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

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AAVLD Plenary Session

Saturday, November 13, 2010
Salon AB

One Health: Opportunities for Veterinary Diagnostic Laboratories

Plenary Sponsor: CAI Computer Aid, Inc. *(Coffee, tea and soft drinks provided)*

07:50 AM **Welcome**
Craig Carter, AAVLD President-Elect – Program Chair

08:00 AM **Keynote. Human-animal medicine: How do laboratories fit into the picture?**
Peter Rabinowitz, MD, MPH, Professor of Medicine, Yale School of Medicine, Director of Clinical Services, Yale Occupational and Environmental Medicine Program, Author of Human-Animal Medicine:, Clinical Approaches to Zoonoses,Toxicants and Other Shared Health Risks, Elsevier, 2010, speaker sponsored by Pfizer, Inc 30

08:30 AM **NBAF and its role in the growing One Health initiative**
Cyril Gay, DVM, PhD, Senior National Program Leader, USDA-ARS, Coordinator for the Animal Health National Research Program 31

09:00 AM **What are the challenges and pitfalls to my laboratory participating in One Health?**
Bruce Akey, MS, DVM, Assistant Dean for Diagnostic Operations and Executive Director of the Animal Health Diagnostic Center, Cornell University 32

09:30 AM **Break**

10:00 AM **Valuing One Health: Opportunities and challenges for veterinary diagnostic laboratories**
Tammy Beckham, DVM, PhD, Director, Texas Veterinary Medical Diagnostic Laboratory System and Director, National Center for Foreign Animal and Zoonotic Disease Defense (FAZD), Texas A&M University 34

10:30 AM **When/where/how veterinarians can build bridges between the diagnostic laboratory and the local health community**
Tanya Graham, DVM, Diplomate ACVP, Associate Director & Professor, Animal Disease Research and Diagnostic Laboratory, South Dakota State University 35

11:00 AM **Walk the talk: The Center for Excellence of Emerging & Zoonotic Animal Diseases (CEEZAD) and One Health**
Juergen Richt, DVM, PhD, Regents distinguished Professor, Eminent Scholar for Kansas Bioscience Authority, Director, DHS Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) 36

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Pathology Scientific Session

Saturday, November 13, 2010
Salon A

Moderators: Tanya Graham and John Adaska
Sponsor: VENTANA® a member of the Roche Group

01:00 PM **Microchip-associated soft tissue sarcomas and massive multi-organ extramedullary haematopoiesis in a house musk shrew (*Suncus murinus*)**
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01:15 PM **Fatal hemorrhagic pneumonia in a dog associated with extraintestinal pathogenic *Escherichia coli**
Geisa Paulin-Curlee, James R. Johnson, Aníbal Armién. 39

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José Ramos-Vara, Margaret Miller. 43

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* Graduate student presentation

Exhibit Hall Open 9:30am - 6:00pm

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Sponsor:

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Erin Mulhern, Andrew Fabian, Emily O'Hearn, Fawzi Mohamed, Amaresh Das, Michael T. McIntosh, Samia A. Metwally 48

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Robert W. Fulton, Jean d'Offay, Lurinda J. Burge, R. Eberle, Kim McCormack, D.L. Step, Jackie Wahrmund, Ben Holland 49

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*Graduate student presentation

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Sponsor:

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William Wilson, Hana Weingartl, Jieyuan Jiang, James Neufeld, Brett Dalman, Peter Marszal, Barbara Drolet, Kristine Bennett, Myrna Miller, Leonard Ateya, Yatinder Binepal, Jacqueline Lichoti, Joseph Macharia, James Mecham 56

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Scott Adams, DVM, PhD, President and CEO of VMRD, Inc.

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Saturday, November 13, 2010
Room: Duluth

Moderators: Catherine Barr and Cindy Gaskill

Exhibit Hall Open 9:30am - 6:00pm

Avian/Wildlife/Exotic Scientific Session

Saturday, November 13, 2010
Rochester

Moderator: Steven Bolin and Amy Swinford

***** Graduate student presentation

Exhibit Hall Open 9:30am - 6:00pm

Sponsor Presentations

Saturday, November 13, 2010
Salon ABC & Rochester

Salon A

Moderator: Charles Moore

6:00-7:00 PM **Computer Aid: National Agribusiness Technology Center (NATC) - The Complete Animal Health Surveillance Solution; AGRAGuard featuring USALims and USAHerds**

Salon B

Moderator: Hemant Naikare

6:00-7:00 PM **Applied Biosystems by Life Technologies: Things that make you go hmmm?**

Rochester

Moderator: John Adaska

6:00-6:30 PM **IDEXX: Driving laboratory diagnostic testing by engaging practicing veterinarians, a companion animal example**
William Goodspeed, Corporate Vice President Livestock and Poultry Diagnostics, Water and Dairy

Salon C

Moderator: Kristy Pabilonia

6:00-6:15 PM **VADDS: LIMS options for frozen budgets**
Joseph J. Bove, President, Advanced Technology Corp

6:15-6:30 PM **Biovet: New developments in diagnostic kits**
Dr. Andre Broes, DVM, PhD, Director, R&D Technical Services Manager

6:30-6:45 PM **Newport Laboratories: Technology, growth, and leadership**
Randy Simonson, General Manager & Chief Operating Officer

6:45-7:00 PM **SDIX: Rapid and cost-efficient Salmonella Enteritidis testing**
Tim Lawruk

Pathology Scientific Session

Sunday, November 14, 2010
Salon B

Moderators: Scott Fitzgerald and Laura Kennedy

11:00 AM **Serum chemistry reference ranges in captive Alaskan reindeer (*Rangifer tarandus*)**
Zoe Purtzer, Gregory Finstad, Antony Bakke, Diane Kazmierczak, Carla Willetto, Steve Kazmierczak. 94

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11:45 AM **Pathology of experimental *Avian Borna virus* infection in psittacines and chickens**
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Exhibit Hall Open 7:00am – 2:00pm

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Sunday, November 14, 2010
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Sponsor:

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Peter Timoney, Carol Bruser, William McCollum, Reed Holyoak, Tom Little. 100

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John Neill, Jeremy Schefers, Chris Chase, Julia Ridpath. 101

08:30AM **Change in predominance of *Bovine viral diarrhea virus* subgenotypes among samples submitted to a diagnostic laboratory over a 20-year time span**
Julia Ridpath, Gayla Lovell, John Neill, Thomas Hairgrove, Binu Velayudhan, Richard Mock. 102

08:45AM **Characterization of an antiviral compound effective against several pestiviruses ***
Benjamin Newcomer, Mylissa Marley, Julia Ridpath, John Neill, Dan Givens. 103

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09:15 AM **Detection and genetic diversity of VP7 in porcine group B rotavirus in the United States***
Douglas Marthaler, Kurt Rossow, James Collins, Jelle Matthijnssens. 105

09:30 AM **Application of the gold standard rabies diagnostic technique for brain material to salivary glands as an estimate of viral shedding and potential evolutionary adaptation to new species**
Michael C. Moore, Rolan D. Davis, Cathleen A. Hanlon. 106

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10:15 AM ***Influenza A virus (H1N1)* in two cats with severe respiratory disease**
Kristy Pabilonia, Angela Marolf, Christina Weller, Michelle Thomas, Andrea Beam, Ellen Miller, Kyoung-Jin Yoon, Barbara Powers. 107

10:30 AM **Genetic and antigenic characterization of recent human-like H1 swine influenza virus isolates**
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***** Graduate student presentation

Exhibit Hall Open 7:00am – 2:00pm

Microbiology Scientific Session

Sunday, November 14, 2010
Salon C

Moderators: Ching Ching Wu and Amar Patil
Sponsor:

08:00 AM **Distribution of *Canine pneumovirus* (CnPnV): Seroprevalence and identification of virus in dogs with acute respiratory disease**
Edward Dubovi, Nancy Zylich, Melissa Laverack, Amy Glaser, Randall Renshaw. 116

08:15 AM **A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *Borellia burgdorferi* outer surface protein A (OspA), OspC and OspF in canine serum**
Bettina Wagner, Heather Freer, Alicia Rollins, Hollis Erb. 117

08:30 AM **Isolation of a new *Streptococcus*-like bacterium from a goat abscess**
Jing Cui, Yan Zhang, Anne Parkinson, Beverly Byrum. 118

08:45 AM **Isolation of a *Streptococcus*-like organism from the milk of a cow with mastitis**
Yan Zhang, Jing Cui, Anne Parkinson, Mary Beth Weisner, Beverly Byrum. 119

09:00 AM **Isolation of a new *Corynebacterium*-like bacterium from a cow with septicemia**
Yan Zhang, David Newman, Jing Cui, Anne Parkinson, Beverly Byrum. 120

09:15 AM **Comparison of fecal culture and direct fecal real-time PCR in the identification of *Mycobacterium avium* subsp. *paratuberculosis* in fecal specimens**
Ching Ching Wu, Tsang L. Lin, Gilles R. G. Monif. 121

09:30 AM **Comparative IS900 and IS1311 direct fecal *Mycobacterium avium* subspecies *paratuberculosis* nested PCR tests: Significance of disparities ◊**
J. Elliot Williams, Pedro J. Pinedo, Gilles R. G. Monif. 122

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Dr. William Fales, University of Missouri

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10:30 AM **Bacterial identification using pyrosequencing for animal pathogens**
Deepanker Tewari, Stephen Cieply, Julia Livengood. 123

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Ronald D. Schultz, Jamie Henningson, Patricia Sharp, Bliss Thiel, Laurie Larson, Murali S. Deshpande, Tamara Davis, H. Jayappa, Teri Wasmoen, N. Lakshmanan 128

◊ USAHA paper

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Epidemiology Scientific Session

Sunday, November 14, 2010
Duluth

Moderators: Suzanne Burgener and Albert Rovira

◊ USAHA paper
***** Graduate Student presentation

Exhibit Hall Open 7:00am – 2:00pm

Molecular Diagnostics Session

Sunday, November 14, 2010
Rochester

Moderator: Kyoung-Jin Yoon and Steve Sells

* Graduate Student presentation

Exhibit Hall Open 7:00am – 2:00pm

Molecular Session

Sunday, November 14, 2010
Salon A

Moderator: Kyoung-Jin Yoon and Steve Sells

***** Graduate student presentation

USAHA/AAVLD Joint Plenary Session

Monday, November 15, 2010
Salon ABCD

One Health: One-way street or are there opportunities for animal agriculture?

Moderator: **Lonnie King,** *DVM, MS, MPA, ACVPM, Dean of The Ohio State University College of Veterinary Medicine*

07:50 AM **Welcome**
Steve Halstead, DVM, USAHA President-Elect - Program Co-Chair

08:00 AM **Keynote: One Medicine - It's all herd health**
Lisa Conti, DVM, MPH, DACVPM, CEHP, Director, Division of Environmental Health, Florida Dept. of Health and Co-Author (with Dr. Peter M. Rabinowitz), Human-Animal Medicine: Clinical Approaches to Zoonoses,Toxicants and Other Shared Health Risks – speaker sponsored by Pfizer. 167

08:30 AM **Emerging infectious diseases: The case for integrating science, medicine and public health**
Gary Simpson, MD, PhD, MPH, College Master and Professor of Internal Medicine and Infectious Disease, Departments of Medical Education and Internal Medicine, Paul L. Foster School of Medicine, Texas Tech University Health SciencesCenter at El Paso, TX 168

09:00 AM **Producer perspective on One Health: What are the implications of being a One Health partner?**
*Mark Engle, DVM, Director, Health and Transportation, PIC North America *169

09:30 AM **Break**

10:00 AM **One Health and the environment: Improving health in a wicked world**
*Katey Pelican, DVM, PhD, Assistant Professor, University of Minnesota; Current principle investigator on a USAID One Health Grant *170

10:30 AM **Global prospective of One Health: Are we missing opportunities?**
Mo Salman, BVMS, MPVM, PhD, DACVPM, Professor of Epidemiology, Director, Animal Health Population Institute, Colorado State University. 171

11:00 AM **Emerging microbial threats: Challenges and opportunities at the human-animal-ecosystem interface**
*James Hughes, MD, FACP, FIDSA, Professor of Medicine and Public Health, Emory University; and former Director of the National Center for Infectious Diseases at CDC . . .*174

11:30 AM **One Health Discussion/Q&A Panel of speakers**
Lonnie King, Moderator

AAVLD Poster Session

3 PM Friday, November 12 through
2 PM Sunday, November 14, 2010

Third Floor Foyer

***** Graduate student presentation

AAVLD Plenary Session

Saturday, November 13, 2010
Salon AB

One Health: Opportunities for Veterinary Diagnostic Laboratories

Plenary Sponsor: CAI Computer Aid, Inc. *(Coffee, tea and soft drinks provided)*

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Peter Rabinowitz, MD, MPH, Professor of Medicine, Yale School of Medicine, Director of Clinical Services, Yale Occupational and Environmental Medicine Program, Author of Human-Animal Medicine:, Clinical Approaches to Zoonoses,Toxicants and Other Shared Health Risks, Elsevier, 2010, speaker sponsored by Pfizer, Inc 30

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Juergen Richt, DVM, PhD, Regents distinguished Professor, Eminent Scholar for Kansas Bioscience Authority, Director, DHS Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) 36

Exhibit Hall Open 9:30am - 6:00pm

Human-animal medicine: How do laboratories fit into the picture?

Peter Rabinowitz, MD, MPH
Associate Professor of Medicine, Yale University School of Medicine

Narrative: In many of its current forms, the concept of "One Health" is long on visionary scope and maddeningly short on tangible specifics and short term action steps for implementation. Yet there is a substantial, although often anecdotal, amount of evidence suggesting that the clinical laboratories could play a key and increasingly important role in diagnosis, prevention, and treatment of diseases overlapping human and animal medicine in a manner that could be considered One Health. This talk will review such evidence, through presentation of cases that involve animals as sentinels for human environmental health hazards, humans as sentinels for animal disease risk, and cases that highlight how little we still know about zoonotic pathogen transmission and its impact on the global burden of illness in both humans and animals.

NBAF and its role in the growing One Health initiative

Cyril Gay, DVM, PhD
Senior National Program Leader, USDA-ARS, Coordinator for the Animal Health National Research Program

No narrative submitted

What are the challenges and pitfalls to my laboratory participating in One Health?

Bruce Akey, MS, DVM
Assistant Dean for Diagnostic Operations and Executive
Director of the Animal Health Diagnostic Center, Cornell University

Narrative:

Every health professional intuitively recognizes the basic wisdom of a One Health approach to medicine. The interconnectedness of the natural world, the web of life, is a concept repeated over and over from the first biology lecture onward. Unfortunately there has been a divergence between human medicine and veterinary medicine over the years in the more advanced countries as, due to many significant advances in controlling infectious diseases, Public Health became more focused on chronic, non-infectious disease control and prevention (heart disease, cancer, diabetes...). Though there were also significant advances in infectious disease control in veterinary medicine, infectious disease control still retains a far greater emphasis in Animal Health.

At the heart of the challenge for veterinary medicine and veterinary diagnostic laboratories to participate in the One Health Initiative are **significant differences in the paradigms of disease control** between Public Health and Animal Health that will have to be addressed. Some of these differences include:

	Public Health	Animal Health
Acceptable Level of Risk	Zero – no Morbidity, no Mortality is the goal	Morbidity/Mortality expected, in fact some Mortality is deliberate (diagnostics, surveillance)
Cost-Benefit Assessment	Willing to expend large amounts of money to even approach basic goal of no Morbidity/Mortality. Negative economic consequences of disease control decisions not a large factor. No legal requirement to pay for economic damage.	Much more limited resources to deal with prevention and control efforts. Negative economic impacts of disease control decisions carry substantial weight. Legal requirement to reimburse for destruction of animals.
Mode of Operation	Human health is paramount. Even a threat to human health, before the first human case happens, justifies strong intervention with Public Health given priority in decision making. Rare use of quarantine.	Need for balance between the maintenance of a safe, but not necessarily sterile, food supply, and zero threat of zoonotic transmission. Quarantine a commonly used tool.

Funding of zoonotic disease surveillance testing is also an issue. The traditional surveillance partners (State and Federal animal health regulatory medicine agencies) have never specifically targeted zoonotic disease testing. Anything other than opportunistic passive surveillance for many zoonotic diseases will require additional funding either through these historical partners or from the Public Health regulatory agencies. Funding for surveillance of zoonotic diseases of Public Health interest will inevitably create some **confusion over responsibilities for reporting and lines of authority**, given the differences in regulatory paradigms outlined above. This confusion will need to be addressed if the efforts are to be successful and sustainable.

Creating a seamless integration of disease testing between the Animal Health and Public Health sectors will require **standardized testing protocols, reagents, proficiency panels and Quality Assurance** requirements for all participating laboratories. The **trend of Public Health laboratories branching out into animal testing should be thoughtfully and critically reviewed** as to the merits and benefits of that approach versus adequately funding those testing capabilities in Animal Health laboratories.

Certainly some of the largest and most critical stumbling blocks to creation of a true One Health culture are the **deficiencies and shortcomings in the ability to collect, store, transmit, integrate and analyze the wealth of information** already collected on a daily basis in Animal Health laboratories. Current Laboratory Information Management Systems (LIMS) are designed to facilitate reporting of test results on a per accession basis and to provide the billing/accounting functions related to that testing. They are not designed to collect or organize data in a manner that would best facilitate surveillance needs. Though small steps have been made, **lack of development and implementation of universal data and messaging standards still prevents all but the most limited interchange of information** between sytems, much less the on-demand aggregation and analysis of that information on a broad scale. The use of powerful and sophisticated analytical tools such as metadata analysis, geospatial and temporal analysis, data mining, modeling and others to provide early-warning indicators or insights into the epidemiology of diseases is simply not currently possible given the Tower of Babel that is laboratory data.

Valuing One Health: Opportunities and challenges for veterinary diagnostic laboratories

Tammy Beckham, DVM, PhD
Director, Texas Veterinary Medical Diagnostic Laboratory and Director, National Center for Foreign Animal and Zoonotic Disease Defense (FAZD), Texas A&M University System

Narrative: The "One Health" concept of addressing global health challenges is highly dependent on an effective multi-disciplinary approach to solving complex problems. Veterinarians, ecologists, medical doctors, wildlife biologists and researchers are but few of the many who must come together in a holistic approach to ensuring animal, human, and environmental health.

Throughout the US, and in many foreign countries, veterinary diagnostic laboratories (VDLs) play a central role in early detection and monitoring of animal, emerging and/or zoonotic diseases. This mission clearly defines and cements their role in the "One Health" concept.

Along these lines, veterinary diagnostic laboratories have a long and successful history of working with their counterparts in human diagnostics and regulatory medicine to ensure timely diagnosis and reporting of disease. History has demonstrated that more often than not, zoonotic diseases are first diagnosed by veterinarians and, without exception, veterinarians continue to play a major role in zoonotic disease investigations and diagnosis.

VDLs will play a major role in successful implementation of the "One Health" concept. As with all new programs, effective implementation of the "One Health" concept will come with both challenges and opportunities. Some of these challenges include: 1) resource limitations; 2) personnel commitment 3) development of clearly defined communication plans as well as roles and responsibilities; and 4) clearly defined and exercised response plans/policies. Despite these obstacles, there are also many exciting opportunities including 1) the opportunity to be a leader in the "One Health" initiative, 2) increased public awareness of the critical role VDLs play in public health and 3) new opportunities/sources for external revenue.

Resource limitations and challenges faced by veterinary diagnostic laboratories include both personnel and information technology infrastructure. Most veterinary diagnostic laboratories are "caseload" heavy and FTE (full-time equivalent) "limited." Heavier caseloads reduce the time available for diagnosticians to participate in programs and communications with their public health/wildlife counterparts. In addition, most veterinary laboratory information management systems are not capable of communicating into one centralized database and electronically alerting regulatory agencies of new disease events. Novel, highly-integrated communication systems are desperately needed to ensure timely communication of disease emergence. Success of the "One Health" approach at the State, National and Global level, will require commitment from veterinary and public health officials to participate in joint-training sessions, and regular communications and discussions regarding policy and response planning. Policy decisions surrounding regulatory actions should be discussed prior to an event and emergency response plans should clearly define communication chains and responsibilities.

Although full-implementation of the "One Health" concept will be challenging to most VDLs, it is this very action that has potential to raise awareness of the critical role that veterinarians and, more specifically, veterinary laboratory diagnosticians play in public health. Increased communications and success stories of early detection and protection of livestock, companion animal, wildlife, and public health will further cement the role of VDLs in the "One Health" Program. The items above, in addition to the expertise and wealth of data located within the VDL network, will ensure that VDLs are key partners in competitive grants funded through "One Health" initiatives. These additional external funding opportunities will help expand the role of the VDLs in public health research and diagnostics and allow them to reach their full potential in contributing to this effort.

When/Where/How veterinarians can build bridges between the diagnostic laboratory and the local health community

Tanya D. Graham, DVM, Diplomate ACVP
South Dakota State University

Narrative:

Zoonotic diseases; animal agriculture (both as a source of protein and as a source of power); social determinants of infectious diseases; bioterrorism; globalization of goods and people; climate change—the list of reasons for developing a *One Health* mindset is endless. Recent international conferences such as the OIE's *Veterinary Education for a Safer World* (12-14 October 2009) and the upcoming *First OIE Global Conference on Veterinary Legislation* (7-9 December 2010) will yield a great deal of information about how future generations of veterinarians need to be educated to meet the needs of society. The *One Health Commission* (established in 2009) is focused on establishing "closer professional collaborations and educational opportunities across the health science professions and their related disciplines." Despite these various efforts, very little discussion has focused on how to educate the world of human health about what veterinarians can bring to the table.

In order to further identify areas in which veterinarians / diagnostic laboratories can interact with the health sciences / medical community at the local level, the following questions were asked in the form of a survey with open-ended answers:

1. What is your position / title / job function in the medical community? (e.g. Physician? Nurse? EMT? Administrator?)
2. At what point in your education were you informed of the educational requirements necessary to be a practicing veterinarian / veterinary researcher / diplomate of various specialty boards in veterinary medicine?
3. In what type of facility(ies) do you practice your profession? (e.g. Hospital? Local health clinic? Private office? Other?)
4. What types of veterinary-related information do you think the health sciences / medical community would find useful? (e.g. Zoonotic diseases? The latest medical topic in the popular press? Animal agriculture? Food safety? Other?)
5. What do you think would be the most appropriate venue(s) for physician / veterinarian interactions on topics of common importance? (e.g. Through continuing education seminars at local clinics / hospitals? Presentations at state / regional medical associations? Joining a listserv-if so which one(s)? Other?)
6. Are there other ways (not previously mentioned) that you believe would promote interactions between veterinarians and the local health sciences/medical community?

The answers / results of this survey will be discussed as well as options for future interactions.

Walk the talk: The Center for Excellence of Emerging & Zoonotic Animal Diseases (CEEZAD) and One Health

Juergen A. Richt, DVM, PhD
Director, Department of Homeland Security Center for Emerging and Zoonotic Animal Diseases (CEEZAD), Kansas State University, Manhattan, KS, USA

Narrative:

The history of integrative thinking between veterinary medicine, human medicine, and environmental and economic domains has slowly evolved through time into comparative medicine and lately to the concept of "One Health". Recognition of interdependence between these entities has increasingly become apparent within the last decade. Although this concept is not new, its implementation requires collaborative efforts involving multiple disciplines.

When applied to emerging and zoonotic animal diseases, the concept of "One Health" is one of the critical strategies that is fully embraced for efficient risk analysis and implementation of control measures. The concept relies on vaccine and diagnostic tools using a transboundary line of attack because of the underlying interconnection between these disciplines and the potential risk for failure without a global approach.

The Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) conducts research, develops technology, and trains a specialized work force to successfully defend US pre-harvest agricultural systems against accidental or intentional introduction of emerging animal pathogens, and especially those with zoonotic potential. The Center's emphasis on foreign, zoonotic and novel emerging pathogens will have a significant impact on both human and animal health.

The CEEZAD's research is concentrated around three themes: vaccines, diagnostics and epidemiology/modeling. The vaccine theme targets the development, testing and validation of vaccines against known and newly emerging threat agents such as Foot and Mouth Disease Virus (FMDV), Rift Valley Fever Virus (RVFV), Animal Influenzas or other newly discovered and economically important threat pathogens. The second theme objective encompasses the development of diagnostic tools to support the vaccine theme and especially DIVA companion test for FMDV, RVFV and influenza, as well as ability to ensure the rapid detection of unknown novel agents whether natural or engineered. The third theme goals involve inter-disciplinary, interagency and international collaboration to support U.S. national policy and emergency responses in case of introduction of threat pathogens. To fully integrate the concept of "One Health", the mission of CEEZAD is supported by a strong emphasis on an education and outreach approach.

Pathology Scientific Session

Saturday, November 13, 2010
Salon A

Moderators: Tanya Graham and John Adaska
Sponsor:

01:00 PM **Microchip-associated soft tissue sarcomas and massive multi-organ extramedullary haematopoiesis in a house musk shrew (*Suncus murinus*)**
Leah Schutt (ACVP Awardee), Patricia Turner. 38

01:15 PM **Fatal hemorrhagic pneumonia in a dog associated with extraintestinal pathogenic *Escherichia coli**
Geisa Paulin-Curlee, James R. Johnson, Aníbal Armién. 39

01:30 PM **Hepatic copper and iron concentrations in periparturient mares dying of hemorrhage from a ruptured uterine artery**
Andrew Allen, Cynthia Gaskill, Lori Smith, Barry Blakley, Uneeda Bryant, Lynne Cassone, Laura Kennedy, Alan Loynachan. 40

01:45 PM **Differential diagnosis and histopathology for infectious causes of encephalitis in cervids**
Scott D. Fitzgerald. 41

02:00 PM **Two cases of pancreatic necrosis/atrophy due to zinc toxicosis in ostriches**
Scott Fitzgerald, Vinicius Carreira, Barbie Gadsden, Emmett Braselton, Tara Harrison. . . 42

02:15 PM **VENTANA**

02:30 PM **Melanocytic antigen PNL2 and tyrosinase in the immunohistochemical identification of canine melanocytic neoplasms: Comparison with Melan A**
José Ramos-Vara, Margaret Miller. 43

02:45 PM **Tissue distribution of avian Borna viral antigen and molecular characterization of avian bornaviral RNA in psittacine birds with natural spontaneous proventricular dilatation disease**
Arno Wünschmann, Kirsi Honkavuori, Thomas Briese, W. Ian Lipkin, Jan Shivers, Anibal Armien. 44

03:00 PM **Ultrastructural findings in the fundus of the eyes of red-tailed hawks (*Buteo jamaicensis*) with naturally acquired *West Nile virus* infection**
Aníbal G. Armién, Arno Wünschamm, Pat Redig. 45

* Graduate student presentation

Exhibit Hall Open 9:30am- 6:00pm

Microchip-associated soft tissue sarcomas and massive multi-organ extramedullary hematopoiesis in a house musk shrew (*Suncus murinus*)

Leah Schutt and Patricia Turner
Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Narrative:

A 16 month-old female house musk shrew (*Suncus murinus*) with a 2-month history of a rapidly growing subcutaneous mass on the dorsum was euthanized and submitted from a research colony to the Laboratory Animal Diagnostic Service for necropsy.

Macroscopic examination identified an irregular, well-demarcated, solid, tan-white **subcutaneous mass on the interscapular dorsum at the center of which was a small cavity containing a microchip implant**. Massive splenomegaly was also noted grossly.

Histologically, the subcutaneous mass was comprised of spindle cells arranged in a storiform pattern of interweaving bundles consistent with a high-grade soft tissue sarcoma with multifocal necrosis. Preliminary immunohistochemical investigation suggested the neoplastic cells were positive for neuron-specific enolase, and rarely smooth muscle actin and negative for cytokeratin, vimentin and desmin, most suggestive of a diagnosis of a poorly differentiated sarcoma or fibrosarcoma. Neoplastic growths at the site of microchip implants have been described in both dogs and laboratory rodents.

Additionally, the degree and extent of organ involvement with extramedullary hematopoiesis noted histologically was impressive in this shrew, involving the hepatic portal tracts, splenic red pulp, renal cortical interstitium, and adrenal medulla.

To our knowledge, this is the first report of a microchip-associated soft tissue sarcoma in a shrew. Splenic extramedullary hematopoiesis has been previously reported in healthy musk shrews and may be physiologically normal in this species.

Fatal hemorrhagic pneumonia in a dog associated with extraintestinal pathogenic *Escherichia coli*

Geisa Paulin-Curlee[1], James R. Johnson[2], and Aníbal Armién[1]
[1]Department of Population Medicine and Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota; [2]Medical Service, Minneapolis Veterans Affairs Medical Center, and Department of Medicine, University of Minnesota, Minneapolis, Minnesota

A 6-month-old, intact, male Golden Retriever presented to the University of Minnesota Veterinary Medical Center with sudden onset of vomiting, lethargy, and labored breathing. The clinical signs became worse over two hours, progressing to hematemesis, epistaxis, and subsequent cardio-respiratory arrest, and the dog was euthanized.

Postmortem examination revealed hemothorax, severe hemorrhagic pneumonia, and hemorrhagic enteritis. Microscopically, all sections of affected lung were almost completely effaced by large hemorrhages, edema, and necrosis, admixed with moderate numbers of degenerated mononuclear cells and colonies of short bacillary bacteria. *Escherichia coli* O6:H31 was isolated in pure culture from lung, spleen, liver, and small intestine. Virulence gene screening by multiplex PCR revealed the presence of ***papG* allele III (P fimbriae adhesin variant), *hlyD* (hemolysin), *cnf1* (cytotoxic necrotizing factor 1 [CNF1]), and *sfaS* (S fimbriae) genes,** which are typically observed in extraintestinal pathogenic *E. coli* (ExPEC). Random amplified polymorphic DNA (RAPD) analysis yielded a genomic profile nearly indistinguishable from that of human archetypal pyelonephritis isolate 536. The isolate was susceptible to 16 of the 23 antibiotics tested, being resistant only to ampicillin, clindamycin, erythromycin, oxacillin, penicillin, rifampin, and ticarcillin. There was no evidence of influenza A virus or canine influenza virus by PCR and the liver was negative for anticoagulant rodenticides.

Hemorrhagic pneumonia in dogs is uncommon, but when caused by *E. coli* is often due to necrotoxigenic strains that produce CNF1 and resemble the *E. coli* strains that cause other types of extraintestinal infections in dogs and cats. Moreover, canine *E. coli* isolated from feces and clinical infections share many similarities with certain human ExPEC isolates, including specific virulence genes, O types, clonal groups, and genomic profiles, raising concerns over a potential zoonotic risk (in either direction) posed by close human-dog contact. The source of the fatal infection in this canine patient was not determined.

Hepatic copper and iron concentrations in periparturient mares dying of hemorrhage from a ruptured uterine artery

Andrew Allen[1], Cynthia Gaskill[2], Lori Smith[2], Barry Blakley[3], Uneeda Bryant[2], Lynne Cassone[2], Laura Kennedy[2], and Alan Loynachan[2]
[1]Departments of Veterinary Pathology and [3]Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada; [2]Livestock Disease Diagnostic Center, College of Agriculture, University of Kentucky, Lexington, KY, USA

Introduction: Periparturient hemorrhage (PPH) due to rupture of one or more arteries that supply blood to the uterus and related structures is one of the most common causes of morbidity and mortality in periparturient mares. Neither the cause nor pathogenesis of PPH is known. A low serum copper concentration has been described in mares suffering with PPH. But, it is important to note that this finding was based on small numbers of mares and that serum copper concentrations can sometimes be a poor predictor of copper reserves in the body as measured by copper concentrations in the liver. Rupture of the aorta and other arteries have been associated with copper deficiency in poultry and pigs, and rupture of an internal abdominal artery has been associated with lower concentrations of iron in the liver of dairy cows. The purpose of this study was to determine if hepatic copper concentration, hepatic iron concentration, or both, were associated with fatal PPH in mares.

Materials and Methods: Portions of liver were collected from periparturient mares submitted to the Livestock Disease Diagnostic Center (LDDC) for postmortem examination between December 2008 and November 2009, inclusive, and diagnosed as dying of hemorrhage from a ruptured uterine artery (PPH GROUP). Portions of liver were also collected from female horses, 10 years of age or older, submitted to the LDDC for postmortem examination between January and June, 2009, inclusive, and diagnosed as dying or euthanized for reasons other than hemorrhage, liver disease, copper toxicity, or iron toxicity (CONTROL GROUP). Portions of liver were stored frozen until digested in nitric acid. Digests were analyzed for copper and iron concentrations, on a wet weight basis, using inductively coupled plasma mass spectrometry. Individual hepatic copper and iron concentrations were classified as being either 'marginal to deficient' or 'adequate' using published and widely used criteria. Age, hepatic copper concentrations, and hepatic iron concentrations of the two groups were compared using a 2-tailed, unpaired t-test.

Results: Five of the 36 mares in this study had hepatic concentrations of copper <4.0 ppm and were classified as marginal to deficient. All 5 of these mares were in the CONTROL GROUP. All mares had hepatic iron concentrations >100 ppm and were classified as having adequate stores of iron. Mean age and mean hepatic copper concentrations of the mares in the two groups were not considered significantly different (P=0.80 and 0.07, respectively). The mean hepatic iron concentrations of the mares in the PPH GROUP (279.5 ppm) was considered significantly lower (P<0.01) than that of the CONTROL GROUP (452.4 ppm).

Discussion: This study found that broodmares dying of PPH had significantly lower concentrations of iron in their livers compared to mares from the same geographic region and of similar age, but dying of other causes. A similar relationship has been documented in dairy cows from upstate New York and northern Pennsylvania dying of internal hemorrhage from a ruptured abdominal artery. **Further, there was no evidence of either copper deficiency in the PPH GROUP or of lower hepatic concentrations of copper in the PPH GROUP compared to the CONTROL GROUP.**

The potential implications of these finding are that (1) fatal hemorrhage from a ruptured uterine artery may be associated with lower iron status in older, periparturient mares and (2) the criteria for assessing deficient, marginal, and adequate iron status in older broodmares may need to be reevaluated.

Differential diagnosis and histopathology for infectious causes of encephalitis in cervids

Scott D. Fitzgerald
Diagnostic Center for Population & Animal Health, Michigan State University, Lansing, MI

Narrative: With the recent expansion of Chronic Wasting Diseases (CWD) from a relatively small geographic region in the central-western United States, to 15 states and provinces throughout the United States and Canada, many pathologists are now evaluating the brains of free-ranging and captive cervids. While the search for prion diseases has been greatly simplified by the development of rapid screening assays, such as immunohistochemical staining and ELISA, efficient and effective surveillance of the many other infectious encephalitides that affect deer, elk and moose is lacking. Many states now have targeted surveillance programs that retrieve and examine the brains from cervids demonstrating CNS signs. Can we provide more information to our clients then simply prion protein negative or positive?

Bacterial encephalitis is relatively common in cervids, since they are susceptible to infections entering the brain either from the retro-orbital area, or at the base of the antlers. In addition to generic suppurative abscesses due to *Staphylococcus* or *Streptococcus spp*., we have found examples of thrombo-embolic meningoencephalitis due to *Histophilus* and *Streptococcus spp*. Another bacterium with CNS tropism is *Listeria monocytogenes* which affects the brainstem area with characterisitic microabscesses. Routine culture, Gram stains, and immunohistochemistry are all useful adjuncts to the diagnosis.

Viral encephalitis in cervids may be caused by rabies (rare), Eastern equine encephalitis, and West Nile virus. These diseases are nearly impossible to differentiate simply by histopathology, although EEE tends to have neutrophils admixed with the predominant mononuclear leukocyte population surrounding vessels and the meninges. Adjunct testing such as *in situ* hybridization, immunohistochemistry, and PCR are all helpful for a definitive etiologic diagnosis.

Fungal encephalitis is secondary to gastrointestinal ulcerations, allowing irregular sized and branching zygomycete hyphal forms entry to the brain by way of the vasculature. Special stains and fungal culture are helpful in these uncommon cases.

Parasitic encephalitis includes aberrant migration of the extra-pulmonary lungworm *Parelophostrongylus tenuis* (endemic in deer) or the raccoon roundworm *Baylisascaris procyonis* to enter and damage the CNS, often with eosinophilic infiltrates present. The ubiquitous environmental contaminant *Toxoplasma gondii* produces a predominantly mononuclear cuffing very difficult to separate from viral encephalitis, unless organisms are found by histopathology, immunohistochemistry, or by PCR.

While all these organisms are encountered in domestic animals, and are more or less familiar to diagnostic pathologists, **this review serves to point out the value added by having trained veterinary pathologists join in targeted wildlife surveillance in order to provide more broad disease interpretation that supplements the positive or negative test results provided by a single molecular test**.

