehydrating, and a peroxidase inhibition step, the slides were placed in citrate buffer and heated to 121°C in a decloaking chamber for 10 min (antigen retrieval). Non-specific binding sites were blocked using a casein buffer. Primary Ab (mouse monoclonal IgG1 clone BC6-4, Research Technology Innovation) was applied at a 1:5000 dilution for 30 min. A horseradish peroxidase-labeled mouse polymer (30 min at room temperature) followed by AEC chromogen (10 min) was used to visualize antibody binding. FAT resulted in positive fluorescent signal in the intestinal villi of the horse. Coronaviral antigen was detected using the IHC in both infected equids but not in the negative control. The reaction was characterized by strong intralesional granular to globular cytoplasmic immunoreactivity in the superficial and crypt enterocytes. Fresh jejunal tissue from the horse tested negative for BCoV by a validated PCR, ruling out BCoV coinfection. Altogether, our results suggest that antibodies directed against BCoV cross-react with ECoV and that this cross-reactivity allows for the detection of ECoV by FAT and IHC in equids with ECoV-associated enteritis. Future studies are required to validate the sensitivity and specificity of these assays.

§ AAVLD Staff Travel Awardee

Poster 12

Necrotizing Enteritis and Hyperammonemic Encephalopathy Associated with *Equine Coronavirus* in Equids

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Equine coronavirus (ECoV) is a Betacoronavirus recently associated clinically and epidemiologically with emerging outbreaks of pyrogenic, enteric and/or neurologic disease in horses in the United States and Japan; however, information on the pathological findings associated with ECoV infection is scant in the scientific literature. In this work we summarize clinical, pathological, immunohistochemical, ultrastructural, and molecular findings in two American miniature horses (cases 1 and 2) and one miniature donkey (case 3) that succumbed to natural infection with ECoV. The animals were 6 months and 7 (donkey) and 12 years old and presented with a variety of clinical signs of acute onset including lethargy, fever, colic, anorexia, and/or neurologic deficits. The animals either died naturally or were euthanized because of lack of response to treatment and poor prognosis, and necropsies were performed. One horse and the donkey (cases 1 and 3) had severe diffuse necrotizing enteritis with marked villous attenuation, epithelial cell necrosis in the villous tips, neutrophilic and fibrinous extravasation into the small intestinal lumen (pseudomembrane formation), as well as crypt necrosis, microthrombosis, and hemorrhage. In case 2, hyperammonemia and encephalopathy with Alzheimer type II astrocytosis throughout the cerebral cortex were diagnosed. ECoV was detected by qPCR in small intestinal tissues, contents, and/or feces, and coronavirus antigen was detected by immunohistochemistry in the small intestine in all cases and by direct fluorescent antibody test in case 1. Coronavirus-like particles characterized by spherical, moderately electron lucent, enveloped virions with distinct peplomeres projecting from the surface were detected by negatively stained transmission electron microscopy in small intestine in case 1, and transmission electron microscopy of fixed small intestinal tissue from the same case revealed similar 85-100 nanometer intracytoplasmic particles located in vacuoles and free in the cytoplasm of unidentified (presumably epithelial) cells. Sequence comparison of a 435-nucleotide segment of the N gene of ECoV showed between 97.9 and 99.0% sequence identity with the NC99 and the Tokachi09 strains. Other enteric infectious agents including *Salmonella* sp., *Lawsonia intracellularis, Clostridium perfringens, Clostridium difficile, Neorickettsia risticii*, and *Rhodococcus equi* were ruled out by specific testing. Altogether, these results indicate that ECoV is associated with necrotizing enteritis and hyperammonemic encephalopathy in equids, although further investigation is needed to better understand the pathogenesis of ECoV infection.

Poster 13

Correlation of *Corynebacterium pseudotuberculosis* Synergystic Hemolysin Inhibition Serologic Test Results with *Rhodococcus equi* ELISA Results in Equine Samples

Ashley Hill, Michelle Davidson

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Corynebacterium pseudotuberculosis is a gram-positive rod-shaped soil-dwelling bacteria that is the etiologic agent of caseous lymphadenitis in sheep and goats (*C. pseudotuberculosis* biovar *ovis*), and of pigeon fever or dryland distemper in equids (*C. pseudotuberculosis* bv *equi*). *Rhodococcus equi*, which is also in the suborder Corynebacterineae, is also a gram-positive soil-dwelling bacterium that is an important cause of pyogranulamatous pneumonia in foals but is rarely pathogenic in adult horses or other animals despite its ubiquity in soil. Diagnosis of *C. pseudotuberculosis* infection can be made directly via bacterial culture of abscess material, or indirectly via serology. The synergystic hemolysin inhibition (SHI) test detects antibodies to exotoxin phospholipase D (PLD) produced by *C. pseudotuberculosis*. Briefly, in synergy with cholesterol oxidase and phospholipase C (both produced by *R. equi*), PLD causes β-hemolysis of erythrocytes cultured in blood agar. Presence of PLD antibodies is manifested as the absence of hemolysis. Although the SHI test includes antigens produced by both organisms, the potential for the *C. pseudotuberculosis* SHI test to cross-react with antibodies to *R. equi* has not previously been reported. Serum samples from 161 healthy horses were tested for antibodies to *C. pseudotuberculosis* using the SHI test and for antibodies to *R. equi* using an ELISA. SHI results were reported as titers, which were log-transformed prior to analysis. Antibodies to *R. equi* were reported as %inhibition values and analyzed in that form. The correlation between SHI results and *R. equi* antibody levels was evaluated using Kendall's tau rank correlation coefficient. Statistically significant correlation between *R. equi* ELISA values and *C. pseudotuberculosis* SHI titers in equine samples was identified. The magnitude of effect does not seem likely to substantially affect SHI test interpretation.

Poster 14

Bovine Coronavirus-Associated Hemorrhagic Colitis in Two Young Adult Beef Cattle # *

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Two, 13-month-old, Angus bulls presented for acute, voluminous, red, watery diarrhea. Both bulls were in excellent body condition, routinely vaccinated, dewormed, and had no history of prior health concerns. One of the bulls died in transit to the hospital and a necropsy was performed at the Oklahoma Animal Disease Diagnostic Laboratory. At necropsy, the contents of the descending colon were watery and dark red with blood clots. Segmentally, the distal colonic mucosa had a cobblestone appearance containing innumerable coalescing petechial and ecchymotic hemorrhages with few, small, elliptical, shallow mucosal ulcers. However, most of the colonic mucosa was within normal limits. Histologically, colonic sections exhibited multifocal loss of colonic glands with scattered remnant glands present in an uneven distribution. The remnant glandular epithelium was either hyperplastic or was exhibiting features of degeneration and necrosis with occasional sloughing of necrotic epithelial cells into the glandular lumens. Expansive sections of the surface mucosa were often re-epithelialized. The surviving bull exhibited frank blood in the descending colon with moderate anemia (PCV 19%), and fully recovered with symptomatic care. Ancillary testing (immunofluorescence, immunohistochemistry, virus isolation) confirmed a diagnosis of *bovine coronavirus* (BoCV) infection in the deceased bull. BoCV-associated, hemorrhagic colitis, or winter dysentery, is a rare cause of severe anemia and death in beef cattle. BoCV should be included as an etiological differential in young, adult beef cattle with acute hemorrhagic diarrhea, possibly resulting in death.

AAVLD Trainee Travel Awardee (Pathology, Virology)
* Graduate Student Poster Presentation Award Applicant

Poster 15

Tritrichomonas foetus Polymerase Chain Reaction: Pooling TF InPouch™ Samples from Bulls

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A real time PCR protocol was evaluated for detecting *Tritrichomonas foetus* in pooled prepucial samples derived from multiple bulls. *Tritrichomonas foetus* is a highly contagious, sexually transmitted protozoan known to cause infertility in cattle. Economic losses result from a reduced calf crop and lower overall weaning weights. *T. foetus* testing is mandatory in many states of the US. In Oklahoma, all bulls over one year of age must be tested any time they change ownership. Prepucial smegma collected in appropriate growth media is the sample of choice for *T. foetus* testing. TF InPouch™ is the most commonly used commercially available culture kit. PCR testing has largely replaced culture testing due to its increased turnaround time however; PCR testing of individual bulls can be cost prohibitive. To better serve the producer, testing of pooled samples from multiple animals has become routine practice in many diagnostic laboratories. Major advantages of pooling samples include lower costs for the producer and reduced need for reagents/labor in the laboratory. This option also incentivizes producers to screen more animals. In this study, two hundred and fifty samples of prepucial scrapings in TF InPouch™ were used. Theses samples were previously classified as positive or negative by individual real time PCR testing. Fifty groups containing one known positive sample pooled with 4 negative samples were tested to evaluate sensitivity and specificity of the "pooled" PCR. Results confirmed a sensitivity of 90% and a specificity of 90% in detecting *Tritrichomonas foetus* in the pooled samples.

Poster 16

Genotypic Testing for Antimicrobial Resistance Determinants Using Multiplex PCR Assays in *Mannheimia hemolytica* Isolated from Nebraska Cattle

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Bovine respiratory disease (BRD) is one of the most prevalent and costly diseases of cattle production in the United States. BRD is multi-factorial with both viral and bacterial etiologies. One of the most significant pathogens associated with BRD is the gram-negative bacterium, *Mannheimia hemolytica*. Multiple antimicrobial therapies are approved for the treatment and control of BRD associated with *Mannheimia hemolytica*, and these drugs are critical to reduce the impact of these pathogens in production systems. A retrospective analysis of phenotypic antimicrobial susceptibility data in *Mannheimia hemolytica* isolated from case submissions to the Nebraska Veterinary Diagnostic Center (VDC) from 2010-2013, indicate that the prevalence of resistant phenotypes using minimum inhibitory concentration broth microdilution assays appears to be increasing. Resistance of isolates to one or more antimicrobial classes approaches 40% of total isolates with some drug classes showing a 20% increase in resistant susceptibility patterns over the three year period. Antimicrobial resistance in pathogens can arise *de novo* from accumulated mutations or via exchange of genetic information from other organisms that harbor resistance genes. To better understand the genetic elements underlying resistant phenotypes and to further development of informative molecular diagnostic tests, multiplex PCR assays were utilized to determine if the presence or absence of resistance genes is correlated with resistance or susceptible phenotypes. Multiplex PCR enables rapid turnaround, can test for the presence of multiple genes in a single assay, and allows for sensitive and specific assay design. To evaluate the utility of this approach in a diagnostic laboratory setting, a large collection of archived *Mannheimia hemolytica* field isolates from case submissions to the Nebraska VDC were evaluated for the presence or absence of genetic antimicrobial resistance determinants for comparative evaluation with phenotypic susceptibility data. Genetic determinants that confer resistance to macrolides, beta-lactams, aminoglycosides, sulfonamides, and tetracyclines were included in multiplex PCR assays to enable high-throughput and rapid testing.

Poster 17

Correlation of Histologic Lesions with *Neospora caninum* Polymerase Chain Reaction (PCR) Findings in Naturally Aborted Bovine Fetuses: 2010-2013

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The parasitic agent *Neospora caninum* is considered an important cause of bovine fetal abortions and is routinely diagnosed in fetal tissues by the presence of characteristic histologic lesions and the identification of the organism by immunohistochemical or polymerase chain reaction (PCR) procedures performed on fetal tissues. In the present study, fetal heart and brain from 244 naturally aborted bovine fetuses obtained over a 4 year period from January 1, 2010 to December 31, 2013 were examined histologically. In addition, fresh heart and brain tissues were also submitted for *N. caninum* PCR procedures in an effort to correlate the presence of histologic lesions with PCR findings. In 199 out of the 210 PCR negative fetuses (94.8%), no histologic lesions were present. In the remaining 11 PCR negative fetuses, histologic lesions consisting of non-suppurative endocarditis, myocarditis, epicarditis and necrotizing encephalitis with gliosis were evident. In this group of 11 fetuses, the fetal abortion was considered due to specific bacterial or viral etiologies in 6 fetuses. The definitive cause of the fetal abortion in the remaining 5 fetuses could not be determined. In the 34 PCR positive fetuses, 23 of the fetuses (67.6%) did not display histologic lesions while 11 of the fetuses (32.4%) displayed histologic lesions similar to the lesions evident in the 11 PCR negative fetuses previously described. The lack of histologic lesions in this group is most likely due to the limited amount of tissue (5 μm thick sections) examined under the light microscope and the characteristic sporadic appearance of the organisms and lesions within the tissues. This study suggests that using only the identification of specific histologic lesions in fetal heart and brain as the sole diagnostic procedure without additional PCR testing can produce erroneous results in the diagnosis of fetal neosporosis.

Poster 18

Wohlfahrtiimonas chitiniclastica – A First Report of an Emerging Zoonotic Pathogen in the US *

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Wohlfahrtiimonas chitiniclastica is an emerging human pathogen that has been identified as the cause of septicemia in humans in Europe and South America. Septicemia due to *Wohlfahrtiimonas chitiniclastica* has been described in adult humans originating from suspected wound infections. Reported cases occurred in homeless patients with poor hygiene and multiple skin lesions. Although the pathogenesis of *W. chitiniclastica* associated disease has not been investigated, wound contamination was considered to be the origin of septicemia in these cases and the fly *Wohlfahrtia magnifica* is believed to play an important role as carrier of *W. chitiniclastica*. Here, we report the first case of a unique disease manifestation of *Wohlfahrtiimonas chitiniclastica* -induced bacterial septicemia secondary to wound myasis in a deer in Michigan, USA. A two-year-old, white-tailed deer was submitted to the Diagnostic Center for Population and Animal Health, Michigan, USA for necropsy with a history of severe drooling and a hard, swollen tongue with gray areas around the edges. On gross examination, the rostral tip of the tongue was gray, dry, friable and easily to be separated from the caudal portion of the tongue. The liver was dark red and swollen, and contained multifocal 1-2-mm white spots randomly distributed throughout the hepatic parenchyma. The spleen was diffusely enlarged and congested. Based on gross examination, bacterial septicemia was suspected as the cause of death of this animal. On microscopic examination, the tongue exhibited severe, locally-extensive, necrotizing inflammation with abundant intralesional Gram negative bacilli. The liver had multifocal, randomly distributed areas of hepatocellular coagulative necrosis. Fibrinoid necrosis of the blood vessel walls and multiple fibrin thrombi were present in tongue, liver, and choroid plexus of the brain. Bacterial isolation from the tongue and liver yielded numerous Gram-negative rod-shaped bacteria, which were initially identified as *Wohlfahrtiimonas chitiniclastica* by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Polymerase chain reaction (PCR) amplifications of the partial 16S rRNA gene and the partial DNA gyrase subunit B (gyrB) gene of *Wohlfahrtiimonas chitiniclastica* were performed. Nucleic acid sequencing from both primers sets confirmed the isolate as *Wohlfahrtiimonas chitiniclastica*. Based on the pathology, bacteriology and molecular results, a final diagnosis of *Wohlfahrtiimonas chitiniclastica* septicemia was made. In conclusion, this case highlights the importance of proper pathology and microbiology diagnostic testing on cases of naturally occurring death which allowed detection of an emerging pathogen, *Wohlfahrtiimonas chitiniclastica*, as cause of an arthropod-borne bacterial septicemia.

* Graduate Student Poster Presentation Award Applicant

Poster 19

Isolation and Identification of *Campylobacter fetus* subspecies *venerealis* from an Aborted Fetus and from Preputial Washes of Bulls

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Bovine genital campylobacteriosis (BGC) is an economically important disease that may affect international trade. BGC is a venereal disease caused by *Campylobacter fetus* subspecies *venerealis*. The disease is characterized by infertility, early embryonic death, and occasional abortion in cows. Infected bulls are usually clinically normal and are the reservoir for infection by natural mating or by artificial insemination using infected semen. Diagnosis of BGC remains a challenge. Traditional cultural methods followed by biochemical tests are not reliable for differentiation of *C. fetus* subspecies *fetus* from *C. fetus* subspecies *venerealis*. Published PCR methods may give false positive or false negative results. Here, we report the successful diagnosis of outbreaks caused by BGC in a bull stud farm and in a dairy farm, using a comprehensive approach combining biochemical reactions and PCR followed by pulsed-field gel electrophoresis. The diagnosis was confirmed by whole genome sequence analysis and by OIE reference laboratory.

Poster 20

Recombinant Baculovirus Expression and Immunoreactivity of *Schmallenberg Virus* Nucelocapsid Protein

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Schmallenberg virus (SBV) is an arthropod-transmitted virus that belongs to the genus Orthobunyavirus of the family *Bunyaviridae*. The virus infects ruminants notably cattle, sheep and goats and causes fetal malformation. SBV has been mostly reported in Europe but there is potential risk for introduction into the US. The genome of the virus consists of 3 segments of negative sense single-stranded RNA: small (S), medium (M), and large (L). The S segment encodes the nucleocapsid (N) protein, which is the most abundant protein that makes up the basic structure of the virus. The primary objective of this study is to express the N protein using the recombinant baculovirus expression system and assess its use as a diagnostic antigen. The coding sequence of the N protein was initially amplified by PCR and the PCR product was cloned into pFastBac, to construct a donor plasmid. The latter was used to create a recombinant bacmid carrying the target N protein sequence. The purified recombinant bacmid was transfected into *Spodoptera frugiperda*, Sf9, insect cells to recover recombinant baculoviruses. The recombinant viruses were used to express the SBV N protein, which was purified by affinity chromatography. Western blot analysis using anti-histidine monoclonal antibody detected the expected size of 29 kDa recombinant protein; and specific immunoreactivity with polyclonal bovine sera, and a monoclonal antibody raised against SBV N protein confirmed antigenicity of the expressed protein. These results suggest the potential suitability of the recombinant N protein as a serodiagnostic antigen for detection of SBV infection in susceptible hosts.

Poster 21

Evaluation of a Cutoff Change for the IDEXX *Mycobacterium paratuberculosis* Antibody Test Kit

Valerie Leathers

IDEXX, Westbrook, ME

Detection of *Mycobacterium paratuberculosis* (MAP) antibodies in bovine serum or milk can help dairy producers identify and remove MAP positive animals, reducing the spread and cost of Johne's disease. This study evaluated the performance of the IDEXX *Mycobacterium paratuberculosis* Antibody Test Kit (IDEXX Kit) with alternative lower test cutoffs in comparison to the performance of the kit with the original test cutoffs and the performance of another commercial MAP Ab kit. Sensitivity and specificity for each of the three approaches was calculated using fecal culture to classify MAP status for the animals. A total of 250 serum samples (from 113 positive and 137 negative animals, by fecal culture) and 133 milk samples (from 31 positive and 102 negative animals, by fecal culture) from dairy cows were evaluated. The IDEXX kit, using the alternative cut-off for milk samples, resulted in a sensitivity of 54.8%, whereas using the original cutoff the sensitivity was 48.4%. Serum sample results evaluated with the alternative cutoff had a sensitivity of 80.5%, while serum sensitivity with the original test cutoff was 72.6%. Specificity for serum and milk was 100% using both cutoffs. When another commercial MAP Ab test kit was evaluated, using the same populations of serum and milk samples, the sensitivity was 41.9% for milk and 76.1% for the serum sample set; specificity was 100% for both serum and milk. The alternative IDEXX *Mycobacterium paratuberculosis* Antibody Test Kit milk and serum test cut-offs increased sensitivity as compared to both the original IDEXX test cutoffs and the other commercial MAP Ab test. The alternative cutoffs had no effect on specificity for either milk or serum. Using the alternative cutoffs with the IDEXX MAP Ab Kit offers enhanced accuracy for identifying MAP antibody positive cows, so they can be removed from the herd, and offers dairy producers an improved tool in the fight against Johne's disease.

Poster 22

Sensitivity and Specificity Evaluation of the VetMAX™ MAP Real-Time PCR Reagents on Fecal Samples from USA Cattle

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Johne's disease is a chronic wasting disease of ruminants, caused by an incurable infection of the intestinal tract by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) which leads to a decrease in production of infected animals. Transmission is predominantly fecal-oral with infected animals shedding MAP into the environment increasing the risk of infection to healthy animals in the same herd. Therefore, the implementation of an accurate diagnostic test for herd management is fundamental to maintain a successful bovine health and production program. Back in 2011, field validation studies were performed in Netherlands to evaluate the VetMAX™ MAP Real-Time PCR Screening kit and a FLI registration was granted by the Animal Health Service registration number: Zul. Nr. FLI - B 566. Similarly, Thermo Fisher Scientific is now pursuing registration in the USDA by planning field validation studies that are required to obtain licensed kit. Thus, the VetMAX™ MAP Real-Time PCR Screening Kit was tested in an external laboratory that uses the Thermo Fisher Scientific MAP workflow in a routinely basis. The sample repository was able to provide a total of 535 bovine fecal samples from diverse origins from USA that were initially characterized by culture (n= 77) positives and (n=458) negatives. On the preliminary results, the Thermo Fisher Scientific MAP workflow was able to call 75 of the 77 MAP positive cultures as positives and the 2 remaining samples as suspects. On the 398 cultured negative samples tested with Thermo Fisher Scientific MAP workflow, 36 samples were classified as positives and 24 as suspects. DNA sequencing as a confirmatory test will be implemented to resolve the discrepant and suspect samples between the VetMAX™ MAP Real-Time PCR Screening Kit and culture. Sensitivity and specificity of the assay will be further discussed.

Poster 23

Vaccinal Prevention of Reproductive Disease Due to *Bovine Viral Diarrhea Virus* ◊

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Bovine viral diarrhea virus (BVDV) is a common cause of reproductive inefficiency and failure in cattle. Reproductive disease due to BVDV infection has been recognized since the virus was first reported and remains a major concern on dairy farms, cow-calf ranches and breeding stock operations. The reproductive sequelae of BVDV infection depends largely on the immune status of the dam and the stage of gestation at which infection occurs; possible adverse outcomes of infection include poor conception rates, early embryonic death, abortion, congenital malformations and the creation of persistently infected (PI) animals. Such animals are critical to the propagation of the virus within populations and as such, are the focus of most control programs. Vaccination against BVDV has been practiced for several decades but there has been a recently renewed focus on providing fetal protection through vaccination. Consequently, the aim of this study was to evaluate the efficacy of BVDV vaccination to prevent reproductive disease by performing a quantitative synthesis of previously published studies. Relevant articles were found by performing a search of four relevant scientific databases (PubMed, CAB abstracts, Agricola and Web of Science) using the search term "BVDV vaccine" and by examining the reference lists of 10 systematic reviews. Inclusion criteria for the meta-analysis mandated that the studies were controlled, primary studies that included necessary data for use in the meta-analysis, such as the number of pregnancies, abortions or fetal infection events in the treatment and control groups. Forty-seven studies in 42 separate manuscripts were identified that matched the inclusion criteria. One study was subsequently excluded from the analysis due to the inadvertent use of a vaccine containing a BVDV contaminant. Risk ratio effect sizes were used in random effects, weighted meta-analyses to assess the impact of BVDV vaccination on three outcomes: pregnancy risk, abortion risk, and risk of fetal infection. Within each outcome, sub-analyses were performed to evaluate the effect of modified live and inactivated or polyvalent and monovalent vaccines, homologous and heterologous or field challenge and vaccination using only bovine studies. The analysis demonstrated that the probability of fetal infection in vaccinated cattle is approximately one-seventh the risk in unvaccinated cattle exposed during gestation. Use of a polyvalent vaccine may further reduce the risk of fetal infection. The risk of abortion is reduced by more than 40% with vaccination (risk ratio = 0.597). Pregnancy risk was significantly improved in vaccinated animals (risk ratio = 1.05) subjected to field exposure relative to unvaccinated animals and was not adversely affected by vaccination when viral challenge was delayed until animals were gestating. This meta-analysis provides quantitative support for the benefit of vaccination in the prevention of BVDV-associated reproductive disease.

◊ USAHA Paper

Poster 24

A Genome-Wide Association Study for the Incidence of Persistent *Bovine Viral Diarrhea Virus* Infection in Cattle

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Bovine Viral Diarrhea Viruses (BVDV) comprises a diverse group of viruses that causes disease in cattle. BVDV may establish both, transient and persistent infections depending on the developmental stage of the animal at exposure. The objective was to determine if genomic regions harboring single nucleotide polymorphisms (SNP) could be associated with presence or absence of persistent BVDV infection. A genome-wide association approach based on 777,000 SNP markers was used. Samples of animals identified as positive (n= 1,200) or negative (n= 1,200) for the presence of BVDV in skin samples (n= 1,200), were used. DNA samples were combined in 24 pools (100 animals per pool). One SNP, significant at the 5% genome wide level (P = 9.41 x 10-8) was detected on chromosome 14, located at position 80,675,176 bases. Fifteen SNP, residing on chromosomes 1, 2, 6, 8, 10, 15, and 18, were moderately associated (P < 1 x 10-5) with persistent BVDV infection. These SNP reside within or in the vicinity of genes involved in several biological processes. Genes, in which the significant SNP, reside are known to be involved in events that moderate immune responses, signal transduction, RNA splicing, and DNA methylation. These processes may contribute to the animal's ability to survive the persistent infection. Genomic regions identified in the present study are necessary to understand the disease in cattle.

Poster 25

A New Level of Standardization in Real-Time PCR with the IDEXX RealPCR™ BVDV Assays

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Real-time PCR has revolutionized diagnostic testing over the past years. While real-time PCR is continuously developing, commercial assays often deliver a set of reagents designed for testing a precise number of samples for a specific target(s). IDEXX RealPCR real-time PCR assays provide a new level of standardization to PCR diagnostics by using reagents in a modular system. The components of this modular system are shared over the entire platform, making it possible to run any pathogen-specific detection mix with a standard master mix and a single positive control. Moreover, the IDEXX RealPCR modular system maintains a single cycling protocol for all assays and shared quality controls and guidelines across the entire platform. The RealPCR BVDV RNA assay has been evaluated using characterized samples and synthetic oligonucleotides. The assay has an analytical sensitivity of ≤ 15 copies / reaction for Type I, Type II and HoBi BVDV with efficiencies of > 95% over at least a 7-log range. The assay displays no cross-reactivity with many common bovine viral pathogens either by in silico analysis or with diagnostic specificity testing. In addition to individual whole blood, serum, plasma and ear notch samples, the assay detects BVDV in sample pools of up to 50 for blood fractions or 25 for ear notches.

Poster 26

Extended Genetic Characterisation of Novel Pestiviruses in the UK

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In the UK, there has been an increase in the genetic variability of BVDV-1 strains in the past two decades in addition to the introduction of a new genotype, BVDV-2, in cattle. There have also been cases of *border disease virus* (BDV) infections in both cattle and pigs. This study aimed to further the genetic characterisation of these novel or unusual ruminant pestiviruses. Classification of pestiviruses is most commonly performed through phylogenetic analysis of a portion of the 5' untranslated region (5'UTR), and on occasion is extended to include partial Npro and E2 regions. Sequence data for the three regions, 5'UTR, Npro and E2 was obtained for three BVDV-1 isolates, subgenotypes -1e, -1f and -1i, two BVDV-2 viruses isolated from cattle, a BDV isolate from cattle and a BDV isolate from pigs. Phylogenetic analyses demonstrated that all 7 isolates clustered with the reference strains of the different species. Bootstrap values were significantly higher in the phylogenetic trees for both the Npro and E2 regions compared to the 5UTR. The BDV strain isolated from a pig herd clustered with subgenotype BDV-1b strains. Sequence data for the E2 region of the BVDV-1 isolates previously typed as subgenotype 1e, 1f and 1i has been generated. It will be interesting to confirm if the typing presented here is preserved when further data from the E2 region of other isolates of these subgenotypes becomes available. This further highlights the need for a united approach to pestivirus classification in the future.