Two cases of pancreatic necrosis/atrophy due to zinc toxicosis in ostriches

Scott D. Fitzgerald[1], Vinicius Carreira[1], Barbie J. Gadsden[1], W.Emmett Braselton[1], Tara M. Harrison[2]
[1]Diagnostic Center for Population & Animal Health, Michigan State University, Lansing, MI;
[2]Potter Park Zoo, Lansing, MI

Two of three captive adult African ostriches (*Struthio camelus*) in a zoological exhibit developed weight loss and weakness. The first ostrich was an 11 year old female which had been losing weight for several months associated with loose feces and a poor appetite. It failed to respond to antibiotics or anthelmintics. Following euthanasia, the pancreas was not grossly visible at necropsy in its normal location adjacent to the proximal duodenal loop. Histologically, the pancreatic acini were diffusely and markedly atrophic with few remaining shrunken and disorganized acini, which were replaced by high numbers of pancreatic ducts separated by extensive interstitial fibrosis. Bacterial culture of the intestinal tract revealed numerous coliforms, but no *Salmonella* were isolated.

The second ostrich was a 13 year old male, which presented after an acute illness characterized by weakness and recumbency. This ostrich was euthanized, and was submitted in better body condition than the first bird. While the pancreas was clearly visible on gross necropsy, histologically the pancreatic acinar cells were moderately degenerate with cytoplasmic vacuoles, scattered pyknotic nuclei, and individual acinar cell necrosis. Culture of the intestines revealed moderate numbers of coliforms, but no *Salmonella* were isolated.

Toxicologic testing by inductively coupled plasma emission spectroscopy (ICP) revealed markedly elevated levels of zinc in liver of both birds, while serum levels of zinc from a third, clinically normal, pen-mate were normal. The first ostrich had hepatic zinc of 441 µg/g (ppm), while the second ostrich had hepatic zinc of 375 µg/g. The Diagnostic Center for Population and Animal Health toxicology laboratory has data from 65 ostriches with a mean liver zinc of 76.4 µg/g, plus or minus one standard deviation of 58 µg/g. Previous reports in the literature report normal hepatic zinc levels in ostrich range from 23 to 86 µg/g; both the affected ostriches zinc levels were significantly higher than the normal range. The third healthy ostrich form this pen had a serum zinc level of 1.66 µg/g.

Zinc toxicosis resulting in pancreatic degeneration and atrophy has been previously reported in a variety of avian species, however, it has rarely been reported in ostriches. Nor have the microscopic pancreatic lesions been thoroughly described in ostrich previously. **Since ratites tend to indiscriminately ingest foreign objects present in their enclosures, it is important to watch for and remove zinc-containing objects such as pennies, batteries, and small pieces of zinc-plated wire or galvanized metal to prevent toxicosis.**

Melanocytic antigen PNL2 and tyrosinase in the immunohistochemical identification of canine melanocytic neoplasms: comparison with Melan A

José Ramos-Vara, Margaret Miller
Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN 47907

We compared PNL2 and tyrosinase immunoreactivity with that of Melan A in formalin-fixed, paraffin-embedded canine primary or metastatic melanocytic neoplasms in 101 dogs. There were 26 cutaneous and 53 oral tumors. Other locations were the eye, lip, lung, and lymph node. Eleven dogs had more than one tumor, so there was a total of 113 specimens. Of the total 113 samples, 106 (94%) were positive for PNL2, 101 (89%) for Melan A, and 90 (80%) for tyrosinase. Six tumors that were positive for PNL2 were negative for Melan A; one tumor, negative for PNL2, was positive for Melan A. Eighty of 113 (71%) tumors expressed all 3 markers; 111 (98%) tumors reacted with at least one of the three antibodies.

Decalcification with formic acid for up to 1 week did not affect immunoreactivity of any marker; however, decalcification with HCl for 1 day or 1 week notably decreased or eliminated immunoreactivity for Melan A and PNL2. Prolonged fixation (up to 2 months) did not affect PNL2 or tyrosinase immunoreactivity, but Melan A reduced after 1 month fixation. None of 120 nonmelanocytic tumors, including epithelial, mesenchymal, leukocytic, and steroid-producing tumors, reacted with antibody PNL2.

This study demonstrates that : 1) **antibody PNL2 is more sensitive than Melan A, and both markers are more sensitive than tyrosinase** in the identification of canine immunoreactivity was melanocytic neoplasms ; 2) **PNL2 does not cross-react with nonmelanocytic neoplasms**; 3) **PNL2 is resistant to prolonged fixation, but sensitive to hydrochloric acid decalcification**.

In summary, PNL2 is an excellent marker for canine melanocytic neoplasms, and, when used in conjunction with Melan A and tyrosinase, the sensitivity approaches 100%.

Tissue distribution of avian borna viral antigen and molecular characterization of avian bornaviral RNA in psittacine birds with natural spontaneous proventricular dilatation disease

Arno Wünschmann[1], *Kirsi Honkavuori*[2], *Thomas Briese*[3], *W. Ian Lipkin*[4], *Jan Shivers*[5], *Anibal* Armien[6]
Department of Veterinary Population Medicine/Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN[1,5,6], Center of Infection and Immunity, Mailman School of Public Health, Columbia University, NY[2,3,4]

A novel avian Borna virus (ABV) was recently implicated as the cause of proventricular dilatation disease (PDD) of psittacine birds. In the current study, tissues of 12 psittacine birds (5 cockatiels, 4 cockatoos, 2 conures, and 1 African gray parrot) were positive for avian bornaviral antigen by immunohistochemistry using a polyclonal serum specific for the viral N protein. Nine of the twelve birds had clinical signs, necropsy findings such as proventricular dilatation and histopathologic lesions such as ganglionitis and encephalitis suggestive of PDD; such lesions were absent in three birds. ABV antigen was largely limited to cells of neuroectodermal origin including neurons, astroglia, and ependymal cells of the central nervous system, neurons of the peripheral nervous system, and adrenal cells in birds with signs of PDD. In addition, arterial and gastrointestinal tract smooth muscle cells were immunopositive in few of these birds. In two of the birds, which lacked typical lesions of PDD, viral antigen had a more widespread distribution and was abundantly present in the above mentioned cells of central and peripheral nervous system, adrenal gland, epithelial cells of the alimentary and urogenital tract, heart, skeletal muscle, and skin. Avian bornavirus genotype I strain was detected in tissues of seven birds from one facility including the three birds without pathologic findings of PDD while fresh tissue was not available for testing from the remaining 5 birds. Our study provides further evidence for a role of ABV in PDD of psittacine bird species.

Ultrastructural findings in the fundus of the eyes of red-tailed hawks (*Buteo jamaicensis*) with naturally acquired *West Nile virus* infection

Aníbal G. Armién[1], Arno Wünschmann[1], Pat Redig[2]
[1]Department of Veterinary Population Medicine/Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; [2]The Raptor Center, University of Minnesota, St. Paul, MN

West Nile (WN) disease in red tailed hawks is frequently characterized clinically by blindness and pathologically by pectenitis, chorioiditis, retinal degeneration and necrosis and retinal atrophy. WN virus antigen is commonly detected in the retina using immunohistochemistry but the pathogenesis of the eye lesions is uncertain. Post mortem histologic, immunohistologic (WNV antigen) and ultrastructual examination of the fundus of the eyes of ten red tailed hawks with naturally acquired WN disease and two red tailed hawks without WN disease was performed. Acute lesions were ultrastructurally characterized by tumefaction and swelling of pigmented retinal epithelial (RPE) cells and neurons and damage of Bruch's membrane in the presence of a lymphoplasmacytic choroidal infiltrate. Proliferation of RPE cells, Müller cell hypertrophy and fibroblast proliferation were evident in chronic lesions. Viral particles were only detected in acute lesions of three hawks although immunohistochemistry demonstrated viral antigen in the retina of all hawks. The ultrastructurally detected viral particles were fairly abundant in the extracellular location, while only few particles were detected within cells. Viral particles were present within or attached to pigmented epithelial cells and photoreceptor cells.

The results suggest that damage of the blood retinal barrier constitutes an early lesion of WNV infection in the eyes of hawks. Detection of viral particles by electron microscopy is difficult possibly due to low quantity of fully assembled virus in retinal cells.

Virology Scientific Session

Saturday, November 13, 2010
Salon B

Moderators: Naomi Taus and Robert Fulton
Sponsor:

01:00 PM **Evaluation of cost effective strategies for the detection of animals persistently infected with BVDV in dairy herds**
Peter D. Kirkland, Andrew J. Read, John House, Rodney J. Davis. 47

01:15 PM **Laboratory validation of a lateral flow device for the detection of *Foot-and-mouth disease virus* in clinical samples**
Erin Mulhern, Andrew Fabian, Emily O'Hearn, Fawzi Mohamed, Amaresh Das, Michael T. McIntosh, Samia A. Metwally 48

01:30 PM ***Bovine herpesvirus-1*: Molecular studies of isolates from clinical cases characterizing field isolates and vaccine strains**
Robert W. Fulton, Jean d'Offay, Lurinda J. Burge, R. Eberle, Kim McCormack, D.L. Step, Jackie Wahrmund, Ben Holland 49

01:45 PM **A multiplex method for the serological detection of swine respiratory pathogens**
Kathy Lin, Chong Wang, Michael Maurtaugh, Kyoung-Jin Yoon, Jeffrey Zimmermann, Patrick Halbur, Xiang-Jin Meng, Sheela Ramamoorthy. 50

02:00 PM ***Porcine reproductive and respiratory syndrome virus* (PRRSV) in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance? ***
Apisit Kittawornrat, Mark Engle, Wayne Chittick, Jeremy Johnson, Devi Patnayak, Chong Wang, Daniel Whitney, Chris Olsen, John Prickett, Trevor Schwartz, Kent Schwartz, Jeffrey Zimmerman 51

02:15 PM **Detection of anti-PRRSV antibodies in oral fluid samples from individual boars using a commercial ELISA ***
Apisit Kittawornrat, Mark Engle, Wayne Chittick, Jeremy Johnson, Chong Wang, Devi Whitney, Chris Olsen, John Prickett, Trevor Schwartz, Kent Schwartz, Jeffrey Zimmerman. .52

02:30 PM **Molecular characterization of influenza A viruses from swine for potential virulence markers ***
Susan Detmer, Daniel Darnell, Srinand Sreevatsan, Marie Gramer 53

02:45 PM **Pen level sensitivity for detecting influenza virus in swine oral fluids ***
Anna Romagosa, Marie Gramer, Montserrat Torremorell 54

*Graduate student presentation

Exhibit Hall Open 9:30am - 6:00pm

Evaluation of cost effective strategies for the detection of animals persistently infected with BVDV in dairy herds

Peter D. Kirkland[1], Andrew J. Read[1], John House[2] and Rodney J. Davis[1]*
[1]Elizabeth Macarthur Agricultural Institute, Woodbridge Rd, Menangle, NSW, 2568, Australia
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Bovine viral diarrhoea virus (BVDV) is now recognised globally as an important pathogen of cattle. With increasing frequency, programs are being developed to control this virus in infected herds. A number of countries have launched eradication programs and some have been successfully completed. The key to ultimately controlling infection is the reliable identification of persistently infected (PI) animals. BVDV diagnosis and detection of PI animals has become extremely reliable with the availability of antigen capture ELISA assays and real time reverse transcriptase PCR (qRT-PCR) assays that each have high diagnostic sensitivity. When combined with appropriate sample types, these assays overcome problems associated with the presence of maternally derived antibodies in young animals.

In countries where testing for BVDV control is supported by an official government sponsored control program, the cost of testing to detect PI animals is of less concern to the individual farmer than in countries such as Australia and the USA where most or all costs are born by the herd owner. A further consideration is the cost for collection of samples. Collection of skin biopsy ('ear notch') or hair samples by the owner has assisted to address the cost of sample collection. In very large herds, the logistics of sample collection can become significant but in dairy herds other sample options can be considered. Bulk milk samples have been used to screen dairy herds for the presence of virus and to monitor antibody levels. However, the value of monitoring for antibody levels can be reduced by the use of vaccines. On the other hand, detection of virus, reflecting the likely presence of one or more PI animals in the milking herd, is heavily influenced by the number of cows that are being milked and the relative volume of milk being produced by the PI animal(s).

As well as monitoring the entire herd using the tank milk, individual milk samples can sometimes be used as a cost-effective way to screen the herd and detect individual PI animals. Individual milk samples are most efficiently tested in pools. However, for this to be practical, there are a number of factors to be considered. These include the herd size and number of individual animals to be tested, the analytical sensitivity of the test used to screen sample pools and its impact on the size of pools that can be used and in turn its influence on the number of individual animals to be tested when a positive pool is detected. Finally, there are also many elements that affect the overall cost to test individual animals and include the cost of sample collection, preparation of pools, retrieval of individual samples for testing and finally the cost of both screening and individual assays. Methods used for the collection of individual samples also need to be considered to minimise the possibility of cross contamination. These issues will be discussed with examples from the testing of large milking herds in Australia.

Laboratory validation of a lateral flow device for the detection of *Foot-and-mouth disease virus* in clinical samples

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Foot-and-mouth disease virus (FMDV) represents one of the greatest threats to the United States livestock industry. Early detection is a key element in disease control and eradication should FMD occur in the U. S. A lateral flow device (LFD) for the detection of all seven serotypes of FMDV was recently developed by Svanova Biotech AB and evaluated in the laboratory on suspensions of vesicular epithelia collected from 67 countries between 1968 and 2008 (Ferris et al., 2009). The diagnostic sensitivity of the LFD for FMDV was 84% compared to 85% obtained by the reference method of antigen ELISA, while the diagnostic specificity was 99.5% for LFD compared to 99.9% for the AgELISA. The device detected FMDV strains from all seven serotypes, but weaker reactions were often evident with SAT 2 strains, thus a separate device was developed to enhance sensitivity for SAT2 strains. The developers recommend the use of the two devices in tandem for penside diagnosis in the field (Ferris et al., 2010).

The purpose of this study was to further evaluate the performance of the LFD on fresh clinical samples representing the major FMDV serotypes and subtypes and to determine the window of detection during the course of infection in bovine, swine, ovine and caprine.

Epithelial tissue, vesicular fluid, oral and vesicular lesion swab, and saliva were collected from bovine, sheep, goats, and domestic and feral swine infected by contact exposure with one of the following FMDV serotypes: A Iraq 09, A24, O1 Brugge, O1 BFS, A22 Iraq, or Asia 1. An instructional video was developed to support field trials of the LFD.

Preliminary results showed that the device correctly identified positive clinical samples 3-16 days post contact (DPC) for bovine, up to 10 DPC for feral and domestic swine, up to 9 DPC for ovine with lesions and 3-4 DPC for caprine. Additionally, the device showed promising performance to use on saliva, and oral and vesicular lesion swabs in bovine.

Our data will assist in defining the overall performance characteristics of the device which will determine the effectiveness and facilitate the policy on the use of this type of diagnostic field assay as well as provide guidance to field practitioners on when and how to use the device during outbreaks and recovery.

Bovine herpesvirus-1: molecular studies of isolates from clinical cases characterizing field isolates and vaccine strains

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Bovine herpesvirus-1 (BHV-1) is a significant virus in bovine respiratory disease (BRD) as well as other diseases including fetal, reproductive tract, neonatal disease, and conjunctivitis along with inapparent infections. There are three BHV-1 subtypes based on antigenic and genomic differences: BHV1.1, BHV1.2a and BHV1.2b. BHV1.3 are now classified as BHV-5. BHV-1 vaccines are often combined with other viruses: parainfluenza-3 (PI-3V), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV) 1a and 2a. Reports of suspected BHV-1 disease in cattle receiving MLV strains raise the issue of differentiating vaccine strains from field strains in diseased cattle or aborted fetuses. At OSU in 2009, three groups of mixed source and transported calves were studied over the initial 60 days for bovine coronaviruses (BCV). Samples were collected weekly from both sentinel and sick animals with serums, nasal swabs, and lung lavage samples along with convalescent serums. All calves were negative for BVDV persistent infection (PI) status. All calves received a two dose regimen of MLV BHV-1 vaccine (and other immunogens) at entry and two weeks later. There were BCV and BVDV isolates in selected calves in these three studies and are described in other reports. There were no BHV-1 isolates found in day 0 samples in any of studies. In the three studies there were BHV-1 isolates from nasal swabs and/or lung lavage samples in calves after vaccination, and were confirmed by BHV-1 PCR. The objectives of this study included: (1) sequencing of genomic regions of BHV-1 in primer product; and (2) perform sequencing of the region to identify homology to reference strains (Colorado/Cooper [respiratory] and K-22 [reproductive]) and vaccine strains the calves received. Two regions were selected for primers and the sequencing was perfomed.

In Study 1, BHV-1 was isolated from nasal swabs of 4 calves postvaccination. Based on homology using a region of the thymidine kinase (UL23) and UL36, three isolates were homologous to the BHV1.2 strain; often referred as the reproductive form, and one isolate was homologous to the vaccine strain. In Study 2, BHV-1 was isolated from nasal swabs and lung lavage samples from 3 calves after vaccination. These BHV-1 were homologous to the vaccine strain based on the UL36 region. Isolates from the Study 3 are now being compared using the sequencing of the two different regions.

This study shows that MLV BHV-1 can be recovered from the nasal swabs and lung lavage samples in vaccinated calves under field conditions. It also draws focus on the pathogenicity of vaccine strains, and epidemiology of BHV1 subtypes: (1) calves can be naturally infected with the genital strain, BHV-1.2 as evidenced by the recovery of virus in the nasal swabs; (2) strains recovered in nasal swabs from calves receiving MLV vaccines given subcutaneous may be of vaccine origin as found in the calves in both Study 1 and 2 ; (3) the MLV BHV-1 strains may also be recovered from the lungs after vaccination as found in the Study 2. Potentially use of molecular studies with sequencing may assist in differentiating BHV-1 strains in cattle.

A multiplex method for the serological detection of swine respiratory pathogens

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Porcine circovirus type 2 (PCV2) and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) are two major contributing factors to the porcine respiratory disease complex (PRDC). Serological monitoring plays an important role in the routine diagnosis of these and other pathogens involved in the PRDC. Micro-sphere-based technology permits the development of multiplex testing to detect antibodies to several pathogens from a single serum sample, thus reducing the need for multiple, individual ELISA tests. In this study, we have developed a duplex assay for the simultaneous detection of antibodies to PCV2 and PRRSV.

Recombinant PRRSV N protein antigen was produced in a bacterial expression system while the PCV2 capsid antigen was produced in a baculovirus expression system. A non-recombinant baculovirus designated as the wild type antigen was included as a control to eliminate background signals. 8ug of PRRSV N antigen, 12 ug of PCV2 capsid antigen and 6 ug of wildtype antigen were coupled to fluorophore dyed beads with distinct spectral addresses. Weekly sera samples from 72 pigs that were experimentally exposed to PCV2, PRRSV or both PCV2 and PRRSV over a 28 day period were used to validate the assay. All sera samples were previously tested using an in-house PCV2 ELISA and a commercial PRRSV ELISA. 2.5 x10 ^{2} beads representing each antigen were incubated with the sera samples in 96 well filter plates. Specific reactivity to each antigen was measured as the median fluorescence intensity (MFI). The magnitude of the PCV2-specific antibody response was measured as the difference between the MFI values for the capsid antigen and the wildtype antigen.

PCV2 exposed pigs sero-converted between day 7 and day 14 while the unexposed controls remained negative throughout the study. The kinetics of the PCV2-specific antibody responses as measured by the MBA was comparable to the in-house PCV2 ELISA which has a sensitivity and specificity of ≥90%. The trend was similar for PRRSV-specific immune responses in the MBA assay which were comparable to the commercial PRRSV ELISA (sensitivity of ≥98%). Multiplexing did not interfere with assay performance or diagnostic sensitivity. Our future work will focus on including the detection of other swine respiratory pathogens in the panel. **Therefore, the MBA is a practical and reliable method for simultaneous detection of antibodies to several agents, thus saving labor and time. This technology could potentially have great utility in sero-epidemiological studies and for herd-profiling.**

Porcine reproductive and respiratory syndrome virus (PRRSV) in serum and oral fluid samples from individual boars: Will oral fluid replace serum for PRRSV surveillance?

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Introduction: Previous experiments used pen-based oral fluids to monitor porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV) 2 infections in wean-to-finish populations [1,2]. The purpose of this study was to determine whether oral fluid samples could be used to monitor individually-housed adult boars for porcine reproductive and respiratory syndrome virus (PRRSV) infection.

Methods: In 3 trials, 24 boars, 5.5 months to 4 years in age, were intramuscularly (IM) inoculated with a modified-live PRRSV (MLV) vaccine (Trial 1), a Type 1 PRRSV isolate (Trial 2), or a Type 2 isolate (Trial 3). Oral fluid samples were collected daily and serum samples were collected twice weekly. Following the completion of the study, samples were randomized and blind-tested for PRRSV by real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

Results: With the exception of 2 boars in Replicate 2, all boars provided oral fluid samples. A total of 2088 oral fluid samples were attempted i.e., 29 days x 24 boars x 3 trials, 1954 (93%) samples were collected. The average volume of oral fluid collected per boar across all trials was 17.9 ml (range: 1 to 39 ml). PRRSV was detected in oral fluids at DPI 1 and all oral fluid specimens were PRRSV qRT-PCR positive at DPI 4. Although PRRSV was detected in both serum and oral fluid specimens through DPI 21, a comparison of matched samples from individual boars showed that oral fluid was equal to serum for the detection of PRRSV at DPI 7 and more likely to be positive than serum on DPI 14 and 21. Overall, oral fluid was superior to serum for the detection of PRRSV using PCR over the 21 day observation period in this study.

Conclusions: The results of this experiment suggest that **individually-penned oral fluid sampling could be an efficient, cost-effective approach to PRRSV surveillance in boar studs and other swine populations.**

Detection of anti-PRRSV antibodies in oral fluid samples from individual boars using a commercial ELISA

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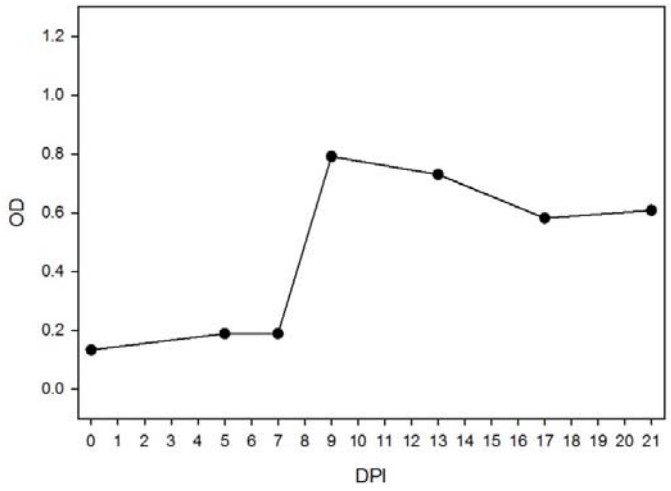
Introduction: Oral fluid samples are of particular interest because of their ease of collection and documented use in surveillance of PRRSV and PCV2 in group-housed pigs under experimental and field settings [1,2] . Furthermore, the previous results reported PRRSV isolation from oral fluids collected up to 21 days post inoculation (DPI) from individually penned boars. Therefore, the purpose of this study was to determine whether oral fluid samples could be used to monitor individually-housed adult boars for anti-PRRSV antibodies.

Methods: In 3 trials, 24 boars, 5.5 months to 4 years in age, were intramuscularly (IM) inoculated with a modified-live PRRSV (MLV) vaccine (Trial 1), a Type 1 PRRSV isolate (Trial 2), or a Type 2 isolate (Trial 3). Oral fluid samples were collected daily and serum samples were collected twice weekly. Following the completion of the study, samples were randomized and blind-tested for anti-PRRSV antibodies by HerdCheck PRRSV X3.

Results: Figure 1 shows OD results over time in oral fluid. Anti-PRRSV antibodies were detected at nine days post inoculation (DPI).

Conclusion: Detection of PRRSV antibodies in oral fluids is a useful diagnostic tool and can be used in oral fluid to monitor PRRSV infection in commercial swine herds.



Molecular characterization of influenza A viruses from swine for potential virulence markers

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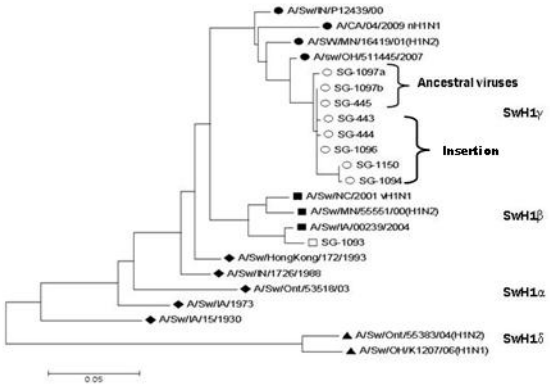
Introduction: As influenza A viruses continue to emerge and evolve through reassortment and antigenic drift in the US swine population, novel strains have arisen over the last decade that have resulted in changes in influenza epidemiology and persistence within endemically infected populations of pigs. The hemagglutinin (HA) gene of all influenza viruses that were detected in samples submitted from one endemically infected farm system (farm M) have been sequenced since 2005. In 2008, a two amino acid (AA) insertion (Glu-Lys) was found at position 156 using the H1 numbering system. The viruses with the insertion were isolated from pigs in specific barns in a multi-site rearing system from farm M that were experiencing increases in piglet mortality. To better understand how this insertion relates to the pathogenicity of these influenza viruses, five viruses with the insertion and three potential ancestral viruses without the insertion were selected for further molecular analysis.

Materials and Methods: The full genomes of eight viruses were sequenced at St. Jude Children's Research Hospital in Memphis, TN. The sequences were analyzed using MEGA 4 with Clustal W alignment (www.megasoftware.net) the three-dimensional models of the antigenic sites were examined using PyMOL (www.pymol.org).

Results: Phylogenetic analysis of the viruses revealed that the isolates clustered tightly together with the highly pathogenic virus A/Sw/OH/51145/2007 (H1N1) in the SwH1 gamma clade (Figure 1). Analysis of the protein sequence revealed that **the two AA insertion at position 156 disrupted the Sb and Sa antigenic sites which are proximal to the HA receptor binding pocket in the three-dimensional protein structure.** This was the only variation in the antigenic sites for two of the five viruses. One virus also had one AA change at position 70. Two other viruses also had AA changes at 74, 139, 162 and 168.

Discussion: Molecular characterization of influenza viruses has become one of the key tools used by researchers to examine the continual changes that are found in viruses isolated from the U.S. swine population. By sequencing the viruses that are endemic in pigs, we can screen the circulating viruses for significant genetic changes, compare multiple viruses isolated from the same population over time, as well as to viruses in available vaccines. These changes will provide a benchmark for evaluating how the virus evolves in swine. This information is critical for understanding the epidemiology of the disease caused by the virus within a farm or production system, especially when the molecular data can be combined with clinical signs and post-mortem findings.



Antigenic site (Total no. AA residues)	SG-443 and SG-1096		SG-444		SG-1094 and SG-1150	
	No. changes	AA position	No. changes	AA position	No. changes	AA position
Sa (13)	0	n.a.	0	n.a.	1	162
Sb (9)	1	156	1	156	1	156
Ca (14)	0	n.a.	0	n.a.	2	139,168
Cb (8)	0	n.a.	1	70	1	74

Table 1. The number and position of AA changes in antigenic sites of HA1 of the viruses containing the insertion compared to the ancestral viruses.

Figure 1. Phylogenetic tree of eight viruses (O) analyzed from farm M.

Pen level sensitivity for detecting influenza virus in swine oral fluids

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Introduction: The use of oral fluids as a monitoring tool for detecting porcine viral pathogens is increasing in North America. Porcine oral fluids are now routinely used for detecting porcine reproductive and respiratory syndrome virus from field samples and detection of influenza virus in oral fluids collected from experimentally infected pigs has also been reported. In this abstract, we report the use of oral fluids to detect influenza A virus in pigs in a pen-based experimental population setting.

Materials and Methods: Samples for this study were collected as part of a transmission study determining rates of influenza A virus (flu) in vaccinated and non-vaccinated pig populations. Briefly, thirty 3-week-old piglets negative for influenza were randomly distributed in separate rooms of 10 pigs each. In this study each room represents an individual pen. The pens were designated as control (C), heterologous vaccinated (HE), and homologous vaccinated (HO). Pigs from group C were not vaccinated against flu. Pigs in the HE and HO pens were vaccinated at arrival and two weeks later with a heterologous commercial vaccine and a homologous vaccine, respectively. Thirteen days after the second vaccine, 3 unvaccinated pigs were infected with A/Sw/IA/0239/2004 H1N1 and at 48 h post infection, one challenged pig was moved into each of the treatment pens. Infection was evaluated by collecting individual nasal swabs from all pigs daily. Three replicates from the same experiment were performed for the HE and HO pens and two replicates for the C pen. Oral fluids were collected daily by using 3-strand twisted cotton ropes tied to the pens for oral fluids to accumulate as the pigs chewed on the ropes. Oral fluids were wrung from the ropes and refrigerated at 4°C for 24 h to allow separation of debris from the fluids. Pen-based oral-fluids and individual nasal swabs were assayed for influenza A virus matrix gene RNA by real time reverse transcription PCR. Statistical analysis were performed to compare PCR results from oral fluids and nasal swabs using Fisher's Exact Test, as well as a logistic regression model to test the predicted probabilities for influenza detection in oral fluids (SAS System, SAS Inst., Cary, North Carolina, v 9.2).

Results: A pen was considered flu positive if there was at least one individual nasal sample positive. A total of 1155 individual nasal swabs were collected and 46 pens were considered positive. One hundred five oral fluid samples were collected throughout the study of which 38 were positive. 82.6 % of the flu RRT-PCR results in oral fluids were in agreement with the pen status results (at least one positive individual sample per pen). The predicted probability of detecting influenza in oral fluids from a pen with 11 pigs was 50% if 1 pig was infected, increasing to 99% if 2 pigs were infected and to 99.9% if 3 to 10 pigs were infected.

Conclusion: Results from our study indicate that pen based collection of oral fluids is a sensitive method to detect flu in infected populations even when within pen prevalence is low.

Microbiology Scientific Session

Saturday, November 13, 2010
Salon C

Moderators: Erdal Erol and Lindsay Oaks
Sponsor:

01:00 PM **Current status on the development of operator safe diagnostic tools for Rift Valley Fever**
William Wilson, Hana Weingartl, Jieyuan Jiang, James Neufeld, Brett Dalman, Peter Marszal, Barbara Drolet, Kristine Bennett, Myrna Miller, Leonard Ateya, Yatinder Binepal, Jacqueline Lichoti, Joseph Macharia, James Mecham 56

01:15 PM **Methods that increase the sensitivity of *Mycobacterium avium* subspecies *paratuberculosis* culture as a diagnostic test using samples from serology positive sheep and goats**
Beth E. Mamer, M. Wayne Ayers, Marie S. Bulgin. 57

01:30 PM **Long-term persistent shedding of *Coxiella burnetii* in dairy milk by naturally infected cows**
Sung G. Kim, Renee R. Anderson, Eun H. Kim, Amy E. Cassano, Nancy C. Zylich, Jin Z. Yu, Edward J. Dubovi 58

01:45 PM **Do the criteria used to interpret the microscopic agglutination test (MAT) for the diagnosis of canine leptospirosis need to be changed?**
Ronald D. Schultz, E. Mukhtar, Laurie J. Larson, Ogi Okwumabua 59

02:00 PM **VMRD: Past, Present and Future**
Scott Adams, DVM, PhD, President and CEO of VMRD, Inc.

02:15 PM **Characterization of a previously unrecognized pneumovirus in domestic dogs with acute respiratory disease**
Randall Renshaw, Nancy Zylich, Melissa Laverack, Amy Glaser, Edward Dubovi. 60

02:30 PM **Acute BVDV infections of Boer goats (*Capra hircus*) with BVDV type 2 isolates: Molecular evidence of horizontal transmission from persistently infected cattle to goats**
Sung G. Kim, Renee R. Anderson, Katrina J. Walker, Steven Yousey, Nancy C. Zylich, Edward J. Dubovi. 61

02:45PM ***Providencia vermicola* septicemia resulting in acute death in a domestic rabbit (*Oryctolagus cuniculus*)**
Lalitha Peddireddi, Carl Myers, Baoyan An, Jianfa Bai, Richard Oberst, M. M. Chengappa, Gary Anderson, Kyathanahalli S. Janardhan. 62

Exhibit Hall Open 9:30am - 6:00pm

Current status on the development of operator safe diagnostic tools for Rift Valley Fever

William Wilson[1], Hana Weingartl[2], Jieyuan Jiang[2], James Neufeld[2], Brett Dalman[2], Peter Marszal[2], Barbara Drolet[1], Kristine Bennett[1], Myrna Miller[1], Leonard Ateya[3], Yatinder Binepal[3], Jacqueline Lichoti[4], Joseph Macharia[4], and James Mecham[1]

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Rift Valley fever (RVF) is a disease of animals and humans that occurs in Africa and the Arabian Peninsula. It is caused by a *Phlebovirus* in the family *Bunyaviridae.* Mosquito-borne epidemics occur during years of unusually heavy rainfall. Domestic cattle, sheep and goats are highly susceptible to infection, which can result in high mortality in young animals and increased abortion in adults. Unapparent infections are quite common in wild ruminants. Infection in humans causes influenza-like symptoms, but can lead to severe complications, including retinopathy, blindness and even death. An international team has been working to develop diagnostic tools that do not pose a health risk to the operator that can be used for the early detection of an introduction of RVF into North America. One of these diagnostic tools is a multiplex real-time RT-PCR that detects all three segments of RVF viral RNA and can distinguish between wild-type and some candidate attenuated vaccine strains. This assay was field tested at the Kenya Agriculture Research Institute and Kenya Department of Veterinary Services. The assay performed well, did not cross-react with Nairobi sheep disease virus and was advantageous over existing assays. Immunological assays based on expressed Gn, N and NSs have also been developed and laboratory evaluated. These interactions along with the assistance of USDA, APHIS will allow the development of internationally harmonized diagnostic tools for RVF.

Methods that increase the sensitivity of *Mycobacterium avium* subspecies *paratuberculosis* culture as a diagnostic test using samples from serology positive sheep and goats

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Caine Veterinary Teaching Center Caldwell, ID 83607

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's disease in ruminant species. This contagious bacterium targets mesenteric lymph nodes and intestines of infected animals, causing a chronic infection that result in wasting and death. MAP are facultative intracellular bacteria that are transmitted from adult animals to young in utero, through colostrum, milk and feces. Mycobacterium infections are cell-mediated diseases; therefore, antibody is not produced consistently while infected animals are alive. MAP bacteria are shed late or not at all in Johne's disease clinical sheep and goats. MAP infected animals cannot be cured, and control depends on detection and removal of positive animals to prevent infection of non-infected animals. A positive MAP culture is the standard to accurately identify animals from a farm. This testing was initiated to improve the sensitivity of diagnostic tests to identify MAP positive animals for elimination from flocks and herds. Ante-mortem and post-mortem tests for MAP suspect animals:

- **MAP culture** of feces and tissues, fresh or frozen prior to set up, using 10 ml sediment inoculum and one year in culture with liquid culture media: BACTEC™ MGIT™ para TB liquid medium.
- Two serology **MAP ELISAs** for testing serum and milk samples: IDEXX Herdchek - 0.250 S/P cutoff and IDEXX Pourquier - 0.300 S/P cutoff.
- **Acid-fast tissue histopathology**.

The majority of our samples come from five cooperator producers. These producers include sheep range flocks/farm flocks/goat herds. Other producers bring thin animals to us for diagnostic assessment. Animals came from three states and 15 farms. Many individual animals that were MAP serology positive are eventually necropsied, and samples are tested with culture and histopathology.

Thirty Herdchek ELISA positive animal tissues were culture positive for MAP. Twenty-four of these animals tested positive with the Pourquier ELISA. All of these animals had at least one tissue with acid fast bacilli (AFB). The majority of samples were set up from frozen tissues. Tissues from two animals were cultured as fresh tissues and in duplicate as frozen tissues. The fresh tissue cultures were positive in 5 to 6 weeks; the frozen tissues were culture positive in 3 weeks. Some of these tissues were set up as: three liquid culture tubes for each animal (lymph nodes, intestinal tissues, feces); and as composite samples-all samples from one animal in one culture tube. Composite cultures required 1.5 months (2 weeks –7 months) longer to become culture positive than three culture tubes for one animal. Intestinal content samples from paucibacillary animals were culture positive one month earlier than their lymph node samples. Lymph node and intestinal tissues from multibacillary animals were culture positive in two weeks, but four of these animals were fecal culture negative. Frozen composite tissues and fecals from three animals were set up in duplicate, with and without yeast/malachite green decontamination (Y/MG). Those set up with the standard method were culture positive in three months; the duplicate samples set up with Y/MG were culture positive in five months.

Only 36 fecal samples were culture positive of 140 Herdchek ELISA positive symptomatic animals. Those that tested positive (35) with the Pourquier ELISA were more likely to be culture positive. One doe that was fecal culture negative, milk and serum ELISA positive at least twice/year with both ELISA tests, was admitted with two bucklings that were necropsied. One of these bucklings was AFB positive/culture positive. **MAP culture sensitivity is improved by freezing any samples from serology positive animals prior to culture, incubating the culture samples at least 10 months, and AFB staining tissue samples prior to culture if only one culture is set up per animal.**

Long-term persistent shedding of *Coxiella burnetii* in dairy milk by naturally infected cows

Sung G. Kim[1], Renee R. Anderson[2], Eun H. Kim[2], Amy E. Cassano[2], Nancy C. Zylich[2], Jin Z. Yu[2], Edward J. Dubovi[2]*

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The shedding of *Coxiella burnetii*, Q fever agent, in bovine milk by naturally infected dairy cows at individual and herd levels was examined using a real-time Trans-PCR assay targeting the *ISlllla* sequence. Greater than 91% of dairy herds (434/473) tested positive for *C. burnetii* based on bulk tank sample testing over a period of 3 years. About 39.4% (254/645) of individual cows from 3 dairy herds were shedding *C. burnetii* based on composite milk testing. The shedding patterns of individual cows were monitored at daily, weekly, and 2-3month term intervals, for a period of 7 days, 6 weeks, and 10 months, respectively. The shedding of *C. burnetii* in milk by infected cows was shown to be consistent throughout the study period. Weekly shedding at quarters of infected cows was also shown to be consistent, but the number of shedding quarters varied between cows. About 61.7% (37/60) of infected cows shed from all 4 quarters and the rest shed from 1 to 3 quarters. **Some of infected cows continued to shed over 2 to 3 lactation periods, suggesting parturition did not clear *C. burnetii* from the infected cows. In contrast to the previous reports, shedding *C. burnetii* in milk by naturally infected cows was consistent for a long term period.**

Do the criteria used to interpret the microscopic agglutination test (MAT) for the diagnosis of canine leptospirosis need to be changed?

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The most common serologic test used for the diagnosis of canine and bovine leptospirosis is the microscopic agglutination test (MAT). The MAT detects IgM and IgG antibody to multiple serovars of leptospira. This test is relatively sensitive and specific. Many laboratories will perform the MAT with serovars *Leptospira autumnalis, L. bratislava, L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae,* and/or *L. pomona*. During the past eight years, two new 4-way canine leptospira bacterins have been licensed to replace the traditional 2-way bacterins that have been used since the 1970's. The 2-way vaccines contained the two most important canine serovars, *L. canicola*, in which the dog serves as the natural reservoir, and *L. icterohaemorrhagiae*. The two new serovars added were *L. grippotyphosa* and *L. pomona,* both capable of causing disease in the dog. The introduction of the new vaccines has created a renewed awareness and interest in leptospirosis in dogs. More dogs are being vaccinated with the 4-way vaccines than were being vaccinated with the 2-way products, and the vaccines are being given annually to dogs at high risk. Due to the addition of the 2-new serovars, the new vaccines cause more cross reactivity on the MAT with certain of the serovars that are used in the test, but that are not in the vaccines and do not cause disease in the dog – namely, *L. autumnalis* and *L. bratislava.* These two serovars are frequently positive, at relatively high titers, in dogs vaccinated with the 4-way vaccines.

In the present study, we examined the MAT to determine:

1. If it would reliably predict the serovar that infected the dog
2. If the-2 way and 4-way vaccine induced antibody to all or most of the serovars tested
3. If a titer of ≥1:800 on a single sample could be used in the vaccinated dogs to diagnose infection, as has been suggested
4. If titers at various times after vaccination with experimental monovalent serovar vaccines cross reacted with multiple serovars and/or induced titers that would be considered indicative of infection
5. If the antibody titers to *L. autumnalis* and/or *L. bratislava*, which are often higher than for other serovars, were affecting the accuracy and the value of the MAT as a test for the diagnosis of canine leptospirosis.

Based on our results, we propose new criteria that could be used to diagnose canine leptospirosis. **The criteria we propose using for diagnosis of disease are:**

1. **MAT titers to only serovars *L. canicola, L. grippotyphosa, L. icterohaemorrhagiae,* and/or *L. pomona* should be used for the diagnosis of canine leptospirosis.**
2. **With a single sample, a titer should be equal to or greater than 1L1600 for one or more of these 4 serovars.**
3. **When acute and convalescent samples are available, the increase in titer to one or more of the 4 serovars should be an 8-fold or greater increase.**
4. **An acute titer of 1:1600 or greater would constitute a positive sample, even if the convalescent sample did not show an 8-fold increase.**

We believe these changes will significantly increase the value of the MAT as a diagnostic test to demonstrate canine leptospiral disease rather than infection with a leptospira serovar that is not able to cause disease.

Characterization of a previously unrecognized pneumovirus in domestic dogs with acute respiratory disease

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During a recent survey of shelter dogs involved in an outbreak of acute respiratory disease, nasal and pharyngeal swab extracts were applied to canine A72 cells to isolate respiratory viruses. Following extended passage in culture, an atypical cytopathic effect was observed in several of the flasks. Extensive testing for canine respiratory agents failed to identify the virus. Testing for viruses that infect other species ultimately produced a positive signal using monoclonal antibodies (Mabs) to respiratory syncytial virus (RSV).

Attempts to amplify the virus using RSV-specific PCR primers were unsuccessful. Degenerate primers for conserved regions in the nucleocapsid (N) and large polymerase (L) genes were designed based on conserved amino acid (aa) sequences within multiple sequence alignments of all viruses in the *Pneumovirinae* subfamily. **Amplification using these primers yielded 3 fragments with sequences that were ~96-97% identical to murine pneumovirus (MPV).** MPV is known to be weakly antigenically related to the RSVs but it is relatively divergent in aa sequence as compared to the more closely grouped human, bovine and ovine RSVs. Further confirmation of the viral antigen in A72 cultures was obtained using a mono-specific polyclonal antibody to MPV.

Generation of sequentially overlapping PCRs was used to determine the sequence of 9 of the 10 genes in the genome of a tissue culture passage 4 isolate. Overall, the nucleotide identity of the canine pneumovirus was ~95% when compared to MPV strains 15 and J3666. The most divergent aa sequences as compared to MPV were in the G protein with ~92% identity and the NS1 and NS2 proteins with ~ 94% identity. Predicted protein sequences with the highest identity were N and M with 98% identity.

Acute BVDV infections of Boer goats (*Capra hircus*) with BVDV type 2 isolates: Molecular evidence of horizontal transmission from persistently infected cattle to goats

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Bovine viral diarrhea virus (BVDV) was detected by real-time RT PCR in 4 Boer goats (*Capra hircus*) from a farm with 75 goats, 34 beef cattle, and 5 sheep. All sheep and 32 beef cattle were negative for BVDV; 2 yearling calves were BVDV positive. Virus isolation from the four PCR-positive goats was not successful, but virus was isolated from the two positive cattle. Those two BVDV positive cattle were confirmed persistently infected in contrast to the 4 goats that were acutely infected. Further PCR amplification and sequencing of the 5' UTR, N^{pro}, and partial C gene of BVD viruses from 4 goats and 2 BVDV isolates from persistently infected cattle revealed that the sequence of one bovine isolate was 100% identical to the sequences of the 4 goat BVD viruses The subsequent phylogenetic analysis showed the BVDV isolate belonged to the BVDV type 2 lineage. **This is the first report providing molecular evidence of horizontal transmission of BVDV from persistently infected cattle to goats, and the finding of BVDV type 2 in goats in North America.**

Providencia vermicola septicemia resulting in acute death in a domestic rabbit (_Oryctolagus cuniculus_)

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Bacteria belonging to the genus *Providencia* are facultative anaerobes and some of its important members are *Providencia stuartii (P. stuartii), Providencia rettgeri (P. rettgeri) and Providencia alcalifaciens (P. alcalifaciens)*. These bacteria are known to cause diseases mainly in humans. *P. stuartii and P. rettgeri* are associated with urinary tract infections while *P. alcalifaciens* is associated with gastroenteritis. In domestic animals, except for a few scattered reports, *Providencia spp*. are usually considered as contaminants and not considered to be significant pathogens. Here we present a case of acute death in a rabbit from which we isolated *Providencia sp.* and further characterized it as *Providencia vermicola*. An adult rabbit with no prior clinical illness was found dead and was submitted for necropsy. Grossly, there were multifocal petechial hemorrhages on the cecal serosa. The colon contained no formed feces. Histologically, the serosa of the cecum and colon were infiltrated by small to moderate numbers of heterophils mixed with a small amount of fibrin. Gram negative rod-shaped bacteria were present within the fibrin. The bacteria isolated from lung, liver, spleen, kidney, and cecum was identified as *Providencia* species based on the initial biochemical analysis. Subsequent molecular evaluation of 16S rRNA and fusA genes by polymerase chain reaction (PCR) followed by sequencing identified the bacterial isolate as *Providencia vermicola* (99.9% sequence match). BLAST search analysis of the sequence obtained also showed a significant homology to *P. rettgeri* (~99.5%) sequences. However, when 150bp of 5′ end variable region sequence alone was included in the BLAST search, the sequence is 100% identical to the *P. vermicola* and was only 96-98% identical to the *P. rettgeri*. We found no reports in the literature implicating *P. vermicola* as a cause of septicemia in rabbits or other animals. Bacteria in the genus *Providencia*, when isolated, should be considered as a significant pathogen. Further, it is important to understand the zoonotic potential of *P. vermicola* as many other bacteria in this genus can infect humans.

Toxicology Scientific Session

Saturday, November 13, 2010
Room: Duluth

Moderators: Catherine Barr and Cindy Gaskill

Exhibit Hall Open 9:30am - 6:00pm

Anticholinesterase agents: Global challenges in terrorism, toxicity, treatment and beyond

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Organophosphates (OPs) and carbamates (CMs) constitute a large class of synthetic chemicals primarily used as pesticides around the world. These compounds are also indicated in human and veterinary medicine. Unfortunately, some OPs have also been used as nerve agents in chemical warfare, and by dictators, extremists, terrorists, and cult leaders. The first OP, tetraethyl pyrophosphate, was synthesized by Phillipe de Clermont in France in 1854. About eighty years later, Gerhard Schrader of Germany synthesized parathion for insecticidal purposes. Prior to, during, and after World War II, OP nerve agents of the G series (tabun, GA; sarin, GB; soman, GD; cyclosarin, GF) and super toxic agents of the V series (English VX, Russian VX, Chinese VX, agent IVA, Novichok-5, Novichok-7) were developed in Germany, the UK, the USA, Russia, and China, and produced and stockpiled in many other countries. Currently more than 25 countries possess nerve agents which can be used as Chemical Weapons Mass Destruction (CWMD). More than 200 OPs and 2 dozen CMs are on the market for a variety of purposes.

On multiple occasions, OP and CM compounds have been encountered in chemical warfare and accidental release, or used by dictators, terrorists, or extremists. To mention just a few incidents: in 1968, >6,400 sheep were killed in the Dugway incident with VX spray in Skull Valley, AZ; in the I980s, 100,000 human casualties occurred in Iran-Iraq conflict; and in 1984, >400,000 people were exposed to methyl isocyanate (precursor of carbaryl) in Bhopal incident; approximately 20,000 people and 4,000 animals died. In the Tokyo subway sarin attacks in 1994-1995, 12 people died, >5,000 were injured and millions were terrified in Japan and around the world. In these incidents, the Gulf War II in 1991, and current US-Iraq and US-Afghanistan conflicts, there is little or no account of deaths or injuries to animals.

Each year, approximately 1 million people are poisoned and several hundred thousand die from OPs and CMs as a result of accidental exposure or malicious intent. Similar data for animals is not available. With either type of insecticide, toxicity is most often acute. OPs can also induce Intermediate Syndrome (IMS) or OP-Induced Delayed Neuropathy (OPIND). In the acute case, although the initial trigger is inactivation of acetylcholinesterase (AChE) enzyme at the synapses in the brain and at the neuromuscular junctions, other systems (e.g. NMDA receptor) are involved in excitotoxicity. Current evidence suggests that many OPs and CMs directly interact with muscarinic acetylcholinesterase receptors (mAChRs) and nicotinic acetylcholinesterase receptors (nAChRs). Ultrastructural-neuropathological alterations are due to excess generation of free radicals, leading to depletion of high-energy phosphates, and ultimately cell death occurs due to apoptosis or necrosis. Death of an animal/human ensues due to paralysis of the respiratory center and muscles. In essence, multiple mechanisms of action are involved.

In the last 75 years of research, significant progress has been made in the development of sensitive biomarkers of exposure/effects and antidotal treatment. We have the life saving antidote, atropine sulfate (mAChR blocker), but the quest continues for a universal AChE reactivator. Hundreds of oximes have been synthesized and tested against OPs, but only five (pralidoxime, trimedoxime, obidoxime, methoxime, and HI-6) are commercially available. Current experimental research proves that K-series oximes are superior when compared to other oximes against OP nerve agents. So far, efforts to develop a universal antidote have been futile due to the diversity and complexity of OP and CM structures.

In summary, our current global challenges are to precisely understand the molecular mechanism of anticholinesterase toxicity, and to develop novel biomarkers of exposure, sensors and detectors, on-site and laboratory analytical methods, and more effective antidotes, in addition to strategic plans to combat terrorists' threats.

Acute avocado (*Persea americana*) intoxication in goats: Two cases

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Avocado (*Persea americana*) is a widely cultivated plant in California and Florida. There are a number of cultivars and hybrids within the *P. americana* species. The toxicity of *P. americana* has been suspected since the early 1900s but detailed observations of clinical intoxication were not described until the 1980s. The leaves, fruit and seeds of the plant are considered to be toxic, with the leaves being the most toxic. Of all of the cultivars and hybrids of *P. americana*, only the Guatamalan variety and the Fuerte hybrid have been shown to be toxic. A variety of animal species have been naturally or experimentally intoxicated by *P. americana* including cattle, goats, horses, mice, rabbits, rats, sheep, ostriches, pet birds and dogs. Persin is believed to be responsible for the toxic effects, which target the lactating mammary gland and the myocardium. Although avocado tree intoxication has been reported in goats from Australia and South Africa, there are no published reports of intoxication in North America. In the first case reported here, a branch from an ornamental avocado tree fell into a neighbor's yard following a freeze in December. Two goats had access to the fallen branch. An approximate 5 year old male, Nubian goat was found dead in the barn and was subsequently submitted for necropsy. Gross and microscopic findings included pulmonary congestion and multifocal, necrotizing, neutrophilic myocarditis. Abundant avocado leaf fragments were identified in the rumen contents by microscopy. In the second case, a herd of 68 Boer goats had been in an avocado orchard for approximately 7 days in mid-March. Seven yearlings died on days 4 and 5 and one 2 to 3 year old lactating doe died on day 6. The doe was necropsied by the herd veterinarian and a variety of formalin-fixed tissues submitted for histopathology. Multifocal, acute myocardial necrosis with moderate pulmonary edema and mild hemorrhage were noted. The rumen contents contained a large number of avocado leaf fragments that were positively identified by microscopy. Unfortunately, no mammary tissues were submitted for evaluation, although three other does in the herd reportedly had partially hardened udders. Small numbers of *Bibersteinia trehalosi* was isolated from the lungs from the goat of Case 1 and was considered an incidental finding. An additional finding in case two included a moderately low liver selenium concentration (0.15 ppm, wet weight). The diagnosis of avocado intoxication relies on a history of ingestion of the plant, clinical signs referable to acute cardiac failure or mastitis, and postmortem cardiac and mammary gland necrosis. **Identification of avocado leaves in rumen contents is important to confirm exposure since analytical testing for persin is not currently available.** Microscopic examination for characteristic leaf morphology can be essential for proper plant identification. Other cardiotoxins such as plant-derived cardiac glycosides, ionophores, selenium excess, and selenium and/or vitamin E deficiencies should be considered in the differential diagnosis.

When every milli-mass unit counts: the application of high resolution mass spectrometry in a veterinary diagnostic laboratory

Mike Filigenzi, Elizabeth Tor, Linda Aston, Robert Poppenga
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Mass spectrometers, generally interfaced to gas or liquid chromatographs, offer powerful capabilities for the detection of toxins in a wide variety of matrices. These instruments have rightfully become workhorses for analytical toxicology in veterinary diagnostic laboratories. One technique involving these instruments has remained a rarity in this setting, though, and that is high resolution mass spectrometry. In the past, this technique required the use of instruments that were either exotic and expensive (such as magnetic sector instruments or ion cyclotron resonance machines) or which were limited in their resolution and quantitative capabilities (such as time-of-flight instruments). In the last few years, a new type of mass spectrometer has hit the market, based on Orbitrap technology. These instruments offer extremely high mass resolution capabilities, accurate mass measurement, ease of use, and price tags that at least begin to approach a range that will not cause a laboratory director to immediately faint. They offer capabilities for both targeted screening and for the detection of unknowns.

One toxin which can benefit from accurate mass analysis is anatoxin-a. Anatoxin-a is a potent neurotoxin produced by several species of blue-green algae. Detection of this toxin in biological matrices is difficult due to the common presence of high concentrations of the amino acid phenylalanine. Both compounds have similar chemical properties, co-elute on HPLC columns and produce similar product ions when analyzed by a single quadrupole or MS/MS technique. LC-MS/MS/MS analysis has been used in this laboratory to prevent mis-identification of anatoxin-a. High resolution mass spectrometry offers another method of separating these compounds since the molecular weight of anatoxin-a is 165.1154 and that of phenylalanine is 165.0790. **Use of an Orbitrap-based instrument for this analysis as well as others, including requirements for mass accuracy and resolution, will be discussed.**

Use of dried blood spot technology in veterinary diagnostic toxicology

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Dried Blood Spot (DBS) technology has been in use for decades for the assessment of the health status of human neonates for congenital defects such as phenylketonuria. DBS sampling has distinct advantages for field testing, for ease of storage and shipping, is amenable for use on patients with large and small blood sample volumes such small birds, small animals, reptiles and amphibians. The methodology is simple. Fifty microliters of whole blood are applied to one or more circles on a special paper card which is then mailed to the diagnostic laboratory for analysis.

We have extended this technology and validated two assays: a) DBS heavy metal panel which includes arsenic, cadmium, lead, mercury, selenium, thallium; and b) DBS chlorinated compounds panel which includes some organochlorine insecticides (4,4'-DDE, 4,4'-DDT, γHCH), and a marker for polychlorinated biphenyls (congener 153). Validated parameters include precision, accuracy, linearity, limits of detection and quantitation. **The limit of quantitation by ICP/MS is 2 ppb for arsenic and thallium, 5 ppb for cadmium and selenium, and 13 ppb for lead and mercury. The limit of quantitation by gas chromatography with electron capture detection (GC-ECD) for the chlorinated compounds is 10 ppb.** These limits of quantitation are sufficient both for diagnostic purposes and also for basic research.

Utilizing this technology, we have demonstrated that neither heavy metals nor chlorinated compounds are responsible for decreasing breeding success in Israeli griffon vultures (*Gyps fulvus*). We have also demonstrated that African penguins (*Spheniscus demerus*) have 10 fold higher concentrations of DDT metabolites (op DDT and ppDDE) than Magellanic penguins (*Spheniscus magellanicus*) from South America.

An immunohistochemical study of equine nigropallidal encephalomalacia

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Introduction. Equine nigropallidal encephalomalacia (NPE) is a neurological disease of horses which has been likened to human Parkinson's disease (PD). It is caused by chronic ingestion of two poisonous plants: yellow star thistle (*Centaurea solstitialis*) and Russian knapweed (*Centaurea repens*). Invariably, paralysis of lips and of the tongue is the first clinical presentation, incapacitating the horse's ability to eat. There is reduced jaw tone, causing the mouth to remain partially open with the tongue protruding. The histopathology of NPE has been well documented based on H&E sections, revealing circumscribed and mostly bilateral necrosis in the substatia nigra and the globus pallidus. Although likened to human PD, there are no published immunohistochemical studies to demonstrate if the two diseases are similar or not. The objective of this experiment was to do an immunohistochemical study of brain tissue from horses confirmed to have died of NPE to determine if this disease is similar to human PD.

Materials and Methods. Case materials were either paraffin-embedded blocks or formalin-fixed brains from equine NPE cases from California and Colorado. Tyrosine hydroxylase and alpha synuclein are markers used for diagnosis of PD and were used in this study, following routine procedures. The primary antibodies used in this study included were monoclonal mouse anti-tyrosine hydroxylase (Clone LNC1, Chemicon/Millipore, Temecula, CA, at 10-30 µg/ml or 1:100 dilution of stock solution), and polyclonal rabbit anti-alpha synuclein (Chemicon / Millipore, Temecula, CA, at 1:1000 dilution of stock solution.

Results. Immunohistochemical staining with anti-tyrosine hydroxylase (TH) confirmed that the lesions are located within the substantia nigra pars reticulata (SNr), with essentially no involvement of the dopaminergic neurons in the SN pars compacta (SNc); and the anterior portion of the globus pallidus (GP), with no involvement of the corpus striatum. The fine TH-positive dopaminergic fibers in the striatum appear relatively intact. However, there is a marked decrease of en-passant TH positive fibers within the GP lesion that is at the same time rimmed with a number of enlarged dystrophic TH+ neurites. Immunostaining for alpha-synuclein did not reveal any abnormal inclusions that resemble Lewy Bodies (LB), a hallmark of human PD.

Discussion/Conclusion. NPE lesions are located within the substantia nigra pars reticulata (SNr), sparing the cell bodies of the dopaminergic neurons in the SN pars compacta (SNc), and in the anterior portion of the GP, with partial disruption of dopaminergic (TH+) fibers passing through GP. These histopathologic features are markedly different from typical human PD brains in which the SNr often appear relatively normal, whereas the number of pigmented dopaminergic neurons in SNc are variably but often markedly reduced in number. Also, no Lewy bodies were seen in these equine NPE brains either in H&E stained sections, or immunohistochemically reacted sections.

NOAEL determination in fish exposed to melamine and cyanuric acid

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During 2007, a large number of pets in the US, Canada, and South Africa died of renal failure caused by a crystal nephropathy which was induced by co-ingestion of melamine (MEL) and cyanuric acid (CYA) in contaminated pet foods. These nitrogen rich triazine compounds were added to feed ingredients to falsely boost the apparent protein levels and were used not only in pet food, but also in livestock and fish feeds. This caused major concern for public health because there was little information available regarding uptake and depletion of MEL or its analogues, such as CYA, in edible tissues. We recently reported the depletions of MEL and CYA in edible tissues in trout and catfish.

Since crystal formation was the cause of the renal failure, we conducted a No Observable Adverse Effect Level (NOAEL) study to provide data for United States Food and Drug Administration's (USFDA) risk assessment for melamine and analogues. The study was designed to determine if a threshold dose could be established for the formation of renal crystals following oral co-administration of MEL and CYA using trout and catfish as non-mammalian models. The starting dose for the study was 20 mg/kg body weight.

In catfish, the NOAELs for crystal formation for single, 4 day or 14 day dosing were 10, 2.5 and 0.5 mg/kg bw, respectively. In trout, the respective NOAELs were 2.5, 2.5 and 0.5 mg/kg bw. Since fish generally excrete chemicals more slowly than mammals, they may provide a "worst case scenario" model for higher risk populations, such as infants or persons with compromised renal function.

Toxicity of the fungal metabolite, pyrrocidine A, to mice

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Pyrrocidines A and B are polyketide-amino acid-derived antibiotics produced by the fungus *Acremonium zeae*, a common seedborne endophyte of corn. Pyrrocidine A exhibits potent activity against Gram-positive bacteria, including drug resistant strains, and displayed significant activity against *Candida albicans*, as well as major stalk and ear rot pathogens of corn. *A. zeae* isolates from corn grown in warmer regions consistently produce pyrrocidines while corn isolates from regions with cold winters seldom produce pyrrocidines. Pyrrocidines have been detected in visibly molded (unmarketable) corn subjected to drought and temperature stress. *A. zeae* is being evaluated for its potential application as a biocontrol agent in protecting corn plants from virulent pathogens, thus inviting the question "What safety information is available for pyrrocidines that could convince regulators that its levels could be increased in corn grain and not pose a safety risk to consumers?" There are no reports of *A. zeae* toxicity to livestock or humans, nor have pyrrocidines been detected in corn based food products. Patented drug candidates, oteromycin, GKK 1032A2, and hirsutellones A, B, and C, exhibit the closest known structural resemblance to pyrrocidines A and B, but there are no reports of mammalian toxicity in the Dictionary of Natural Products database.

In 2007 the Haschek laboratory reported that the cytotoxicity of pyrrocidine A to human HepG2 cells was more potent than known *Fusarium* mycotoxins, deoxynivalenol, fumonisin B_1, moniliformin and zearalenone. However, pyrrocidine A toxicity was not identified in mice at doses up to 10 mg/kg body weight based on clinical signs, organ weights, and gross and microscopic lesions.

To evaluate higher doses, adult, male, Swiss-Webster mice were administered pyrrocidine A at 0 (DMSO control), 10, 100 or 250 mg/kg by intraperitoneal injection (n = 5/group). Pyrrocidine A at 10 mg/kg, was not lethal and did not cause clinical signs, while 100 mg/kg and 250 mg/kg resulted in death within 24 hours. Pyrrocidine A at 25 mg/kg and at 50 mg/kg caused death in 1 mouse in each group within 12 hours. No significant gross or histological lesions were seen.

In Summary: **Pyrrocidine A is cytotoxic to mammalian cells in culture. In mice, doses of 10 mg/kg (i.p.) do not cause acute toxicity, while doses of 25 mg/kg or greater can be rapidly fatal.** Future studies will attempt to more accurately identify the toxic dose and characterize target organ toxicity.

Monensin toxicosis in a group of 25 horses

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Monensin is routinely used as a feed additive in cattle and poultry rations. Monensin is one of several ionophore antibiotics that can form reversible lipid-soluble complexes with sodium and potassium, and transport them across biological membranes. Horses are the most sensitive domestic species to monensin toxicosis with a LD50 of 2 to 3 mg/kg bodyweight.

A group of 25 horses broke into a shed and had access to feed that was supplemented with monensin at 800 g/ton. Approximately 6 to 8 hours later, a 7 year old Quarter Horse gelding became lethargic and tetraparetic and, on physical examination, had an irregular heart rate. The horse was treated symptomatically with iv fluids, but died 3 to 4 hours later. A field necropsy revealed epicardial hemorrhages and white/red streaking of the ventricular myocardium. Histopathologically, there were rare foci of mild acute cardiomyocyte degeneration. Approximately 24 hours after exposure, two more geldings developed severe cardiac arrhythmias, lethargy and recumbency and were administered iv fluids, selenium, vitamin E and flunixin meglumine. In addition, all remaining 24 horses were given activated charcoal 36 and 60 hours after exposure. One of the two clinically affected horses was euthanized approximately 48 hours after exposure to monensin due to poor prognosis. Acute cardiomyocyte degeneration and necrosis were noted on histopathological examination. The other clinically affected horse remained lethargic, but started eating and drinking. A third horse collapsed suddenly and died 4 days after exposure to monensin.

A sensitive and selective liquid chromatography-mass spectrometry method for the detection of monensin in various biological matrices was developed. **The LC-MS/MS method is suitable for rapid analysis for monensin at a detection limit of 1 ng/g.** Stomach contents, urine, liver and serum from clinically affected horses, and serum from clinically unaffected horses were collected and analyzed. Stomach contents from two horses that died 12 and 48 hours after exposure contained 1 and 6 mg/kg of monensin. Five serum samples, two from clinically affected and three from clinically unaffected horses contained between 5.3 and 52 ng/ml of monensin. These results show that while the feed contained a very high concentration of monensin, the levels in the stomach contents were very low. Furthermore, monensin was detected in both clinically affected and unaffected horses and, therefore, does not indicate the severity of clinical disease. There is a relative lack of data about serum and stomach content monensin concentrations in suspect or known exposures in horses. **While the results show a lack of correlation between serum or stomach content monensin concentrations and clinical progression or outcome, a powerful LC-MS/MS diagnostic tool for establishing a definite diagnosis of ante- and post-mortem monensin exposure has been developed** which, through ongoing diagnostic testing, will provide valuable data regarding monensin exposures and will assist in defining the interpretive criteria for intoxications.

Avian/Wildlife/Exotic Scientific Session

Saturday, November 13, 2010
Rochester

Moderator: Steven Bolin and Amy Swinford

***** Graduate student presentation

Exhibit Hall Open 9:30am - 6:00pm

Establishment of the Northeast Wildlife Disease Consortium

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The Northeastern United States is a hotspot for emerging infectious diseases; West Nile Virus and Lyme Disease were detected here before anywhere else in the country. Eastern Equine Encephalitis and Tularemia persist in wildlife here and sporadically claim human lives. Dense human populations and major ports like Boston and New York, where both human and animal travelers pour into the country, combine to increase the risk of emerging diseases in our region. Despite our apparent vulnerability, however, the Northeast does not have a designated wildlife disease laboratory capable of investigating potential disease outbreaks or conducting surveillance to anticipate the emergence of new diseases before they become widespread. To address the lack of a region-wide entity capable of detecting and responding to wildlife disease events, we propose a **Northeast Wildlife Disease Consortium** (NWDC). The NWDC would function as a collaborative effort of the Northeast states, federal wildlife agencies, and non-profit groups. The NWDC would provide expertise and state-of-the-art diagnostics for the Northeast, and would assist in disease surveillance and investigation throughout the region. The consortium would draw on the respective strengths of its veterinarians, researchers, public health officials and wildlife managers to detect, diagnose and respond to disease outbreaks. Because disease rarely respects state borders, the consortium would be a bridge between the Northeast states, facilitating cross-border collaborations. Specifically, the goals of the Cooperative are to provide:

- Active and passive surveillance of diseases and contaminants in live and dead wildlife
- Coordination of diagnoses of wildlife disease outbreaks and mortality events
- Expertise in wildlife-domestic animal disease transmission
- Training for veterinarians, scientists and wildlife managers
- Accessible database of regional wildlife disease outbreaks for use by human and animal health professionals

The consortium will draw on the regional expertise in the Northeast including and administered by Tufts Cummings School of Veterinary Medicine. The consortium will initially be comprised of the diagnostic laboratories and wildlife services from the region including: Department of Pathology and the Wildlife Clinic, Tufts Cummings School of Veterinary Medicine (TCSVM); Animal Health Diagnostic Center (AHDC), Cornell University College of Veterinary Medicine; Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL), University of Connecticut; and New Hampshire Veterinary Diagnostic Laboratory (NHVDL), University of New Hampshire. The Northeast Wildlife Disease Consortium (NWDC) will seek to preserve and protect regional biodiversity and ecosystem health by offering wildlife diagnostic services, expertise, and cutting edge research on the interplay of wildlife, domestic animal, and human health in meeting the initiative of One Health.

Serological diagnosis of *Mycoplasma ovipneumoniae* infection in Rocky Mountain bighorn sheep and domestic sheep by monoclonal antibody-based competitive inhibition ELISA.

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Mycoplasma ovipneumoniae infection is associated with population limiting respiratory disease in free-ranging Rocky Mountain bighorn sheep. Serology could provide a practical and consistent "live animal" test for *M. ovipneumoniae* infection in both bighorn and domestic sheep and would not be affected by culture or PCR-based agent detection method limitations such as intermittent/variable shedding by the host or maintaining agent viability during sample transit. The most widely used *M. ovipneumoniae* serologic test is the indirect hemagglutination assay (IHA) based upon whole bacterial cells, which is difficult to standardize in the laboratory and can potentially detect antibodies to closely related agents such as *Mycoplasma arginini*. To increase standardization and specificity of *M. ovipneumoniae* serologic testing we report herein development of a competitive inhibition ELISA (cELISA) assay based upon a *M. ovipneumoniae*-specific monoclonal antibody.

Analytical validation studies showed sera from bighorn sheep and domestic sheep experimentally infected with *M. ovipneumonaie*, serum from BALB/c mice immunized with whole *M. ovipneumoniae*, and monoclonal antibody (MAb) F141.224.2.1, produced from BALB/c immunized mice, bound a 71 kDa antigen from whole *M. ovipneumoniae* cells as indicated by immunoblot analysis. MAb 141.224.2.1 was specific for *M. ovipneumoniae* and did not bind to closely related agents *M. agalactia, M.capricolum, M. mycoides, M. putrifacions*, and *M. arginini*. A cELISA based upon MAb 141.224.2.1 correctly classified pre-inoculation and temporal post-inoculation sera from experimentally infected bighorn and domestic sheep and there was an appropriate decrease in percent inhibition during end-point dilution of cELISA positive serum. Sera from free-ranging bighorn sheep shown positive using *M. ovipneumoniae*-specific PCR had mean percent inhibition of 85% (+/- 7.5%).

Diagnostic validation was implemented using a set of sera from 218 free-ranging Rocky Mountain bighorn sheep (76 positive and 142 negative samples) defined as *M. ovipneumoniae* positive by clinical disease (presence or absence of pneumonia in a group) and seropositivity using *M. ovipneumoniae* indirect hemagglutination assay. MAb 141.224.2.1 cELISA showed a distinct bimodal distribution of negative and positive sera with histogram analysis. **A cutoff was determined of "≥50% inhibition = positive" and "<50% inhibition = negative" based upon 3 standard deviations from the mean percent inhibition of negative sera. Using this cutoff the performance analysis of the cELISA showed 88% sensitivity, 99.4% specificity, and 95.6% agreement.** Ongoing validation of the *M. ovipneumoniae* cELISA with sera from free-ranging Rocky Mountain bighorn sheep is in progress. The analytical and diagnostic validation studies for the *M. ovipneumoniae* cELISA indicate a rapid, simple, easily standardized serological assay for accurate identification of *M. ovipneumoniae* infection versatile for domestic and wildlife ovine species.

Establishment of culture conditions for survival of *Histomonas meleagridis* in transit

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Histomonosis (blackhead disease), caused by *Histomonas meleagridis*, has been reported in domestic turkeys, poultry, and numerous wild birds. Outbreaks have become more frequent and severe in commercial operations due to the ban of nitroimidazole products. Although *H. meleagridis* is readily cultured in several media preparations, parasite culture is not often used for diagnosis. Establishing protocols for transporting live *H. meleagridis* cultures would assist diagnosticians and allow for banking of cultures for future research. To establish these conditions, we infected domestic turkeys with histomonads, euthanized the birds 10 days post-infection and allowed carcasses to incubate at room temperature for either 2 or 24 hrs. Following incubation, samples of cecal contents (0.5 g) were placed in Dwyer's media and held at 4, 25, or 30 C for 6, 18, 24, 48, 72, 96, or 120 hrs. Samples were placed in a 40 C incubator at the specified times and examined daily for histomonad growth by light microscopy. **Positive histomonad growth was detected from cecal samples obtained from the 2 hr incubated carcass and from cultures held at 30 C for 6, 18, 24, 48 and 72 hrs.** No growth was seen from cultures held at 25 C or 4 C or at any temperature from the carcass allowed to incubate for 24 hrs at room temperature. **These results were validated by the positive identification of live histomonads in Dwyer's media submitted via overnight courier by a poultry veterinarian during a histomonosis outbreak at a North Carolina turkey farm.**

Isolation of *Avian influenza virus* from samples containing both avian influenza and Newcastle disease viruses

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Avian influenza viruses (AIVs) are important zoonotic pathogens that can infect many species including humans. Waterfowl are the main natural reservoir of all influenza A viruses and all 16 HA and 9 NA subtypes have been found in aquatic birds. These birds can serve as a source of the viruses to many other species and regular surveillance of AIV in waterfowl is important in tracking the circulating AIVs of epidemiological importance. In addition to AIV, waterfowl may be co-infected with other viruses such as the paramyxoviruses, which are frequently isolated from waterfowl. Of these, paramyxovirus type-1 (APMV-1) or the Newcastle disease virus (NDV) is of particular importance because of high virulence of certain strains of this virus for domestic poultry. In routine surveillance of waterfowl for AIV, we encountered a number of cloacal samples that were positive for AIV by real-time RT-PCR but did not yield AIV by inoculation in embryonating chicken eggs. On further testing, these samples were also positive for NDV when tested by conventional RT-PCR. We hypothesized that if both NDV and AIV are present in a sample, NDV may overgrow the AIV yielding false negative AIV results. We treated such samples with chicken anti-NDV polyclonal antiserum and then inoculated in embryonating chicken eggs. Several samples were found to be positive for different subtypes of AIV (H1N1, H3N8, H4N1, H4N2, H4N6, H5N2, H2N2, H7N1, and H7N2). These results demonstrate that, in the presence of mixed infection with NDV and AIV, it is imperative to remove the influence of NDV so a true picture of AIV prevalence can be gained. An additional benefit is obtaining information on the circulation of NDV in these birds, which can be further studied to evaluate their epidemiological and ecological significance.