Poster 27

A Serologic Survey of *Bovine Leukosis Virus* (BLV), *Bovine Viral Diarrhea Virus* (BVDV) and Johne's in Oklahoma/Arkansas Beef Herds ◊

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The Oklahoma Animal Disease Diagnostic Laboratory (OADDL) initiated an ELISA panel in the fall of 2013 to test for three low-morbidity bovine diseases. The program was designed to provide producers with a financial incentive to screen their herds and provide data for culling or retention of heifers. The ELISA panel consisted of *Bovine Viral Diarrhea Virus* (BVDV- antigen capture ELISA, IDEXX), *Bovine Leukosis Virus* (BLV- ELISA antibody, VMRD), and Johne's disease (MAP- ELISA antibody, IDEXX). All 1,120 cattle sera tested in the program were from 26 beef herds in Central and Eastern Oklahoma and Western Arkansas. The testing showed the number of animals infected with BLV is higher than the average of 17.1% found in our region in a NAHMS study from 1999. Overall 39.1% of animals tested positive for BLV with individual herds ranging from 1.2% to 100% positive. Twenty-five percent of herds tested had at least one animal that was positive for Johne's, which is higher than the national estimated average of 8%; and 4.2% of the herds had at least one animal test positive for BVDV, which is slightly lower than the 8.8% found in a 2007-08 USDA study. The individual herd incidence varied, however, from 0%-1.7% for Johne's and 0%-7.1% for BVDV. It is important that producers, veterinarians, and diagnostic laboratories work together to identify animals with chronic disease in order to implement management practices that improve production and profitability. This data is invaluable for decisions on culling or retention/expansion, especially during the ongoing drought conditions, and provides current antibody and antigen detection information for the region.

◊ USAHA Paper

Poster 28

BVD Isolation from Multivalent Vaccines and Cross Neutralization Antibody Response to BVD Vaccine Viruses and Field Isolates

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Current commercially available vaccines against *Bovine Viral Diarrhea Virus* (BVD) contain strains that are 20 - 40 years old. The efficacy of these vaccines against contemporary field strains is unknown. Testing of these strains through serum neutralization can be used in order to assess the potential efficacy of these vaccines. Vaccine strains are largely proprietary and modified from their natural state. However, targeted isolation of a single BVD virus from a multivalent vaccine using an "antiserum cocktail" containing antibodies against PI3, BHV-1, BRSV, and type-specific BVD can be used to isolate these viruses from the commercially available vaccines. The isolated vaccine virus can then be serially passaged and sequenced by 5' UTR sequencing to confirm identity and purity. In order to investigate the serotypic relationship of the vaccine viruses, BVD-negative calves were vaccinated with eight BVD strains isolated from four commercially available vaccines. Monospecific antisera against the 8 viruses was collected four weeks post inoculation. The calves were then vaccinated with the complementary BVD strain in the vaccine to generate multivalent antibodies, representative of those generated by vaccination in the field. The sera generated from this model were then used in neutralization testing against an array of contemporary BVD viruses representing genotypes 1a, 1b, 2a and 2b. The data generated from these assays allows quantification of the potential efficacy of vaccines and possibly the need for additions or modifications to improve protection against BVD.

Poster 29

A Retrospective Analysis of *Mycoplasma bovis* Lung Infection in Dairy and Beef Cattle with Pneumonia

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Prevalence of *Mycoplasma bovis* lung infection among cattle with pneumonia was evaluated using immunohistochemistry (IHC). Lung sections archived from 1999 to 2013 from cattle diagnosed with pneumonia were tested using a *M. bovis* specific monoclonal antibody. The samples which were IHC positive were graded as mild, moderate, and heavy based on the intensity of reaction. The culture results were analyzed for bacterial and viral coinfections. Out of 378 samples examined 235 samples (62.1%) were positive for *M. bovis* IHC. Out of 235 positive samples 42.5 % (100) had mild reactivity, 31.9% (75) had moderate reactivity and, 24% (58) had heavy reactivity. Positive staining was noted in large airway epithelium, submucosal glandular epithelium and within necrotic debris. Incidence of co-infections with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Trueperalla pyogenes* was detected more than viral infections such as BHV1, BHV 4, BVD, PI3, and BRSV. Both beef and dairy adults had lower mycoplasma prevalence (43.75%) compared to young animals (67%). Beef cattle had a higher prevalence (74%) of *Mycoplasma* infection compared to dairy cattle (60%). This study indicates that *Mycoplasma* lung infections are prevalent among cattle with pneumonia and may predispose animals to co-infection with other bacterial of viral pathogens.

Poster 30

Osteoarthritis in a Bovine Abortion Associated with *Ureaplasma diversum*

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A third trimester Holstein fetus was submitted to California Animal Health and Food Safety (CAHFS) at University of California, Davis from a 450 cow dairy that had 4 recent mid to late term abortions. The submitted fetus was in fully haired with erupted teeth, consistent with late gestation but was proportionally small with a crown to rump length of 58 cm and weighing 8.1 kg, consistent with early third trimester development. These findings were suggestive of in utero growth impairment. A severe bilateral coxofemoral arthritis with thickened, eroded articular surfaces was identified on necropsy. Routine ancillary diagnostic tests performed to identify an infectious cause were unremarkable other than elevated serum immunoglobulin levels. Histological examination of multiple tissues detected lymphoid depletion in lymph nodes, thymus and spleen; and mild lymphoplasmacytic conjunctivitis. The coxofemoral joints had multifocal articular cartilage erosion exposing the subjacent trabecular bone with surface fibrinonecrotic and neutrophilic exudate. Chronic fibrosing lymphoplasmacytic synovitis was present. Frozen samples of synovium were sent to an accredited laboratory for *Ureaplasma diversum* PCR testing and were positive. According with the literature, *U. diversum* abortions in cattle usually occur in the third trimester. Typically, there are placental lesions characterized by thickening of intercotyledonary areas with fibrosis and occasional fibrinous exudate, hemorrhage and mineralization. The amnion is frequently the most affected portion. Retained placentas are common (placental membranes were not submitted in this case). Fetuses are often grossly unremarkable. Microscopic lesions typically include non-suppurative placentitis, lymphocytic pneumonia and diffuse alveolitis. The recent literature describes cases of *U. diversum* cattle abortions with joint lesions as the primary finding. This case and the recent literature supports the practice of more thorough examination of fetal joints for lesions consistent for *Ureaplasma* arthritis. Synovial cultures or PCR testing on articular soft tissues could be confirmatory. *U. diversum* is slow growing with special culture media requirements. Interestingly, *U. diversum* abortion in cattle is infrequently reported in the United States, although in Ontario, Canada, a substantial portion of abortions have been attributed to this infection. A recent retrospective survey of bovine abortion submission to CAHFS from 2007-2012 found that 8.2% (110/1344) of cases had lesions suggestive of an infectious cause but no agent could be demonstrated with routine diagnostic procedures.

Poster 31

Hoof Sloughing in Beef Cattle Following Transportation: A Preliminary Clinical Report

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Fifteen animals out of a shipment of approximately 200 animals sloughed one or more hoof walls while in lairage at a slaughter establishment and were euthanized. Animals displayed a spectrum of clinical signs including increased respiration rate, abdominal breathing, lameness, and reluctance to move. All animals in the group were fed high-concentrate finishing rations and were administered zilpaterol hydrochloride according to label directions. Two of the affected animals were necropsied by slaughter facility personnel. Samples were collected and mailed to the Kansas State Veterinary Diagnostic Laboratory. The most significant gross pathological lesion observed was sloughed hoof horn with severe necrosis and hemorrhage in the underlying laminar dermis of the affected hooves. Histopathologically, there was diffuse necrosis of primary epidermal laminae. The underlying dermal corium was also necrotic and contained degenerate collagen admixed with cellular and karyorrhectic debris. Distal limbs without sloughed horn were also submitted and a varying degree of necrosis and hemorrhage was observed in these on cross-section. The serum lactate and creatine phosphokinase levels were elevated. No bacterial or viral pathogens were detected. Other potential causes of hoof sloughing such as selenium or ergot alkaloid toxicity, or traumatic causes were ruled out during diagnostic investigation. Apart from the hoof loss, the observed clinical signs and biochemical anomalies are similar to a condition known as fatigued pig syndrome. A definitive causative agent was not identified in these cases and it is mostly a multifactorial and rare condition with a complex etiology. The loss of hoof walls and exposure of P3 as described herein has a profound impact on the welfare of cattle and more research needs to be performed to better understand any metabolic, biomechanical, and pathophysiological changes that could have lead to this rare event of hoof sloughing

Poster 32

Evaluation of MALDI-TOF as a Tool for Speciation of *Mycoplasmas* in Selective Broth Culture

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Identification of *Mycoplasma* sp. at the genus level is easily accomplished at the bench, while species level identification requires specialized methods such as PCR and fluorescent antibody (FA) tests. As bacterial identification by MALDI-TOF mass spectrometry is largely replacing standard identification methods for many common pathogens, we chose to investigate the accuracy of MALDI-TOF identification of *Mycoplasma* species from selective mycoplasma broth media. A previous study indicated that MALDI-TOF showed great promise for species and even sub-species identification, but this relied on a custom database. Our study's MALDI-TOF identifications are based on the standard Bruker database so that the results would apply to all labs with the Biotyper system. We included clinical isolates received in the Indiana Animal Disease Diagnostic Laboratory from multiple species of origin. All isolates were tested by sequencing, FA and MALDI-TOF. Our laboratory developed a modified tube extraction method for preparation of the cultures, and yielded scores ranging from 1.70 (the acceptable species identification cut-off based on the previous study) to over 2.0 (the acceptable species identification cut-off according to Bruker). We successfully identified isolates at the species level from the following species of origin: canine, avian, bovine and ovine. The only limitation encountered was the database itself, indicating that *Mycoplasma* sp. identifications on the MALDI are reliable, and a "no reliable identification" result is most likely due to absence of the appropriate species in the database. Laboratories interested in using MALDI-TOF for species-level identification of *Mycoplasma* sp. will need to expand their database with custom entries until the Bruker database is updated to meet these needs.

Poster 33

The Importance of Continued Prion Research: An Enigmatic Case of Chronic Wasting Disease in a Captive Red Deer (*Cervus elaphus*)

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A 22-month-old, female red deer (*Cervus elaphus*) was found positive for the Chronic Wasting Disease (CWD) abnormal prion protein in the obex and the retropharyngeal lymph node (RPLN) by immunohistochemical staining. Microscopic lesions of spongiform encephalopathy and immunohistochemical staining patterns and intensity were similar to those in CWD-positive elk and experimentally infected red deer. Western blot and enzyme-linked immunosorbent assay were positive for a TSE consistent with CWD. The red deer was in good body condition and the only clinical sign observed was recumbency the day of death. The red deer did not appear to demonstrate advanced clinical signs of CWD infection despite severe CWD prion accumulation within the brain and RPLN and widespread distribution throughout multiple other tissues. An epidemiological investigation was completed on the captive herd from which this red deer originated with no obvious biosecurity deficiencies revealed. Approximately 70% of the herd, including this red deer's life-long enclosure cohorts, has tested negative for CWD prion infection via immunohistochemical staining methods on obex and RPLN. Certain aspects of this case are enigmatic including the young age of this red deer in correlation with the severe and widespread CWD prion accumulation, the fact that none of the lifelong cohorts were infected, and the source of the infection. CWD and other prion diseases are not completely understood. Spontaneous prion disease has been described in bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep, and creutzfeldt-jakob disease (CJD) in humans. The methods for spontaneous classification in BSE, scrapie, and CJD have not produced evidence of spontaneous CWD. Continued research in CWD prion disease is warranted for sound protection and management of captive herds, free-ranging herds, and human health.

Poster 34

Equine Herpesvirus-9 (EHV-9) Meningoencephalitis in a Thomson's Gazelle

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EHV-9, originally designated *gazelle herpesvirus-1*, is the newest member of the group of equine herpesviruses and is genetically closely related to EHV-1. Naturally occurring EHV-9 infection has been reported to cause lethal encephalitis in Thomson's gazelles, a polar bear and a giraffe. Experimental infection suggests that EHV-9 has a wide host range, including hamsters, mice, horses and goats. A 4-year-old female Thomson's Gazelle at the Baton Rouge Zoo was found acutely lethargic with head-shaking and seizures. Cytology of cerebrospinal fluid revealed mononuclear pleocytosis, and granulomatous meningoencephalitis was suspected. A week after the onset of clinical signs she was found dead in her habitat and was submitted for postmortem examination. At necropsy, no gross abnormalities were noted except for meningeal hyperemia. Microscopic evaluation demonstrated multifocal nonsuppurative meningoencephalitis characterized by moderate perivascular and leptomeningeal lymphohistiocytic infiltration, neuronal necrosis, satellitosis, gliosis and microglial nodules. Eosinophilic intranuclear inclusion bodies were rarely seen in the necrotic neurons. Polymerase chain reaction demonstrated the presence of a herpesvirus, which DNA sequencing determined to be EHV-9. Zebra and other equine species are thought to be asymptomatic natural hosts/reservoirs for EHV-9. The present gazelle had shared the habitat with zebras. Transmission of the virus from these zebras was therefore suspected; however, tissues of three zebras submitted for necropsy from the same location did not contain EHV-9. Nevertheless, this case is a reminder that the arrangement and biosecurity measures of equine and non-equine exhibits in zoos and nature parks should be carefully planned to prevent EHV-9 transmission between species.