Different routes of transmission of low pathogenicity avian influenza viruses in chicken layers

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In order to develop better control measures against avian influenza it's necessary to understand how the virus transmits in poultry. In a previous study in which the infectivity and transmissibility of the pandemic H1N1 virus was examined in different poultry species, we found that no or minimal infection occurred in chicken and turkeys intranasally inoculated with the virus. However, we demonstrated that the virus can infect laying turkey hens by the intracloacal and intrauterine route causing decreased egg production. Such novel routes of exposure to the virus have not been previously examined in chickens and could explain outbreaks of low pathogenicity avian influenza (LPAI) causing drops in egg production in chicken layers. In the present study, 46 week-old chicken layers were infected with by the intranasal (IN) (n=10), intracloacal (IC)(n=10) or intrauterine route (IU)(n=10) with 10^6EID_{50} of one of two LPAI viruses: a chicken adapted virus (A/Ck/CA/1255/02 H6N2) and a live bird market isolate (A/Ck/NJ/1220/97 H9N2). Only hens IN inoculated with the H6N2 virus presented mild clinical signs consisting of depression and anorexia. However a decrease in eggs laid was observed in all inoculated groups compared to control hens. Hens inoculated with the H6N2 virus produced 48-52% less eggs, and hens inoculated with the H9N2 virus produced 32-35% less eggs, with no difference between the routes of inoculation. All chickens became infected with the H6N2 virus when exposed by any of the three routes. This was demonstrated by seroconversion and virus shedding. In the IN inoculated group, at four days after inoculation 8/8 hens shedded virus through the oropharyngeal route and 3/8 through the cloacal route. In the IC inoculated group, 5/8 hens shedded virus through the oropharyngeal route and 4/8 through the cloacal route. In the IU inoculated group, 4/8 hens shedded virus through the oropharyngeal route and 6/8 through the cloacal route. All hens from all three groups presented antibodies to the virus at 14 days and transmitted virus to contact hens. On the other hand, only 1 or 2 hens from each of the groups inoculated with the H9N2 virus shedded virus or seroconverted and did not transmit the virus to contacts. **In conclusion, LPAI viruses can also transmit in chickens through other routes besides the intranasal route, which is considered the natural route of exposure. However this transmission also depends on the virus.**

Comparison of NAHLN and NVSL rRT-PCR test results for 2007-2009 wild bird surveillance specimens

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Potential roles played by migratory waterfowl and water birds in the spread of H5N1 highly pathogenic avian influenza (HPAI) virus from Asia to Europe, the Middle East, and Africa prompted a large-scale surveillance program to detect HPAI H5N1 in wild aquatic birds in the U.S. The program is a cooperative effort between the U.S. Departments of Agriculture (USDA), the National Animal Health Laboratory Network (NAHLN), and State Wildlife Agencies. Cloacal (CL) and oropharyngeal (OP) swabs were collected from apparently healthy and hunter-killed birds and tested for presence of avian influenza virus (AIV) by the real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) assay at NAHLN laboratories. Swab specimens were screened for AIV by the matrix (M) rRT-PCR assay. Positive M specimens were subsequently tested by the H5 rRT-PCR subtyping assay and all H5 positive specimens were shipped to the National Veterinary Services Laboratories (NVSL) for confirmatory testing by rRT-PCR, virus isolation (VI), and virus characterization. This USDA program represents the first large scale, multi-agency AI surveillance program using a rRT-PCR NAHLN screening/NVSL confirmation testing procedure. The rRT-PCR assay is a screening tool and has a higher diagnostic sensitivity and lower diagnostic specificity than VI, the gold standard. A comparison between NAHLN and NVSL rRT-PCR H5 test results for specimens collected by USDA Wildlife Services (WS) was conducted to evaluate the degree of agreement and to identify possible factors that might contribute to disagreement.

Swabbing methodology was described at the beginning of each surveillance period (April 1 – March 31) in the WS Procedure Manual and based on the WS project objectives for the biological year. Specimens testing positive for H5 by rRT-PCR at the NAHLN laboratories were submitted to NVSL as presumptive positive (pp) specimens. Cloacal and OP swabs from a single bird were collected and placed in the same vial of brain heart infusion broth and submitted for testing. Pooling of samples did not occur during 2007-09 biological years, even though this was a common practice in the 2006 biological year. A total of 406 H5 pp specimens were received in 2007, 729 in 2008, and 653 in 2009 for confirmation rRT-PCR testing and VI. Of the 406 pp specimens submitted in 2007, a selected subset of 280 pp specimens from 31 different NAHLN laboratories were evaluated in more detail to determine the degree of agreement and possible source of disagreement. Shipping conditions and possibility of specimen degradation were analyzed.

The percentage of submitted pp specimens confirmed positive at the NVSL was 76.6% (311 of 406) for 2007, 73.38% (535 of 729) for 2008 and 80.55% (526 of 653) for 2009. In addition, 78% (216 of 280) of the selected subset of 2007 specimens were confirmed as positive. The difference in mean cycle threshold (Ct) values between the 2007 subset samples that agreed and those that disagreed is -5.6 (95% *CI*: -6.8, -4.4) for specimens refrigerated (4C) prior to shipping. Differences in Ct values could be indicative of specimen degradation or decrease in sensitivity. The Ct values for the 2007 subset showed no evidence of specimen degradation during shipment or decrease assay sensitivity by confirming laboratory.

The rRT-PCR assays are screening tools and might produce a false positive result. Low pathogenicity AI (LPAI) viruses are shed normally in low titers from wild waterfowl, the natural reservoir for AI, resulting in higher and less reproducible Ct values. Factors that might contribute to disagreement of test results include; inconsistent repeatability of positive results at assay endpoint, difference in assay sensitivity of screening and confirming assays, difference in rRT-PCR instrumentation, RNA extraction procedure and chemistry, nonspecific false positive result, false positive resulting from low level contamination, nonspecific inhibitors and sample degradation. Further assay evaluation and validation efforts are in progress.

Transmission and diagnosis of *Foot- and- mouth disease virus* (FMDV) in wildlife: Pronghorn antelope (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*)

Brenda C. Donahue[1], Gordon Ward[1], Tom Gidlewski[3], Matt McCollum[2], Jack Rhyan[2]
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Pronghorn antelope (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*) were tested for their susceptibility to O_1 Manisa Foot-and- Mouth Disease virus (FMDV) and their ability to transmit virus to cattle and of cattle to transmit to them. Animals were inoculated by the intradermal lingual route and allowed to transmit the virus to naïve animals by direct contact. These contact infected animals were then introduced into rooms with additional naïve animals which were observed daily. Samples of blood, serum, nasal swabs and oral swabs were taken daily along with periodic probang samples. Samples were tested by virus isolation in cell culture and by real time RT-PCR for the presence of virus. Serum samples were tested by ELISA for antibodies to 3 ABC and 3D proteins and for neutralizing antibodies by virus neutralization.

Transmission of FMDV was demonstrated between pronghorn antelope, and interspecies transmission was demonstrated from pronghorn antelope to cattle and cattle to pronghorn antelope. Virus was detected from pronghorn antelope blood more reliably than from either nasal or oral swabs. Thirty percent of the pronghorn antelope showed no evidence of shedding of virus in nasal or oral swabs while all the animals had at least 2 days of viremia during the 8 days post exposure (DPE). Results obtained from probang antelope samples taken 28 days and greater after exposure were negative indicating no evidence of a carrier state.

Transmission of FMDV was also evident between mule deer, and interspecies transmission was demonstrated from mule deer to cattle and cattle to mule deer. Virus was detected from mule deer blood early during infection until 8 DPE by virus isolation. Viral shedding was evident in both cattle and mule deer, where 90% of mule deer demonstrated viral shedding from oral swabs and 100% from nasal swabs.

All pronghorn antelope and cattle seroconverted with neutralizing antibodies detected by14 DPE using virus neutralization. By 14 DPE, 40% of pronghorn antelope had positive 3ABC ELISA readings and 20% were positive for 3D ELISA while 50% of cattle had positive readings for 3ABC and 100% for 3D. By 21 DPE, all animals were positive for both ELISA's. These positive responses dropped to 80% by 58 DPE for the pronghorn antelope but not cattle.

Twenty-five percent of mule deer and 50% of cattle began to seroconvert by 10 DPE. By 21 DPE, all mule deer and cattle were positive for FMD antibodies by 3D and 3ABC ELISA and remained positive throughout the remainder of the experiment.

In this study we determined that intra- and interspecies transmission of FMDV O_1 Manisa occurred between pronghorn antelope and cattle, and mule deer and cattle. Evaluation of current diagnostic assays to detect FMDV infection in pronghorn antelope found blood to be a more reliable sample than nasal or oral swabs. Nasal and oral swabs proved to be adequate samples for mule deer by rRT-PCR. Both pronghorn antelope and mule deer developed antibodies to structural and non-structural proteins serving as evidence of FMD virus infection.

Novel *H1N1 Influenza A virus* infection in a captive cheetah in California, 2009

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In November of 2009 the California Animal Health and Food Safety (CAHFS) laboratory detected the pandemic H1N1 2009 virus (H1N1pdm) in a privately owned cheetah (*Acinonyx jubatus)*. Presumptive diagnosis was made using the real-time PCR protocols provided by the National Animal Health Laboratory Network (NAHLN) under the approved protocol deviation for testing samples originating from non-porcine species. The positive PCR results were confirmed by virus isolation, followed by complete genome sequence analysis of the pandemic virus isolate performed at the St. Jude Children's Research Hospital. The two nasal swab samples tested were obtained under guidance of a public health worker 2-3 days after the onset of the clinical signs from Cheetah A, and 4-6 days after onset of clinical signs from companion Cheetah B. Clinical signs included nasal discharge, wet cough, anorexia, ptyalism and lethargy. At the time of sampling, the two remaining cheetahs in the facility had already recovered from their respiratory illness and were not sampled.

Full genome sequencing of one of the isolates showed that it did not have any unique molecular signatures as compared to H1N1pdm viruses isolated from humans. **To assess the pathogenicity of the isolate, five ferrets were experimentally infected with 1 x 10^6 $TCID_{50}$ of the novel H1N1 by the intranasal route.** The clinical and virologic course of the infection was typical of other H1N1pdm viruses with the infected animals losing approximately 6% of body weight before recovering. Virus was detected in the nasal washes of all animals at day 3 and 5 post infection, but all had cleared the virus by day 7 post infection. **Taken together these data highlight the reverse zoonotic potential of H1N1pdm viruses and show in this case severe consequences for zoological parks.**

Sponsor Presentations

Saturday, November 13, 2010
Salon ABC & Rochester

Salon A
Moderator: Charles Moore

6:00-7:00 PM **Computer Aid: National Agribusiness Technology Center (NATC) - The Complete Animal Health Surveillance Solution; AGRAGuard featuring USALims and USAHerds**

Salon B

Moderator: Hemant Naikare

6:00-7:00 PM **Applied Biosystems by Life Technologies: Things that make you go hmmm?**

Rochester
Moderator: John Adaska

6:00-6:30 PM **IDEXX: Driving laboratory diagnostic testing by engaging practicing veterinarians, a companion animal example**
William Goodspeed, Corporate Vice President Livestock and Poultry Diagnostics, Water and Dairy

Salon C

Moderator: Kristy Pabilonia

6:00-6:15 PM **VADDS: LIMS options for frozen budgets**
Joseph J. Bove, President, Advanced Technology Corp

6:15-6:30 PM **Biovet: New developments in diagnostic kits**
Dr. Andre Broes, DVM, PhD, Director, R&D Technical Services Manager

6:30-6:45 PM **Newport Laboratories: Technology, growth, and leadership**
Randy Simonson, General Manager & Chief Operating Officer

6:45-7:00 PM **SDIX: Rapid and cost-efficient Salmonella Enteritidis testing**
Tim Lawruk

Pathology Scientific Session

Sunday, November 14, 2010
Salon B

Moderators: Scott Fitzgerald and Laura Kennedy

11:00 AM **Serum chemistry reference ranges in captive Alaskan reindeer (*Rangifer tarandus*)**
Zoe Purtzer, Gregory Finstad, Antony Bakke, Diane Kazmierczak, Carla Willetto, Steve Kazmierczak. 94

11:15 AM **Causes of morbidity and mortality in moose (*Alces alces*) from Minnesota**
Arno Wünschmann, Anibal Armien, Mike Schraege, Erika Butler, Michelle Carstensen. 95

11:30 AM ***Clostridium perfringens* type C and *Clostridium difficile* combined enter-typhlo-colitis in foals**
Francisco Uzal, Santiago Diab, Patricia Blanchard, Janet Moore, Lucy Anthenill, Glenn Songer. 96

11:45 AM **Pathology of experimental *Avian Borna virus* infection in psittacines and chickens**
Hulimangala Shivaprasad, Herbert Weissenbock, Sharmon Hoppes, Patricia Gray, Susan Payne, Ian Tizard. 97

***** Graduate student presentation

Exhibit Hall Open 7:00am - 2:00pm

Experimental infection of white-tailed deer (*Odocoileus virginianus*) with *Epizootic hemorrhagic disease virus* serotype 7 (Israel)

Mark G. Ruder[1], Andrew B. Allison[1], Sabrina N. McGraw[2], Deborah L. Carter[2], Steven V. Kubiski[1], Daniel G. Mead[1], David E. Stallknecht[1], Elizabeh W. Howerth[2]

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Introduction: In Israel in 2006, epizootic hemorrhagic disease virus (EHDV) serotype 7 was the cause of an intense and widespread epidemic that affected 105 cattle herds (dairy and beef) with morbidity ranging from 5 – 80% in the dairy herds. Although mortality was < 1%, a 10-20% drop in milk production resulted in significant economic impacts. The susceptibility of potential North American vectors and vertebrate hosts to infection with EHDV-7 is not known. This information is essential when attempting to understand the risk of establishment of this exotic orbivirus in North America. Our primary objective was to determine if white-tailed deer (*Odocoileus virginianus*) (WTD), the most abundant and widely distributed wild ruminant in the United States, are susceptible to infection with EHDV-7.

Materials and Methods: Eight, hand-reared, eight-month-old WTD were used for this study. The virus (EHDV ISR2006/04) used in the trial was obtained from the Institute for Animal Health, Pirbright Laboratory and was originally isolated from a cow in Israel. Tissue culture supernatant was passaged through a single deer via subcutaneous and intradermal inoculation. Blood harvested from this animal on post-inoculation day (PID) 6 was used to inoculate six additional animals via the same route. Animals were monitored twice daily for clinical signs. Blood was collected on PID 0, 3, 5, 6, 7, 10, 12, 18, 25, 32, 39, and 46 for virus isolation and titration, serology, a complete blood count, and coagulation assays. At the time of death or euthanasia, each animal was necropsied and tissues were collected for virus isolation and titration, and histopathology. Viral isolates were identified via serotype-specific RT-PCR.

Results: All six EHDV-7 inoculated deer became infected and exhibited varying degrees of clinical disease. Rectal temperature was variably increased between PID 5 - 7. Common clinical signs between PID 4 -12 included inappetence, rough hair coat, depression, erythema of non-haired regions, and hyperemia of conjunctiva and oral mucosa. Reluctance to rise, recumbency, bleeding tendencies, and oral hemorrhages were also observed. Four of six animals died or were euthanized due to severity of disease, one animal on PID 5 and the remaining three on PID 7. Gross and microscopic findings in these animals were consistent with previous reports of EHDV-1 and -2 infections in WTD[2,3]. An intestinal perforation in the animal that died on PID 5 contributed to death. All deer had a detectable viremia on PID 3 that peaked on PID 5 or 6. Deer surviving infection had precipitating and neutralizing antibodies present by PID 10. Hematologic abnormalities, including decreased platelets, total protein and leukocyte count, were consistent with previous reports of EHDV-1 and -2 infection in this species[2].

Discussion/Conclusion: **These results demonstrate that WTD are susceptible to infection and severe clinical disease with EHDV-7**. Further, the clinical similarities observed in this study with disease caused by endemic EHDV serotypes highlight the importance of serotype-specific diagnostics.

Fatal Herpesviral Infection in an Adult Dog

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A 9-year-old, spayed female Bichon Frisé was presented to the Diagnostic Center for Population and Animal Health for necropsy. Upon initial presentation to the referring veterinarian, the primary complaint was vomiting and diarrhea. Prior to death, physical examination revealed pale yellow mucous membranes with petechia and bilateral oculonasal discharge. Terminally, the rectal temperature was 97.6°F. Lateral abdominal radiographs showed a slightly enlarged liver. Evaluation of serum chemistry was consistent with liver damage {increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST)}.

At necropsy, icteric sclera and oral mucous membranes were noted. The liver was mottled red and tan. Petechiae were present on the gingival mucous membranes and the serosal and mucosal surfaces of the stomach, jejunum, and urinary bladder. Other findings included icteric sclera and oral mucous membranes.

Microscopically, the normal hepatic cord architecture was disrupted by multifocal to coalescing, random areas of hemorrhage and pale eosinophilia, with faint retention of hepatocellular architecture and loss of individual cellular detail (coagulative necrosis). Hepatocytes adjacent to the necrotic areas occasionally contained eosinophilic, intranuclear viral inclusions that displaced the nuclear chromatin to the periphery. Necrotic foci and intranuclear viral inclusions were also seen within the adrenal glands, and jejunum crypt epithelial cells. Fresh samples of liver were negative for canine adenovirus-1 by PCR and positive for canine herpesvirus-1 (CHV-1) by direct fluorescent antibody (FA) testing and by PCR. Sequencing of the PCR product confirmed the amplification of a portion of the CHV-1 thymidine kinase gene. Herpesviral nuclei acid was localized within hepatocytes in the periphery of the areas of necrosis with *in situ* hybridization (ISH). Canine parvovirus and canine distemper virus were not detected with immunohistochemistry on sections of small intestine. Aerobic culture of the liver did not reveal any bacterial organisms.

The combination of gross and histopathologic findings, along with positive FA, PCR, and ISH, indicate that this dog suffered fatal systemic infection with CHV-1. This alphaherpesvirus typically causes fatal liver and renal necrosis and hemorrhage in young puppies between the ages of 1-3 weeks, with infection occurring as the puppies pass through the birth canal. Puppies that are transplacentally infected *in utero* may be aborted or stillborn. If puppies that are infected after 3 weeks recover, the virus becomes latent, often residing in the trigeminal or lumbosacral ganglia. Adult dogs with viral reactivation secondary to immunosuppression usually present with mild, self-limiting conjunctivitis, vaginitis, and posthitis. A case of a dog with disseminated CHV-1 infection secondary to chemotherapeutic immunosuppression was recently published. In contrast, this Bichon Frisé had no known prior history of immunosuppression (stress, corticosteroid administration, chemotherapy), and the bloodwork was not supportive of such. **This represents the first case of systemic CHV-1 infection in an immunocompetent dog.**

Cranial osteochondromas in free-ranging white-tailed deer (*Odocoileus virginianus*)

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Other than cutaneous fibromas, neoplasia is uncommon in wild white-tailed deer and boney tumors seem to be among the least common type. We present a case series of wild white-tailed deer with osteochondromas, a neoplasm rarely reported in this species and not diagnosed at the Southeastern Cooperative Wildlife Disease Study until 2003. We received five female white-tailed deer with cranial masses, from November, 2003 until December, 2008. The deer were from Georgia, Mississippi, South Carolina and West Virginia. The heads were radiographed and the masses were sectioned using a band saw. Each deer had a single, large, immovable mass, from 6 x 6 x 9 cm to 17 x 24 x 14 cm, that protruded from the flat bones of the skull. In four deer, the frontal bones, maxillary and/or nasal bones were affected, whereas in a fourth the mass protruded from the ramus of the mandible. There was no evidence of metastasis, though there was local destruction of bone and protrusion into the nasal cavities of three deer. The deer with a mandibular mass was emaciated, presumably due to an inability to chew. The remaining deer were in good nutritional condition and were clinically normal when collected by hunters. The masses were decalcified using a formic acid solution and Hematoxylin & Eosin stained sections were examined microscopically. The common finding of endochondral ossification beneath an expanding cap of disorganized hyaline cartilage was consistent with the diagnoses of osteochondroma. Of the boney tumors occurring in white-tailed deer osteochondromas seem to be the most common. In addition this tumor appears to have a strong age and sex predilection for middle-aged, female deer.

Characteristics of *Geomyces destructans* infection of bat skin as demonstrated by scanning electron microscopy of naturally infected tricolored and little brown bats.

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The skin of two little brown bats (*Myotis lucifugus*) and four tricolored bats (*Perimyotis subflavus*), with grossly visible lesions consistent with white-nose syndrome, were examined by scanning electron microscopy. All bats were euthanized and immediately fixed in 10% neutral buffered formalin then post-fixed in glutarldehyde. Skin samples from the muzzle, patagium and body of each bat were dehydrated by critical point drying and sputter-coated with gold prior to microscopy.

Growth of hyphae on the muzzle was generally diffuse, but proliferation on the patagium was much more multifocal. Hyphae were tightly adhered to the surface of the stratum corneum and occasionally infiltrated the stratum corneum, extending under the edges of individual cornified epithelial cells. Hair follicles of the muzzle and patagium were frequently invaded by hyphae. Areas with more dense hyphae had some hair shafts with adherent clumps of fungal elements, but invasion or damage of the hair shafts was not observed. Densely furred areas from the trunk had little growth of fungus. The characteristics of the infection did not differ appreciably between the little brown bats and tricolored bats examined. Erosion or inflammation of the epidermis was not evident in any of the bats.

Sporogenesis was observed on all the bats examined and conidia were very numerous in some individuals. The epidermis of the muzzle in some individuals was paved by dense mats of conidia.

Our findings illustrate the tight adherence of hyphae to the skin, paving of epidermal surfaces by conidia, the lack of damage to hair shafts and only mild damage to the stratum corneum itself. Adherence is a critical factor in the pathogenesis of dermatophytosis and might also be an important factor in infections by *G. destructans*.

Treponema-associated ulcerative mammary dermatitis in dairy cows

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Ulcerative mammary dermatitis (UMD), a new disease entity, has been described in the United Kingdom (UK) ("Foul Udder", "Intertrigo", "Necrotic Dermatitis of the Udder"), California ("Udder Sores") and Iowa ("Ulcerative Mammary Dermatitis"). The dermatitis involves the median cleft and anterior skin fold of the udder of lactating dairy cows, usually high producers, and often in outbreaks where housing is in cubicles (UK) or freestalls (US). Clinically, the syndrome initially presents as matting of the hairs with foul-smelling, brown viscid exudate and, if untreated by antibiotics, progresses to raised or craterous, red, moist or scabbed skin ulcers. Comparative histologic studies show that UMD has similar features to Papillomatous Digital Dermatitis (PDD, Digital Dermatitis, Hairy Heel Warts, Footwarts). These features include: loss of stratum corneum, ulceration of dermal papillae, invasion of stratum spinosum and papillary dermis by dense mats of spirochete-dominant bacteria, epidermal hyperplasia, parakeratosis and papilliform hyperkeratosis**. In California, a herd of 850 Holstein dairy cows was studied. Morbidity varied from 10% in first lactation heifers to 48% in high producing groups. One of 4 affected cows biopsied had diagnostic lesions of UMD, material from which was genetically analysed; the remaining 3 cows had non-specific ulceration. In Iowa, a herd of 150 Ayreshire dairy cows was studied. Morbidity was 20%. One affected cow biopsied had diagnostic lesions of UMD, material from which was genetically analysed. In the UK, 7 Holstein-Friesian dairy farms were studied. One to 2 affected cows from each farm were biopsied. Three biopsied cows had diagnostic lesions of UMD; the remaining 5 cows had non-specific ulceration. Material from other cows with UMD, from other farms, was genetically analysed.**

Several novel spirochetes have been isolated or detected from UMD lesions. Collectively, genomic studies have shown diverse *Treponema* phylotypes, most were closely related to those isolated from PDD (*T. denticola*-like, *T. phagedenis*-like, *T. medium*-like) as well as unique and non-cultivable phylotypes. Although the precise relationship of UMD to PDD is yet undefined, the histologic and microbiologic similarities of the respective lesions suggests that the two entities may be related. Observations that UMD and PDD co-exist on the same farm supports this notion.The etiology of the two entities is probably polybacterial but is also undefined. However, experimental PDD studies have shown that *Treponema* spp. are primary and dominant invaders and that the disease is transmissible by direct contact. It seems, therefore, that given optimal environmental conditions, pathogens of PDD-affected hind feet could theoretically infect mammary skin by contact juxtaposition of the foot of the underside leg near the front of the udder when a cow lies down.

Camelid neoplasia and congenital proliferative lesions: a 10 year retrospective study, 2001-2010

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Examination of archived camelid submissions to Colorado State University Diagnostic Laboratories (CSU-VDL) in the period of Jan. 2001 to Jun. 2010 confirms that neoplasia in camelids is heterogeneous and affects both llamas and alpacas of wide age-range. The following table summarizes the different types and numbers of tumors and proliferative lesions encountered in both species and the location of these different neoplasia. To our knowledge mast cell tumor, liposarcoma, carcinomatosis, ocular squamous cell carcinoma, osteosarcoma, hemangiosarcoma, sarcoid-like oral sarcoma, and chromophobe pituitary adenoma are among neoplasia that have not been previously reported in camelids.

Table 1: Summary of neoplasia in llamas and alpacas submitted to CSU-VDL from 2001 to 2010.

Tumor type	Total number	Alpacas	Llamas	Location
Lymphoma	20	15	5	Multicentric
juvenile		6		
Soft tissue sarcoma	8	3	5	Skin, oral, visceral
Squamous cell carcinoma	7	4	3	Ocular, oral, skin, urethral
Fibroma/fibropapilloma	9	7	2	Skin, head, oral
Intestinal adenocarcinoma	6	4	2	Jejunum, ileum, rectum
Mammary adenocarcinoma	4	2	2	Mammary gland
Osteosarcoma	3	1	2	Scapula, jaws
Uterine adenocarcinoma	2	1	1	Uterus
Melanoma	2	1 Malignant	1	Eye, perianal
Lipoma/Liposarcoma	2	0	2 (1 malignant)	Subcutis, chest
Myxoma	1	0	1	Skin, canthus
Keratinizing ameloblastoma	1	1	0	Maxilla
Mucoepidermoid carcinoma	1	1	0	Maxilla
Mast cell tumor	1	0	1	Lip
Cystadenoma	2			
Pancreatic		0	1	Pancreas
Salivary		1	0	Salivary gland
Thyroid adenoma	1	1	0	Thyroid gland
Pituitary adenoma	1	1	0	Pituitary gland
Gemistocytic astrocytoma	1	1	0	Brain
Pilomatricoma	1	1	0	Skin, back
Gastric leiomyoma	1	0	1	Stomach
Giant melanocytic nevus	1	1	0	Periauricular and brain
Melanocytic alveolar Hyperplasia	1	0	1	Lungs*(TVMDL)
Hemangiosarcoma	1	0	1	Abdominal

Hypocalcemic syndrome associated with neurological signs and pulmonary edema in pigs

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Neurological and respiratory disorders in pigs are typically associated with bacterial or viral infections. Herein, we report two cases in which pigs presented with muscles tremors, sudden death, recumbency, tremor and pulmonary edema associated with hypocalcemia. Hypocalcemia is often overlooked as a cause of neurological signs and pulmonary edema in swine and can result from ration formulation errors.
Case #1 was composed of 6 grow-finish pigs (35-45 kg). Pigs in case #2 were 7 weeks old (n = 5). Serum was collected from all pigs prior to euthanasia and the calcium level was decreased in all pigs (4.8 – 6.2 mg/dl, reference value 7.1-11.6 mg/dl). One animal in case #1 was given 50 ml of 20% calcium gluconate intramuscular and fully recovered. The serum calcium level of this treated pig was within reference range (8.4 mg/dl) post-treatment. Pulmonary edema was the only gross necropsy finding. Bones were collected from all pigs for bone ash analysis at Michigan State University. In case #1, femurs were submitted and bone ash values were within reference range, ranging from 64-67% (reference range 63-70%). In case #2, ribs were submitted and bone ash values were decreased, ranging from 49.2 – 52.2% (reference range 55-65%). Analysis of serum vitamin D (25-hydroxyvitamin D) was performed in case #2, and yielded an undetectable serum level in 5 pigs and a concentration of 10 nmol/L in one pig. Results below 4-5 nmol/L indicate vitamin D deficiency. Four pigs in case #1 had histological lesions compatible with a mild metabolic bone disease in ribs, characterized by marrow fibrosis, decreased cortical bone thickness, and cartilage retention. There was no evidence of bacterial or viral meningitis in any pig. Pigs from case #2 had a concurrent Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection. Taking into consideration all the above information, hypocalcemia and hypocalcemia associated with hypovitaminosis D was judged to be the cause of neurological signs in those pigs.

Bone ash analysis is a useful tool to quantitatively assess bone density. One reason why bone density was reduced in case #2, but not in case #1 is that femur were submitted in case #1 and ribs in case #2. According to Wolff's law, weight bearing bone (e.g.: femur) tends to decrease density less than non-bearing bone (e.g.: ribs). If bone ash analysis is not readily available, veterinarians and pathologists are encouraged to assess bone density by manually checking the breaking strength of ribs on post-mortem examination. However, "softness" of bone is subjective, and requires extensive swine necropsy experience. Assessments of response to treatment with exogenous calcium or measurement of serum calcium level in live affected pigs are also supportive of diagnosis of hypocalcemia. Vitamin D and calcium level can also be measured on feed sample. **In summary, hypocalcemia should be considered as differential diagnosis for neurological diseases and pulmonary edema in pigs.**

Phytase deficient diet as a cause of bone fractures in pigs

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Phytase is an enzyme which liberates phytic phosphorus from grain, which enhances the availability of phosphorus for absorption. Due to a steep increase in price of feed grade inorganic phosphorus and a desire to decrease the amount of phosphorous in swine manure, the addition of phytase and reduction of phosphorous in swine diets has become an effective and common practice in the swine industry. Loss of phytase activity, however, results in a deficiency of phosphorus available for the growing pig. Here we report outbreaks of bone fractures resulting from phytase deficient diets in two different pig farms. In the first case the pigs were growing slowly and one or two pigs had to be euthanized each day because they were going down and/or fracturing legs. Two pigs, 120 and 180 days-old, were euthanized on the farm and submitted for necropsy. In the second case eleven pigs were submitted to the diagnostic laboratory for necropsy. These pigs were from a barn of 1,200 pigs approximately 110 days of age. The clinical problem started five days prior to presentation and the farm was losing four to nine pigs/day. Clinically, 6 of 11 pigs could not use their rear legs, were weak, screamed in pain and were attacked by other pigs. The pigs also had multifocal purple discoloration of the skin which was assumed to be trauma-induced. The other 5 pigs were found dead. In the two pigs from first case, there were multiple rib fractures with calluses, the ribs did not break cleanly or snap when broken, and there was a complete fracture of the left femur of the large pig. Also, the spinous processes of the lumbar and thoracic vertebrae could be cut near their origin from the dorsal arch with a knife. In all 11 pigs from the second case, the ribs did not snap when they were broken. Instead, they folded with no sound. Four pigs had fractures involving vertebrae, acetabulum, scapula, ribs and radius, and/or ulcers on the femoral head. In some pigs there were pneumonia and purple discoloration of skin which were associated with bacteria. Histological changes in the ribs from both the cases were equivocal. The third metacarpal bone from all the pigs was analyzed for total bone ash. The per cent ash ranged from 30.62 to 37.46 and is lower than the reported normal values. In the first case, phytase was not added to the feed by the manufacturer. In the second case, the premix containing phytase was improperly stored and outdated. **We conclude that the lack of/loss of phytase activity in the feed led to reduced amount of available phosphorus resulting in the observed bone abnormalities.** Diet correction by addition of monocalcium phosphate resulted in resolution of the clinical signs in 10 days. No long-term impact on growth was observed.

Bone marrow pathology in young calves with idiopathic haemorrhage syndrome

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Introduction: An unexplained haemorrhagic syndrome (bleeding calf syndrome, bovine neonatal pancytopenia) of calves less than 1 month old has been recorded in several European Countries since 2007/2008. The first cases were reported in Germany and cases have also occurred in other countries including the Netherlands, France, the UK, Ireland and Belgium. The presenting clinical signs are of external or internal haemorrhage. Clinical pathology on affected calves demonstrates thrombocytopaenia, neutropaenia and occasionally other dyscrasias.

Materials and Methods: Histological examinations of bone marrow were performed on 85 calves submitted to VLA for investigation of unexplained haemorrhagic syndrome, forming part of a multidisciplinary approach. Criteria for inclusion in the study were: (1) less than 4 weeks of age (2) no BVDV RNA detected and (3) clinical features of unexplained haemorrhage *eg* from injection or tagging sites, nares, rectum, skin; and/or pancytopenia with thrombocytopenia [$< 20x10^9$/L]; and/or necropsy findings of unexplained haemorrhage at one or more sites. The standardised necropsy sampling protocol included fixation of bone marrow from standardized sites from femoral cavity, sternebrae 1 – 3 and ribs 6 – 8 in 10% neutral buffered formalin. Sternebrae and ribs were decalcified for 6-8 hours in a 'rapid decalcifying' solution before processing routinely to produce H&E sections. Immunohistochemistry for porcine circovirus 2 (PCV2) was performed on femoral bone marrow from 16 randomly selected calves meeting the criteria for inclusion and 2 unaffected calves with BVDV-associated thrombocytopenia.

Results: Marked trilineage hypoplasia (aplastic anaemia) involving extensive depletion of erythroid and myeloid precursors and megakaryocytes was observed in 75 (88.2%) calves although in the majority of cases small and occasional larger foci of haemopoiesis were also present. Areas of haemopoiesis were particularly striking in femoral cavity bone marrow in 4 calves. In ribs, large clusters of up to 20 adjacent megakaryocytes could be found in subcortical and occasionally intracortical sites in contrast to unaffected calves. Haemopoietic cell depletion was most accurately assessed in sternal samples due to the even distribution of haemopoiesis at this site in bone marrow samples from age matched unaffected calves. Three calves (3.5%) had evidence of regenerative responses and 1 calf (1.2%) had combined marked depletion of both late erythroid series cells and megakaryocytes. Two calves (2.4%) had evidence of DIC and in 4 calves (4.7%) no evidence of underlying bone marrow pathology was detected. No labeling for PCV2 was observed in the case calves or BVDV-infected calves.

Discussion/Conclusion: The predominant bone marrow lesion in this series of young calves with haemorrhagic presentation was trilineage hypoplasia. Severe depletion of megakaryocytes provides an explanation for the thrombocytopenia and haemorrhages. Trilineage hypoplasia in cattle and other species has been documented in association with genetic, viral (*eg* retroviruses, herpesviruses, parvoviruses, circoviruses), toxic (*eg* T2 toxin, chemotherapeutic agents) and immune-mediated (T cell) aetiologies. The latter includes many human cases previously categorised as idiopathic. **Sternum is the site of choice for detection of lesions of trilineage hypoplasia in young calves with idiopathic haemorrhagic syndrome.**

Copper deficiency and hair loss syndrome associated with high mortality in California mule deer (*Odocoileus hemionus californicus*)

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Over two hundred deer died between March and June of 2009 in resident mule deer herds in Tuolomne County near Yosemite National Park. Ten deer were submitted to the California Animal Health and Food Safety Laboratory System, Davis for necropsy and/or histopathology. Deer were from herds experiencing hair loss from which an exotic louse, *Bovicula tibialis*, was identified. Diagnostic work-up included: ELISA for clostridial alpha, beta and epsilon toxin; culture for Salmonella, aerobic bacteria and Mycoplasma; PCR for West Nile virus (WNV), Bluetongue virus (BTV) and Epizootic Hemorrhagic Disease virus (EHDV); and immunochemistry for adenovirus, *Toxoplasma gondii*, *Neospora caninum* and coronavirus; serology for antibodies to EHD and BT viruses; cholinesterase activity in the brain; nitrate screen; heavy metal and selenium analysis; and direct electron microscopy for enteric viruses.

In addition to pediculosis in some deer, the most significant findings were selenium deficiency in 9/10 and copper deficiency in 8/10. Other findings included verminous pneumonia in 9/10, myocarditis in 4/10, mild encephalitis in 2/10, and vasculitis in 2/10. Body hair and serum were analyzed for copper and selenium from five live deer that were captured from a herd experiencing mortality and hair loss in the region. All five serum samples had copper levels within the acceptable range for deer, but copper levels in all hair samples were suboptimal. All whole blood and hair samples had suboptimal selenium levels. **Copper and selenium deficiencies were highly associated with hair loss and pediculosis in this mortality event.**

Serum chemistry reference ranges in captive Alaskan reindeer (*Rangifer tarandus*)

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Few studies have established serum chemistry reference ranges in captive reindeer. Serum chemistry analysis is an important diagnostic tool for evaluating the health of individual animals and to establish reference ranges for herd health management. Reindeer have evolved in high latitude ecosystems and have unique physiology, which merits study. The purpose of this study was to establish reference ranges for 18 serum chemistry analytes in captive Alaskan reindeer (*Rangifer tarandus*) and assess potential differences based on age, gender, reproductive status and annual cycle.

Blood samples were collected from 91 reindeer through the reindeer research program farm, University of Alaska, Fairbanks. Samples were collected during winter, spring and summer seasons from females, males and steers. Females were divided into three categories: non-pregnant, pre-parturition and post-lactation. Calves were sampled at 40 days of age. Analysis of serum biochemical analytes was performed using commercially available standard procedures. Serum protein concentrations were measured by densitometric scanning of proteins following agarose gel electrophoresis. Reference ranges were calculated as the central 95% confidence interval.

Significant differences were observed between adults and calves for ten analytes: albumin, alkaline phosphatase, alanine aminotransferase, urea, creatinine, glucose, gamma globulins and total protein. In addition, there were significant differences between pre-parturition and post-lactation females for five analytes: alkaline phosphatase, alanine aminotransferase, urea and total calcium.

The reference ranges for common biochemical analytes reported in this study provide baseline data for future studies of captive and free-ranging reindeer and can aid veterinarians working with this species. Subsequent studies will make it possible to monitor trends in the analytes tested. Changes in these analytes may correlate with changes in nutritional status, new and emerging diseases and potential ecosystem perturbation.

Causes of morbidity and mortality in moose (*Alces alces*) from Minnesota

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The moose population in Minnesota is decreasing. Diseases are considered to contribute to this decline. Current data regarding diseases in Minnesota's moose are lacking. The carcasses of 32 moose were submitted to the Minnesota Veterinary Diagnostic Laboratory for necropsy including histopathology and ancillary testing (e.g. parasitology, bacteriology, and toxicology). Twenty seven animals [10 adults (≥ 3 years), all four subadults, twelve of fourteen calves (≤ 1 year), and one animal of unrecorded age] had an inadequate nutritional state. Brains of 18 moose (4 adults, 3 subadults and 11 calves) had evidence of larval migration (presumptive *Parelaphostrongylus tenuis* infection) that was associated with significant histologic brain lesions in 13 moose. Fourteen moose (8 adults, 2 subadults and 4 calves) had significant hepatitis consistent with *Fascioloides magna* infection. Significant winter tick (*Dermacentor albipictus*) infection was detected in 9 animals (1 adult and 8 calves). Vehicular trauma was the reported or suspected cause of death in 4 moose while one calf likely died due to complications of a presumptive predator-induced wound. The cause(s) of disease remained undetermined in 2 adult animals. **In conclusion, concurrent significant parasitic infection is highly prevalent in Minnesota's moose population and affects all age groups. Continuing postmortem work up of moose is required to obtain more data and should include moose that are sick or died of natural causes and moose that died of anthropogenic trauma (hunter and vehicle killed). Efforts to conduct necropsies of radio-collared moose are essential to reduce data bias as a result of random submissions.**

Clostridium perfringens type C and *Clostridium difficile* combined enter-typhlo-colitis in foals

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Clostridium perfringens type C is one of the most important agents of enteritis in newborn animals, including foals. The disease is mediated by beta toxin, a highly trypsin sensitive metabolite, which explains why newborn animals, that have low levels of trypsin in the intestine, are particularly susceptible to this infection. *C. difficile* is considered the principal infectious cause of antibiotic-associated diarrhea in adult humans and it is now recognized as an important cause of enterocolitis in horses of all ages. While infections by *C. perfringens* type C or *C. difficile* are frequently seen in foals, we are not aware of any report describing combined infection by these two microorganisms in foals or in any other animal. We present here six cases of foal enterocolitis associated to *C. difficile* and *C. perfringens* type C infection. Six foals of ages ranging between one and seven days were submitted for necropsy examination to the San Bernardino branch of the California Animal Health and Food Safety laboratory system. The six animals had a clinical history of acute hemorrhagic diarrhea followed by death; none of these animals had received antimicrobials. Postmortem examination revealed severe hemorrhagic and necrotizing entero-typhlo-colitis. Histologically, the superficial mucosa of both the small intestine and colon presented severe diffuse necrosis and hemorrhage, and it was covered by a thin pseudomembrane composed by desquamated epithelial cells, mixed leukocytes, fibrin, cell debris and large number of large Gram positive bacilli. Thrombosis was observed in veins and arteries of the lamina propria and submucosa. Immunohistochemistry of small intestinal and colonic sections of all foals showed that most large bacilli in the sections were positive for *C. perfringens*. *C. perfringens* beta toxin and *C. difficile* toxins A/B were detected in small intestinal and/or colonic contents of all animals by ELISA. A rich culture of *C. perfringens* (identified as type C by PCR) was isolated from the small intestine and/or colon of 5 animals. *C. difficile* (typed as A-/B+ by PCR) was isolated from the small intestine in 4 out of the 6 cases. This report suggests a possible synergism of *C. perfringens* type C and *C. difficile* in foal enterocolitis. Because none of the foals had received antibiotic therapy, the predisposing factor for the *C. difficile* infection remains undetermined; it is possible that the *C. perfringens* infection acted as a predisposing factor for *C. difficile*. **This report stresses the need to perform a complete diagnostic work up in all cases of foal digestive disease, even when one possible etiological agent has already been identified.**

Pathology of experimental *Avian Bornavirus* infection in psittacines and chickens

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Proventricular dilatation disease (PDD) was first recognized in psittacines in the late 1970's. PDD has been observed in more than 80 species of psittacines but has also been observed in some non psittacine species. PDD is one of the most common and often fatal diseases of psittacines. Clinical signs of PDD include anorexia, regurgitation, passing of undigested seeds in the feces, diarrhea, lethargy, loss of weight and neurological signs as well as sudden death. PDD is characterized by dilation of the proventriculus in most cases, with associated microscopic changes such as lymphoplasmacytic ganglioneuritis involving the gastrointestinal tract, nonsuppurative encephalomyelitis, peripheral neuritis and ganglionitis, myocarditis, adrenalitis and lesions in the eye and skin.

The cause of PDD remained unknown till 2008 when Kistler *et al.* and Honkavuori *et al.* independently reported on the recovery of a novel Bornavirus from birds with PDD. The virus was named Avian Bornavirus (ABV) because it was quite distinct and shared only 65 % nucleotide sequence with the well known Borna disease virus (BDV) of mammals. Based on nucleotide sequence analysis of numerous ABV isolates from psittacines, 6 distinct genotypes designated ABV1 to ABV6 have been identified.

PDD was successfully reproduced in Patagonian conures (*Cyanoliseus patagonus*) inoculated with ABV4 by the intramuscular route. At examination 66 days post inoculation (p.i.), **typical lesions of PDD such as dilated proventriculus and lymphoplasmacytic ganglioneuritis of the crop/esophagus, proventriculus, gizzard, intestine, adrenalitis, myocarditis, encephalomyelitis and neuritis were observed.**

In another experiment cockatiels (*Nymphicus hollandicus*) which were healthy carriers of ABV4 were inoculated intramuscularly and orally with another strain of ABV4 and examined between 92 and 110 days p.i. revealed unusual lesions. Most of the birds had dilated proventriculi but in contrast the microscopic examination showed unusually severe and widespread lesions. **These included massive lymphocytic inflammatory infiltration and lymphoid nodule formation within and around the ganglia through out the gastrointestinal tract. In addition there were similar lesions in the kidneys, spleen, liver, adrenal medullary regions, heart, pancreas, testes and ovary.** Immunohistochemistry of the various organs demonstrated ABV antigen not only in the nervous tissue but also in the mononuclear inflammatory cells infiltrating the various organs.

Several 5-day-old ABV and specific pathogen free chickens were inoculated either intracerebrally or intramuscularly with ABV4. The chickens have remained clinically normal six weeks p.i. The chickens will be observed till they are about 120 days-old and will be euthanized if no clinical signs develop and tissues will be examined microscopically.

Virology Scientific Session

Sunday, November 14, 2010
Salon A

Moderators: Peter Timoney and Julia Ridpath
Sponsor:

08:00 AM **Comparative susceptibility of selected cell lines for the primary isolation of *Equine arteritis virus***
Peter Timoney, Carol Bruser, William McCollum, Reed Holyoak, Tom Little. 100

08:15 AM **Vaccination of cattle persistently infected with BVDV does not cause a change in the consensus sequence of the structural proteins of the viral quasispecies**
John Neill, Jeremy Schefers, Chris Chase, Julia Ridpath. 101

08:30AM **Change in predominance of *Bovine viral diarrhea virus* subgenotypes among samples submitted to a diagnostic laboratory over a 20-year time span**
Julia Ridpath, Gayla Lovell, John Neill, Thomas Hairgrove, Binu Velayudhan, Richard Mock. 102

08:45AM **Characterization of an antiviral compound effective against several pestiviruses ***
Benjamin Newcomer, Mylissa Marley, Julia Ridpath, John Neill, Dan Givens. 103

09:00 AM **Singular PCV2a or PCV2b infection results in apoptosis of hepatocytes in clinically affected gnotobiotic pigs ***
Avanti Sinha, Kelly M. Lager, Chong Wang, Tanja Opriessnig. 104

09:15 AM **Detection and genetic diversity of VP7 in porcine group B rotavirus in the United States***
Douglas Marthaler, Kurt Rossow, James Collins, Jelle Matthijnssens. 105

09:30 AM **Application of the gold standard rabies diagnostic technique for brain material to salivary glands as an estimate of viral shedding and potential evolutionary adaptation to new species**
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09:45-10:15 AM **BREAK**

10:15 AM ***Influenza A virus (H1N1)* in two cats with severe respiratory disease**
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10:30 AM **Genetic and antigenic characterization of recent human-like H1 swine influenza virus isolates**
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***** Graduate student presentation

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Comparative susceptibility of selected cell lines for the primary isolation of *Equine arteritis virus*

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Historically, isolation of equine arteritis virus (EAV) has been attempted in different primary and secondary kidney cell cultures, as well as in a range of continuous or diploid cell lines of horse, rabbit, hamster and monkey origin. Whereas isolation of the virus from horses experimentally infected with the Bucyrus strain of EAV has seldom presented a problem, attempts to isolate EAV from *bona fide* outbreaks of EVA using the same cell systems have not always met with similar success. This is especially the case when attempting virus isolation from the semen of carrier stallions.

The following study was undertaken to determine the relative susceptibility of a range of commonly used cell lines for the primary isolation of EAV from assorted clinical/necropsy specimens from cases of natural or experimentally acquired infection.

A total of 5 cell lines were used in the study, 4 of which were obtained directly from the American Type Culture Collection, Rockville, MD, USA. These comprised the LLC-MK_2 (ATCC CCL7) rhesus monkey kidney cell line, RK-13 (ATCC CCL37) rabbit kidney cell line, Vero (ATCC CCL81) African green monkey cell line, and the equine dermis (ATCC CCL 57) cell line. The fifth cell culture system was another line of RK-13 cells, designated RK-13 (KY) of very high passage history which had been employed in this laboratory for many years for the virological and serological diagnosis of EAV infection. All of the cell lines were used within a 10 passage range. Whereas each line was confirmed free of bacterial/mycoplasma contaminants, all of them were positive for non-cytopathic bovine viral diarrhea (BVD) virus.

A total of 59 specimens, comprising secretions, body fluids or tissues from cases of natural or experimental EAV infection were evaluated for infectivity in the selected cell lines. The specimens were screened for infectivity by inoculation of 3-4 day-old confluent monolayers and a minimum of 2 x 25 cm^2 flasks per inoculum. In the case of each specimen, all five cell lines were inoculated on the same day using the same source material.

Under the conditions of the study, the primary isolation rate was highest in the RK-13 (KY) cell line, regardless of source of the inoculum. The next most sensitive was the ATCC derived RK-13 cell line, followed by LLC-MK_2, E. dermis and Vero cells. Respective sensitivities of the different cell lines for the primary isolation of EAV varied depending on whether the source material was nasopharyngeal swabs, buffy coats, body fluids, tissue homogenates or semen. Greatest differences was noted when attempting isolation from semen.

The isolation rate for the 30 semen samples tested was 100% - RK-13 (KY), 90% RK-13, 80% - LLC-MK_2, 74% - E. dermis and 43% - Vero. The majority of virus isolates were made on initial passage in cell culture, irrespective of cell line. In several cases, EAV was not detected until second passage in culture; this was most evident when using Vero cells.

The results of this study clearly illustrate the importance of selecting the appropriate cell line for the primary isolation of EAV. This is especially significant when attempting isolation from semen. Of 5 cell lines studied, the high passage RK-13 (KY) provided greatest sensitivity for detection of virus. Both RK-13 cell lines were superior to the other 3 lines, especially E. dermis and Vero, neither of which should be recommended for the primary isolation of EAV from semen, nasopharyngeal swabs or buffy coat specimens. It is worth emphasizing that most isolations of EAV, especially in both RK-13 cell lines were made on initial passage in cell culture.

Vaccination of cattle persistently infected with BVDV does not cause a change in the consensus sequence of the structural proteins of the viral quasispecies

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Bovine viral diarrhea virus (BVDV) is a ubiquitous viral pathogen of cattle worldwide. An interesting aspect of these viruses is the great amount of sequence diversity that exists amongst strains in circulation in livestock herds. This diversity impacts diagnostic testing and control by vaccination. The driving force behind change in sequence is not known but the inaccurate replication of the genomic RNA by a viral RNA polymerase without proof-reading capabilities is believed to be a major source of errors. Additionally, immune pressure on structural proteins is also thought to play a role in generating differences between strains.

To test the hypothesis that immune pressure on the virus results in the selection of new viral structural protein sequences from the quasispecies population, cattle persistently infected with BVDV (PI) were vaccinated with commercially available killed virus vaccines and the nucleotide and amino acid sequences of the structural proteins of the persistent viruses were determined at various times post-vaccination. To do this, six calves infected in utero with the same progenitor virus were divided at random into two groups of three animals, with a seventh calf acting as a non-vaccinated control. The first group of three animals received the recommended dose of Triangle 4 Plus that contained a BVDV type 1a virus while the second group received the recommended dose of Triangle 4 that contained BVDV types 1a and 2. The calves were vaccinated on days 0 and 21 and were bled on day 0 and then at weekly intervals through day 35. Viral RNA was purified from the serum using the Qiagen Viral RNA purification kit. The sequences encoding the structural proteins were amplified by PCR and were sequenced.

The sequences of the structural proteins of the persistent viruses on Day 0 were amplified and compared. The seven calves used in this study were all derived from the same progenitor virus and thus all viruses had a very high degree of sequence similarity (>99%). There were slight differences in nucleotide sequence noted between viruses but this consisted solely of point mutations. Next, the Day 35 viruses were subjected to PCR amplification and DNA sequencing in the same manner and these sequences were compared to those of Day 0. **This comparison showed that there were no sequence differences between the 2 bleeding dates in any of the viruses. The immune responses of these calves following vaccination did not result in changes in the structural proteins of these viruses.**

This was a surprising result because it was hypothesized that virus replicating in the presence of an immune response would develop protective changes in the nucleotide and amino acid sequences of the immunodominant proteins. This experiment was done using killed vaccines containing type 1a and type 2 viruses while the persistent viruses were type 1b. It is not known whether a type 1b killed vaccine would have more of an effect because a vaccine of this type was not available at the time of the experiment.

Change in predominance of *Bovine viral diarrhea virus* subgenotypes among samples submitted to a diagnostic laboratory over a 20-year time span

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While the causative agent of bovine viral diarrhea was initially categorized as one species, phylogenetic analysis revealed that these viruses belong to two different species, bovine viral diarrhea virus type 1 (BVDV1) and BVDV2. Further analysis led to the recognition of multiple subgenotypes within both species (BVDV1a through BVDV1k and BVDV2a and b). From the first characterization of noncytopathic BVDV in 1957 until the 1980s, BVDV isolations reported in the literature belonged exclusively to the BVDV1 species. Most lab reference strains and vaccines strains in use from the 1960s to the 1990s belonged to the BVDV1a subgenotype. In 1994 it was recognized that a syndrome known as hemorrhagic disease resulted from infection with a genetically distinct group of pestiviruses, BVDV2. Recognition of antigenic differences between BVDV1 and BVDV2 strains led to the development of bivalent BVDV vaccines, in the 1990s, that contained antigens derived from both BVDV1a and BVDV2a strains. Surveys conducted between 1994 and 2008, report three BVDV subgenotypes circulating among cattle in the United States; BVDV1a, BVDV1b, and BVDV2a. The average percent prevalence of BVDV1a, BVDV1b, and BVDV2a strains reported in surveys prior to 2001 were 21%, 43%, and 36%, respectively. Surveys conducted on viruses isolated after 2001 reported decreasing percentages of BVDV1a and BVDV2a strains with BVDV1b strains accounting for 75% to 100% of samples. Comparison of these surveys is confounded by differences in geographic location, method of collection, and type of sample used in survey. The purpose of this study was to determine if there was a shift in the prevalence of BVDV subgenotypes in the same geographic region in samples collected by the same laboratory over time. To this end, submissions to the Texas Veterinary Medical Diagnostic Laboratory, Amarillo, Texas, were analyzed over a 20-year time span. The same methods of sample collection, storage, and virus isolation were used over the span of the study. BVDV strains isolated in years 1988, 1998, and 2008 were genotyped and prevalence of BVDV1a, BVDV1b, and BVDV2a strains were determined. A total of 155 strains were genotyped based on comparison of sequences from the 5' untranslated region (5' UTR). Of these strains, 66 were isolated in 1988, 44 in 1998, and 44 in 2008. Of the 1988 strains, two were re-isolations of BVDV1a vaccine strains and one sample had a mixture of BVDV strains. Of the remaining strains, 51% were BVDV1a strains, 41% were BVDV1b strains, and 8% were BVDV2a strains. Four of the 44 strains isolated in 1998 were re-isolations of BVDV1a vaccine strains. Of the remaining strains, 31% were BVDV1a strains, 53% were BVDV1b strains, and 16% were BVDV2a strains. Of the 44 strains isolated in 2008, 12 were re-isolations of BVDV1a vaccine strains. Of the remaining strains, 18% were BVDV1a, 61% were BVDV1b, and 21% were BVDV2a. **The proportion of field strains identified as BVDV1a showed a steady decrease over the observed time span.** The preponderance of BVDV1a strains among the earliest reported BVDV isolations, also suggests that BVDV1a strains might have been more prevalent in the 1960s than they are now. While the reason for the decline in BVDV1a strains cannot be established from this study, it should be noted that vaccines based on BVDV1a antigens have been available since the 1960s, while vaccine containing BVDV2a antigens have only been available since the 1990s. At present there are very few vaccines that contain BVDV1b antigens.

Characterization of an antiviral compound effective against several pestiviruses

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Introduction: The *Pestivirus* genus of the *Flaviviridae* family consists of four separate species: bovine viral diarrhea virus (BVDV) type 1 and type 2, classical swine fever virus and border disease virus (BDV). Classification of several other viral isolates as pestiviruses has been proposed due to their genetic and structural relatedness to the current member viruses, including HoBi virus and pronghorn virus (PhV). An aromatic cationic compound, 2-(2-benzimidazolyl)-5-[4-(2-imidazolino)phenyl]furan dihydrochloride (DB772), has previously been shown to inhibit both BVDV 1 and BVDV 2 in vitro at concentrations lacking cytotoxic side effects. The compound also inhibits BVDV replication in vivo. The aim of this study was to determine the scope of antiviral activity of DB772 among the pestiviruses.

Materials and Methods: Isolates of BDV, HoBi, or PhV were tested for in vitro susceptibility to DB772. A 24 well plate was seeded with ovine fetal turbinate cells (BDV and PhV) or MDBK cells (HoBi). After incubating for 24 hours, DB772 was added to the wells to achieve a concentration of 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.10, 0.05, 0.02, 0.01, or 0.006 μM DB772 except for the positive control well containing no DB772. The plates were then infected with the appropriate virus at a multiplicity of infection of 0.5 and incubated for four days. The plates were then frozen and wells assayed for the presence of virus by virus isolation and titration (BDV) or PCR (HoBi, and PhV). Each virus was tested in triplicate.

Results: Complete inhibition of BDV was seen when DB772 was included in the culture media at concentrations of 0.05 μM and higher. A concentration of 0.02 μM DB772 was sufficient to decrease viral titers of BDV by 3 log scores. Pronghorn virus was completely inhibited at concentrations of 0.20 μM. Partial inhibition at lesser concentrations was not detected. A concentration of 0.05 μM DB772 was sufficient to completely inhibit HoBi virus replication.

Discussion/Conclusion: DB772 effectively inhibits all pestiviruses studied at concentrations of 0.20 μM or less. As cytotoxicity is not seen until concentrations of DB772 exceed 60 μM, a wide therapeutic window exists. This antiviral compound represents a potential new therapeutic and/or preventative for use in pestivirus infections.

Singular PCV2a or PCV2b infection results in apoptosis of hepatocytes in clinically affected gnotobiotic pigs

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Introduction: Porcine circovirus type 2 (PCV2) can be further subdivided into two main subtypes known as PCV2a and PCV2b. Porcine circovirus associated disease (PCVAD) may be manifest clinically as respiratory disease, failure to thrive, and diarrhea. There are still many gaps in our knowledge about the pathogenesis of PCVAD including information on the degree to which PCV2 induces apoptosis in different tissues. The objectives of this study were to determine whether PCV2 is associated with apoptosis by using hepatic tissues from pigs experimentally-infected and clinically affected with PCVAD, determine if there were differences between PCV2a and PCV2b in inducing apoptosis, and if there was any difference between the apoptotic detection systems used.

Materials and Methods: Forty-eight gnotobiotic pigs were separated into five groups based on inoculation status and development of clinical disease: (1) Non-inoculated, healthy (n=4), (2) PCV2a, healthy (n=10), (3) PCV2a, clinically affected (n=6), (4) PCV2b, healthy, (n=13) and (5) PCV2b, clinically affected (n=15). Formalin-fixed and paraffin-embedded sections of liver from all pigs were analyzed for signs of apoptosis [presence of single strand DNA breaks in the nucleus or presence of intra-nuclear cleaved caspase 3 (CCasp3)] by TUNEL assay and CCasp3 immunohistochemistry (IHC). In addition, the tissues were also tested for presence of cytoplasmic and intra-nuclear PCV2 antigen by an IHC assay.

Results: Specific CCasp3 IHC and TUNEL labeling was detected in the nucleus of hepatocytes in PCV2a and PCV2b infected pigs with significantly ($P<0.05$) higher levels of apoptotic cells in clinically affected pigs with abundant amounts of PCV2 antigen. No significant differences were observed between PCV2a and PCV2b genotypes and TUNEL assay and CCasp3 IHC stain. Sixteen of twenty-one (76%) clinically affected PCV2 inoculated pigs and eleven of twenty-seven (40%) clinically unaffected PCV2 inoculated pigs had moderate-to-severe hepatic lesions characterized by severe diffuse lymphohistiocytic hepatitis associated with degeneration and loss of hepatocytes. Specific CCasp-3 staining and TUNEL labeling was detected in the nuclei of hepatocytes in PCV2a and PCV2b infected pigs with significantly ($p<0.05$) higher levels of apoptotic cells in clinically affected pigs. Clinically affected pigs also had significantly ($p<0.05$) higher levels of PCV2 staining compared to non-affected pigs.

Discussion: This work was done to further advance knowledge on the role of PCV2a and PCV2b genotypes in apoptosis of hepatic cells in gnotobiotic pigs and to determine if there were differences between apoptotic detection systems. We observed that PCV2a- and PCV2b-experimentally-infected gnotobiotic pigs when developed clinical disease associated with severe hepatitis, had abundant amounts of PCV2 antigen associated with inflammatory cells and hepatocytes, and the apoptosis occurring in hepatocyte-like cells was similarly detected by CCasp3 stains and the TUNEL assay.

Conclusion: In conclusion, PCV2a or PCV2b infection results in apoptosis of hepatocytes in clinically affected gnotobiotic pigs with no difference between genotypes in causing apoptosis and no difference in apoptotic detection systems.

Detection and genetic diversity of VP7 in porcine group B rotavirus in the United States

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Rotavirus is a major cause of viral gastroenteritis in young animals. Rotaviruses are classified into seven different groups (A-E). Group A-C rotavirus infections have been associated with humans and animals while D-E have only been detected in animals. Until recently, Group A was considered the major cause of rotavirus diarrhea in pigs. Group B rotavirus (GBRV) has been described in calves and humans in south central and Southeast Asia. GBRV infections have been considered to be uncommon in pigs. From post-mortem evaluations, it became evident that more than group A rotavirus was involved in pre- and post-weaning porcine enteritis. To further clarify the extent of GBRV as a cause of enteritis in pigs, we developed a PCR test for VP7 of GBRV to complement our existing PCR tests for group A and group C rotaviruses (GARV and GCRV respectively). The rotavirus PCR tests were used on clinical samples submitted to the University of Minnesota Veterinary Diagnostic Lab.

Primarily small intestinal homogenate was used in the investigation as well as fecal samples. The age of pigs in the study group ranged from 1-2-days-old to 14-16-weeks old. Samples were also routinely screened for Transmissible Gastroenteritis Coronavirus (TGEV) by PCR. One hundred and seventy three samples were tested over a period of one month. Eighty-one samples (46.8%) were PCR positive for GBRV, 107 (61.8%) were positive for GARV, 100 (57.8%) were positive for GCRV, and 3 (1.7%) were positive for TGEV. Group B rotavirus viral protein seven sequences were compared to previously published sequences. From the 81 samples positive for GBRV, we obtained valid VP7 sequences for 69. The remaining 13 samples showed evidence of mixed infections and could not be accurately analyzed. The 69 GBRV VP7 sequences were deposited into GenBank. In conjunction with the Rotavirus Classification Working Group (RCWG), a nucleotide cut-off value of 80% for VP7 of group B rotavirus relatedness is suggested. The group B sequencing results indicate GBRV has been a common/pre-existing undiagnosed disease entity in pigs for a long time. GBRV was identified in samples from fourteen states. Thorough investigation identified the true genetic diversity of group B rotavirus in swine rather than a rush to focus on a single case and an implication of an "emerging new disease".

The results of this study indicate: 1.) PCR is an excellent tool to identify and classify rotavirus infections in pigs, 2.) new findings may represent better testing capabilities rather than "a new emerging disease" and 3.) group B rotavirus is a common and significant cause of enteritis in pre- and post-weaning pigs throughout the U.S.

Application of the gold standard rabies diagnostic technique for brain material to salivary glands as an estimate of viral shedding and potential evolutionary adaptation to new species

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Introduction: The direct fluorescent antibody technique is the Gold Standard for primary rabies diagnosis and is performed on fresh brain material from rabies-suspect animals (and humans). Through the application of this technique, there are between 6,000 and 10,000 animal rabies cases diagnosed every year in the US. Most of these cases occur in wildlife species. There are a number of diverse rabies virus variants which are sustained through transmission primarily within a single reservoir species. These variants are uniquely adapted to a particular species. Although exposure may result in a clinical case of rabies among individual animals of other species, it is rare to detect sequential or serial transmission of a rabies virus variant outside of its primary reservoir species. However, in view of the high genetic diversity of RNA viruses, there are innate opportunities for current variants to rapidly adapt to new host populations. For example, a rabies virus variant previously associated with Big Brown bats in the Flagstaff Arizona area is now circulating and maintained in striped skunks in this locale. This recent viral adaptation event highlights the need for enhanced surveillance to estimate the potential for emergence of rabies virus variants within new hosts. In this report, our investigative hypothesis is that the presence and quantity of rabies virus in the salivary glands of the primary reservoir host will be greater in incidence and amount than in other naturally infected non-reservoir species. If a rabies virus variant is reliably detected and in high amounts within the salivary glands of a particular spillover species, then this could signal that this is a permissive species for this rabies virus variant and/or that the rabies virus variant is capable of emerging in this species through viral adaptation.

Materials and Methods: In this pilot study, we applied the direct fluorescent antibody (dFA) test to fresh salivary glands from naturally infected rabid animals diagnosed in our laboratory to estimate the potential for emergence of rabies virus variants within new hosts.

Results: Among the right and left salivary glands examined from nearly 180 naturally rabid animals, the dFA test was positive for rabies virus antigen in the majority of skunks infected with the South-Central skunk rabies virus variant (n=339 positive (99%) of 343 glands examined). Moreover, the quantitative ranking of amount of antigen was an average of 3.1 of a maximum score of 4.0 in these samples. The one raccoon examined which was positive for the raccoon rabies virus variant was also positive for viral antigen in the salivary glands and the amount and distribution was abundant. The number of spillover species examined to date is limited. Nevertheless, it is interesting to note that among 25 cats, 7 dogs, 6 cows, and 1 horse which were naturally infected with the South-Central skunk rabies virus variant, very few samples were positive by dFA on the salivary glands and, among the positive samples, very little rabies antigen was detected. Conversely, three foxes, one coyote, and one bobcat were positive for rabies virus antigen in the salivary glands and virus antigen was relatively abundant in amount and distribution.

Discussion/Conclusion: Although this is a preliminary study, the results are intriguing for the extrapolation of the differential risk of viral adaptation and emergence among these spillover species. Continued investigation is warranted and ideally would include the quantification of live rabies virus through cell culture or mouse inoculation, as well as the detection and quantification of viral genomic material through the reverse-transcription polymerase chain reaction assay. The application of these tools to naturally, and indeed also experimentally, infected animals would be illuminating from the perspective of viral evolution, adaptation, and emergence, and also provide information directly applicable to the estimation of public health risk of rabies transmission from and among these various species, according to the unique characteristics of particular rabies virus variants.

Influenza A virus (H1N1) in two cats with severe respiratory disease

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An 8-year old Maine Coon cat presented to a general practitioner on November 9, 2009 with a five day history of inappetance, lethargy and tachypnea. Both owners reported a history of influenza-like illness (ILI) 2 weeks prior. The veterinarian obtained an oral swab and submitted the sample for influenza A real-time reverse-transcription polymerase chain reaction (rRT-PCR). The sample yielded **suspect positive results for influenza A virus (matrix gene) and for the pandemic H1N1 virus (N1 subtype specific)**. In addition, a serum sample was submitted and was positive for influenza A antibodies by agar gel immunodiffusion (AGID) and positive for H1-specific antibodies by hemagglutination-inhibition (HI).

An 11-year old spayed female domestic short hair cat became inappentant on October 2, 2009. Within several days, the cat developed an intermittent cough. The owner reported that a member of the household had tested positive for influenza A virus and was home ill for a few days prior to the onset of illness in the cat. An oropharyngeal swab collected on November 20 and trans-tracheal wash fluid collected on Novmeber 24 were negative for influenza A virus by rRT-PCR. Serum collected on November 25 was **positive for influenza A antibodies by AGID and H1-specific antibodies by HI**. Additionally, serum was collected from two cats from the same household. Both of these samples were negative for influenza A antibodies.

On the basis of physical exam findings, imaging, diagnostic testing and owner history ILI, **a diagnosis of H1N1 infection was made for both cats**. Even though oropharyngeal swabs were collected for the first case 5 days after onset of illness, only suspect positive results were obtained by rRT-PCR. This is likely due to a very low level of virus present in the sample, likely related to decreased viral shedding at this time point in infection for this species. For the second case, samples were not obtained until seven weeks after onset of illness, so it is not surprising that rRT-PCR results were negative.

Both cats were positive for antibodies to H1N1 influenza virus, providing a diagnosis for these cases. Due to the high transmission rate of H1N1 influenza virus between humans, it was surprising that the other cats in the second household remained antibody negative after exposure to the infected cat and owner. In this household, the owner reported that the infected cat was favored over the other cats in the household and spent significant amounts of time on the owner's lap during the owner's period of illness. This contact likely increased the exposure of the cat to the H1N1 virus and resulted in infection. **In this situation, it appears that close contact from human to cat was important to transmission of the H1N1 virus.** Additionally, the infected cat did not transmit the virus to the remaining cats in the household. This may be due to minimal contact between the sick cat and the other cats or indicate that the low viral shedding of the sick cat was not high enough to cause infection.

Genetic and antigenic characterization of recent human-like H1 swine influenza virus isolates

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Recently, unique human-swine reassortant swine influenza viruses (SIV) were identified that possessed human hemagglutinin and neuraminidase genes with an internal gene constellation similar to those commonly found in triple reassortant SIV. While both H1N1 and H1N2 SIV are currently circulating in U.S. herds, H1N2 accounts for the vast majority of contemporary human-like H1 SIV (δ-cluster) isolates. These new SIV have become endemic in U.S. swine herds and currently account for 35% of the H1 SIV isolated at Newport Laboratories in 2010.

Genetic analysis of the hemagglutinin gene of 2009-2010 δ-cluster isolates identified five distinct sub-clusters with approximately 95-100% sequence similarity within sub-clusters and 91-94% similarity between sub-clusters. Eighteen δ-cluster isolates were antigenically characterized using a recently described high throughput serum neutralization (HTSN) assay utilizing a panel of antisera generated against contemporary H1 SIV isolates. H1 antisera included three of the five sub-clusters of δ-cluster isolates, as well as α, β and γ SIV clusters. Antisera generated against representatives of α, β and γ clusters failed to neutralize any of the 18 δ-cluster viruses. Only 56% of the δ-cluster isolates were neutralized by at least one δ-cluster antiserum. A majority of the viruses not neutralized by δ-cluster antisera were found in one sub-cluster.

Given the high percentage of isolates not neutralized by reference antisera, the viruses and antisera were further characterized by the hemagglutination inhibition (HI) assay using antisera generated from three δ-cluster isolates. HI assay results were in good agreement with the HTSN assay with 61% of the isolates positive for antibodies (HI titer >40) to at least one δ-cluster antiserum.

This study indicates that δ-cluster SIV comprise a large proportion of currently circulating influenza and that significant genetic and antigenic heterogeneity exists within δ-cluster isolates. The inability of antisera generated against three δ-cluster isolates to recognize a significant proportion of contemporary δ-cluster isolates suggests that either multiple representatives of this cluster may be required in commercial vaccines or that herd specific vaccines may be required to protect swine.

Effect of swab type, collection media, and storage on the detection of influenza a virus in porcine nasal secretions

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Clinical samples from humans and animals require collection systems that are easy to use and will safely stabilize and preserve microbial RNA/DNA during transportation from distant collection sites to diagnostic centers. An influenza (flu) detection study was conducted to compare the performance of commonly used rayon tipped swab with liquid Stuart's transport media (RTLS) and flocked swabs with PrimeStore Molecular Transport Medium™ for collecting and transporting nasal secretions from pigs experimentally infected with H1N1 influenza A virus.

The animal phase of this study was conducted in accordance with the approval of the Institutional Care and Use Committee of the University of Minnesota. Twenty two 3-week-old swine from a herd free of flu, *Mycoplasma hyopneumoniae* and PRRSV were randomly assigned to 2 treatment groups. Ten swine per treatment group were inoculated 2 ml intratracheally and 2 ml intranasally with $10^{5.5}$ $TCID_{50}$/mL of influenza A/Swine/IL/02450/2008 (H1N1). Two swine were sham inoculated and served as negative controls. Nasal swabs were taken from each pig at 1, 3, and 5 days post-inoculation (dpi) using both the RTLS and PrimeStore MTM™ collection systems. RTLS swabs were tested immediately after collection for flu by virus isolation (VI) on MDCK cells. If virus isolation attempts were negative, the RTLS swabs were tested for flu by matrix gene RRT-PCR. PrimeStore MTM™ swabs were held for 2 weeks at 4˚C and frozen for 6 weeks at -20˚C prior to testing for flu by RRT-PCR. VI was not performed on PrimeStore MTM™ swabs because the transport solution is designed to safely inactivate viruses and bacteria and preserve the released RNA and DNA. Flu detection results were compared for both media types.

A total of 66 specimens were obtained and placed in each collection medium. **Both the RTLS and PrimeStore MTM™ performed similarly, with 100% agreement in flu detection results** for each pig at 1and 3dpi. Thirty-seven pigs were positive for flu by VI on RTLS collected nasal swabs and PCR on PrimeStore MTM™ collected nasal swabs. Seven pigs at 1dpi and two pigs at 3dpi were negative for flu on both RTLS nasal swabs by VI and PCR and also on PrimeStore MTM™ swabs by PCR. All RTLS flu VI positive nasal swabs were quantified by virus titration and therefore it was determined that RRT-PCR detected influenza virus from swabs collected in PrimeStore™ MTM at levels as low as 56 $TCID_{50}$/mL of virus (C_T value 31.29). The only differences in flu detection performance were found on 5dpi, where 2 RTLS nasal swabs were negative for flu by both VI and PCR, but were positive for flu in PrimeStore™ medium by PCR.

PrimeStore Molecular Transport Medium™ provides a suitable, safe, collection, handling, and preservation system that can be used to detect flu in porcine nasal secretions. PrimeStore™ even detected flu RNA in samples from pigs at 5 dpi, a time when shedding is decreased or non-detectable by VI or PCR using RTLS swabs. A larger field study to compare pecimen collection systems from naturally infected pigs is recommended and will commence in Summer/Fall 2010.