AAVLD Trainee Travel Awardee (Pathology)

Poster 35

New Directions on *Caprine Arthritis Encephalitis Virus*/Ovine Progressive Pneumonia Virus Serology

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Caprine Arthritis Encephalitis virus (CAEV) and *Maedi-Visna virus*/Ovine Progressive Pneumonia virus (MVV/OPPV) are genetically and antigenically closely related. These viruses are transmitted from infected adults to younger animals via respiratory secretions, and to a lesser extent, from dam to kids/lambs via colostrum and milk. Historically the control strategies for these diseases have centered on serologically testing animals followed by culling or by segregating positive animals and orphaning newborns. Among tests available to identify positive animals, ELISA and AGID are the most commonly used serological tests. Various commercial ELISA test kits are available for the detection of antibodies to CAEV/MVV/OPPV. Three separate sheep flocks have experienced cross-reactivity to cELISA and AGID possibly due to recent use of chlamydia bacterin for abortion control. This presentation describes the use of "Elitest" ELISA for detection of antibodies to CAEV/MVV/OPPV. This test was used to confirm and eliminate this cross-reactivity and is now being used in an OPPV Eradication Trial in collaboration with the USDA, Minnesota (MN) Board of Animal Health, MN Lamb and Wool Producers and the MN Veterinary Diagnostic Laboratory and College of Veterinary Medicine.

Poster 36

A Multiplexed Fluorometric Immunoassay (MFIA) for the Detection of Antibodies to *Actinobacillus pleuropneumoniae* 1-9-11, 2, 3-6-8-15, 4-7, 5, 10 and 12

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Actinobacillus pleuropneumoniae (APP) remains an important swine respiratory pathogen in many countries worldwide. Fifteen APP serotypes corresponding to 9 serogroups have been identified so far (APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, APP12, APP13, and APP14). The surveillance of swine herds for APP mostly relies on the detection of serotype or serogroup specific antibodies. Various serological assays have been developed for that purpose. The most sensitive and specific are indirect ELISA using highly purified long chain lipopolysaccharides (LC-LPS) as antigen. Presently, in order to detect antibodies to the 9 serogroups, as many ELISA have to be performed. Testing for several serogroups at the same time in a single assay (multiplex testing) could save a lot of labor, time, and cost. Multiplexed Fluorometric Immunoassay (MFIA) is a method recently introduced in veterinary medicine. MFIA is using suspensions of microspheres (beads) with unique internal fluorescent dyes. The beads are coupled to their surface with unique antigens. Bead sets and samples are added to microtitre plates. Antigen-antibody complexes formed during incubation are then detected through successive incubations with biotinylated anti-immunoglobulins (Ig) followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubations are followed by wash steps to remove unbound sample constituents and reagents. MFIA plates are read and analyzed using a microtiter plate fluorescence analyser. The intensity of the R-phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI) which denotes how much reporter fluorescence of a given microsphere set carries. An S/P ratio of the SA-PE on the antigen-coated microspheres above provided threshold indicates that antibodies to the corresponding antigen are present in the sample. We have developed a 7-plex MFIA using magnetic beads (Magplex®, Luminex) to detect antibodies to APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, and APP12 LC-LPS in swine serum samples. Preliminary results suggest that the assay sensitivity and specificity are between 95 and 100% of those of the corresponding serogroup specific LC-LPS ELISAs.

Poster 37

Association Between O Serogroups and Pathotypes of *Escherichia coli* Encoding F18 Subtypes Isolated from Diarrhoeic and Edema Disease piglets in Korea

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The aims of this study were to compare the associations between O serogroup and pathotypes of pathogenic *E. coli* encoding F18 fimbrial subtypes and to see if the STEC O121: F18new variant has a potential to vaccine candidate for pigs. From 2008 to 2013, two hundred F18 positive *E. coli* (northern (n = 59), middle (n = 59) and southern area (n = 82)) were isolated from postweaning diarrhea (PWD) and edema disease (ED) piglets from 180 farms throughout South Korea. All bacteria were cultured on sheep blood agar (Asan, Korea) for 18 h at 37 °C and hemolysis was determined. Bacterial DNA was extracted and tested for genes for adhesins (F4, F5, F6, F18, F41, Paa, AIDA and EAE) and toxins (LT, STa and STb, EAST1 and STX2e). The determination of O antigens was carried out and F18 subtypes were determined by real-time PCR described previously. Briefly, real-time PCR was performed using a Rotor-gene 3000 (Corbette Research) with reaction volumes of 20 μL, containing 2 μL template, 2x SensiMastermix (TAKARA, Japan), 1 μM each primer and 0.5 μM each probe. After 95 °C for 1 min, there were 40 cycles of 95 °C for 20 s and 60 °C for 25 s. The isolate showing the value of Ct < 40 was determined as positive for each variant. Two hundred F18-positive *Escherichia coli* were isolated from PWD and ED pigs throughout South Korea. The serogoup of O139 and O121 were associated with F18ab (39/43) and F18new variant (12/20), respectively. However, F18ac positive isolates belonged to various serogroups such as O157 (8, 5.8%), O14 (7, 5.1%), O35 (6, 4.4%), and O182 (5, 3.6%). Most virotypes encoding F18 subtype were F18ab:AIDA:STX2e (18/43), F18new variant:STX2e:EAST1 (7/20) and F18ac:STa (8.0%, 11/137). F18ab subtype was highly associated with AIDA (79.1%, $p < 0.001$) rather than Paa (18.6%). However, F18new variant and F18ac were associated with hemolysis ($P < 0.01$). The results showed that F18 subtyping is helpful towards understanding vaccine development and diagnosis of PWD and ED in pigs.

Poster 38

A Multiplexed Fluorometric Immunoassay for the detection of antibodies to *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV), *Swine Influenza Virus* (SIV), and *Porcine Circovirus* (PCV2)

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV), and *Porcine Circovirus* (PCV2) are three major swine pathogens. Detection of antibodies to these viruses is performed routinely for monitoring herd status or optimizing vaccination protocols. Testing for the 3 viruses at the same time in a single assay (multiplex testing) could potentially save a lot of labor, time, and cost compared to traditional methods. Multiplexed Fluorometric Immunoassay (MFIA) is a method recently introduced in veterinary medicine. MFIA is using suspensions of microspheres (beads) with unique internal fluorescent dyes. The beads are coupled to their surface with unique antigens. Bead sets and samples are added to microtitre plates. Antigen-antibody complexes formed during incubation are then detected through successive incubations with biotinylated anti-immunoglobulins (Ig) followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubations are followed by wash steps to remove unbound sample constituents and reagents. Internal controls consisting in Ig bead set and anti-species Ig bead may be incorporated into the assay to evaluate sample suitability and assay function respectively. MFIA plates are read and analyzed using a microtiter plate fluorescence analyser. The intensity of the R-phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI) which denotes how much reporter fluorescence of a given microsphere set carries. An S/P ratio of the SA-PE on the antigen-coated microspheres above provided threshold indicates that antibodies to the corresponding antigen are present in the sample. We have developed a 4-plex MFIA using magnetic beads (Magplex®, Luminex) to detect antibodies to PRRSV type 1, PRRSV type 2, SIV and PCV2 antibodies in serum samples. Detailed results will be presented at the meeting.

Poster 39

Development of an Indirect Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Antibodies Against *Porcine Epidemic Diarrhea Virus* (PEDV)

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Porcine epidemic diarrhea virus (PEDV) is a Coronavirus infecting swine and causing acute diarrhea in all age groups. PEDV was first identified in Western Europe in the 1970s and then spread to other European countries and Asia. In the Americas PEDV was identified in the USA in May 2013 and has since been confirmed in at least 30 states, causing high mortality in young piglets. Moreover PED outbreaks have also been reported recently in Mexico, Canada, Dominican Republic, Peru and Colombia. PCR assays are now available to detect PEDV in intestinal tissues, feces, oral fluids and environmental samples. While numerous PCR assays have been deployed, very few serological assays have been developed and validated. Well-validated, high-throughput serological assays to detect PEDV antibodies in serum or oral fluid samples would provide additional valuable diagnostic tools for controlling the virus. Therefore, we developed an enzyme-linked immunosorbent assay (ELISA) based on recombinant expression of a full length PEDV nucleoprotein (NP) for the detection of PEDV antibodies in swine serum samples. The NP gene was cloned and expressed as a 51kDa, 6x His tag protein which reacted to PEDV positive sera and a 6x His-specific monoclonal antibody via immunoblotting. The test was evaluated for sensitivity and specificity for the serodiagnosis of PEDV antibodies in serum samples of known status. Known PEDV negative sample sets included samples from selected high biosecurity herds with no history of PEDV and archived serum samples collected prior to the emergence of PEDV in the US and Canada. In addition, samples testing positive for the related *swine coronaviruses*, TGEV and PRCV were evaluated to confirm PEDV ELISA specificity. Known positive samples were collected from pigs that were naturally infected at least 3 weeks prior to collection. A receiver operating characteristic (ROC) curve analysis of the ELISA results showed an estimate of both sensitivity and specificity of over 95%. These results indicated that the purified NP is a useful antigen for the serodiagnosis of PEDV and that the ELISA is a sensitive and specific test for detecting antibodies to PEDV. This assay will be useful for monitoring and controlling the spread of the disease and in seroprevalence studies.

Poster 40

Development and Diagnostic Application of Monoclonal Antibodies Against *Porcine Epidemic Diarrhea Virus* (PEDV)

Aaron Singrey, Steve Lawson, Faten Okda, Xiaodong Liu, Travis Clement, Julie Nelson, Jane Christopher-Hennings, Eric A. Nelson

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Porcine epidemic diarrhea virus (PEDV) was first described in Europe with more recent and severe outbreaks in Asia. PEDV was first documented in the U.S. in May 2013 and has since been confirmed in at least 30 states and additional countries. PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. Due to the recent emergence of PEDV in North America, availability of specific monoclonal antibodies (mAbs) is limited. Therefore, the purpose of this study was to develop high quality, readily available reagents for detection of PEDV antigen in research and diagnostic applications. Ten hybridoma clones producing mAbs against the PEDV nucleoprotein and two producing spike-specific mAbs were isolated. Most mAbs were of the IgG1 isotype though several IgM mAbs were also produced. Further characterization of epitope specificity was performed using immunoprecipitation and immunoblotting methods. The SD6-29 and SD17-103 mAbs recognized native PEDV nucleoprotein on Western blots while the SD67-41 and SDH37-11 mAbs recognized native PEDV spike protein on Western blots. Fluorescein conjugated mAbs were prepared for direct fluorescence applications including verification of virus isolation attempts, virus titrations and the fluorescent foci neutralization (FFN) assay. The FFN assay allows relatively rapid determination of neutralizing antibody levels in swine serum and milk samples. Virus neutralization titers of PEDV naïve pigs, as determined by the FFN assay, were <1:20 while most previously infected animals demonstrated titers of 1:80 to 1:1280 by 3 weeks post-exposure. The mAbs and related reagents produced in this project should be of substantial value in the detection of PEDV antigen in a variety of applications including: early verification of virus isolation and in virus titrations; immunohistochemistry staining of fixed tissues and fluorescent antibody staining of fresh tissues; development of field-based antigen capture assays such as lateral flow devices; and ELISA applications (competitive ELISA and antigen capture). They are also a key component of the FFN assays for assessment of neutralizing antibodies produced following PEDV exposure. Evaluation of neutralizing antibody responses may provide insight into protective immunity. The FFN assay is currently being used as a tool in efforts to understand duration of immunity and identify the most effective feedback and management strategies.

Poster 41

Development of a Fluorescent Microsphere Immunoassay (FMIA) and a Blocking ELISA for Detection of Antibodies Against *Porcine Epidemic Diarrhea Virus* (PEDV)

Steve Lawson, Faten Okda, Xiaodong Liu, Aaron Singrey, Travis Clement, Jane Christopher-Hennings, Eric A. Nelson

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PEDV is an enveloped, single-stranded, positive-sense RNA virus infecting swine and is a member of the *Coronaviridae* family. PEDV was first identified in the U.S. in May 2013 and since has been confirmed in at least 30 states and additional countries. PCR assays provide an important tool in control of the virus; however, well-validated, high-throughput serological assays would provide a valuable additional diagnostic tool for the swine industry. In response to these needs, we developed a fluorescent microsphere immunoassay (FMIA) and a blocking, enzyme-linked immunosorbent assay (bELISA) for the detection of antibodies against PEDV. Both tests use expressed, full length PEDV nucleoprotein (NP) as an antigen. For the FMIA, a two-step carbodiimide coupling procedure was used to couple PEDV-NP protein to Luminex™ microspheres. For the bELISA, monoclonal antibodies against the PEDV-NP (SD6-29 & SD17-103) were biotinylated and served as the competitive, detection moiety. The tests were optimized in a checkerboard fashion to maximize signal-to-noise ratios using serum of known serostatus. Known PEDV negative sample sets included samples from selected high biosecurity herds with no history of PEDV and archived serum samples collected prior to the emergence of PEDV in the U.S. Next, multiple comparison analysis was performed to assess sensitivity, specificity and testing agreement against previously developed indirect ELISA and IFA assays. Receiver operating characteristic analysis (ROC) was performed using swine serum samples, n=1420 for FMIA and n=1186 for bELISA. Results of ROC analysis for FMIA showed estimated sensitivity and specificity of 98.2% and 99.2%, respectively. The bELISA showed a sensitivity and specificity of 98.2% and 98.9%, respectively. Inter-rater (kappa) agreement, a statistical measure of test agreement, was calculated to be 0.932 between FMIA and IFA, 0.945 between bELISA and IFA and 0.941 between FMIA and bELISA. Similar comparative kappa values were observed between the FMIA, bELISA and the indirect ELISA. This demonstrated a significant level of agreement between all tests. Additionally, none of the known positive TGEV or PRCV samples tested (n= >50) was shown to cross-react on either test. These high throughput assays should be of value in controlling the spread of PEDV and in seroprevalence studies. Ongoing studies include adapting the assays to oral fluid and milk testing.