Development of an in process control filtration-assisted chemiluminometric immunoassay to quantify *Foot and mouth disease virus* (FMDV) non structural proteins in vaccine antigen batches

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In many countries, Foot and Mouth Disease (FMD) is controlled by vaccination and surveillance against non structural proteins (NSPs); therefore vaccines are required not to induce antibodies against NSPs. Vaccine purity is evaluated by repeated inoculation of naïve cattle, an expensive and time consuming protocol that raises several animal welfare concerns. We have developed an in process control filtration-assisted chemiluminometric immunoassay (FAL-ELISA), to detect and quantify NSPs in vaccine antigen batches regardless its volume and composition. Samples are filtered through PVDF-filter microplates pre-coated with a monoclonal antibody against NSPs. Filtration removes all unbound components in the sample and captured NSPs are detected by anti-NSP conjugate followed by incubation with the substrate, luminol/peroxide. Analytical detection limit was 2 ng for purified NSP and about 4 ng for vaccine antigen batches spiked with NSP, which makes this assay sensitive enough to be applied to purity control of FMD vaccines. Vaccine components did not interfere with the antibody and substrate reactions in the assay. FAL-ELISA is an alternative for the in vivo tests, observing the objective to Replace, Reduce and Refine the use of animals for quality control of immunobiologicals.

Generation of stable, DNASE/RNASE resistant, encapsulated, external controls for foreign animal disease testing with real time, reverse transcriptase PCR

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To monitor nucleic acid extraction procedures and PCR-based diagnostics, there is a need for stable cost effective internal and external controls (ICs, ECs). The use of such controls can greatly reduce the risk of obtaining false negative reactions due to poor assay performance, the presence of PCR inhibitors in a clinical sample, or poor quality nucleic acid extractions. Such controls may also serve as reference standards and proficiency test samples for monitoring assay performance and technical capability. Naked nucleic acid controls cannot fully meet these needs as they are less stable and more susceptible to nuclease mediated degradation, thus making it difficult to obtain predictable and quantifiable control data. RNA controls/standards to support RT-PCR applications are particularly susceptible to degradation.

In recent years new options have emerged including use of a recombinant plant picornavirus (King et al., 2007) or use of commercially available Armored RNA (Pasloske et al., 1998), a custom RNA control encapsulated *in vitro* in a coliphage-like particle. Production of these controls is often expensive or technically challenging. To support real-time-PCR (rRT-PCR) applications, such controls incorporate a protected nucleic acid target that contains primer and probe binding sequences that are appropriate to a particular diagnostic assay. Rinderpest is an infectious viral disease of cattle, buffalo, yak and numerous wildlife species which in the past caused serious economic devastation. Rinderpest is anticipated to be declared globally eradicated with only small areas of Somalia still harboring disease in the recent past (Normile, 2008). Because of this, use of attenuated strains to generate positive controls or reference standards is precluded. At the same time, diagnostic readiness needs to be maintained. To bridge this gap we have genetically engineered an encapsulated, defective, synthetic control containing the primer and probe binding sites for the Rinderpest virus (RPV) and no other part of the RPV genome.

The resulting control was found to be stable at 4^{o}C and -20^{o}C. Limits of detection and reproducibility were determined, and the control was found to be stable in a variety of sample types making it suitable as an internal control for sample extractions. We have applied this technology to develop extraction controls, proficiency test samples, and reference standards for rRT-PCR diagnostic assays to detect other high-consequence transboundary animal diseases including classical swine fever and foot-and-mouth disease. The use of recombinant bacteriophage can be an efficient and cost effective means of generating internal or external controls for monitoring performance of PCR-based diagnostic assays

Comparison of Idexx 2XR and X3 kits for the detection of antibodies to *Porcine reproductive and respiratory syndrome virus*

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Porcine Reproductive and Respiratory Syndrome (PRRS) is an economically important disease affecting the U.S. swine industry. The most common test used by diagnostic laboratories for herd profiling of PRRS antibodies is Herdcheck PRRS 2XR ELISA available from Idexx Laboratories. However, this test has been shown to have non-specific positive reactions (up to 1.5 %) in certain populations of gilts and sows, which are referred to as "Singleton reactors" (Munoz *et al*., Idexx laboratories). Recently, Idexx Laboratories in Europe have released a new, modified ELISA (PRRS X3) that claims up to a 90% reduction in Singleton reactors. According to Idexx, the new kit has 99.9% specificity and 98.8% sensitivity and provides early detection of PRRS antibodies.

At University of Minnesota Veterinary Diagnostic Laboratory, we compared the performance of PRRS X3 kit with that of PRRS 2XR kit using different types of field samples. The X3 kit showed up to 85% reduction in Singleton reactors. Both kits showed 93% agreement with X3 kit being slightly more sensitive (statistically non-significant). These results indicate that **the new X3 kit can be used in place of 2XR kit for eliminating Singleton reactors**.

Comparison of *Equine arteritis virus* antibody detection by cELISA and serum neutralization

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Equine arteritis virus (EAV) is a contagious disease of horses with signs that include fever, anorexia, conjunctivitis, nasal discharge, edema and abortion with death occurring in young foals. USDA APHIS and OIE define a horse as seropositive for EAV if the serum neutralization (SN) test is positive with a titer ≥1:4. However, determining the SN titer is time-consuming and requires extensive laboratory facilities, equipment, and technical expertise. Further, SN titer interpretation is difficult with some sera because of non-specific cellular cytotoxicity. Another serologic test would be useful and among the several described, none are reported to have requisite sensitivity and specificity relative to SN. To this end, a competitive ELISA (cELISA) was developed using EAV G_L protein-specific monoclonal antibody 4B2 as the blocking antibody (Cho HJ, Entz SC, Deregt D, Jordan LT, Timoney PJ, McCullum. Detection of antibodies to equine arteritis virus by a monoclonal antibody-based blocking ELISA. Can J Vet Res 64:38-43, 2000). This test has been improved by changing the monoclonal antibody to 17B7 and several other test format variables. The relative specificity of the new cELISA was 99.8 % in a comparison with 2223 SN negative sera and the sensitivity was 95.5 % when evaluated with 246 SN positive sera (≥1:4 titer). These results indicate that this new cELISA may be a useful adjunct to the SN test pending further validation with SN positive field sera.

Microbiology Scientific Session

Sunday, November 14, 2010
Salon C

Moderators: Ching Ching Wu and Amar Patil
Sponsor:

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Edward Dubovi, Nancy Zylich, Melissa Laverack, Amy Glaser, Randall Renshaw. 116

08:15 AM **A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *Borellia burgdorferi* outer surface protein A (OspA), OspC and OspF in canine serum**
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08:30 AM **Isolation of a new *Streptococcus*-like bacterium from a goat abscess**
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08:45 AM **Isolation of a *Streptococcus*-like organism from the milk of a cow with mastitis**
Yan Zhang, Jing Cui, Anne Parkinson, Mary Beth Weisner, Beverly Byrum. 119

09:00 AM **Isolation of a new *Corynebacterium*-like bacterium from a cow with septicemia**
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09:15 AM **Comparison of fecal culture and direct fecal real-time PCR in the identification of *Mycobacterium avium* subsp. *paratuberculosis* in fecal specimens**
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09:30 AM **Comparative IS900 and IS1311 direct fecal *Mycobacterium avium* subspecies *paratuberculosis* nested PCR tests: Significance of disparities ◊**
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09:45 AM **TREK: Antimicrobial Resistance**
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10:00-
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10:30 AM **Bacterial identification using pyrosequencing for animal pathogens**
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10:45 AM **Evaluating *Tritrichomonas foetus* samples with high CTs**
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11:00 AM **Comparisons of 4 methods for the detection of canine *Giardia***
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11:30 AM **Comparison of five enzyme immunoassays (EIAs) for detection of anti hepatitis E virus (HEV) immunoglobulin G (IgG) in swine**
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11:45 AM **Efficacy of *Canine influenza virus* (CIV) (H3N8) vaccine in dogs co-infected with CIV and *Streptococcus equi* ss *zooepidemicus***
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◊ USAHA paper

Exhibit Hall Open 7:00am - 2:00pm

Distribution of *Canine pneumovirus* (CnPnV): Seroprevalence and identification of virus in dogs with acute respiratory disease.

Edward Dubovi, Nancy Zylich, Melissa Laverack, Amy Glaser, Randall Renshaw
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A novel pneumovirus closely related to murine pneumovirus was recently identified, among other respiratory pathogens, during the course of a research study of respiratory disease in dogs in a shelter population in the Northeast US. To gain a better understanding of the potential contribution of this virus to canine respiratory disease we developed a microtiter serum neutralization assay to detect antibody in serum and a real-time RT-PCR assay based on the nucleocapsid sequence for detection of virus. Nasal swab samples submitted during the shelter study were analyzed retrospectively by RT-PCR. The virus was identified in 71% (N=100) of the dogs sampled in the shelter study at day 5 or if ill. A subset of dogs was sampled at entry (day 0) and at day 5 or if ill (N=74). Two dogs tested positive for virus at day 0 with C_t values near the assay endpoint at 40 cycles while one dog was positive with a C_t value of 36.54. Analysis of the samples collected at day 5 or ill revealed that 37 dogs became virus positive, and 2 of the dogs with detectible virus at day 0 remained virus positive (53%).

To determine if dogs outside of the shelter environment had exposure to CnPnV, 119 serum samples from dogs ranging in age from 14 weeks to 13 years submitted for routing vaccine titer analysis from multiple locations in the US and Canada were tested for antibody to CnPnV at two fold dilutions from 1:4 to 1:48. Positive titers of 4 to >48 were identified in 81 samples (68%) from most regions from which samples were analyzed. To determine how frequently the virus could be detected in dogs with respiratory disease, nasal swab samples submitted by private practitioners from dogs with respiratory disease from diverse regions of the US were tested using the CnPnV PCR assay. A total of 216 samples were analyzed and 46 were positive for CnPnV RNA with C_t values ranging from 21 to the assay endpoint of 40 cycles (21.3%) Together, the data suggest that CnPnV is a common virus infection of dogs and virus can readily be identified in clinical samples obtained from dogs with acute respiratory disease from multiple geographic regions. The role the virus may play in causing acute respiratory disease in dogs is currently under investigation.

A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *Borellia burdorferi* outer surface protein A (OspA), OspC and OspF in canine serum

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Introduction: Lyme disease is caused by infection with the spirochete *B. burgdorferi*. It is a zoonotic, vector-borne disease affecting humans, dogs, and horses and possibly other mammalian species. The bacteria are transmitted to the mammalian host by infected ticks (*Ixodes*). Lyme is the most common vector-borne disease in the United States and Europe. The serological detection of antibodies to *B. burgdorferi* is commonly performed by ELISA followed by a confirmatory Western blotting (WB) procedure.

Material and methods: *B. burgdorferi* OspA, OspC and OspF proteins were expressed in *E. coli* and purified by Ni-affinity chromatography. The recombinant proteins were coupled to multiplex beads of three different fluorescent color codes which provided the matrix for the assay. The assay was measured in a Luminex analyzer. Two sets of canine sera were used for the validation of the multiplex assay. First, sera from 79 dogs with known ELISA and WB results were used to establish the conditions of the assay. These samples were selected to provide similar numbers of sera ranging from negative to high positive results by WB and included sera from vaccinated and/or naturally infected dogs. Second, a total of 188 canine serum samples that were not tested previously were used for further multiplex assay validation. All samples were analyzed in parallel for antibodies to *B. burgdorferi* antigens by WB. The presence (positive) or absence (negative) of serum antibodies to the 31 kDa (OspA), 22 kDa (OspC) and 29 kDa (OspF) was determined blindly by a technician who was not aware of the multiplex assay results. The WB results provided a 'relative gold standard' for each antigen.

Results: A high correlation was observed for detection of antibodies to *B. burgdorferi* in single and multiplex assays (n=79) resulting in Spearman's rank correlations of 0.93, 0.88 and 0.96 for OspA, OspC and OspF, respectively. The multiplex assay results for each antigen were then compared to the presence or absence of serum antibodies to the corresponding *B. burgdorferi* protein detected by WB (n=188) using a ROC curve analysis. The areas under the curves were 0.93 for OspA, 0.89 for OspF, and 0.82 for OspC. In addition, multiplex assay interpretation ranges for antibodies to all three *B. burgdorferi* antigens were established by a likelihood analysis.

Conclusion: The new fluorescent bead-based multiplex assay provides a sensitive and fully quantitative platform for the simultaneous evaluation of antibodies to *B. burgdorferi* OspA, OspC and OspF. The multiplex assay distinguishes between antibodies that originated from vaccination or natural exposure to *B. burgdorferi*. Compared to conventional testing by ELISA and WB, multiplex technology is less time consuming and allows high throughput of samples.

Isolation of a new *Streptococcus*-like bacterium from a goat abscess

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Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH 43068

A 3-month-old female Boer goat with facial lumps was presented to a veterinary clinic. Physical examination indicated that both the submandibular and prescapular lymph nodes were swollen. The abscess sample aspirated from the submandibular lymph node was submitted to the Animal Disease Diagnostic Laboratory for confirmation of caseous lymphadenitis. After 24-hours of incubation, a heavy growth of two bacterial organisms was isolated from aerobic culture. One of the bacteria from the aerobic culture was an *Actinobacillus*-like organism. The other isolate was not identifiable using routine microbiologic methods. In addition to the aerobic culture, a third bacterium type was isolated from an anaerobic culture and was identified as *Fusobacterium necrophorum*. **DNA sequencing analysis on the 16S rRNA gene of the unidentifiable isolate indicated that it is most closely related to *Streptococcus minor* (94% identical) and is a member in the genus of *Streptococcus* that has never been reported**.

Isolation of a *Streptococcus*-like organism from the milk of a cow with mastitis

Yan Zhang, Jing Cui, Anne Parkinson, Mary Beth Weisner, Beverly Byrum
Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH 43068

A milk sample from 3-year-old Jersey cow with chronic mastitis was submitted to the Animal Disease Diagnostic Laboratory for culture and sensitivity. After 24-hours of incubation in both aerobic and anaerobic conditions, a heavy and pure growth of a bacterium was isolated. The colony was pinpoint, smooth, non-haemolytic, and round. The bacterium was Gram positive and morphologically resembled a *Corynebacterium* species. Conventional microbiologic methods failed to identify the organism**. DNA sequencing analysis on the 16S rRNA gene indicated that the organism is closely related to *Streptococcus suis*. Phylogenetic studies demonstrated that the organism is a new species in the genus of *Streptococcus* and has never been reported**.

Isolation of a new *Corynebacterium*-like bacterium from a cow with septicemia

Yan Zhang, David Newman, Jing Cui, Anne Parkinson, Beverly Byrum
Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH 43068

A Holstein cow developed respiratory disease three weeks after calving. The animal did not respond to treatment and was submitted to the Animal Disease Diagnostic Laboratory for euthanasia and necropsy. The most remarkable gross and histological findings included: multifocal, moderate to marked fibrinous bronchopneumonia with occasional pulmonary parenchymal abscesses and severe, diffuse suppurative mastitis involving multiple quarters. Other pathologic changes were: moderate, focally extensive vegetative valvular endocarditis and multifocal, portal hepatitis with moderate, multifocal centrilobular hepatic vacuolar change. Viral pathogens were not detected by direct florescent antibody or viral isolation. Heavy growth of two bacterial types was isolated from both lung and mammary gland. One of the bacteria was identified as *Arcanobacterium pyogenes.* The second was not identifiable using conventional microbiologic methodology. **DNA sequencing analysis of the 16S rRNA gene indicated that the organism was a new species belonging to the genus *Corynebacterium*. The bacterium was most closely related to *Corynebacterium diphtheria* (95% identical) and *Corynebacterium hansenii* (94% identical). The etiologic significance of the organism was not clear, but might have contributed to the clinical disease.**

Comparison of fecal culture and direct fecal real-time PCR for detection *of Mycobacterium avium* subsp. *paratuberculosis* in fecal specimens

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Heat-shock proteins are present in all organisms under normal temperatures. Heat-shock proteins (HSP 90, HSP 70, and HSP 60) are induced by cells in response to raised temperature, starvation, oxygen radicals, toxins, and viral and bacterial infections. When an organism is phagocytized by a neutrophil, heat-shock protein production by the initiating organism is significantly increased. Heat-shock protein 60 (HSP 60) is the dominant antigen induced by mycobacterium. The real-time PCR test (Tetracore®)measures heat-shock protein X (presumably hsp60).

The fecal samples were obtained from two dairy herds that participated in The Florida Johne's Disease Dairy Herd Prevention Program. Eight hundred and seventy-one fecal specimens from dairy cows were analyzed in a comparative study of methodologies using the Trek® fecal culture and Tetracore® real-time direct fecal PCR diagnostic systems. The Trek® (fecal culture) and the Tetracore® (real-time PCR) Map Diagnostic Systems were utilized in accordance with their respective manufacturers' instructions. Of the 112 positive fecal cultures, 35 (31.3%) were positive for Map by real-time PCR. Of the 717 negative fecal cultures, real-time PCR detected 61 (8.5%) as being positive for Map. The kappa value between the two tests was 0.242 [0.17; 0.31], indicating poor agreement between the fecal culture and real-time direct fecal PCR tests. **Thus, the herd management decisions regarding the status of Johne's disease infectivity are better made based on multiple tests for detection of Map in a given fecal specimen.**

Comparative IS900 and IS1311 direct fecal *Mycobacterium avium* subspecies paratuberculosis nested PCR tests: Significance of disparities

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Mycobacterium avium subspecies paratuberculosis (Map) is theorized to be an evolutionary adaptation from *Mycobacterium avium* subspecies *avium* (Ma). That, between Map and *M. avium*, pathogenic mycobacterium exist whose insertion sequences do not necessarily completely mesh with the IS900 insertion sequence is to be anticipated. IS1311 is present in *Mycobacterium avium* subspecies *avium* as well as Map. To challenge the hypothesis of genomic polymorphism, two direct fecal nested polymerase chain reaction (PCR) tests based upon the IS900 and the IS1311 insertion sequences were constructed and tested in parallel in four United States Department of Agriculture (USDA) Laboratory Certification tests.

The sensitivities for P90-P91 and J1-J2 IS900 direct and nested primers were 21.7% and 76.7% where as those for the IS1-IS2 and IS3-IS4 primers were 38.3% and 86.7%. Given that the IS1311 based primers, IS1/IS2, identify only 6-8 copies where as the P90/P91 primers based upon the IS900 sequence identify 14-18 copies, there should have been no reason to anticipate that the IS1311 based primers would exhibit superior sensitivity unless the sequences being sought had greater representation.

The ability of 1311 base insertion sequence primers to better identify Map in the USDA laboratory certification tests over IS900 base insertion sequence primers argues for the existence of a degree of genetic polymorphism among culture-positive isolates of Map used in USDA's laboratory certification testing.

Bacterial identification using pyrosequencing for animal pathogens

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Bacterial identification using genetic sequencing is fast becoming a confirmatory tool for both the clinical and the diagnostic microbiologists. Use of genetic sequencing for bacterial identification in veterinary diagnostic microbiology is still expanding but the procedure is quiet cumbersome. Pyrosequencing technology for sequencing is a unique method for short-read DNA sequencing. Its ease of use, sequence validation and flexibility makes it easy for diagnosing bacterial infections rapidly. In this study, we have compared microsequencing with pyrosequencing for identifying bacterial organisms.

Forty five bacterial strains commonly encountered in veterinary diagnostic microbiology laboratories as animal pathogens spanning 21 different bacterial families were sequenced using 16S rRNA gene with pyrosequencing and microsequencing. Pyrosequencing was performed using V1 and V3 region of 16S rRNA. The amplifications frequently yield a sequence for each isolate spanning approx. 20-50 bp. Typical run for pyrosequencing can be completed in 15 minutes for 1-24 samples as opposed to several hours needed for microsequencing spanning 500bp gene fragment.

Pyrosequencing was able to resolve and identify most organisms at least to the genus level, and for up to 50% isolates included in the study, it was also able to identify organisms correctly up to the species level. Microsequencing using 500 bp performed better in species level identification. Cost of pyrosequencing was lower compared to microsequencing, making pyrosequencing a viable first choice for performing veterinary laboratory testing and bacterial genotypic identifications.

Evaluating *Tritrichomonas foetus* samples with high Cts

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The demand for *Tritrichomonas foetus* (TF) testing is greater than ever before due to increased recognition of the disease and the rapid expansion of state import regulations. Most states accept a single PCR or 3 cultures for regulatory purposes, making the classification of a single TF PCR sample critical. Although many labs utilize the same primers and probe for real-time PCR detection of TF, there is not a standardized procedure for the processing of samples. Due to the ramifications of both false positive and false negative results, our laboratory has established a suspect range for TF with recommendations for retesting the animal. This result is somewhat unsatisfactory, especially when testing an animal for shipping purposes; therefore, further examination of samples in the suspect range was performed.

Previous AAVLD presentations have established that proper handling of the sample is critical for accurate test results. Death of the TF organism is believed to release DNases that can decrease the sensitivity of TF PCR, leading to high Cts in a truly positive animal. This can happen as a result of prolonged incubation in the InPouch™ TF (BioMed Diagnostics) or incubation at suboptimal temperatures.

Our laboratory has been performing TF PCR based on the McMillen assay (McMillen, L. and Lew, AE, 2006, Vet. Parasitology 141:204-215) for approximately one year. To date, of 757 samples tested we have had 56 positives and 16 suspects, with the suspect range in our lab defined as a Ct >37. One animal that was resampled and retested came up as suspect on both samples submitted. At that time we decided to test both samples with a conventional PCR designed to detect a broad range of trichimonads. Sequencing results of that amplification demonstrated that the organism was a recently described tritrichimonad, *Simplicimonas moskowitzi* (Cepicka, I., et. al. 2010, Protist 161:400-433). Our laboratory is now performing a restrospective study on samples that were previously classified as suspect for TF. These samples are being evaluated for repeatability on our assay as well as being amplified for sequencing. Thus far we have successfully amplified 6 suspect samples for sequencing, with 3 determined to be *Simplicimonas moskowitzi*, representing two different animals. As time permits, we will also examine samples classified as positive, focusing on those with Cts in the 34-37 range.

At this time, we have evidence that the McMillen assay, typically used for TF PCR can cross-react with a recently described related organism *Simplicimonas moskowitzi*. **Setting strict Ct guidelines may falsely classify animals- a diagnosis that could be costly and detrimental to the herd. It is for these reasons, samples yielding high Ct's should be regarded with caution before classification as positive or negative.**

Comparisons of 4 methods for the detection of canine *Giardia*

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Numerous methods are available for the detection of *Giardia* in canine fecal samples. This study examined the concordance and agreement between 4 detection methods in 202 fecal samples. Detection methods were the modified double centrifugation technique (MDCF) with Sheather's sugar, fluorescent monoclonal antibody on concentrated samples (FAB), coproantigen detection (SNAP), and conventional PCR. Samples were selected from those submitted to the Colorado State University Veterinary Diagnostic Laboratory. All samples were refrigerated upon reciept and processed within 5 days. Estimates of cysts/gram of feces were also determined. Overall, *Giardia* was detected in 102/202 samples. Cohen's Kappa statistic for multiple tests indicated substantial agreement for all tests except PCR. The SNAP and MDCF had the best agreement (Kappa = 0.78; 95% CI: 0.69-0.87), although they detected fewer positive samples than FAB (FAB = 102; SNAP = 78; MDCF = 73). These false negatives tended to be associated with low cyst numbers. PCR detected the fewest positives of all the methods used. This is in agreement with previous studies showing that even in cyst-positive samples, PCR is not always capable of ampifying available DNA. Thus, it appears PCR is best reserved for determination of genotype rather than primary detection of infected animals.

Better detection and further characterization of *Erysipelothrix* spp. isolates from erysipelas outbreaks in vaccinated pig farms using novel diagnostic tools

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Introduction. *Erysipelothrix rhusiopathiae* is associated with erysipelas in pigs which if uncontrolled can result in substantial losses on the farm and at the time of slaughter. Commercial vaccines are available and commonly used. However, outbreaks of clinical disease in grow-finish pigs and breeding animals continue to occur. Timely and accurate diagnosis of erysipelas is important as effective treatments are available. However, the disease needs to be distinguished from other causes of septicemia and sudden death such as Sal*monella choleraesuis, Actinobacillus suis* and others. At the Iowa State University Veterinary Diagnostic Laboratory, diagnosis of *Erysipelothrix* spp. is commonly done by isolation from enriched tissues, immunohistochemistry (IHC), and multiplex real-time PCR assay. For further characterization, serotyping is commonly done. Recently a novel multiplex PCR for identification of surface protective antigen (SPA) A, B1, B2, and C has been developed. The objective of this study was to utilize the different diagnostic tools to characterize *Erysipelothrix* spp. isolates from the environment and pigs from recent erysipelas outbreaks in vaccinated farms in the Midwest U.S.

Materials and methods. Samples were collected in six Midwest swine operations from 2007 to 2009. Pig tissue samples were collected from 1-3 pigs on each site. Environmental samples (manure, feed, central line water, oral fluids and swabs collected from walls, feed-lines, air inlets, exhaust fans and nipple drinkers) and vaccine samples were collected following the isolation of *Erysipelothrix* spp. from clinically affected pigs. All *Erysipelothrix* spp. isolates obtained were further characterized by serotyping. Selected isolates were further characterized by PCR assays for genotype (*E. rhusiopathiae*, *E. tonsillarium*, *E*. sp. strain 1 and *E*. sp. strain 2) and surface protective antigen (*spa*) type (A, B1, B2 and C).

Results. All twenty-six isolates obtained from affected pigs were *E. rhusiopathiae*, specifically, serotypes 1a, 1b, 2 and 21. In environmental samples, 56 isolates were obtained and 52/56 were *E. rhusiopathiae* (serotypes 1a, 1b, 2, 6, 9, 12 and 21), 3/56 were *E.* sp. strain 1 (serotypes 13 and untypeable) and one novel species designated as *E.* sp. strain 3 (serotype untypeable). Four of six vaccines used on the sites were commercially produced products and contained *E. rhusiopathiae* serotype 1a. Of the remaining two vaccines, one was an autogenous vaccine and contained *E. rhusiopathiae* serotype 2 and one was a commercially produced inactivated vaccine and was not further characterized. All *E. rhusiopathiae* isolates were positive for *spa*A. All *E*. sp. strain 1 isolates and the novel *E.* sp. strain 3 isolate were negative for all currently known *spa* types (A, B1, B2 and C).

Discussion. Results indicate that *Erysipelothrix* spp. can be isolated from the environment occupied by clinically affected pigs; however, the identified serotypes in pigs may differ from those in the environment on selected sites. The vaccine strain and the isolates identified in clinically affected pigs were identical on one of five sites but dissimilar on four of five sites suggesting that re-evaluation of vaccine efficacy using recent field strains may be warranted.

Conclusion. The novel tools for characterization of *Erysipelothrix* spp. aided in identification of a novel species and will be useful for future epidemiological and vaccine failure investigations.

Comparison of five enzyme immunoassays (EIAs) for detection of anti hepatitis E virus (HEV) immunoglobulin G (IgG) in swine

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Introduction: Hepatitis E virus (HEV) causes acute hepatitis in humans. HEV is widespread in the pig population and pigs are considered an important reservoir for zoonotic transmission of HEV-induced disease. There are currently four known genotypes (1-4) of HEV and one recognized serotype. Swine HEV serological studies are often conducted with *in-house* assays based on peptides and recombinant proteins of different HEV genotypes and are mainly limited to research laboratories. The objective of the current study was to determine the diagnostic accuracy of two currently available commercial EIAs , two modified ELISAs and one in-house ELISA on samples from pigs experimentally inoculated with HEV genotype 3 (human or swine origin) and HEV genotype 4 (human origin).

Materials and Methods: A total of 84 serum samples (12 obtained from gnotobiotic pigs and 72 obtained from conventional pigs) were tested by five different assays. Twenty-seven of the 84 samples were control samples from non-inoculated pigs, 24/84 samples were obtained from pigs experimentally inoculated with human genotype 3 (US-2 strain), 9/84 samples were obtained from pigs experimentally inoculated with porcine genotype 3 (Meng strain), and 24/84 samples were obtained from pigs experimentally inoculated with human genotype 4 (Taiwan strain TW6196E). For the comparison, all samples were tested with one of four enzyme-linked immunosorbent assays (ELISAs) or a strip line immunoassay. The coating antigens varied from assay to assay. Plate-specific cutoffs were used. The strip line immunoassay results were read both visually by eyes and by digital-interpretation. For the statistical analysis, receiver operator characteristic (ROC) analysis and kappa statistic were used.

Results: All four ELISAs detected anti-HEV antibodies in pigs experimentally inoculated with HEV genotype 3 or 4 with varying sensitivity. All 27 control samples (27/27) were negative with all five EIAs. In all experimentally-inoculated pigs, seroconversion was detected for most individual pigs across all EIAs. The overall seroconversion rate for ELISA-1, ELISA-2 and the strip-line immunoassay was 61.4% (35/57), 56.1% (32/57) for ELISA-3, and 63.2% (36/57) for ELISA-4. Kappa analysis showed an almost perfect agreement among tests (0.88-1). The ROC analysis showed that the assay with the greatest sensitivity and specificity was ELISA-4 when the optimized cut-off was used; however, the sample number was low and results may vary with a greater sample set.

Discussion: Very similar sensitivities and 100% specificity were obtained regardless of whether the capture antigen in the different EIAs originated from HEV genotype 1 or 3. Since the capture recombinant antigens were based on human HEV genotypes 1 and 3, the data suggests that these five EIAs are effective for detecting antibodies against HEV viruses representing the main genotypes. This is in agreement with previous data indicating that only one HEV serotype exists.

Conclusion: All five EIAs used in this study were found to be similar in identifying anti-HEV antibodies in pigs experimentally inoculated with HEV genotype 3 and 4 strains and can be adapted for routine usage for determination of HEV exposure of pigs in veterinary diagnostic laboratories.

Efficacy of *Canine influenza virus (H3N8)* vaccine in dogs co-infected with CIV and *Streptococcus equi zooepidemicus*

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Canine Influenza virus (CIV) was first reported in racing greyhounds in Florida. Crawford, Dubovi and their colleagues later discovered that the epizootic spread of respiratory disease in the general dog population was caused by CIV, an H3N8 influenza virus that is closely related to equine influenza virus.

Subsequently, there were reports of H3N8 equine influenza virus infection in dogs in the UK and Australia in 2006 and 2007 respectively. Since that time, no further H3N8 influenza virus infections have been seen either in the U.K or in Australia. However, in the U.S., CIV infections of dogs have occurred throughout the country, possibly due to interstate transport of racing greyhounds.

CIV infections have also been reported in shelters, commercial kennels, and doggy day care centers. Outbreaks have been limited to the affected facilities, and CIV has not spread throughout the general canine population, therefore, most dogs remain serologically negative and susceptible to CIV disease. In susceptible dogs, severity of disease can vary from subclinical to severe disease with mortality. As with all influenza viruses, secondary bacterial infections and/or underlying conditions (e.g. immunosuppression) contribute to disease severity after viral infection.

In the current study, the only licensed (conditional) CIV vaccine, a product of Intervet/Schering Plough Animal Health, was tested for efficacy in reducing severity of disease caused by a combination of CIV and *S equi zooepidemicus* experimental challenges. **It was found that co-infection led to more severe disease than infection with either agent alone. Importantly, CIV (H3N8) vaccine was shown to significantly reduce the severity of disease and viral shedding in co-infected dogs.** These findings are important because most, if not all, CIV infected dogs will also be infected with a variety of opportunistic and secondary bacterial pathogens commonly associated with respiratory infection.

To achieve protection, it is important that dogs be vaccinated at least three weeks before exposure to CIV. This killed vaccine requires two doses be given not less than two weeks apart. Solid immunity can be expected approximately seven days after the second dose. **To ensure protection, dogs at risk of CIV, such as those that routinely go to doggy day care, are frequently boarded, are shown, or go to training facilities regularly, should be given two doses of CIV (H3N8) vaccine two weeks apart, and then held for seven days before being placed in contact with other dogs.**

Epidemiology Scientific Session

Sunday, November 14, 2010
Duluth

Moderators: Suzanne Burgener and Albert Rovira

08:00 AM **Experimental exposure of naive dairy calves to *Mycoplasma bovis* in naturally contaminated bedding sand – risk of transmission**
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08:15 AM **Significance of heavy fecal shedding of *Mycobacterium avium* subspecies *Paratuberculosis* (Map): Comparison of fecal culture, real-time and nested PCR testing ◊**
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08:30 AM **First report of contagious ecthyma in bighorn sheep in Utah**
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08:45 AM **Development of an ELISA for detection of bovine pregnancy**
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09:00 AM **Estimating the value of implementing whole herd *Bovine viral diarrhea virus* (BVDV) testing strategies in U.S. cow-calf herds when BVDV herd status is unknown**
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09:15 AM **Pen-based oral fluid sampling for PRRSV using an optimized PRRSV PCR assay is highly effective for the detection of virus in low prevalence populations**
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09:30 – 10:00 AM **BREAK**

10:00 AM **Colostral transmission of *Bluetongue virus* nucleic acid in California dairy calves ***
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10:15 AM **Estimates of diagnostic test sensitivities and specificities: What confidence do we really have? ***
Stephane Guillossou, H. Morgan Scott, Juergen A. Richt. 138

10:30 AM **Evidence of viral transmission and nasal shedding among beef calves exposed to a calf persistently infected with *Bovine viral diarrhea virus***
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◊ USAHA paper
* Graduate Student presentation

Exhibit Hall Open 7:00am - 2:00pm

Experimental exposure of naive dairy calves to *Mycoplasma bovis* in naturally contaminated bedding sand – risk of transmission

David J. Wilson[1], Anne Justice-Allen[2], Greg Goodell[3], Thomas J. Baldwin[1], Ramona T. Skirpstunas[1], Kimberly B. Cavender[1]

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Introduction: *Mycoplasma* spp., most commonly *M. bovis*, can infect all ages of cattle, and can cause pneumonia, septicemia, arthritis, and death. Adult cows may also contract mastitis, metritis, or agalactia. This experiment evaluated whether bedding sand naturally contaminated with *M. bovis* was associated with transmission of mycoplasma to naive dairy calves.

Materials and Methods: A closed dairy herd was screened resulting in 99% probability that the herd was free of mycoplasma in calves. Neonatal calves (n = 12) were purchased, blocked by weight and height, and randomly assigned as controls (n = 6) bedded with quarry sand, or exposed (n = 6) bedded with sand naturally contaminated with *M. bovis* (confirmed by PCR) from another farm. Calves were housed in calf hutches, fed commercial milk replacer and calf starter, with strict biosecurity and separation between groups; sand was added twice daily. Serum and nasal and ear swabs were collected weekly and tracheal swabs were collected monthly. Calves were euthanized for humane reasons if they had severe disease signs or else at the end of the 15 week study. A complete necropsy with diagnostic testing for mycoplasma and other evidence of disease was performed.

Results: Exposed group sand cultured mycoplasma-positive during weeks 1, 5, 6, 7, 11. The other weekly samples including all samples from control sand were mycoplasma-negative. Exposed group calves were bedded on mycoplasma-positive bedding for 138 total calf-days. Because of the intermittently positive bedding status, the total survival days for exposed calves after their first exposure to mycoplasma was a higher number, 385 days.

All 94 sera were anti-*M. bovis* antibody-negative. All 67 nasal and ear swabs and 16 tracheal swabs from all calves were mycoplasma culture-negative. Two calves died and 3 were euthanized before the end of the study; the remaining 7 calves were euthanized after 15 weeks.

No control or exposed calves had any gross lesions of mycoplasma infection. All post-mortem PCR (n= 36) and culture (n = 36) tests on lung, retropharyngeal lymph node, and trachea from all 12 calves were negative for *Mycoplasma* spp. The PCR could differentiate *M. bovis* if positive.

Using test sensitivity and sequential probability, the probability of each calf being detected positive at least once if they had become infected with mycoplasma beginning 4 weeks after exposure was calculated. For the 9 calves that survived beyond 25 days of age, probabilities of detection were between 96.5% and 99.3%.

Discussion/Conclusion: There was no evidence that *Mycoplasma bovis*-positive bedding sand was a source of infection to naive dairy calves, at least under the conditions of this study. Because of the possibility of infection through teat ends, further studies in lactating cows are still needed.

Significance of heavy fecal shedding of *Mycobacterium avium* subspecies *paratuberculosis* (Map): Comparison of fecal culture, real-time and nested PCR testing

Gilles R.G. Monif [1], Tsang L. Lin[2], J. Elliot Williams[1], Ching Ching Wu[2]
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Demonstration of heavy *Mycobacterium avium* subspecies *paratuberculosis (Map)* shedding has been incorporated into proposed herd management scheme for the control Johne's disease in dairy and beef cattle. Heavy shedding can be shown to correlate with advanced gastrointestinal disease and systemic dissemination. Animals identified as heavy shedders are considered primary disseminators of pathogenic mycobacterium into the environment.