Poster 42

Diagnostic Performance of a Commercial PEDV Indirect Fluorescent Antibody Test in 12-Well Glass Slide Platform

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Since *porcine epidemic diarrhea virus* (PEDV) was detected for the first time in US swine in April 2013, it has caused significant economic impact to the US swine industry, estimating loss of 5 to 7 million piglets within a year. A variety of intervention strategies, such as 'feedback', strict biosecurity and extensive disinfection of farm facilities and vehicle, as well as disease surveillance, have been utilized to minimize the impact and further spreading of PEDV. Despite of such effort, PED has been identified in 30 states as of May 2014. One of the tools commonly used for disease surveillance is serology. A commercial antibody assay has not been made available in the US although a limited number of veterinary diagnostic laboratories including Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) have provided serologic testing for PEDV using in-house antibody assays to practicing veterinarians. Recently, Veterinary Medical Research and Development, Inc. (VMRD) has introduced a commercial indirect fluorescent antibody (IFA) assay in 12-well format on a slide platform for PEDV (Catalog #: SLD-IFA-PEDV) which can be used to assess serologic status of pigs for PEDV exposure. The following study was conducted to evaluate the diagnostic performance of this new test. A set of experimental and field serum samples previously tested by in-house IFA, serum neutralization assay and ELISA were used in the assessment. All those serum samples were randomized and tested in a blind fashion on both VMRD IFA slides and ISUVDL IFA plates (96-well platform) after 1:40 dilution or serial 2-fold dilutions. At the time of submitting this abstract, IFA testing were completed on a total of 60 serum samples (40 sera from experimentally infected pigs with varying antibody titers, 5 from PEDV naïve experimental pigs at various ages, 15 field serum samples from sows with various PED status). Some of PEDV antibody negative field serum samples were positive for either TGEV or PRCV antibody on a commercial TGE/PRCV differential ELISA. At 1:40 dilution, both VMRD IFA and ISUVDL IFA tests resulted in 100% concordance without false positive or false negative results. Positive samples produced good cytoplasmic staining on individually virus-infected cells. Background staining on VMRD IFA was minimal if not absent. At the time and with the samples tested, VMRD IFA slides appeared to be a convenient antibody test reagent for PEDV with acceptable performance, which could be useful in laboratories where high-throughput testing is not required or it has received a relatively small number of swine submissions for testing.

Poster 43

Effect of Pretreatment on Detection of PRRSV in Swine Oral Fluid by qRT-PCR

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Collection and testing of oral fluid samples for disease surveillance is increasing in swine. However, it has been recognized that the oral fluid matrix presents a challenge to PCR detection, most often in the form of false negative results (1). The objective of this study was to evaluate the effect of specific sample treatment or processing factors on PRRSV qRT-PCR performance. A total volume of 2.2 liters of oral fluid was collected from a group of ~250 5-week-old pigs that had been vaccinated 15 days prior with a modified-live PRRSV vaccine (Ingelvac PRRS® MLV, Boehringer Ingelheim Vetmedica). In the laboratory, the samples were pooled, stirred continuously while aliquoted into 25 ml volumes, stored at -80°C. The following treatment/processing factors were evaluated in the study: thaw temperature - samples were thawed at 4°C or 25°C for 24 hours; diluent - samples were diluted 1:2 with either Trizol® or nuclease-free water (total volume = 1.5 ml); sonication - samples were either sonicated in a water bath sonicator for 10 minutes or not sonicated; temperature - samples were sonicated or, if not sonicated, held for 10 min at either 4°C or 25°C; hold temperature - temperature at which the sample was held (4°C vs 25°C) until PRRSV RT-PCR assay was performed. Temperature was monitored throughout the process using NIST-certified thermometers that recorded the maximum and minimum temperatures. Samples were tested at the Iowa State University Veterinary Diagnostic Laboratory. Viral RNA was extracted using the Ambion® MagMAX™ Viral RNA kit. PCR was performed using the Tetracore EZ-PRRSV™ MPX 4.0 RT-PCR. The experiment allowed for direct comparisons of the factors listed above. Overall, there were 32 treatments with 4 samples each, along with 1 negative control per treatment. The process was replicated 5 times, producing a total of 800 PRRSV RT-PCR results including both known positive and negative samples. Testing of 640 known positive samples after treatments produced 85 false negatives (86.7% sensitivity). Testing of 160 negative controls produced 1 false positive (99.4% specificity). Binary test outcomes were analyzed using logistic regression with SAS® Version 9.3. Significant factors were thaw temperature ($p < 0.0001$), and the interaction between diluent and sonication temperature ($p = 0.04$). This study identified that specific pre-extraction factors can significantly impact PRRSV qRT-PCR performance. Additional research is needed to continue the process of assay improvement. References: 1. Chittick et al.: 2011, J Vet Diagn Invest 23:248-253.

Poster 44

Development and Validation of the VetMAX™-Gold SIV Subtyping Kit

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Swine influenza virus (SIV) is a highly contagious viral infection of pigs, resulting in significant economic losses in the swine industry. SIV subtypes are defined by the surface glycoproteins: hemagglutinin and neuraminidase, with H1N1, H3N2, and H1N2 representing the predominant subtypes in swine. We have validated an SIV subtyping workflow consisting of high throughput nucleic acid purification, SIV detection, and SIV subtyping from porcine nasal swab samples. The VetMAX™-Gold SIV Subtyping Kit is a pair of single-well real-time RT-PCR assays to differentiate the H1, H3, N1 and N2 alleles. When performed in conjunction with the VetMAX™-Gold SIV Detection Kit to screen samples for SIV as well as monitor sample isolation and inhibition, the VetMAX™-Gold SIV Subtyping Kit provides a robust method for subtyping the predominant SIV subtypes in swine. The SIV subtyping workflow was evaluated with 169 SIV-positive and 150 SIV-negative porcine nasal swab field samples and virus isolates originating from diverse geographic regions in the US. The SIV status and subtype of each sample was confirmed prior to the start of the study. The characterized positive samples processed in this study consisted of the H1N1, H3N2, H1N2, and H2N3 genotypes, with the majority of samples representing strains circulating within the swine population in the last five years. Collaborator laboratories purified the viral nucleic acid using the MagMAX™-96 Viral RNA Isolation Kit (AM1836) and MagMAX™ Express instruments. Xeno™ RNA was spiked into each nucleic acid isolation to serve as an extraction control and samples were first tested with the VetMAX™-Gold SIV Detection Kit to verify that the Xeno™RNA amplified within the acceptable range, prior to performing the H1/H3 and N1/N2 SIV subtyping reactions on the AB 7500-Fast Real-Time PCR System. RNA isolated from diagnostic nasal swab samples of known SIV status (n=319) were used to determine diagnostic sensitivity and specificity of VetMAX™-Gold SIV Subtyping Kit. The assays produced >97% sensitivity and specificity for identifying the SIV subtype from nasal swab samples. This study indicates that RNA isolated from diagnostic porcine nasal swab samples, tested with the VetMAX™-Gold SIV Subtyping Kit in conjunction with the VetMAX™-Gold SIV Detection Kit, provides an economical and rapid solution for SIV subtype identification.

Poster 45

Efficacy of a Live Attenuated Vaccine in Pigs Persistently Infected with CSFV

Sara Muñoz[1], Marta Perez[1], José Alejandro Bohorquez[12], Rosa Rosell[13], Artur Summerfield[4], Nicolas Ruggli[4], Mariano Domingo[15], LLilianne Ganges[1]

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Classical swine fever (CSF) causes major losses in pig farming. Various degrees of disease severity can be observed, ranging from acute to chronic or subclinical forms. The latter are most problematic for disease control by elimination of infected animals and vaccination. Efficient live attenuated vaccines against CSFV are used routinely in endemic countries. However, despite intensive vaccination programs in these areas for more than 20 years, CSF has not been eradicated. Molecular epidemiology in these regions suggests that the virus circulating in the field has evolved under the positive selection pressure exerted by the immune response to the vaccine, leading to new attenuated viral variants. Recent work by our group demonstrated that a high proportion of persistently infected piglets can be generated by early post natal infection with low virulent CSFV. Considering the epidemiologic importance of persistent CSFV infections in endemic areas, we studied the immune response to a live attenuated CSFV vaccine in 6-week-old persistently infected pigs following early post-natal infection. CSFV-negative pigs were vaccinated as control. The humoral and interferon gamma responses as well as the CSFV RNA load were monitored during 21 days post vaccination. None of the persistently infected piglets developed a detectable immune response against the heterologous vaccine virus. These results have important implications for disease control by vaccination in endemic areas.

Poster 46

Subspecies Level Identification of Lancefield group C *Streptococcus* using MALDI-TOF Biotyper

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A comparative study was conducted to evaluate the accuracy of species level identification of Lancefield group C *Streptococcus* using Bruker MALDI-TOF Biotyper and TREK Sensititre system. The *Streptococcus* spp. used for the current study were isolated from one bovine and 19 equine clinical samples submitted to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) during the months of February to May 2014. Bacterial colonies grown overnight were identified using the MALDI-TOF Biotyper and the Sensititre system. The *Streptococcus* spp. were further characterized biochemically based on standard sugar utilization tests and Lancefield grouping. MALDI-TOF results agreed with standard sugar utilization tests for all the *S. equi* subspp. tested (18/18) while Sensititre results agreed with standard sugar utilization tests for only 17 out of the 18 isolates tested. One equine isolate identified as *S. dysgalactiae* subsp. *equisimilis* by MALDI-TOF was also confirmed by 16S rDNA sequencing. However, Sensititre identified the same isolate as *S. agalactiae*. The only bovine isolate used in the study was identified as *S. dysgalactiae* subsp. *dysgalactiae* by MALDI-TOF and confirmed by 16S rDNA sequencing. However the same isolate was identified as *Streptococcus* beta group-G by Sensititre. The Bruker MALDI-TOF Biotyper in addition to having a faster turnaround time was also successful in accurately identifying 100% (20/20) of the Streptococcal isolates tested in this study while TREK Sensititre system was successful in detecting only 85% (17/20) of isolates tested.

Poster 47

Identification of Toxigenic *Clostridium difficile* Isolates from Fecal Samples

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Toxigenic *Clostridium difficile* is an important cause of enterocolitis in many veterinary species, and can be identified by the presence of one or both toxins: Toxin A, an enterotoxin, and toxin B, a cytotoxin. Isolation of *C. difficile* from fecal samples is not enough to diagnose the cause of diarrhea, as non-toxigenic isolates may be present in the feces of clinically normal animals. Detection of toxins A and/or B is important to differentiate a disease-causing toxigenic strain from a non-toxigenic strain, and this is most commonly accomplished through use of a tissue culture cytotoxicity assay (CTA) or an enzyme linked immunosorbent assay (ELISA). Polymerase chain reaction (PCR) assays can detect the toxin genes, but may not correlate well with the production of toxin. A commercially available ELISA has been shown to be equally as useful as the gold standard CTA for detecting *C. difficile* toxins in equine and porcine fecal samples. For canine samples, it has been suggested that the ELISA shows increased sensitivity when performed on bacterial isolates instead of feces. The ELISA may be preferable to the CTA due to its simplicity and speed, unlike the CTA which requires live cell culture and 48 hours before a negative result can be reported. An algorithm combining toxin testing of fecal samples with toxin testing of *C. difficile* isolates cultured from the fecal samples has been touted as a means to improve sensitivity and specificity. For example, if a fecal sample did not have a detectable concentration of toxins, toxin detection assays could be performed on the isolate. To determine if the ELISA performs as well on isolates as it has been shown to perform on fecal samples, we compared the CTA, a commercially available ELISA, and PCR on a set of equine, canine, and porcine *C. difficile* isolates from the archives of the Indiana Animal Disease Diagnostic Laboratory. Additionally, because MALDI-TOF is becoming a common method for bacterial identification and accurately identifies *C. difficile* isolates, a MALDI-TOF-based proteolytic assay is evaluated in this study as a novel means for *C. difficile* toxin detection. The results of this study will help to update the current protocols for identification of toxigenic *C. difficile* in cases of enterocolitis.

AAVLD Trainee Travel Awardee (Bacteriology/Mycology)

Poster 48

The Center for Veterinary Medicine Veterinary Laboratory Investigation and Response Network Proficiency Testing Program

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The Center for Veterinary Medicine's Veterinary Laboratory Investigation and Response Network (Vet-LIRN) is working in collaboration with the Center for Food Safety and and Applied Nutrition's Institute for Food Safety and Health and Iowa State University to prepare quality samples for a proficiency testing (PT) program. The goal of the collaboration is to help Vet-LIRN laboratories maintain preparedness and competency by developing a robust PT program. This program helps Vet-LIRN laboratories demonstrate their skills and identify weaknesses and opportunities for improving performance. All PT samples are prepared according to ISO standards. To date, the collaboration has offered four complete PTs that focused on both microbiology and chemistry. There were three rounds of the PT for the determination of *Salmonella* spp. in dog fecal samples. It is important that laboratories across the country have methods for analysis of *Salmonella* spp. in dog fecal samples since there have been a number of outbreaks and cases of human salmonellosis correlated with exposure to contaminated animal feed. Household companion animals are potential carriers of *Salmonella* and they can be symptomatic or asymptomatic, and a potential source of zoonotic infections. By completing the three PTs analyzing dog fecal samples for various types of *Salmonella*, the laboratories improved their results each time with the final round showing laboratories correctly identifying six of the eight samples with 100% accuracy and the remaining two samples with greater than 85% accuracy. Vet-LIRN also conducted a chemical PT to determine concentrations of copper (CU), iron (Fe), manganese (Mn), and zinc (Zn) in beef and goat liver samples. The objective of this PT was to evaluate the testing proficiency of participants on the quantitation of elements in beef and goat liver, a potential testing matrix in a diagnostic laboratory. Overall, 12 laboratories for Cu (80%), 7 for Fe (64%), 9 for Mn (90%) and 10 for Zn (91%) showed acceptable laboratory performance represented by a score of ≤ 2.00.

Poster 49

Salmonella Prevalence in Pets – Federal Department of Agriculture's Collaboration with 11 Veterinary Diagnostic Laboratories

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Pet food has been recalled numerous times in recent years due to *Salmonella enterica* contamination, which sometimes has also resulted in human illness. The effect of *Salmonella*-contaminated pet food on dog health is generally unknown and, as a result, salmonellosis in pets may be under-reported. Additionally, the serotypes of *Salmonella* associated with salmonellosis in dogs have not been adequately characterized. The Center for Veterinary Medicine's (CVM) Veterinary Laboratory Investigation and Response Network (Vet-LIRN), in collaboration with 11 cooperative agreement laboratories, investigated the prevalence of *Salmonella* in symptomatic and asymptomatic pet dogs. The 11 laboratories harmonized their culture methods and tested dog fecal samples over a 2 year period, isolating and identifying *S. enterica*. Serotyping was confirmed by the National Veterinary Services Laboratories of the USDA Animal and Plant Health Inspection Service, Ames, Iowa. Of the 2476 fecal samples tested, 62 (2.5%) dogs were positive for *Salmonella*. Of the 62 positive dogs, only 27 (44%) had diarrhea upon presentation. Additionally 2 dogs (3%) presented with bloody stools but no diarrhea. Thus, the majority of *Salmonella*-positive dogs in the study were asymptomatic. All participants (owners and veterinarians) completed an extensive feeding and husbandry questionnaire. Results showed that raw food was fed to 11 of the positive dogs (18%). Some regional differences in prevalence were also noted. This study suggests that an asymptomatic *Salmonella* carrier stage, with potential zoonotic implications, may exist in pet dogs. Data from this study will help CVM prioritize investigations of foodborne diseases that adversely affect both animal and human health. The data will also help us rank and streamline future food safety-related surveillance efforts.

Poster 50

Quality System Management - The Next Level

Terry L. Dobrovolsky[1], Jennifer Havens[2]

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The Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) built a Quality Management System (QMS) based on multiple Microsoft Access databases which were supported by Microsoft Excel pivot tables and reports. As the volume of data grew larger and report requests became more complex, the databases were in danger of becoming corrupt; therefore TVMDL sought a comprehensive, easy-to-use compliance management system. Industry-leading vendors were evaluated, and Qualtrax was chosen based on customized system flexibility and functionality. With the new compliance management system from Qualtrax, QMS and compliance management efficiency and accuracy have increased. Considering the volume of documents and processes managed through the QMS, making the shift from the Access databases to Qualtrax was a large project. Qualtrax allows for flexible onboarding and the option to transition in stages which has made the process manageable. TVMDL now manages document control, employee testing of procedures and processes through an automated, traceable software solution. A new employee, for example, is assigned required training and testing based upon their position within the organization, and within Qualtrax he or she takes tests which are then tracked and documented to completion. This functionality facilitates compliance as the system documents staff acknowledgement that processes are understood and followed. Related to this seamless functionality, Standard Operating Procedures (SOPs) are linked to training and a document trail is stored in Qualtrax. Another time saving automated workflow allows easy transfer of document management and responsibility when there are changes in responsibilities or staffing such as a departmental transfer or someone leaves the agency. As this occurs, all documents managed by the individual are listed and reassignments can be processed automatically, avoiding the need to update each document individually. Additional workflows that have been implemented include Internal Audits, Client Feedback, Corrective /Preventive Action Requests, Management Review Action Items, Requests to Add or Retire Tests, and Requests to Add or Retire Equipment. Qualtrax has been a game changer for TVMDL, taking their quality program to new levels. Quality Assurance staff experience efficiencies allowing them to focus on value added activities versus manual management of documents and process adherence. Managers now have the capability to pull reports relevant to their processes and lead accountability efficiently. Audit preparation and reporting time is at an all-time low as the associated workflow can be accessed utilizing a tablet, allowing for data collection directly into the report. Finally, the overall quality of managing compliance through the QMS is optimized and owned by the entire lab, enhancing the quality-focused culture throughout.

Poster 51

Characterization of Bromethalin, Desmethylbromethalin and Photodegradation Products by GC-MS and GC-MS/MS

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Bromethalin is a neurotoxic rodenticide formulated as solid bait blocks, pellets, or bait stations for hand placement in and around homes and commercial buildings and inside transport vehicles for the control of house mice, roof rats, and Norway rats. Off-target poisoning events involving humans, companion animals and wildlife have been reported though. Once consumed, bromethalin is metabolized to its bioactive metabolite desmethylbromethalin. Both the parent compound and its metabolite are lipid soluble and capable of crossing the blood-brain barrier. Desmethylbromethalin's mode of action is the uncoupling of mitochondrial oxidative phosphorylation. The reduction in adenosine triphosphate production and the subsequent disruption of sodium-potassium gradients leads to cerebral edema and increased cerebrospinal fluid pressure. Owing to its lipophilicity, desmethylbromethalin is predominantly detected in adipose tissue following an exposure; however, it has also been detected in other soft tissues. A diagnostic limitation to the detection of the parent compound bromethalin is its rapid photodegradation in sample extracts. Accordingly, the purpose of this study was to characterize bromethalin, desmethylbromethalin and their physicochemical breakdown products by GC-MS and GC-MS/MS to provide additional surrogate markers to screen for bromethalin exposure. In this study, neat bromethalin and desmethylbromethalin were chromatographically separated using a splitless injection with an injection port temperature of 225°C. Mass spectra were obtained for each compound and fragmentation patterns analyzed. The N-methyl group or nitro group rearrangements were found to be likely contributors to three proposed mechanisms for fragmentation of bromethalin in solvent extracts. Physicochemical breakdown of bromethalin produced at least four unique products with discernible mass spectra. We report here the development of gas chromatographic strategies that facilitate detection of bromethalin and its breakdown products, as well as their Multiple Reaction Monitoring analysis by tandem quadrupole mass spectrometry. The developed methodology is applicable to extracted tissue samples such as liver and kidney.

Poster 52

Investigation of Melamine and Cyanuric Acid Deposition in Pig Tissues Using LC-MS/MS Methods

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A collaborative study between four laboratories was conducted with two major goals: (i) to develop LC-MS/MS methods to quantify melamine (MEL) and cyanuric acid (CYA) in various pig tissues at or above level of concern (2.5 mg/kg) and (ii) to analyze residues of MEL and CYA in various tissues from pigs that had been exposed to these chemicals alone and/or in combination for 7 or 28 days. The four methods developed were modified versions of previously reported procedures and were concluded to be suitable to address objectives of the study. Pigs treated with 200 mg/kg BW/day CYA daily for 7 days did not accumulate significant residue concentrations in muscle, liver and kidney. Treatment with 200 mg/kg bw MEL daily for 7 or 28 days caused residues of MEL in muscles (3-13 ppm), liver (2.8-14.1 ppm) and kidney (9.4-27.2 ppm). Treatment with a combination of MEL+CYA at 100 mg/kg bw of each triazine daily for 7 days caused residues of both MEL (26-59 ppm in muscle, 30-49 ppm in liver and 367-6,300 ppm in kidney) and CYA (1.8-5.8 ppm in muscle, 2.6-6.5 ppm in liver and 303-7,100 ppm in kidney). Treatment with a combination of MEL+CYA at 1, 3 or 10 mg/kg bw for 7 days did not result in residues greater than the level of concern in all tissues tested. Pigs dosed with 33 mg/kg bw of MEL+CYA daily contained residues above the level of concern in kidney but not in loin, ham or liver. Deposition of MEL and CYA in tissues depends on type of pig tissue (muscles, liver and kidney), amount of MEL and CYA given and type of administration (MEL and/or CYA alone or in combination).

Poster 53

Comparison of Zilpaterol Concentrations in Bovine Urine and Hair Specimens

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Zilpaterol (Zilmax®) is a β-adrenergic agonist and is used as a supplement in cattle feed. Through β-adrenergic receptor stimulation, zilpaterol acts as an anabolic agent in animals causing increased muscle mass and lipolysis. Although the U.S. Food and Drug Administration had approved the use of zilpaterol in cattle, the use of zilpaterol or other β-agonists off-label or illegally in livestock continues to be a problem. Drug testing in animals commonly involves screening urine or serum samples; however, zilpaterol has a rapid rate of elimination and a short half-life. β-Adrenergic agonists, such as zilpaterol, have been shown to accumulate in melanin-containing biological matrices, which can include melanin in hair. Therefore, development of a method to detect zilpaterol in hair samples would be a non-invasive matrix to use to monitor abuse of the drug. This study compares the concentrations of zilpaterol in urine and hair samples from two calves fed Zilmax®. Charolais, crossbred calves (18 months old) were fed 96 mg Zilmax®/head/day for 26 days. Three days after withdrawal of the drug, hair and urine samples were collected for analysis. Urine samples (20 μL), including positive and negative controls, were applied directly to an ELISA specific for zilpaterol detection. Hair samples required extraction prior to ELISA analysis. Briefly, this new method developed for hair extraction involved the following: 1) approximately 0.1 g minced hair sample was weighed for extraction, 2) hair sample is decontaminated using a methanol rinse, 3) 2 mL of methanol is added to the hair sample and incubated overnight in a water bath at 60 degrees celsius, 4) sample is then sonicated at room temperature for 1 hour, 5) the methanol extract is aliquotted and evaporated to dryness under N2, and 5) the sample residue is reconstituted in 0.3 mL phosphate-buffered saline for ELISA analysis. The average percent recoveries of fortified urine and hair (positive control) samples were within the ± 20% of theoretical acceptance criteria. When comparing the concentrations of zilpaterol in the urine and hair samples, the hair samples contained the highest concentration. Zilpaterol concentrations were 5.87 ± 0.18 ppb and 25.10 ± 0.04 ppb in the urine, while the hair samples quantitated at 33.60 ± 0.03 ppb and 35.88 ± 0.04 ppb. This study highlights the potential for using hair samples as a non-invasive matrix to monitor for zilpaterol in animals for which the drug is being used off-label.

Poster 54

Detection of the Small Molecule Strychnine Using MALDI-TOF MS

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Strychnine is an extremely toxic alkaloid that acts as a competitive antagonist of the inhibitory neurotransmitter glycine, causing CNS stimulation which can lead to convulsions, seizures, and eventually death. It has historically been used as a rodenticide. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a soft ionization technique in which a target molecule is mixed with a matrix and allowed to crystallize on a target plate and then pulsed with a laser. Traditionally, this technique has been used for identifying very large molecules such as proteins, polymers, or whole microorganisms and it was commonly thought that matrix interferences would prevent detecting small molecules. In this study, it has been shown that strychnine (334 m/z) can be detected using MALDI-TOF MS. Initially, a 1000 ppm strychnine standard was prepared in acetonitrile. Serial dilutions were made from the 1000 ppm standard to find the limit of detection. Commercially available gopher bait containing strychnine was extracted and analyzed, as well as the stomach content from a known case of strychnine poisoning in a dog. Briefly, the extraction procedure involved: 1) vortexing approximately 1g of bait or stomach content with 5 mL of chloroform for 1 minute, 2) centrifuging the sample drying the supernatant under nitrogen gas, 3) reconstituting with 50 μL of 0.1% trifluoroacetic acid: 50% acetonitrile: 49.9% water, 4) then mixing 5 μL of reconstituted sample with 5 μL of matrix, spotting the sample on the target plate and analyzing by MALDI-TOF MS. Strychnine was detected in the neat standards, gopher bait, and stomach contents at a molecular weight of 335 m/z (M+H)+. This study shows that it is possible to detect strychnine, a small molecule, using MALDI-TOF MS and that it could potentially be used as an analytical tool to detect the toxic alkaloid in biological samples.

Poster 55

Rapid Detection of Ractopamine Using MALDI-TOF Mass Spectrometry §

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Ractopamine hydrochloride (Paylean™, Optaflexx™) is a beta-adrenergic agonist that acts as an anabolic agent and is used as an additive in animal feed to enhance muscle leanness and weight gain in livestock. Although approved for use in some animals, abuse of this drug is noted to occur in livestock show and racing animals, making detection of this compound in a veterinary diagnostic setting essential. The purpose of this study was to determine if matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was an effective way in which small molecules, such as ractopamine, could be detected. In the past, MALDI has been primarily used for rapid identification of proteins; however, by using major peaks from the matrix as a mass calibrator, small molecules are able to be detected that would otherwise be too small to analyze using this analytical technique. Ractopamine standards were prepared in acetonitrile at concentrations ranging from 1000 ppm to 1 ppt. MALDI-TOF MS analysis of the standards was achieved by spotting 1 μL of the standard that had been mixed 1:1 with alpha-cyano-4-hydroxy-cinnamic acid matrix. The standards were analyzed in positive ion mode with optimal isotopic resolution of peaks achieved at a low laser intensity, suggesting that ractopamine is ionizes well using this method. Ractopamine was detected at 302 m/z (M+H+) at concentrations ranging from 1000 ppm to 100 ppb. The highest signal intensity was observed with the 100 ppm standard, with an average signal-to-noise of 1088. An approximate limit of detection for ractopamine was determined to be 100 ppb. This study shows that small molecules, such as ractopamine, can be rapidly and accurately detected using MALDI-TOF MS.