Given the importance of heavy shedding in determining whether or not to cull an animal, the potential that uneven distribution of Map in the feces can influence the quantity of organism identified by fecal culturing was analyzed in a prospective, blinded study using comparative fecal culture (Trek® Diagnostic System), direct hspX real-time PCR (Tetracore® Map Diagnostic System) and nested IS1311-based PCR (FecaMap® Diagnostic System). Separate samples were used for each test. The fecal cultures and hspX real-time PCR tests were done at the Animal Disease Diagnostic Laboratory at Purdue University. The nested, fecal Map PCR tests were done at the Map diagnostic laboratory of, College of Veterinary Medicine, University of Florida. Of the 327 fecal specimens analyzed by all three systems, 22 animals were identified as heavy shedders based upon culture results of their feces. Of the 22 culture-positive fecal samples identified as coming from "heavy shedders", only 7 fecal samples were positive for Map by both real-time and nested PCR tests. Five fecal specimens were positive for Map by either real-time or nested PCR test. **Ten fecal specimens (45%) failed to be confirmed by both PCR tests.**

Unevenly distribution (or Clumping) of Map in the fecal samples can cause quantitative misrepresentation of the degree of fecal shedding within a given fecal specimen. Decision makers may be well advised to seek additional confirmation of "heavy shedding" status before culling an animal from the herd based upon this criteria alone.

First report of contagious ecthyma in bighorn sheep in Utah

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Introduction: Contagious ecthyma (CE) is a disease caused by a parapox virus, commonly referred to as contagious pustular dermatitis, orf, soremouth, and scabby mouth. It affects domestic sheep and goats worldwide, causing pustules followed by scabbing on the muzzle, face, and lips. This is the first report of CE in a bighorn sheep in Utah.

Materials and Methods: In December 2008, a 2 ½ year old male Rocky Mountain bighorn sheep in a campground in northeastern Utah was reported to the Utah Division of Wildlife Resources (UDWR), observed with a bloody nose. Ulcerated papular lesions, including frank red blood were present on the markedly swollen lips and muzzle, with crusting around all edges of the lesions. The ram was in poor body condition and appeared unable to eat. There were approximately 30 sheep located in the vicinity including an adult female with scarring and small scabs covering the muzzle area, still in good body condition; no other detectable lesions or scarring were observed on other bighorn sheep. The extent of or how recently they may have been in contact with domestic ruminants including sheep was unknown.

The ram was euthanized by UDWR personnel and portions of the muzzle and oral mucosa were submitted to the Utah Veterinary Diagnostic Laboratory (UVDL). Histopathological sections were made of the junction between the lesions on the lips and muzzle and normal skin.

Results: Sections of haired skin had diffuse acanthosis and hyperkeratosis with numerous intracorneal vesicles and pustules. Some hair follicles were abscessed. Moderate numbers of bacterial colonies were observed among superficial keratinized epithelial cells. There was multifocal intracellular edema of keratinocytes of the stratum spinosum. The superficial dermis was infiltrated with numerous lymphocytes, plasma cells, neutrophils and macrophages. The history, gross lesions, and microscopic lesions observed histopathologically confirmed the diagnosis of CE.

Discussion/Conclusion: In 1982, 21 Rocky Mountain bighorn sheep were translocated from Whiskey Basin, Wyoming into Sheep Creek Canyon, Utah. From 1995 to 2000, this Utah population declined from approximately 100 to 33 adults; new Rocky Mountain bighorn sheep were introduced from Almont and Basalt, Colorado from 2000-2002. This population of bighorn sheep is highly visible to the public and lesions associated with CE were never reported prior to December 2008. It is unknown how the disease was introduced. The virus can lay dormant in soil and scabs for at least 12 years. This area has been utilized by this population of bighorn sheep since their initial release, therefore if the virus had lain dormant for years, whatever conditions caused the infection to become active are unknown. Salt blocks or highway salt have been reported as potential sources of CE, but no artificial salt sources have ever been administered to this population.

Development of an ELISA for detection of bovine pregnancy

Katherine Velek, Shona Michaud, Kate Boucher, Lori Plourde, Nevena Djuranovic, Anna Rice Christopher Egli, Peter Welles,Valerie Leathers

Introduction: Accurate and timely detection of pregnancy in dairy cows is an essential component of today's reproductive management programs. Veterinarians and farmers use early detection of non-pregnant (open) cows to enable faster rebreeding and shorten the calving interval, thereby maximizing milk production and revenue for the farm. The cost of keeping cows open for additional days is estimated at up to $5 per day[1]. IDEXX Laboratories, Inc. is developing an ELISA for the accurate detection of pregnancy as early as 28 days post breeding, which provides veterinarians and dairy farmers with another tool for the early identification of open cows.

Materials/methods: The IDEXX bovine pregnancy ELISA detects the presence of early pregnancy-associated Glycoproteins (PAGs) in bovine serum or plasma as a marker for determination of pregnancy in cows. The assay uses an anti-PAG antibody coated onto the solid phase to bind PAGs that may be present in the sample. A second anti-PAG antibody, coupled with biotin is used as the detection reagent along with streptavidin-horseradish peroxidase (SA-HRP). TMB substrate is used as a colorimetric indicator for PAG containing samples, and the enzymatic reaction is stopped with an acid stop solution. After reading the plate at 450nm, wells with color development above the assay threshold are considered positive, indicating a pregnant animal, while wells with little or no color development indicate non-pregnant animals.

Preliminary evaluations of the bovine pregnancy ELISA have been conducted with serum and plasma samples obtained from 54 bull bred Holstein heifers. Blood samples were taken every three days post breeding for the first 45 days of pregnancy and then every two weeks until calving. After calving, samples were taken from 25 of the cows once weekly for 10 weeks. Transrectal ultrasound was performed between days 27 and 35 post breeding to confirm pregnant status, and once weekly thereafter through 60 days of pregnancy. Fifteen control heifers were not bred, and were bled every three days for 30 days.

Plasma and serum samples were tested on the IDEXX bovine pregnancy ELISA to determine the accuracy of pregnancy detection between days 25 and 30 post breeding as well as the specificity of the test when an animal was not successfully bred. Pregnant animals were also tested throughout the course of gestation and post-partum to monitor PAG levels during pregnancy, and to determine the rate of decline in PAG levels after calving.

Results: All 15 heifers in the control group, as well as seven heifers in the experimental group that were not successfully bred, were considered open by the ELISA, resulting in 100% specificity for the test. Forty five of 47 specimens (96%) from confirmed pregnant cows were considered positive by ELISA by day 28 after breeding. After 30 days post breeding, all of the 47 of the cows determined to be pregnant by ultrasound indicated positive results on the ELISA. Samples tested after calving suggest that the results of the ELISA return to baseline levels 30-60 days post partum.

Significance: Preliminary evaluations of the IDEXX bovine pregnancy ELISA indicate that the test could be a useful adjunct to existing reproductive management programs. It offers a reliable method to distinguish between pregnant and open animals 28-30 days after breeding, and throughout the course of pregnancy. Veterinarians could use the test during regular herd checks to evaluate individual animals when it is too soon after breeding to palpate accurately. In addition, veterinarians may also recommend the test for use when farmers do not have regular access to a veterinarian or an experienced palpator. As with any diagnostic test, the IDEXX bovine pregnancy ELISA should be used under the guidance of a veterinarian as part of the farm's overall health and reproductive management program.

Reference: French, P. D. and R. L. Nebel. 2003. The simulated economic cost of extended calving intervals in dairy herds and comparison of reproductive management programs. Journal of Dairy Science 86 (Suppl. 1):54 (abstract).

Estimating the value of implementing whole herd *Bovine viral diarrhea virus* (BVDV) testing strategies in U.S. cow-calf herds when BVDV herd status is unknown

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Numerous tests are available to identify cattle persistently infected (PI) with bovine viral diarrhea virus (BVDV). However, veterinarians seldom know the true BVDV status of individual herds; therefore, the decision to implement a whole-herd BVDV test relies upon multiple factors (e.g. reproductive history, pathology results) used to determine the likelihood of a herd being BVDV positive. A spreadsheet model utilizing Monte Carlo simulation methods was constructed to estimate the value of whole-herd BVDV test strategies among individual cow-calf herds. Three common BVDV tests (immunohistochemistry, antigen-capture ELISA, and real-time reverse-transcriptase polymerase chain reaction) were incorporated to evaluate 12 different testing strategies analyzed as either a single test (3) or as two-test sequential strategies (9) interpreted in series. Additionally, 3 herd sizes (50, 100, and 500 cows) and 3 probabilities of herds being of BVDV positive status (0.077, 0.19, and 0.47) were evaluated. **Regardless of test strategy or herd size, testing was economically advantageous to BVDV positive herds. When herd status was unknown an economic benefit was more frequently realized when the pre-test probability of a herd being BVDV positive reached 0.47 compared to 0.19 and 0.077. Specific rankings of test strategies differed across herd size and pre-test probability of BVDV positive status; however, strategies containing two tests run in series usually illustrated higher economic returns when compared to a single test.** Selecting the appropriate testing strategy for the situation is important and veterinarians must be confident that the probability of herd BVDV positive status is high prior to performing whole-herd tests.

Pen-based oral fluid sampling for PRRSV using an optimized PRRSV PCR assay is highly effective for the detection of virus in low prevalence populations

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Introduction: The objective of this study was to estimate the probability of detecting one PRRSV viremic pig in a pen of ~25 pigs using a single oral fluid sample from the pen.

Materials, Methods, design: This study was conducted in a confirmed PRRSV negative (serology and PCR) finishing site. All animal housing, handling, and veterinary care was approved and supervised by Murphy-Brown L.L.C. and were in compliance with PQA plus and the guidelines published in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (*Federation of Animal Science Societies, Savoy, IL). Oral fluid samples were collected from 36 pens (~25 pigs per pen) in one barn for 3 consecutive days (Study Day 1, 2, 3). On Study Day 4, one pig from each pen (n = 36) was removed, placed in isolation in a separate building on the site, and vaccinated with a commercial modified-live PRRSV vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim Vetmedica), as per manufacturer's recommendations. One oral fluid sample was collected from each pen (36 + the vaccinate pen) for the next 4 days (Study Day 4, 5, 6, 7). On the evening of Study Day 7 (4th day post vaccination), one vaccinated pig was placed in each of the 36 pens of PRRSV-negative animals. The following morning (Study Day 8), one oral fluid sample was collected from each pen. During this 30 minute collection period, the vaccinated pig was observed for the number of interactions with the rope and the results were recorded as a dichotomous outcome (Y/N) for each one minute interval. Oral fluid samples (30 minute collection) were then collected daily from each of the 36 pens for Study Days 9 through 15. All samples were completely randomized prior to testing and then assayed using a quantitative PRRSV RT-PCR optimized for the oral fluid matrix.

Results: Of the 36 oral fluid samples collected on Study Day 8 (14 hours post placement of vaccinates), 23 (64%) samples were PRRSV qRT-PCR-positive. Of the 252 samples collected over the 7 days after Study Day 8, 195 (77%) tested positive. These data suggest that pen-based oral fluid sampling for PRRSV using an optimized PRRSV PCR assay[1] is highly effective for the detection of virus in low prevalence populations. Sample size recommendations for PRRSV surveillance in the field need to be investigated further, but the data presented here corroborate previous reports[2,3,4] suggesting that oral fluid sampling is a cost-effective alternative for PRRSV surveillance.

Colostral transmission of *Bluetongue virus* nucleic acid in California dairy calves

Christie Mayo[1], *Beate Crossley*[2], *Sharon Hietala*[2], *Richard Breitmeyer*[3], *Ian Gardner*[1], *N. James MacLachlan*[1]
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Bluetongue virus (BTV) is the cause of bluetongue (BT), an economically important, emerging disease of ruminants transmitted by various species of *Culicoides* midges. The global distribution of BTV has recently altered, especially in Europe invaded by at least 8 different BTV serotypes since 1998. The strain of BTV serotype 8 that invaded northern Europe is virulent in cattle as well as sheep, and also in free-ranging and captive species of non-African ungulates. This strain of BTV-8 produces congenital infections in cattle with some frequency, which is unusual when compared with other "field" strains of BTV where transplacental transmission has previously been considered to be largely or exclusively a property of laboratory-adapted strains of BTV, particularly certain live attenuated vaccine viruses. This strain of BTV-8 also infected calves fed virus-spiked colostrum, consistent with earlier experimental studies documenting oral BTV infection of calves. The goal of this study was to reevaluate the epidemiology of BTV infection in California, a region enzootic for BTV infection for at least 60 years. A total of 123 calves from ten dairy herds were enlisted from December, 2008 to March, 2009 (BTV infection typically occurs in California between July-November) and tested until at least December 2009. These dairies represent four geographically distinct regions of the state. Monthly collections of serum and whole blood from each calf were analyzed respectively by BTV-specific cELISA and qRT-PCR assays. Dams of calves that were viral RNA positive by qRT-PCR assay at initial sampling were also evaluated by cELISA for serological evidence (seroconversion) of BTV infection.

Bluetongue viral RNA was detected in sentinel calves at two distinct time periods: 1. among newborn calves during the putatively transmission free period of January-June; 2. during the seasonal transmission period of July-December. During the latter period, sixteen calves tested positive ($Ct \leq 35$) for BTV RNA, and all of these calves seroconverted after becoming qRT-PCR positive confirming that they had been productively infected with BTV. In contrast, five of six calves that were qRT-PCR positive 1-3 days after birth in January-June were positive until 2 month of age when they all became negative; these 5 calves also became seronegative with loss of colostral antibodies, indicating that they were not productively infected with BTV. BTV serotype 11 was isolated from the sixth perinatally infected calf, which remained seropositive for the duration of the study. Dams of all of the qRT-PCR-positive newborn calves that were available for sampling were seronegative to BTV, precluding the possibility of vertical BTV transmission and congenital infection. Pooled colostrum was suspected to be the cause of perinatal infection of the virus-positive calves detected during the BTV-free period and sequence analysis of BTV RNA (gene segment 10) from one calf and the colostrum it received confirmed homology of the amplicons from the two sources. This data confirms both localized, vector-mediated, seasonal transmission of BTV as well as dissemination of BTV and or viral nucleic acid to newborn calves via colostrum. **The role of perinatal BTV infection in the epidemiology of BTV infection is uncertain, as viral nucleic acid was only transiently detected in the blood of individual calves and BTV was isolated transiently from only one calf. These findings confirm results of earlier experimental studies in which oral transmission was demonstrated among calves and this study confirms, for the first time, the natural dissemination of BTV and/or BTV nucleic acid in colostrum, as determined by surveillance monitoring of a sentinel cohort of dairy calves on commercial farms in California.**

Estimates of diagnostic test sensitivities and specificities: What confidence do we really have?

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Introduction: A standard practice in validation of a diagnostic test is to report the sensitivities and the specificities along with a confidence interval for each. This has the potential to be confusing as a multitude of methods for estimation of confidence intervals for proportions have been reported in the literature (e.g., Newcombe, 1998; Brown, 2001). Because of the discrete nature of the underlying distributions that these methods are based upon, the nominal 95% confidence cannot be achieved exactly and this can lead to poor coverage and produce inappropriate intervals.
The objective of this communication is to illustrate the chaotic behavior of these methods with the aid of some previously reported sensitivities and specificities, and then to propose some simple rules to guide veterinary diagnostician in reporting confidence intervals.

Materials and Methods: Previously reported sensitivities and specificities of several diagnostic tests were retrieved (Guillossou, AAVLD proceedings 2006, 2009), 95% confidence intervals were estimated using the most commonly used methods (Brown, 2001), and the results were compared. These methods included the Wald (i.e., the asymptotic or standard interval), the Wilson (or, score interval), the Agresti-Coull interval, the Jeffrey interval (or, Bayesian method), and the Clopper-Pearson interval (also referred to as the exact interval). The exactitude of a confidence interval can be estimated through the coverage probability, which represents the proportion where the interval contains the true value of interest.

Results: The first example illustrates the impact of estimates from a small number of samples. The sensitivity of an antibody ELISA for PCV2 applied to fecal samples was reported to be 51.9% on 27 samples arising from clinical pigs; this is consistent with seroconversion following the excretion of the virus. In this example, 95% confidence interval upper bounds ranged from 57.7% (exact) to 62.1% (Wald) and coverage probability from 93.4% (Bayes) to 96.6% (exact). For the second example with a larger sample size, the proportion observed was close to 100%. The sensitivity of an antigen ELISA for BVDV used on ear notches was reported to be 98.8% on 249 persistently infected cattle. The 95% confidence interval lower bound ranged from 96.4% (Agresti -Coull) to 97.4% (Wald). The coverage probability, ideally equal to 95%, ranged instead from 79.9% (Wald) to 98.9% (exact). The best-fitted coverage probability was observed with the Agresti Coull method.

Discussion/Conclusion: As expected, the erratic behavior of the coverage probability for 95% confidence intervals was confirmed for estimates arising from small sample sizes; however, coverage probabilities were equally erratic in studies with large sample sizes and sensitivity or specificity estimates close to 100%. With the development of new diagnostic assays and the optimization of the existing ones, high test performances close to 100% are more frequently being reported. In these cases, the selection of the method to report the confidence interval is particularly of importance. Interestingly, the exact 95% interval calculation, while relying on "exact" methods, does not actually provide an "exact" coverage probability. It is often described as conservative and leads to bad estimators. As illustrated in this communication, and confirmed in the literature, the Agresti-Coull interval presents a satisfactory compromise between computational requirements and the coverage probability. Ideally, the effects of coverage probability should be investigated and the most appropriate method chosen and applied to the analysis before reporting the results.

Evidence of viral transmission and nasal shedding among beef calves exposed to a calf persistently infected with *Bovine viral diarrhea virus*

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Persistently infected (PI) cattle are the reservoir of bovine viral diarrhea virus (BVDV); however, data regarding transient infection (TI) among non-PI populations is minimal. Although the reference standard for BVDV diagnosis is virus isolation (VI) on buffy coat, serum provides practicing veterinarians with a more practical sample. Study objectives consisted of 1) estimating the onset and duration of TI (post-exposure to a PI calf) based on serum VI and 2) the potential of TI cattle to shed BVDV. Two 21 day studies were performed where one PI calf was commingled with an antibody diverse non-PI cattle population from various origins (n=12 and n=15, respectively). Samples were collected every other (VI) or every fourth (nasal swabs) day. Virus isolation on serum and nasal swabs failed to detect BVDV among non-PI cattle. However, despite minimal clinical illness (n=1), BVDV transmission was demonstrated as 78% (n=21) of non-PI calves displayed a four-fold rise in antibody titer to the homologous BVDV PI strain, 81.5% (n=22) displayed a transient positive serum real-time reverse transcription-polymerase chain reaction (rRT-PCR) outcome to BVDV, and 74.1% (n=20) displayed a transient positive rRT-PCR result on nasal swabs. **Median days of positive serum rRT-PCR onset was 10.0 days post exposure to PI (range: 6-21) and duration of positive rRT-PCR was 3.0 days (range: 1-9)**. These data suggest that non-PI cattle can become TI despite minimal clinical disease and possess the potential to transmit BVDV.

Evaluation of a blocking ELISA to detect antibodies against influenza virus in swine sera

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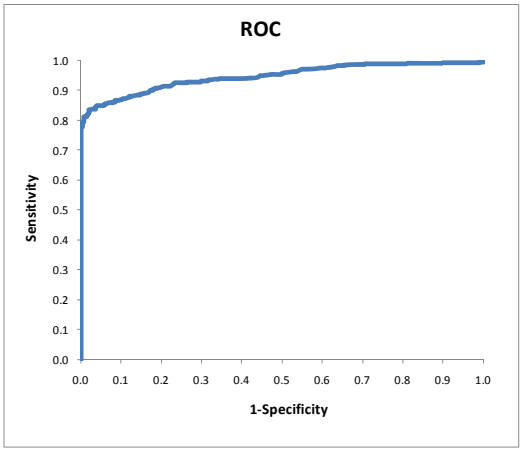
A blocking ELISA originally developed to detect antibodies against influenza in avian serum (FlockChek™ AI MultiS-Screen Ab Kit, IDEXX Laboratories) has been recently used in experimentally inoculated pigs (1) but not in figs infected in field conditions. In this study, we evaluated the accuracy of this test with field samples and we obtained sensitivity and specificity estimates for different cut-off values.

Samples were obtained from farms with known status for influenza virus infection. A set of 308 known negative samples from 10 cases was used to estimate diagnostic sensitivity. On the other hand, 508 samples from 11 herds with documented history of recent influenza virus outbreaks were used to estimate sensitivity. The results of an ROC analysis are shown in the following figure.



The results showed a good discrimination between positive and negative samples. Based on the ROC analysis, the cut-off value that maximized sensitivity and specificity was 0.8, with sensitivity of 0.84 and specificity of 0.97. The estimated sensitivity and specificity for other cut-off values are reported in the following table:

Cut-point	Sensitivity	Se Lower 95% CL	Se Upper 95% CL	Specificity	Sp Lower 95% CL	Sp Upper 95% CL
0.60	**0.72**	0.68	0.76	**1.00**	0.99	1.00
0.70	**0.79**	0.75	0.82	**1.00**	0.98	1.00
0.80	**0.84**	0.80	0.87	**0.97**	0.95	0.99
0.90	**0.92**	0.89	0.94	**0.78**	0.73	0.82
1.00	**0.99**	0.97	0.99	**0.31**	0.26	0.36
1.10	**0.99**	0.98	1.00	**0.03**	0.01	0.05

Based on these results and on a previous study (1), the following cut-off values were set for interpretation of this test at the University of Minnesota Veterinary Diagnostic Laboratory: <0.6 = positive, 0.6 to 0.9 = suspect, >0.9 = negative. In conclusion, **the FlockChek™ AI MultiS-Screen Ab Test Kit can be used for detection of influenza antibodies in pigs.**

Evaluation of the PrioCHECK® Toxoplasma AB Porcine ELISA for surveillance of Toxoplasma infections in pigs

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Toxoplasmosis is caused by *Toxoplasma gondii*, a protozoan parasite which belongs to the family of Sarcocystidae. T. gondii displays a wide host spectrum including a variety of livestock, wild animals and pets. Among the parasitic zoonoses, toxoplasmosis has the highest incidence in humans. In the EU, it is considered to be an under-detected and underreported disease. Although reporting of cases of infection is mandatory, little is known about the epidemiological situation in the member states. Therefore, the European Food Safety Authority has published in 2007 recommendations for optimising the surveillance and monitoring of *Toxoplasma* in humans, animals and foodstuffs, including advice on methods for detection and identification of the parasite in food and animals (e.g. serology).

Here, we report on the evaluation of the PrioCHECK® Toxoplasma Ab porcine ELISA for detection of antibodies to *Toxoplasma* in meat juice and serum or plasma samples of pigs. The PrioCHECK® Toxoplasma Ab porcine is based on microplates coated with a cell culture derived *Toxoplasma* tachyzoite extract as capture antigen. The sample panels used for evaluation of diagnostic sensitivity of the ELISA consisted of 50 *Toxoplasma* positive serum samples and 33 positive meat juice samples. Sample status had been either confirmed by a human Toxoplasma ELISA with a different secondary antibody, a commercial ELISA and/or by a Western blot or IFAT. The diagnostic specificity was determined by testing 272 *Toxoplasma* negative serum samples and 116 negative meat juice samples. In addition, porcine samples with other parasite infections in pigs like *Trichuris suis*, *Oesophagostomum*, *Strongyloides*, *Hyostrongylus*, mixed infection *Oesophagostomum* & *Hyostrongylus*, *Trichinella spp.* were tested to assess the analytical specificity of the ELISA. **Evaluation of the serum samples resulted in a diagnostic sensitivity of 98.0% (95% confidence interval: 94.1-100%) and a diagnostic specificity of 99.6% (95% confidence interval: 98.9-100%). With the meat juice samples, the ELISA showed a diagnostic sensitivity of 97.0% (95% confidence interval: 91.1-100%) and a diagnostic specificity of 100% (95% confidence interval: 89.0-100%).** In the tests of the analytical specificity with a total of 9 samples from animals infected with other parasites no cross-reactivity was observed in the ELISA. The analytical sensitivity of the ELISA was determined by performing serial dilutions of three *Toxoplasma* positive serum samples with different antibody titres. One strongly positive serum sample could be diluted 3200-fold before the signal dropped below the cut-off of 15% positivity. The second strongly positive serum sample could be diluted 800-fold and a weak positive sample could still be diluted 400-fold.

This evaluation study demonstrates that the PrioCHECK® Toxoplasma Ab porcine ELISA is able to detect *Toxoplasma* infected pigs in serum, plasma and meat juice samples with high sensitivity and specificity without showing any cross reactivity towards other parasitic infections. Therefore, the PrioCHECK® Toxoplasma Ab porcine ELISA is a valuable tool for monitoring, surveillance and certification purposes of Toxoplasma infections in pigs.

A field and laboratory investigation on swine diseases in the Republic of Haiti

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Classical swine fever (CSF) re-entered the Republic of Haiti in 1996 and has become endemic. Outbreaks of teschovirus encephalomyelitis (TE) in pigs were first detected in February 2009, and porcine teschovirus (PTV) type 1 was isolated from brain samples of sick pigs. There are currently no vaccines commercially available for TE in Haiti. The devastating earthquake which struck the capital city Port-au-Prince in January 2010 had severe impacts on animal health. TE has spread to other regions including areas near the border with the Dominican Republic, and now TE, rather than CSF, has become the main reason for swine losses in Haiti. Immunosuppressive effects of disease agents such as porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV), circulating in the Haitian swine population in addition to CSF virus (CSFV) and PTV, may have facilitated the expression of PTV. Adding commercially available vaccines for these agents to the on-going CSF vaccine program may be effective in the control of TE.

The purpose of this investigation was to determine the presence of all swine diseases in TE-affected regions in Haiti. A field review was conducted in April 2010. Thirty-five premises mostly of backyard pigs were investigated. Estimated morbidity and mortality due to all diseases were 30% and 10%, respectively. Major clinical signs observed were central nervous system disorders and pigs of all ages were affected. Few lesions were observed at postmortem examination. Diagnostic samples (109 serum, 109 blood and 63 individual tissue samples) were collected from 111 sick and normal pigs and sent to USDA National Veterinary Service Laboratories (NVSL) for testing.

Of the 109 serum samples, 54 were positive for antibodies to PTV (49.5%), 8 were positive for antibodies to PRRSV (7.3%), 13 were positive for antibodies to swine influenza virus (SIV) H3N2 (11.9%), 24 were positive for antibodies to SIV H1N1 (22.0%), and 44 were positive for antibodies to CSFV (40.4%). Of the 44 samples positive for antibodies to CSFV, 30 were collected from CSF-vaccinated pigs, 11 from non-vaccinated pigs and 3 were from pigs of unknown vaccination status. Positive PCR results for CSFV were obtained from 15 of the 109 serum samples and 5 of these PCR-positives were confirmed by virus isolation and an avidin biotin complex assay using monoclonal antibody to CSFV. In addition, PTV was detected from 6 of 8 brain samples and 6 of 8 spinal cord samples; PCV-2 was detected from 6 of 8 tonsil samples; and *Streptococcus suis* was isolated from a brain sample. The above results indicate that multiple disease agents are present in Haitian swine population in addition to CSFV and PTV. Further laboratory tests are being conducted and a panviral microarray is being utilized for the detection of emerging viruses.

Risk management tool for continuous improvements in laboratory safety

Roger Parker
Texas Veterinary Medical Diagnostic Laboratory, College Station, TX

Even critical issues such as **laboratory safety** have a tendency to deteriorate without intentional intervention. A **formal risk management procedure** can ensure that safe laboratory practices are continually advanced and incorporated in routine and special activities. A recommended risk management tool involves five defined but overlapping steps to holistically mitigate potential risks in a workplace or activity. The first step is "**Identify Hazards**". In this step, plausible hazards are considered by activity managers to proceed to the second step of "**Assess Hazards**". This step is a lengthy and deliberate process that attempts to characterize potential hazards for estimated **probability** (or **likelihood**) of occurrence and predicted **severity** (or **impact**) should those hazards occur. These two factors of probability and severity interact to determine risk levels whereby various assessed hazards can be compared for priority of prevention or control. The third step is "**Develop Controls**". This step is an advanced brain-storming period facilitated by a safety manager with leaders of the involved laboratory section or event. Control measures are considered for available resources and return on investment. Ranked courses of action are proposed to decision-maker(s) for the fourth step of "**Implement Controls**". During this step, affected people must receive appropriate information and be trained to modify actions (including use of protective equipment). This step also identifies responsible supervisors that will have acknowledged roles in the fifth step of "**Supervise & Evaluate**". In addition to supervisory oversight of risk controls, this step includes periodic review to prevent complacency and to update procedures in light of new information and technology.

In summary, leaders are responsible for anticipating and managing risk by **planning**. Using a formal risk management procedure offers inclusion of safety input from all work levels, risk communication transparency, and quicker enrollment of all affected. It facilitates a safety climate of encouragement and justified motivation rather than traditional enforcement or compliance. During the briefing, two specific examples of **risk management worksheets** will be highlighted: **rabies infection prevention** and **eye protection**.

Clostridium sordellii and *Clostridium chauvoei* sudden death outbreaks in periparturient adult and young small ruminants

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Seven *Clostridium* species cause acute, sudden and fatal disease in sheep, goats, and other species of domestic animals when unvaccinated. Insufficiently vaccinated animals exposed to large numbers of the *Clostridium* bacilli under conditions where the animals are stressed often result in disease. Pathogenesis and death is caused by the many exotoxin proteins produced by the vegetative growing anaerobic bacteria in these animals. *Clostridium* species spores naturally inhabit the soil, requiring an anaerobic environment to change from the spore stage to the vegetative stage which produces the exotoxins that kills the animal. Some *Clostridium* species are somewhat aerotolerant increasing in numbers in the wet soil. Clostridia diseases can usually be controlled and prevented by immunoprophylaxis (vaccination) prior to the onset of exposure. The *Clostridium* species included in different combinations in cattle, sheep and goat vaccines are: *Clostridium chauvoei*; *Clostridium novyi*; *Clostridium haemolyticum*; *Clostridium septicum*; *Clostridium sordellii*; *Clostridium perfringens*; and, *Clostridium tetani*. Many Clostridia vaccines are approved only for cattle, not sheep and goats. The vaccine most commonly used in small ruminants is *Cl. perfringens* types C&D/ tetani bacterin-toxoid (CD&T).

Five *Clostridium* outbreaks were identified in 2008 and 2010 in small ruminants that coincides with wet warm winters and owners not using vaccine that protects against the seven *Clostridium* species including *Cl. perfringens* types C&D toxoid-a *Clostridium* 9- way for small ruminants.

Diagnostics for these pathogens include symptoms, necropsy and bacterial identification.

- Symptomatic tissues from sudden death animals set up for anaerobic culture.
- Direct impressions of tissues gram stained for the presence of large gram positive rods.
- Only tissues with gram positive rods tested with commercial species specific fluorescent antibody (FA) conjugates to identify the four species of *Clostridium* that can be identified with direct FA: *Cl. chauvoei*, *Cl. novyi*/ *Cl. haemolyticum*, *Cl. septicum* and *Cl. sordellii.*

2008 Case 1. Sudden death in three month old dairy goat kids whose dams were vaccinated with Clostridium 8 way without *Cl. sordellii*. Fresh intestinal tissues revealed large gram-positive rods and were FA positive for *Cl. sordellii.*

2008 Case 2. Sudden death in three-five month old meat goat kids whose dams were vaccinated with CD&T. Large gram-positive rods observed in the tissues and FA positive for *Cl. sordellii.*

2010 Case 1. Sudden death in periparturient range ewes vaccinated with Covexin 8 without *Cl. sordellii* at late fall shearing. Fresh intestinal tissues revealed large gram-positive rods and FA positive for *Cl. sordellii.* Over 100 of 3000 ewes died in 3-5 days before all the remaining ewes were treated by feeding tetracycline and revaccinated with *Clostridium* 9 way for cattle. Bacterial cultures negative for *Cl. perfringens.*

2010 Case 2. Sudden death in yearling range ewes vaccinated with Covexin 8 without *Cl. sordellii* at late fall shearing. Fresh intestinal tissues revealed large gram-positive rods and were FA positive for *Cl. chauvoei.* 100 of 400 ewes died in 3-5 days before these ewes were revaccinated with *Clostridium* 8 way for sheep. Cultures negative for *Cl. perfringens.*

2010 Case 3. Sudden death in periparturient range does on cull onions vaccinated with Covexin 8 without *Cl. sordellii* in the fall. Fresh intestinal tissues revealed large gram positive rods and FA positive for *Cl. sordellii.* Over 10 does died in this outbreak.

Vaccination guidelines and vaccines for small ruminants should be updated to include the importance of vaccination for all the *Clostridium* species.

Molecular Diagnostics Session

Sunday, November 14, 2010
Rochester

Moderator: Kyoung-Jin Yoon and Steve Sells

08:00 AM **Identification and characterisation of a type A influenza virus causing infection in commercial poultry and people**
George Arzey, Peter D. Kirkland, K. Edla Arzey, Melinda Frost, Aeron C. Hurt, Yi-Mo Deng, Dominic Dwyer, Mala Ratnamohan, Paul Selleck.* 147

08:15 AM **Evaluation of a microarray system for the rapid typing of type A influenza viruses**
Melinda Frost, Xingnian Gu, Edla Arzey, Rodney J. Davis, Peter D. Kirkland. . . .. .148

08:30 AM **Development of a multiplex PCR test to differentiate *Salmonella choleraesuis* field isolates from a live attenuated vaccine**
Mark Felice, Simone Oliveira. 149

08:45 AM **Development of calf diarrhea pathogen panel nucleic acid purification and detection workflow**
Mangkey Bounpheng, Megan Schroeder, Hemant Naikare, Binu Velayudhan, Carlos Estevez, Loyd Sneed, Amy Swinford, Sandra Rodgers, Alfonso Clavijo. 150

09:00 AM **Comparison of DNA purification and detection workflows for *Tritrichomonas foetus* detection**
Mangkey Bounpheng, Hemant Naikare, Loyd Sneed, Janell Kahl, Jennifer Meier, Feng Sun, Megan Schroeder,Alfonso Clavijo. 151

09:15 AM **Implementation and coordination of a real-time reverse transcriptase PCR for *Classical swine fever virus* and *Foot and mouth disease virus* detection in the National Animal Health Laboratory Network: Five years of data**
Patricia S. Glas, Lizhe Xu, Barbara M. Martin, Kate R. Schumann, Michael T. McIntosh. ..152

09:30 AM **Development of multiplex real-time PCR assay for detection and differentiation of *Moraxella ovis*, *Moraxella bovis* and *Moraxella bovoculi***
Huigang Shen, Lucas Funk, Tanja Opriessnig, Annette O'Connor. 153

09:45 – 10:15 AM **BREAK**

10:15 AM **Rapid molecular typing of *Epizootic hemorrhagic disease virus* (EHDV) by multiplex one-step rRT-PCR**
Alfonso Clavijo, Feng Sun, Indumathi Srinath, Renata Ivanek. 154

10:30 AM **Identification of molecular targets for diagnosis of bovine tuberculosis ***
Ailam Lim, Juan Pedro Steibel, Steven Bolin. 155

* Graduate Student presentation

Exhibit hall Open 7:00am - 2:00pm

Identification and characterisation of a type A Influenza virus causing infection in commercial poultry and people

George Arzey[1], Peter D. Kirkland[1], K. Edla Arzey[1], Melinda Frost[1], Aeron C. Hurt[2], Yi-Mo Deng[2] Dominic Dwyer[3], Mala Ratnamohan[3] and Paul Selleck[4]

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Type A Influenza viruses are important pathogens of many species, including birds, dogs, horses, humans and swine. Most species of animals and birds are infected by a limited range of haemaglutinin (H) and neuraminidase (N) subtypes of influenza A viruses. Ducks are an exception in that infections with all of these virus subtypes have been recorded but mostly without evidence of disease. In commercial chickens, infections are most frequently due to viruses from the H5 and H7 subtypes. Such infections in chickens are notifiable because of the propensity of H5 or H7 virus strains, often initially of low pathogenicity, to mutate and give rise to "high pathogenicity" strains which cause severe disease and spread rapidly through a poultry population. While most human infections have been associated with viruses from subtypes H1, H2 or H3, occasionally people have been infected after contact with infected birds, the most notable being viruses from the H5N1 group and, infrequently, a H7 or H9 virus.

In March 2010, a chicken farm in New South Wales, Australia, experienced a slight increase in mortality and a 15% drop in egg production over a period of several days. Autopsy of dead birds revealed swollen kidneys and various degrees of visceral gout. No respiratory signs were evident. Cloacal and tracheal swabs were collected from a number of birds and submitted to the laboratory for testing for type A influenza virus infection. All samples were positive in a type A influenza real time reverse transcriptase PCR (qRT-PCR). Subsequent testing in both H5- and H7-specific qRT-PCR assays gave negative results.

A recently developed influenza A typing microarray assay allowed rapid identification of the virus to subtype. Surprisingly, an H10N7 was identified. This virus was present in swabs to apparently high levels and was readily isolated in both embryonated chicken eggs and in cell culture. Several segments of the genome of this virus have been fully sequenced, allowing confirmation of the virus as a 'low pathogenicity' strain and providing an insight into the phylogeny of the virus. Serology with both a type A pan reactive blocking ELISA and HI tests confirmed widespread infection in birds on the farm.