§ AAVLD Staff Travel Awardee

Poster 56

Three Decades of Terrestrial Rabies in Wyoming ◊

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Rabies is a fatal encephalitis in humans, domestic and wild animals and is one of the oldest known deadly viral diseases. It is caused by a rhabdovirus of the genus Lyssavirus. There are two general rabies cycles; 1) terrestrial rabies, consisting of antigenic variants circulating in different species in distinct geographical regions, and 2) bat rabies that circulates in the bat population and occasional infects other mammals. In the United States, 8 variants of terrestrial rabies occur in the reservoir species of coyote, fox, raccoon, or skunk. Geographic distribution of the variants is largely defined by the population dynamics of the reservoir host and the ecology of the habitat such as natural corridors and natural barriers to movement. Spillover into non-reservoir hosts does occur, but does not typically result in sustained transmission in the new species. Wyoming geography offers a unique opportunity to study the dynamics of rabies over time and spread into new areas. Wyoming is a headwaters state and incursions of terrestrial rabies into the state occurs along river drainages from surrounding areas with circulating virus. These river drainages provide the riparian habitat needed to support the reservoir host. We describe 3 decades of rabies in Wyoming from the initial case in 1984 and establishment of the north central skunk variant in northeastern part of the state. Subsequent years found spread of north central skunk rabies in the Big Horn Basin, and central parts of the state. Cases in the Colorado River drainage of south western Wyoming caused concerns of spread into this rabies-free river basin. However, skunk rabies failed to establish a continuing cycle in these areas, perhaps because of the lack of prevalent continuous skunk habitat. In addition, isolated incidents of rabies in unexpected places have occurred due to bat rabies virus, presumably due to skunk contact with infected bats. Animals species diagnosed with rabies in Wyoming include skunk, bats, cats, horses and cows, dogs, raccoons, fox and a squirrel. In 2011, the first incursion of south central rabies variant occurred from Colorado and in 2014 from Nebraska.

◊ USAHA Paper

Poster 57

Acute, Necrotizing, Hemorrhagic, Interstitial Pneumonia in a Florida Panther (*Puma concolor coryi*) Caused by a Rickettsial Organism

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Florida panthers are an intensively managed, highly endangered subspecies of puma found in the Southeastern United States. A 2.5 year old, male, free-ranging, radio-collared, Florida panther was located following the detection of a mortality signal, and was found recumbent but alert by staff members of the Florida Fish and Wildlife Conservation Commission. The panther was immobilized for physical examination in the field, at which time no significant abnormalities were noted. Further evaluation at a referral veterinary hospital revealed only dehydration, a mild elevation in bilirubin, and a moderate interstitial pattern on thoracic radiographs. The panther recovered from anesthesia but died 6 hours later. Gross necropsy findings included good nutritional condition, severe pulmonary edema and pleural effusion, a 10 mm in diameter atrial septal defect, hepatomegaly, and lymphadenomegaly. A severe, acute, necrotizing, fibrinous, hemorrhagic, interstitial pneumonia was noted on histopathology. Intravascular histiocytes frequently contained numerous intracytoplasmic, faintly basophilic, rod-shaped organisms. Transmission electron microscopy (TEM) revealed numerous intracytoplasmic, pleomorphic, rod-shaped rickettsial bacteria with trilaminar cell walls, averaging 1.09 μm by 0.36 μm, within circulating histiocytes. Endothelial cells and alveolar epithelial cells adjacent to infected histiocytes were frequently disrupted. Aerobic bacterial culture, virus isolation, and PCR testing for apicomplexan protozoa on lung tissue were negative. Feline leukemia virus and feline immunodeficiency virus were not detected by ELISA snap tests.

Poster 58

Myocarditis and Arteritis with Intralesional Lankesterella-like Protozoa (Apicomplexa: *Lankesterellidae*) in an American Cliff Swallow (*Petrochelidon pyrrhonota*)

Federico Giannitti[1], Jenny Carlson[2], Bradd Barr[1], Dan Famini[3], Scott Fish[1], Michael J. Yabsley[4], Barbara Shock[4]

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One 8-week-old, male, hand-raised, orphan, American cliff swallow housed in an outdoor aviary in California showed progressive non-specific clinical signs for 12 days in July 2013. The bird was euthanized and submitted to the California Animal Health and Food Safety laboratory at UC Davis for pathologic examination and diagnostic work-up. Necropsy was performed and tissue samples were fixed in 10% buffered formalin for histopathology. Microscopically in the heart there was extensive histiocytic, lymphocytic and plasmacytic myocarditis with cardiomyocyte necrosis, and frequent protozoa in the cytoplasm of macrophages and myocytes, and also free in the interstitium. Intracellular parasites were ovoid to piriform and slightly curved, up to 4.37 μm in length and 1.8 μm in width, with hypereosinophilic cytoplasm, a 1-μm-diameter basophilic nucleus, and were surrounded by a clear vacuole, compatible with protozoal zoites. Round 20-μm-diameter pale eosinophilic structures with internal flocculent amphophilic material (presumably meronts) were identified multifocally in the cytoplasm of endothelial cells lining the ascending aorta. Adjacent segments of the aorta showed extensive mural inflammatory cell infiltrates of lymphocytes, plasma cells, and macrophages in the tunica intima. Macrophages and circulating monocytes contained intracytoplasmic zoites that showed weak cross reactivity with anti-*Toxoplasma gondii* immunohistochemistry. The bird also had conjunctivitis with mucosal infiltrates of lymphocytes, macrophages and plasma cells, and occasional *Cryptosporidium* species on the mucosal surface. Numerous ancillary tests for other infectious and protozoal agents were unremarkable. Transmission electron microscopy of the heart revealed both intra and extracellular zoites characterized by a double membrane, a central nucleus flanked by a single paranuclear body, and all features of apical complex compatible with apicomplexan sporozoites. DNA was extracted from frozen and formalin-fixed paraffin-embedded sections of the heart and PCR was performed with primers, which amplify a portion of the Apicomplexan 18S rRNA gene. A 795-bp sequence was amplified from both sections and it was 96% similar to *Lankesterella valsainensis* (GenBank DQ390207) with BLAST analysis. Thus, the agent was classified as a *Lankesterella*-like protozoan. *Lankesterella* spp. are apicomplexan coccidian protozoa that parasitize amphibian and reptile hosts. A lankesterellid protozoa closely related to *Lankesterella minima,* was previously amplified from *Parus caeruleus*, a passerine bird, from Spain. To the best of our knowledge this is the first molecular and pathologic characterization of a *Lankesterella*-like protozoan infection in swallows.

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Our Life Technologies™ Animal Health portfolio offers products and services for farm animal diagnostics, designed to help understand diseases and the well-being of livestock communities at a more fundamental level.

Our range of diagnostic tools including ELISA, PCR test systems and sample preparation solutions covers most economically important diseases across all major production animal species.

The entire range of Prionics™ farm animal diagnostic solutions has recently become part of the trusted Life Technologies™ product portfolio. Be sure to look for the trusted products brands, BOVIGAM™, PrioCHECK™, and ParaCHECK™ along with VetMAX™ and MagMAX™.

We believe that better diagnostics equals better animal health. Go to lifetechnologies.com/animalhealth to learn more.

National Institute for Animal Agriculture

Booth **302**

13570 Meadowgrass Drive, Suite 201
Colorado Springs, CO 80921
www.animalagriculture.org
Contact: Katie Ambrose
719.538.8843
katie.ambrose@animalagriculture.org

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

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

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

Omega Bio-Tek, Inc.

Booth **108**

400 Pinnacle Way Suite 450
Norcross GA 30071
Website: www.omegabiotek.com
Contact: 770.931.8400
info@omegabiotek.com

Omega Bio-Tek manufactures a complete line of DNA|RNA isolation kits utilizing magnetic beads and silica filter technology for both high throughput facilities and individual labs. With DNA and RNA products for serum, swabs, saliva, stool, blood and more, we offer a selection to meet your workflow and instrumentation requirements. Our Mag-Bind line of products can be used with open liquid handling instruments to help you increase throughput and reduce waste. By offering components separately and in kits you will never be forced to purchase unnecessary reagents. Our specialists can help you find which of our 700 nucleic acid products fit your needs.

PRI Bio

Booth **104**

700 Industrial Drive
Dupo, IL 62239
www.pri-bio.com
Contact: Jim Laarman
618.286.5000
jlaarman@progressive-recovery.com

PRI Bio is a global leader in Alkaline Hydrolysis Technologies (Tissue Digesters) and Effluent Decontamination Systems servicing Veterinary Research and Diagnostic Labs; Zootonic Research Facilities; Centers for Disease Control; and Pharmaceutical R&D and Manufacturing operations. For more than 30 years, PRI has advanced the science in hazardous liquid and solid waste treatment, specializing in the design and manufacture of sterilization and digester equipment. PRI's thermal products are currently used in24 countries worldwide, with over 3,000 units installed.

PRI Bio's flexible and modular Digester and EDS designs are specified for Biological Safety Labs (BSL 2, 3, 3E, 3Ag & BSL4). PRI's equipment can operate in batch or continuous flow modes, vertical or horizontal orientation, thru-the-floor or thru-the-wall configurations, with or without Containment Bioseals, and even with wet or dry discharge options. PRI Bio offers AAVLD facilities the industry's largest array of design and configuration solutions for effluent and tissue treatment.

Explore us at www.pri-bio.com.

Qiagen, Inc.

Booth **107/109**

19300 Germantown Road
Germantown, MD 20874
www.qiagen.com
Contact: Nevena Djuranovic
207.239.7962
nevena.djuranovic@qiagen.com

QIAGEN is the leading global provider of sample and assay technologies that are used to transform biological materials into valuable molecular information. QIAGEN markets more than 500 products around the world, selling both consumable kits and reagents and automation systems to four customer classes: Academia (life sciences research), Applied Testing (veterinary testing, food safety and forensics), Molecular Diagnostics (human healthcare), and Pharma (pharmaceutical and biotechnology companies).

SAGE

Booth **101**

2455 Teller Road
Thousand Oaks, CA 91320
www.sagepub.com
Contact: Lisa LaMont
805.410.7239
lisa.lamont@sagepub.com

SAGE is a leading international publisher of journals, books, and digital media for academic, educational, and professional markets. Since 1965, SAGE has helped educate a global community spanning a wide range of subject areas including business, humanities, social sciences, and science, technology, and medicine. Visit us at www.sagepub.com.

Tetracore, Inc.

Booth **200**

9901 Belward Campus Drive, #300
Rockville, MD 20850
www.tetracore.com
Contact: Ashley Bottomly
240.268.5400
abottomly@tetracore.com

Company Representatives: Dr. William Nelson, Rolf Rauh, John Kelly, and Ashley Bottomly

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

Please visit our booth to see the T-COR™ 8 – our latest real-time PCR thermocycler. Featuring 8 independent sample wells with multiplex capability, it is small, highly portable, completely self-contained, and has a 4-hour battery life. Our qPCR assay for the differentiation and detection of NA and EU PRRSV strains, the multi-platform EZ-PRRSV MPX 4.0, has been shown to have superior coverage and performance than any other commercially available reagent in several studies. Also available is our new product EZ-PED/TGE/PDCoV MPX 1.0, for the detection and differentiation of Porcine Epidemic Diarrhea, Transmissible Gastroenteritis, and Porcine deltacoronavirus.

Thermo Scientific

Booth **206**

12076 Santa Fe Drive
Lenexa, KS 66215
www.thermofisher.com
Contact: Jennifer Lorbach
800.871.8909
jennifer.lorbach@thermofisher.com

From the industry-leading Thermo Scientific™brand comes a comprehensive array of veterinary-specific solutions. Built on the strength and proven performance of Thermo Scientific™ Sensititre™ ID/AST products, Thermo Scientific™para-JEM™ Johne's testing reagents and high quality Remel media products, we deliver a full range of animal-specific formulations. From manual AST products and culture media to automated instrumentation, our products are designed to give you unmatched quality and performance at every step of your workflow.

VMRD, Inc.

Booth **201**

425 NW Albion Road
PO Box 502
Pullman, WA 99163
www.vmrd.com
Contact: Ed Felt
800.222.8673
vmrd@vmrd.com

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

Zoetis

Booth **304**

100 Campus Drive
Florham Park, NJ 07932
www.zoetis.com
Contact: Chris Demiris
973.443.3139
chris.demiris@zoetis.com

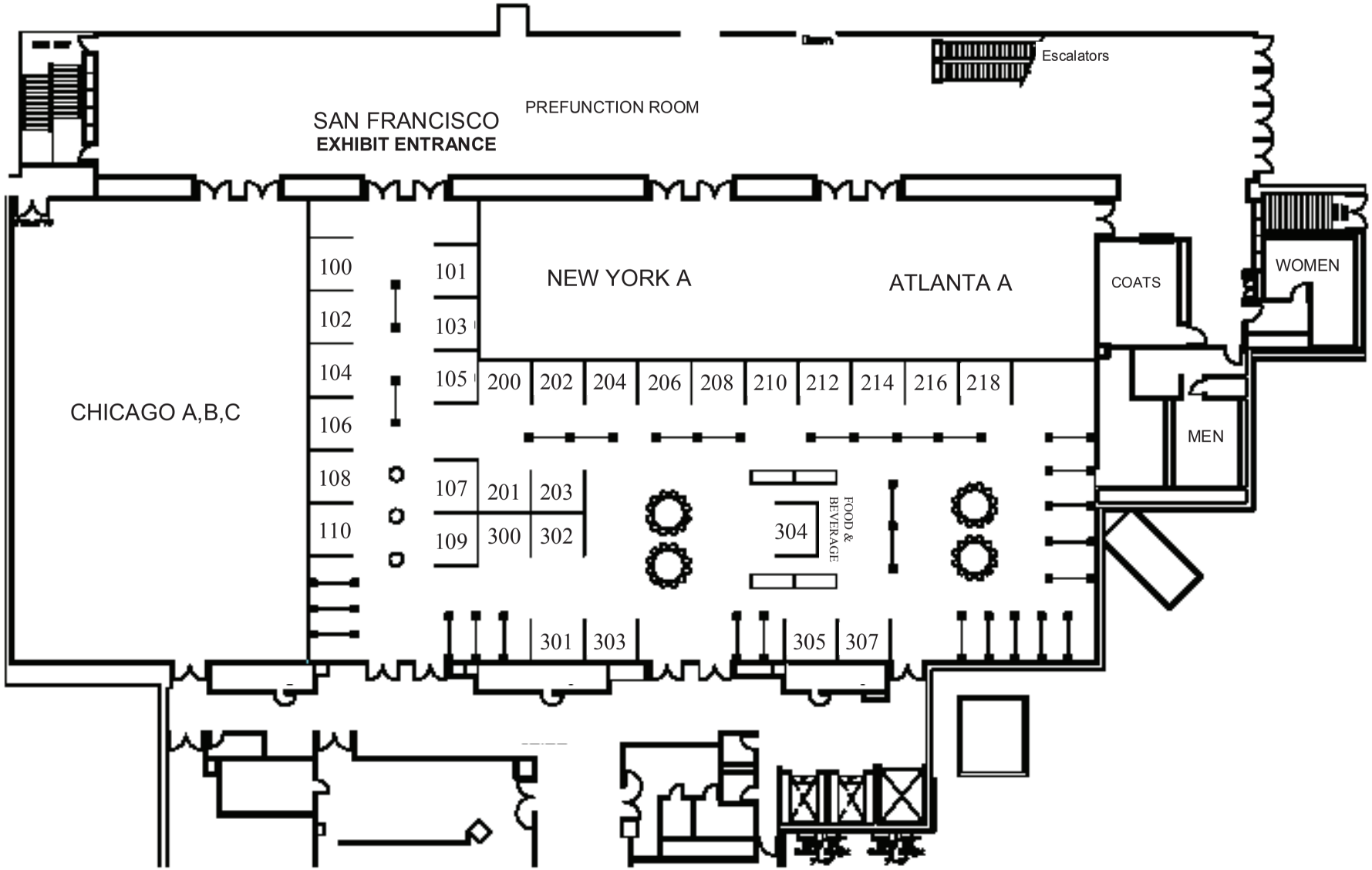
Zoetis (zô-EH-tis) is the leading animal health company, dedicated to supporting its customers and their businesses. Building on more than 60 years of experience in animal health, Zoetis discovers, develops, manufactures and markets veterinary vaccines and medicines, complemented by diagnostic products and genetic tests and supported by a range of services. Zoetis offers a broad menu of diagnostics test for Small animals, Equine and Poultry. Zoetis is also a leader in Canine Reproduction services and products, with a dedicate storage center for Equine and Canine reproduction. In 2013, the company generated annual revenue of $4.6 billion. With approximately 9,800 employees worldwide at the beginning of 2014, Zoetis has a local presence in approximately 70 countries, including 28 manufacturing facilities in 11 countries. Its products serve veterinarians, livestock producers and people who raise and care for farm and companion animals in 120 countries. For more information, visit www.zoetisus.com.

AAVLD/USAHA
Upcoming Meetings

October 22-28, 2015
Providence, Rhode Island

October 20-26, 2016
Greensboro, North Carolina

Sheraton Grand Ballroom



EXHIBITS AND POSTER SESSION

San Francisco - Ballroom Level

2014 EXHIBITORS

Booth #	Exhibitor
202	Abaxis, Inc. - Animal Health
103	AbD Serotec Bio-Rad Company
203	Advanced Technology Corp. VADDS
300	Biolog, Inc.
214	bioMerieux
212	Bioplastics/Cyclertest, Inc
106	Biovet Inc.
204	Bruker Daltonics
218	CABI
210	CEDARLANE Laboratories
102	Computer Aid, Inc.
110	ECL2/Q-Pulse
208	GeneReach Biotechnology Corporation
105	GlobalVetLINK
100	Hardy Diagnostics
301/303	IDEXX Laboratories
216	LabWare, Inc
305/307	Life Technologies
302	Nat'l Institute for Animal Agriculture
108	Omega Bio-Tek
104	PRI Bio (Progressive Recovery, Inc.)
107/109	QIAGEN, Inc.
101	SAGE
200	Tetracore, Inc.
206	Thermo Scientific
201	VMRD, Inc.
304	Zoetis

Exhibit Hall Schedule

Saturday, October 18
9:00 am—6:00 pm

Sunday, October 19
9:00 am—1:00 pm

Poster Session

Posters are available in San Francisco.
9:00 am- 6:00 pm Saturday, Oct 18
9:00 am-12:00 pm Sunday, Oct 19

Authors present 3:00-4:00 pm
on Saturday, Oct 18, 2014

SPONSOR PRESENTATIONS

Saturday, October 18, 2014			
IDEXX	6:00-6:30 pm	Atlanta A	Introduction to the new IDEXX after RealPCR ™ Modular System
Sunday, October 19, 2014			
QIAGEN	12:00-1:00 pm	Benton AB	Customer Perspectives on Successful Utilization of QIAGEN reagents and instruments.

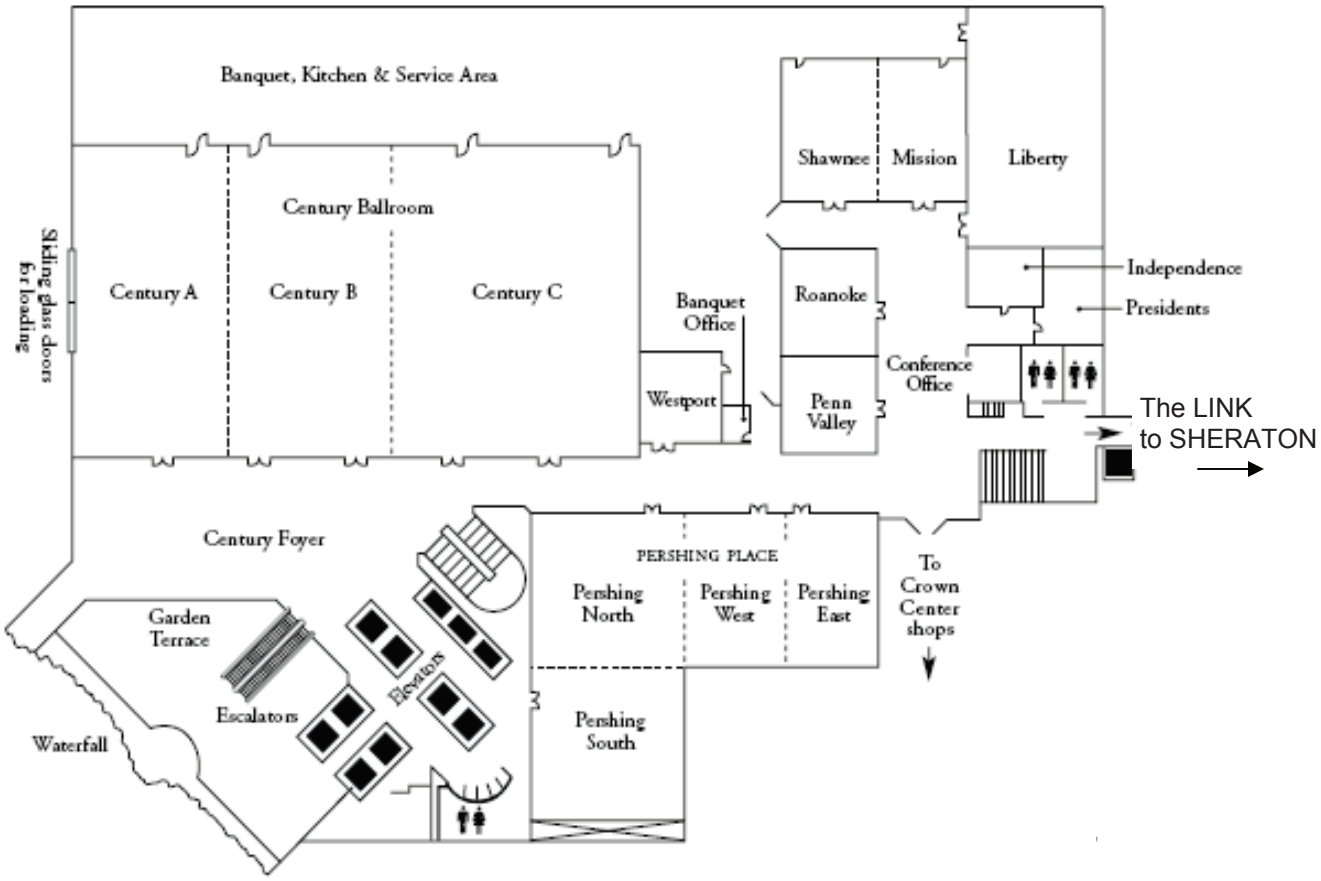
Upcoming AAVLD/USAHA meetings:

Providence, Rhode Island October 22-28, 2015
Greensboro, North Carolina October 20-26, 2016

NOTES

Westin Crown Center

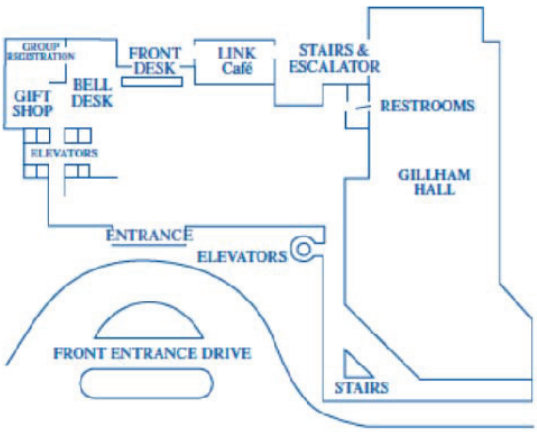
BALLROOM LEVEL



Meeting Rooms Scheduled:

- Century Ballroom A B C
- Shawnee/Mission
- Liberty
- Westport
- Penn Valley
- Presidents
- Pershing East

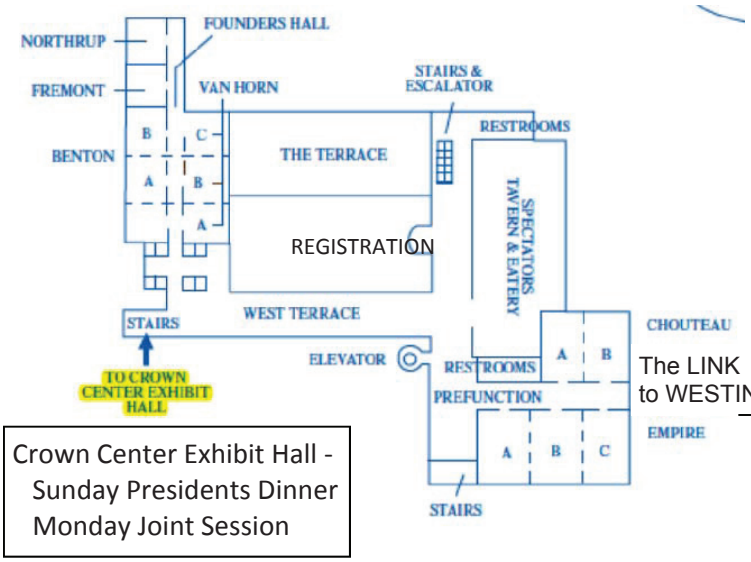
Sheraton
Kansas City Hotel at Crown Center



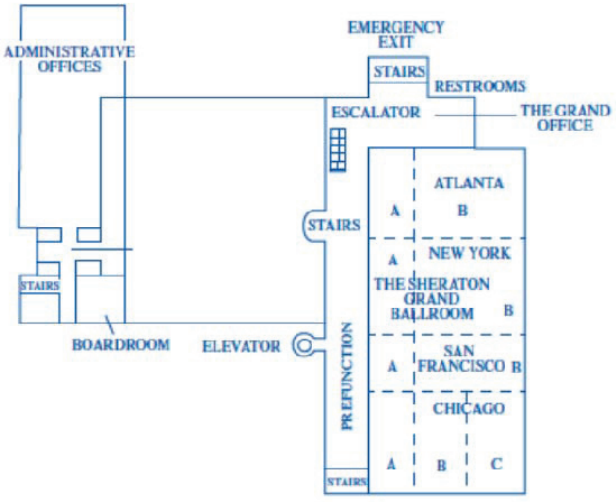
LOBBY LEVEL

MEZZANINE LEVEL

The LINK to Westin Hotel
Registration - The Terrace
Meeting Rooms
- Empire A B C
- Chouteau A B
- Benton A B
- Fremont
- Northrup
- Van Horn A B C



Crown Center Exhibit Hall -
- Sunday Presidents Dinner
- Monday Joint Session



BALLROOM LEVEL

Grand Ballroom -
- Atlanta
- New York
- Chicago

Plenary and Scientific Sessions

San Francisco - AAVLD Exhibits

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