A few weeks later, a number of workers at the poultry abattoir receiving healthy birds from the farm developed conjunctivitis and some experienced a systemic illness consistent with influenza. All affected workers were located in one part of the processing plant. Swabs from 2 people gave positive results in the type A influenza qRT-PCR. The microarray assay detected RNA from a H10 virus in one of these samples.

This presentation will review the use of the diagnostic aspects of this investigation, especially the use of the microarray assay, characteristics of the H10N7 virus and epidemiological studies that have been undertaken on the affected and neighbouring farms in an effort to identify the source of the virus.

Evaluation of a microarray system for the rapid typing of type A influenza viruses

Melinda Frost, Xingnian Gu, Edla Arzey, Rodney J. Davis and Peter D. Kirkland
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Type A Influenza viruses are important pathogens of many species, including birds, dogs, horses, humans and swine. In several species, and particularly in poultry, identification of the virus subtype is an important consideration when considering the management and/or regulatory response to a diagnosis of an influenza infection. In the past, identification of virus subtype and later pathotype took many days and often weeks, depending on the speed with which the virus could be isolated. Following the widespread adoption of molecular based diagnostic assays it has been possible to complete detection and preliminary identification of a virus very quickly, often in a matter of hours. Consequently, in poultry, by running multiple assays concurrently, it has been possible to identify a virus to H5 or H7 subtype at the same time as the primary recognition of the involvement of an influenza virus. Although recognition of the NA subtype has also been possible by the use of specific assays (eg N1), such subtype-specific assays have not been adopted as frequently as assays specific for the H subtype.

In Australia, samples from wild bird surveys have initially been screened by real time RT-PCR (qRT-PCR) using an Influenza A pan-reactive assay targeting the matrix gene. Positive samples are then tested in H5 and H7 specific assays and also submitted for virus isolation. RNA from samples that are positive in the matrix assay is also used to generate nucleic acid sequence data from the HA and NA genes. This data in turn is used to identify the virus subtype and also generate additional phylogenetic data. However, there have often been difficulties in isolating virus from these wild bird samples due to a range of factors including sample quality, low levels of infectious virus and rapid loss of infectivity after collection. Further, it has often not been possible to amplify sufficient viral RNA to complete sequencing. In an attempt to overcome some of these limitations, a Type A Influenza specific microarray has been evaluated.

Microarray based assays developed to detect the HA and NA genes of Type A Influenza viruses have been produced and converted into a commercially available assay. Both H and N types can be identified concurrently in a microplate based array. Comparison with sequencing results has shown that the microarray produces reliable results and, for a significant proportion of samples with low levels of Influenza RNA, is able to identify virus subtype information when no sequence data has been generated. The assay has also been applied to the identification of virus in an influenza outbreak in commercial poultry and quickly identified infection with a novel virus subtype. Nevertheless, there are still some wild bird samples that could not be typed using any of the methods available. A modification involving further amplification steps has been introduced and has overcome some of these limitations. The data obtained from surveillance studies in wild birds will be presented and the advantages and disadvantages of the microarray based characterisation discussed in further detail.

Development of a multiplex PCR test to differentiate *Salmonella choleraesuis* field isolates from a live attenuated vaccine

Mark Felice, Simone Oliveira
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Introduction. Commonly used to control swine salmonellosis, the Argus live vaccine is attenuated by mutations in the *cya* and *crp* genes. This vaccine strain is often isolated from systemic sites following vaccination, and differentiation between field and vaccine isolates is needed to rule out systemic infection by virulent *Salmonella*. We have occasionally isolated *S. choleraesuis* resembling the Argus vaccine strain from systemic sites in non-vaccinated pigs, suggesting that serotyping and biochemical profiles are not be enough to differentiate vaccine and wild strains. In this study, we have developed and validated a multiplex PCR to improve the identification of the Argus vaccine strain in clinical samples.

Materials and Methods. The multiplex PCR contained newly designed primers targeting the Argus vaccine mutation at the crp gene and control primers detecting the FlinC gene previously described by Chiu et al. (2005). The multiplex PCR was validated by testing 101 *S. choleraesuis* field isolates and 115 suspect *S. choleraesuis* Argus vaccine strains (based on serotyping and biochemical profile) obtained from clinical samples. Specificity of the test was defined by testing DNA extracted from *S. krefeld*, *S.worthington*, *S. braenderup*, *S. brandenburg*, *S. saint paul*, *S. dublin*, *S. enteritidis*, *S. derby*, *S. typhimurium*, *S. agona*, *S. newport*, *S. mbandaka*, *S. montevideo*, *S. tennessee*, *S. hartford*, *S. thompson*, *S. bareilly*, *S. ohio*, *S. infantis*, and 15 additional bacterial pathogens commonly isolated from swine. Field veterinarians in charge of swine herds originating vaccine strains were contacted to confirm herd vaccination status.

Results. PCR primers targeting the *crp* gene produced 610 bp band in virulent *S. choleraesuis*, which was absent in the Argus vaccine. The absence of the *crp* amplicon was unique to the Argus vaccine. Previously described *S. choleraesuis*-specific primers were added to create a multiplex PCR test, producing bands of 610 and 963 bp in *S. choleraesuis*, a band of 963 bp in the Argus vaccine, and a band of 610 bp in all other *Salmonellae* (Fig. 1). Bands were absent in all unrelated pathogens tested except *E. coli*. 103 isolates were classified as field strains and 113 isolates were identified as vaccine strain. The use of the Argus vaccine was confirmed for 39 of 40 isolates for which this information was available. One suspected Argus vaccine isolate based on serotyping (*Salmonella sp.* 6,7: non-motile) and biochemical profiles was isolated from a herd not using the vaccine. This isolate was identified as a field isolate based on the multiplex PCR results and confirmed as a field strain. Another suspected Argus isolate based on serotyping (*Salmonella sp.* 6,7: non-motile) tested negative by the multiplex PCR test but originated from a herd using the Argus vaccine. This isolate is suspected to have been misidentified due to suppressed motility, which has been previously reported to cause other group C1 *Salmonellae* to be incorrectly identified as serotype 6,7: non-motile.

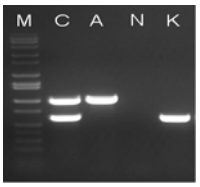

Fig. 1. Agarose gel showing possible multiplex PCR outcomes. From left to right, molecular weight marker, *S. choleraesuis* (C), Argus vaccine (A), negative control (N), and *S. krefeld* representing all other *Salmonellae* (K).

Conclusion. Validation results demonstrate the accuracy and effectiveness of this novel multiplex PCR test for Argus *Salmonella* vaccine identification. During the validation process, the multiplex PCR test produced results conflicting with the initial serotyping results. These differences in results were resolved by re-serotyping the samples, further demonstrating the accuracy of this test over more traditional methods.

Development of calf diarrhea pathogen panel nucleic acid purification and detection workflow

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Calf diarrhea (scours) is a primary cause of illness and death in young calves. Significant economic losses associated with this disease include morbidity, mortality, and direct cost of treatment. Multiple pathogens are responsible for infectious diarrhea; these include but are not limited to Bovine coronavirus (BCV), Bovine rotavirus A, *Cryptosporidium*, and *Giardia intestinalis*. Identification and isolation of carrier calves are essential for disease management. Current methods for calf diarrhea pathogen identification include electron microscopy for BCV and rotavirus and organism detection for *Cryptosporidium* and *Giardia intestinalis* by the direct fluorescent antibody test.

To improve our calf diarrhea pathogen identification process, we have developed a workflow consisting of an optimized fecal nucleic acid purification, and TaqMan® multiplex RT-PCR for single tube concurrent detection of Bovine coronavirus and rotavirus A, *Cryptosporidium,* and *Giardia intestinalis*. Fecal nucleic acid is purified using the MagMAX™ Nucleic Isolation technology and purified pathogen nucleic acid is detected using an optimized multiplex RT-PCR. The multiplex RT-PCR consists of four TaqMan assays targeting the respective pathogens. Highly conserved sequences of Bovine coronavirus and rotavirus A were independently analyzed for signature sequence selection; *Cryptosporidium* and *Giardia intestinalis* assays were selected from publications with acceptable validation data. These assays are further multiplexed with an internal control RNA to monitor nucleic acid purification efficiency, detect the presence of reaction inhibitors, and determine assay reagents functionality.

The analytical sensitivity of the optimized workflow was estimated to be 10 $TCID_{50}$ for Bovine coronavirus and rotavirus A and 20 oocysts and cysts for *Cryptosporidium* and *Giardia intestinalis*, respectively, in "spike-in" fecal experiments using serial dilutions of each pathogen. Analytical specificity was confirmed using Canine and Feline coronavirus, *Giardia muris,* and non-infected bovine purified nucleic acid. The workflow can be completed in approximately 3 hours and can detect all four pathogens concurrently in one single PCR. The results demonstrate that this workflow provides an economical and rapid solution for calf diarrhea pathogen identification which is critical for improved disease prevention and management.

Comparison of DNA purification and detection workflows for *Tritrichomonas foetus* detection

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Bovine trichomoniasis is a cattle venereal disease caused by the protozoan *Tritrichomonas foetus (T. foetus)*. Trichomoniasis causes infertility, abortions, and diminished calf crops resulting in significant economic loss for livestock producers. Regulatory testing of bulls for the pathogen is highly recommended prior to the breeding season and mandatory before cattle movement in many states including Texas. Standard testing programs require multiple InPouch cultures of genital scrapings (preputial smegma) followed by visual microscopic identification and confirmation by PCR. Alternatively, one PCR test can be used directly from InPouch cultures after 2 days incubation. Essential to successful PCR are optimized nucleic acid purification and identification protocols. Currently, a number of nucleic acid purification methods are used (i.e., boiling, glass fiber filter, magnetic beads) for *T. foetus* DNA purification. In order to increase sample throughput, we evaluated the performance of Applied Biosystems' *T. foetus* TaqMan workflow consisting of the automated magnetic bead based DNA purification technology (MagMAX ™ reagents and Kingfisher Magnetic Particle Processor) and VetMAX ™ *T. foetus* reagents containing an internal positive control to streamline and expedite our *T. foetus* identification process.

The *T. foetus* TaqMan workflow and our current workflow (Boil PCR, boiling and PCR reagents) were compared for the evaluation of *T. foetus* detection in 231 InPouch culture smegma samples. The samples were first analyzed using the Boil PCR method; samples with Ct <40 (n=139 samples) and no amplification (n=92 samples) were then used for the *T. foetus* TaqMan workflow. Boil PCR negative samples (n=92) were confirmed negative by InPouch culture microscopic examination. For the Boil PCR method, samples with ≥35 and <40 Ct or aberrant amplification curves (suspect samples) were re-analyzed using glass fiber filter purification (QIAamp DNA Mini Kit); upon re-analysis, samples with <35 Ct were identified as positive and samples ≥35 Ct were identified as negative.

Results show that both workflows were highly specific as supported by the lack of amplification signal in all PCR containing nucleic acid from InPouch culture negative samples (n=92). The Boil PCR method required 18% (25/139 samples) re-analysis with QIAamp DNA purification and PCR for suspect samples; re-analysis resulted in 16 positives and 9 negative identifications (final results). The Boil PCR method also resulted in 3 false negatives (Ct 35-40) with respect to the *T. foetus* TaqMan workflow (Ct <35 for respective samples). The *T. foetus* TaqMan resulted in 3.5% (5/139 samples) suspect samples with ≥35 and <40 Ct; the concurrent amplification of the internal positive control obviated follow-up/secondary DNA purification. Four of these samples were inclusive in the Boil PCR 18% re-analysis sample set; 1 sample was initial Boil PCR positive. Furthermore, 3 and 1 of the 5 suspect *T. foetus* TaqMan samples are inclusive in final Boil PCR negative and positive samples, respectively. Overall, four *T. foetus* TaqMan sample identifications were discordant with final Boil PCR identifications. **In conclusion, the *T. foetus* TaqMan automated MagMAX ™ protocol quickly (~40 mins) provides PCR ready purified DNA in 96 well format, enabling easy and streamlined subsequent PCR setup. Complimentary VetMAX ™ reagents provides reliable sensitive and specific *T. foetus* detection in InPouch culture samples.**

Implementation and coordination of a real-time reverse transcriptase PCR for *Classical swine fever virus* and *Foot and mouth disease virus* detection in the National Animal Health Laboratory Network: Five years of data

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Foot-and-mouth disease (FMD) and classical swine fever (CSF) are high-consequence animal diseases that do not occur presently in the United States. To prepare for possible introduction of foreign animal diseases into the United States, the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA, APHIS) and National Institute of Food and Agriculture (NIFA) formerly known as the Cooperative State Research, Education, and Extension Service (CSREES) formed a partnership with State veterinary diagnostic laboratories and the American Association of Veterinary Laboratory Diagnosticians (AAVLD) to create the National Animal Health Laboratory Network (NAHLN). The principles of the network include implementation of standardized, rapid diagnostic techniques, training personnel, employing modern equipment, developing and using quality standards, and regularly proficiency testing. In the fall of 2003, the "train-the-trainer" program was started to provide a coordinated system for training and transferring technology such that member laboratories of the NAHLN could implement standardized methods for the diagnosis of foreign animal diseases. Annual proficiency testing provides a review of the performance of the laboratories using standardized test methods. Participating laboratories have increased from the initial 12 laboratories in 2004, to 38 state and federal laboratories in 2009. The number of assay variations increased from the original four methods using either vitrified master mix or a wet master mix and the same PCR chemistry on one single tube extraction, to more than 16 variations using manual and automated extraction methods and different PCR chemistries. In 2004, 24 participants completed 96 tests on FMD virus (FMDV) and CSF virus (CSFV) detection; in 2009, 170 participants completed almost 500 tests on FMDV and CSFV detection using PCR**. By using the train-the-trainer program as a method for implementing standardized, validated assays to new laboratory participants, the pass/fail or success rate on standardized proficiency tests for FMDV and CSFV detection by real-time PCR remained relatively unchanged (between 90-95%). The train-the-trainer program of sharing knowledge continues to be an effective method for standardizing methods across the country as new disease assays are introduced into the NAHLN as measured by the successful completion of national proficiency tests for foreign animal diseases.**

Development of multiplex real-time PCR assay for detection and differentiation of *Moraxella ovis*, *Moraxella bovis* and *Moraxella bovoculi*

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Introduction. The exact relationship between *Moraxella bovis* (*M. bovis*), *M. ovis* and *M. bovoculi* and their respective and collective roles in the pathogenesis of infectious bovine keratoconjunctivitis (IBK) is still unclear. Better detection methods for these organisms in diagnostic labs would contribute to improved understanding of the pathogenesis of IBK. It is important to use a method that is sensitive but also specific for all the three organisms. The objective this study was to develop a sensitive and specific *Moraxella* spp. multiplex real-time PCR assay for detection and differentiation of *M. ovis*, *M. bovis* and *M. bovoculi* on field isolates submitted to veterinary diagnostic labs.

Materials and methods. For the *Moraxella* multiplex real-time PCR assay primers and probes were designed for *M. bovoculi*, *M. bovis* and *M. ovis*. Samples were considered positive if the C_T value was less than or equal to 40. The PCR was evaluated on three reference strains for *M. ovis, M. bovoculi*. and *M. bovis* obtained from ATTC. In addition, 57 eye swabs (lacrimal secretions) were collected during an outbreak of IBK in 2008. The lacrimal secretions were inoculated on blood agar plates to isolate any organisms present. Isolates and swabs were subjected to the *Moraxella* spp. multiplex real-time PCR. To further confirm the results obtained with the assay a multiplex conventional PCR assay was developed capable of amplifying and differentiating the 16S rRNA gene from *M.* bovoculi, *M. bovis* and *M. ovis*. Furthermore, all PCR products of obtained with the *Moraxella* spp. multiplex conventional PCR were submitted for sequencing. For samples negative by the *Moraxella* spp. multiplex conventional PCR but positive by the multiplex real-time PCR, the 16S rRNA gene was further amplified using a universal PCR method. The obtained sequences were evaluated using the Basic Local Alignment Search Tool (BLAST).

Results. By using the *Moraxella* spp. multiplex real-time PCR assay, all three reference strains could be identified by the corresponding probes. With the *Moraxella* spp. multiplex conventional PCR, products of 1859bp, 1541bp and 1849bp were amplified from *M. bovoculi, M. bovis* and *M. ovis* isolates, respectively. There were no-cross reactions between different species in both assays. Among the field isolates, 44/57 isolates were identified as *M. bovoculi,* 9/57 were *M. bovis* and 4/57 were found to be negative for all three species. With the *Moraxella* multiplex conventional PCR, 44/57 strains were determined to be *M. bovoculi* , 7/57 were *M. bovis,* and 6/57 were negative. All samples positive with the *Moraxella* multiplex conventional PCR assay (51/57), were identical to those obtained with the real-time PCR and the coincidence rate between the two assays was found to be 96.5%. The sequencing results revealed 100% consistency with the real-time PCR results indicating that the isolates were identified correctly by the *Moraxella* spp. multiplex real-time PCR assay.

Discussion. The *Moraxella* spp. multiplex real-time PCR assay developed in this study was found to be specific for *M. bovis*, *M. ovis* and *M. bovoculi* with no cross-reaction between the different species. Furthermore, it was found that the prevalence of M. *bovoculi* was higher than that of *M. bovis* and *M. ovis*, and *M. ovis* was not detected in IBK cases in the present investigation.

Conclusion. The *Moraxella* spp. multiplex real-time PCR assay developed in this study could be used for detection and differentiation of *M. bovis*, *M. ovis* and *M. bovoculi* in field strains isolated from IBK cases. Further evaluation of the assay directly on eye swabs is in progress.

Rapid molecular typing of *Epizootic hemorrhagic disease virus* (EHDV) by multiplex one-step rRT-PCR

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Captive breeding of white-tailed deer is a growing and important segment of the Texas economy, contributing to the vitality of many rural areas of the state. The direct economic impact of the deer industry on the Texas economy combining breeding and hunting components is approximately $652 million annually. Epizootic hemorrhagic disease virus (EHDV) is one of the most important causes of highly infectious noncontagious hemorrhagic disease in wild and captive deer populations in the United States. Recently, we reported a group-specific real-time polymerase chain reaction (PCR) assay that detects all 8 serotypes of EHDV *(Clavijo A., et al., "An improved real-time polymerase chain reaction for the simultaneous detection of all serotypes of Epizootic Hemorrhagic Disease virus". JVDI, 22:(4) July 2010)*. This PCR assay is reliable, simple, and facilitates the detection of EHDV irrespective of the serotype. However, it does not provide specific serotype information, which may be important in disease epidemiology and disease control through vaccination. In addition, the recent emergence of the new serotype of EHDV type 6, and the possible coexistence or circulation of multiple serotypes, suggest that the geographic distribution of this virus in North America may be changing. Continued monitoring of the distribution of serotypes of EHDV is therefore important.

Currently, virus isolation and the virus neutralization test using monospecific reference sera are required for serotyping EHDV. Both methods are time consuming and expensive. In this report we describe a rapid, sensitive and specific assay for the direct identification and typing of EHDV serotypes 1-8 in cell culture and clinical samples using a multiplex one-step reverse transcriptase PCR (rRT-PCR) . This assay uses a set of well characterized primers derived from genome segment 2 (L2) of EHDV for the specific serotype identification. This newly developed multiplex PCR provides a simple and effective alternative to serotyping, thereby eliminating the time required for virus isolation and the cost of maintaining reference sera and high-quality cell culture supplies. Furthermore, the serotype identification contributes valuable information to epidemiological understanding related to the distribution and ecology of EHDV.

Identification of molecular targets for diagnosis of bovine tuberculosis

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Previously, we have conducted a microarray-based transcriptional profiling study using antigen stimulated white blood cells (WBC) from cattle that were either infected or not infected with *Mycobacterium bovis.* The purpose was to identify potential molecular markers that could be exploited for diagnosis of bovine tuberculosis (bTB). In that study, 91 genes were found that likely showed altered levels of expression between infected and non-infected cattle. To date, we have validated altered expression for 14 of the 91 genes, using quantitative real-time PCR. For validation, RNA was extracted from antigen stimulated WBC from 25 cattle that either had bTB (n=5) or that did not have bTB, as determined at post mortem examination. The cattle that did not have bTB were reactors on the primary caudal fold intradermal skin test (CFT) and non-reactors on the secondary comparative cervical skin test or the whole blood gamma interferon assay (single reactors [n=10]), or were reactors on both primary and secondary tests (double reactor [n=10]). The 25 cattle were a mixture of animals used in the previous microarray study and animals sampled after the conclusion of the microarray study. The majority of the 14 genes were involved in various immune response pathways such as cytokine and chemokine signaling, antigen processing and presentation, and arachidonic acid metabolism.

For cluster analysis, quantitative real-time PCR derived expression ratios (-ΔΔCt) of select genes of the study animals relative to comparable genes of pooled healthy control animals were used. The analysis was based on 14 differentially expressed genes; which allowed clustering of bTB infected cattle and non-infected cattle. Two separate clusters were formed, one of which contained all of the post mortem positive cattle and the other contained all of the double reactors, all of the latter were post mortem negative. Some cattle that were only CFT reactors, and were from bTB positive farms, clustered with bTB positive cattle, while other CFT reactors clustered with the double reactors. Linear discriminant analyses were used to select the minimum number of genes required to provide the best separation between groups of infected and non-infected cattle. Various combinations of genes were assessed to determine their merit as classifiers (predictors) of bTB infection. Results of this analysis showed that the expression ratio (-ΔΔCt) of as few as three out of the 14 differentially expressed genes would provide sufficient statistical power to discriminate cattle with bTB from cattle not infected with bTB. **The gene targets identified in this study have been shown to be differentially expressed in bTB infected and non-infected cattle; thus, those genes might be used to develop a rapid and sensitive diagnostic assay for bTB.**

Variation in *Bluetongue virus* qRT-PCR assay results in experimentally inoculated blood samples of sheep, cattle and alpaca

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Bluetongue is a vector-borne viral disease that affects domestic and wild ruminants. The epidemiology of this disease has been recently altered due to climate changes, with resultant spread into geographic areas in which bluetongue has not historically been reported. Various qRT-PCR assays are used to detect bluetongue virus (BTV); however, PCR assays have not been validated for use with New World camelid species which are known to have a high hemoglobin concentration. Hemoglobin has been documented as an important PCR inhibitor. In this study sheep, cattle and alpaca blood was experimentally spiked with BTV serotypes 10, 11, 13 and 17 and analyzed in 10-fold dilutions by qRT-PCR to determine if species had an impact of viral nucleic acid recovery and assay performance. A separate experiment to assess the influence of alpaca blood on Ct values of the qRT-PCR assay consisting on spiked alpaca blood subsequently diluted in 10-fold series in sheep blood was performed. All experiments were performed in dilutions from 10^{-1} to 10^{-8} and in triplicate. **Results showed that BTV-specific nucleic acid recovery from alpaca blood was approximately 1-2 logs lower compared to sheep and cattle blood and, results were similar for the 4 BTV serotypes analyzed**. These findings should be taken into consideration in future studies of BTV and other diseases diagnosed by qRT-PCR that affect alpaca and other New World camelids.

Comparison and optimization of two previously described real-time RT-PCR assays for the detection of *Equine arteritis virus* in equine semen samples

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Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA) of horses and other equids. A variable percentage (up to 10–70%) of acutely infected stallions can become persistently infected and continue to shed virus in their semen. Virus isolation (VI) is currently the World Organization for Animal Health (OIE)-approved gold standard for the detection of EAV in semen and is the prescribed test for international trade. Sensitive standard RT-PCR, RT-nested PCR, and real-time RT-PCR (rRT-PCR) assays have been developed for the detection of EAV nucleic acid in semen, and these assays are being increasingly used for routine diagnostic purposes. The primary objective of this study was to compare the analytical sensitivity of two previously described TaqMan® fluorogenic probe-based one-tube real-time RT-PCR assays (Balasuriya *et al.* 2002, J. Virol. Meth. 101(1-2):21-28 [T1] and Mankoc *et al.* 2007 J. Virol. Meth. 146(1-2):341-354 [T2]). The second objective was to use the most sensitive rRT-PCR assay to determine the best combination of commercial RNA extraction kit and commercial one-step rRT-PCR kit for the detection of EAV RNA in semen of carrier stallions as compared to the traditional VI.

The analytical sensitivity of T1 and T2 rRT-PCR assays was determined either with *in vitro* transcribed (IVT) RNA or with RNA isolated from 10-fold dilutions of tissue culture fluid (TCF) containing EAV using three different commercial one-step rRT-PCR kits (QuantiFast [Qiagen], TaqMan One-Step [ABI] and TaqMan EZ [ABI]). RNA was extracted from ten-fold serial dilutions of TCF using two commercial kits (MagMax [ABI] and BioSprint [Qiagen]). Finally, the viral RNA extracted from four hundred and nine equine semen samples using the same two RNA extraction kits were tested with QuantiFast [Qiagen], and TaqMan EZ [ABI] and compared to the VI results.

The T1 rRT-PCR assay had a higher sensitivity than the T2 rRT-PCR for detection of IVT RNA as well as EAV RNA extracted from TCF and semen samples. The T1 rRT-PCR assay had the highest analytical sensitivity when used in combination with the QuantiFast kit (Qiagen). When RNA extracted from semen samples was tested with the T1 assay in combination with different rRT-PCR kits, the highest sensitivity was achieved by RNA extracted with MagMax kit (ABI) in combination with QuantiFast rRT-PCR kit (Qiagen) which was more sensitive than the attempted VI in cell culture. Eighty three out of four hundred and nine semen samples tested positive for EAV nucleic acid by T1 rRT-PCR using the aforementioned kits, whereas only 80 semen samples were positive for EAV by VI. **The data from this study clearly demonstrate that the T1 assay is more sensitive than the T2 rRT-PCR assay for detection of EAV nucleic acid in equine semen samples. The T1 assay sensitivity can be further influenced by commercial rRT-PCR assay kits used for amplification of the nucleic acid. The combination of magnetic bead nucleic acid extraction technique along with QuantiFast kit (Qiagen) could significantly improve the sensitivity of the assay. Furthermore, comparison of the sensitivity of the T1 assay with VI showed that the molecular assay provides an equivalent or higher sensitivity. Therefore, this optimized T1 rRT-PCR assay provides a fast and reliable means of virus nucleic acid detection in equine semen samples and could be considered as an alternative or at least a complementary molecular diagnostic to the VI for EAV detection.**

Development and validation of a quantitative PCR for the detection of *Actinobacillus suis*

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Introduction: *Actinobacillus suis* is an emerging pathogen considered to be a new threat for swine production. The isolation of this pathogen from tissues submitted to the University of Minnesota Veterinary Diagnostic Laboratory (MVDL) increased considerably in the past few years. In 2006, a 16s rRNA gel-based PCR was developed and since then requests for *A. suis* PCR testing have raised significantly.[1] This tool has been highly employed in protocols attempting to eradicate *A. suis*. However, complete elimination of the pathogen has not been achieved[2,3] Limitations of this gel-based PCR include the non-specific amplification of *A. equuli* and the lack of bacterial quantification. Therefore, a sensitive and more specific quantitative diagnostic tool is needed to improve eradication protocols. The objective of this study was to develop and validate a sensitive and specific quantitative real-time PCR assay for the detection of *Actinobacillus suis* in clinical samples.

Materials and Methods: Forward and Reverse primers and a TaqMan probe specific for the amplification and detection of an *A. suis* housekeeping gene involved in thiophene oxidation (*Thdf*) were designed using the *Primer 3 Software*. Real-time PCR conditions were optimized utilizing an ATCC *A. suis* reference strain. This strain was also utilized to evaluate the analytical sensitivity of the real-time PCR by testing 10-fold dilutions of extracted DNA. The analytical specificity was evaluated by testing 29 unrelated bacterial species frequently isolated from swine in addition to *A. equuli*, which generated non-specific amplifications with the gel-based 16S rRNA PCR. The stability of the targeted gene sequence among *A. suis* field isolates was evaluated by testing 70 different isolates representing 12 genotypes circulating among U.S swine herds.

Results: The newly developed real-time PCR detected a minimum of 1.75×10^2 CFU/ml, which is as sensitive as the 16S rRNA gel-based PCR that can detect a minimum of 7.2×10^2 CFU/ml. The *Thdf* real-time PCR was highly specific, detecting exclusively *A. suis* isolates. There was no non-specific amplification of *A. equuli* DNA, as previous observed with the gel-based PCR. The new real-time PCR amplified the *Thdf* gene from all 70 *A. suis* field isolates tested, indicating that this housekeeping gene is highly conserved among different strains circulating in U.S. swine herds.

Discussion: A highly specific quantitative real-time PCR for detection of *A. suis* in clinical samples was developed. This new test will allow swine veterinarians to perform a more comprehensive comparison of different control and eradication protocols. The new real-time PCR, which is more specific than the previous gel-based PCR, will allow a better characterization of prevalence at weaning. Quantification of *A. suis* in tonsil swabs will also provide a more detailed characterization of treatment effect, and will allow swine veterinarians to evaluate the cost-benefit of each protocol. We expect the new *A. suis* real-time PCR to be an important aid in *A. suis* control and eradication.

Development of a quantitative PCR assay for the detection of *Mycoplasma hyorhinis* in clinical samples

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Introduction: *Mycoplasma hyorhinis* has recently emerged as an important cause of mortality in nursery pigs. Approximately 50% of the cases with polyserositis received at the Minnesota Veterinary Diagnostic Laboratory have the involvement of this pathogen based on isolation or detection by PCR. It is not clear whether that reflects a real increase in the prevalence of this disease or is the result of an improved ability to detect *M. hyorhinis* due to the introduction of PCR. Isolation of this pathogen requires a specific media and can take up to 7 days to grow. Immunohistoquemistry assays have been developed, but they have proven to be less sensitive than isolation. There are currently no serological tests commercially available for this pathogen. Several protocols for detection of *M. hyorhinis* DNA by gel-based PCR have been published, but the majority of them are for the detection of *M. hyorhinis* in cell cultures. There are no quantitative PCR protocols available for this pathogen.

Materials and Methods: Forward and Reverse primers and a TaqMan probe specific for the amplification and detection of the 16S rRNA were designed using the Primer3 Software. Real-time PCR conditions were optimized utilizing the *M. hyorhinis* ATCC 17981. This strain was also utilized to evaluate the analytical sensitivity of the real-time PCR by testing 10-fold dilutions of extracted DNA. The analytical specificity was evaluated by testing 19 unrelated bacterial species frequently isolated from swine, including *M. hyopneumoniae, M. flocculare and M. hyosynoviae*. A total of 45 clinical samples submitted to the Minnesota Veterinary Diagnostic Laboratory for *M. hyorhinis* testing were analyzed with the real-time PCR. These samples were from animals that presented typical lesions found in *M. hyorhinis* cases such as: pericarditis, pleuritis, arthritis and peritonitis. The reactions were carried out in the ABI 7500 fast real-time PCR system at 95°C for 3 min, 40 cycles of 95°C for 15 sec and 54°C for 50 sec. All samples were run in triplicates, and H_20 was employed as the negative control in all reactions.

Results: The newly developed quantitative PCR detected a minimum of 40 x 10^4 CFU/reaction, compared to the current gel-based PCR employed by the Veterinary Diagnostic Laboratory of the University of Minnesota for clinical samples, which can detect a minimum of 16 x 10^5 CFU/reaction. The quantitative PCR was highly specific, detecting exclusively *M. hyorhinis* isolates. A total of 21 samples were positive for *M. hyorhinis* when tested with the real-time PCR. However, when the same samples were tested using the currently employed *M. hyorhinis* gel-based PCR, only 14 samples were found to be positive.

Discussions: A sensitive and specific quantitative real-time PCR for detection of *M. hyorhinis* was developed. General aspects of infection of this pathogen are reported in the literature, although there is limited information on aspects regarding the epidemiology, control and eradication. With this newly developed real-time PCR a better understanding of the characteristics of *M hyorhinis* infection in modern swine production will be achieved. Uses of this tool involve the characterization of the prevalence of this pathogen in swine herds as well as the quantification of the bacteria to evaluate control and treatment protocols.

Molecular Session

Sunday, November 14, 2010
Salon A

Moderator: Kyoung-Jin Yoon and Steve Sells

Superior detection of North American and European porcine reproductive and respiratory syndrome viruses using a single, simpler commercial multiplex real-time quantitative reverse transcriptase–PCR

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important infectious diseases of swine world wide. Detection of the causative agent, PRRS virus (PRRSv), continues to be a diagnostic challenge in to 2010, primarily due to the heterogeneity of field isolates, and the lack of sensitivity of currently employed PCR assays. Detection is further complicated by the development of persistently infected swine which can act as sources of continued infection in which viral loads are low and variable, and no often detected in currently employed assay in the US. Moreover, European (EU) lineage PRRSV isolates, which are genetically divergent from North American (NA) isolates, introduced into NA swine further complicate efforts to diagnose this disease in North America.

In this study, a commercial multiplex real-time reverse transcriptase PCR (qRT–PCR) assay developed and licensed for use in Europe was validated for the detection, differentiation, and quantification of PRRSv in field samples from North America and samples from pigs experimental infected with NA PRRSv strains. **This assay employs a proprietary sample lysis buffer, retains sensitivity when utilizing whole blood and does not require a conventional nucleic acid extraction step.** The assay employs a superior PRRSv detection system which can be followed by a sensitive typing assay. Sensitivity and specificity was compared to two commercially available reagents utilized for the detection of PRRSv in North America field samples. **The multiplex assay sensitivity was found to be higher (1-3 logs) than currently employed assays and capable of detecting more positive samples.** This assay is more amenable to sample pooling as compared to currently available assays given the PCR inhibitors commonly found in biological samples such as blood, saliva and tissue.

Here we demonstrate the superiority, ease and utility of a novel Multiplex Real-time Quantitative Reverse Transcriptase–PCR assay for detection and differentiation of NA and EU PRRSv in both the diagnostic and research samples.

Evaluation of FTA® card as a sample collection system for detection of PRRSV by RT-PCR

Albert Rovira, Daniel Linhares, Michele Leiferman, Yin Jiang and Montserrat Torremorell
Veterinary Diagnostic Laboratory, University of Minnesota, Minnesota

Introduction: FTA® cards consist of a cellulose-based matrix paper containing chemicals that lyse the cells in the sample while preserving the nucleic acids. Because these chemicals render bacteria and viruses inactive, samples embedded in FTA® cards are not infectious and can be safely shipped internationally. Data from a previous study has shown that PRRSV is detectable by RT-PCR from FTA® cards (1). However this study failed to properly assess the diagnostic sensitivity of RT-PCR in samples embedded in FTA® cards. The objective of this study was to evaluate FTA® cards as a sample collection system for detection of porcine reproductive and respiratory syndrome virus (PRRSV) by RT-PCR.

Materials and Methods: To estimate the analytical sensitivity of this method, serum and oral fluid samples were spiked with PRRSV, serially diluted, and tested directly and after absorption in FTA® cards. To further investigate the effect of this collection method in the sensitivity of the test, 8 pigs were experimentally infected with PRRSV while 4 control pigs were sham-inoculated. Two infected pigs and one control pig were euthanized at 7, 14, 21 and 28 days post inoculation. For each pig, serum, blood, oral fluids and tissue samples were tested for PRRSV directly and after absorption in FTA® cards.

Results: PRRSV RNA could be detected from samples embedded in FTA® cards by RT-PCR. However, results from spiked samples showed a 100-fold increase in the limit of detection. Fresh serum, blood, lung, tonsil and lymph node samples from each control pig were negative for PRRSV by PCR. Collective oral fluid samples from the pen housing the control pigs were also negative. All these samples were also negative when collected on FTA® cards. On the other hand, all serum, blood and tissue samples from experimentally infected pigs were positive for PRRSV by RT-PCR, both when run directly and when collected in FTA® cards. In addition, 11 out of 11 oral fluid samples from experimentally infected pigs were positive when tested directly while only 3 of them were positive when collected on FTA® cards.

Discussion: PRRSV RNA can be detected from samples embedded in FTA® cards by RT-PCR. However, the analytical sensitivity for samples collected in FTA® cards is lower than for samples tested directly. This did not seem to affect the diagnostic sensitivity for serum, blood and tissue samples from experimentally infected pigs. In contrast, the diagnostic sensitivity for oral fluids was decreased.

Optimizing ante mortem detection of *Mycoplasma hyopneumoniae* in pigs

Erin Strait[1], Cassandra Roe[1], Nyssa Levy[1], Carly Dorazio[2], Mike Kuhn[3]
[1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, USA;
[2]TriOak Foods, West Burlington, IA; [3]Pfizer Animal Health, New York, NY

Introduction: *Mycoplasma hyopneumoniae* (*M. hyo*) is an important cause of pneumonia in swine around the world. *M. hyo* can be challenging to identify during the early stages of infection due to its often slow spread within a population. Currently, ante mortem diagnosis relies primarily on detection of serum antibodies that may take weeks to months to become detectible by ELISA, or on nested PCR from This study sought to optimize testing for ante-mortem detection of *M. hyo.*

Materials and Methods: Samples were collected as part of a vaccine study in which 2 seeder pigs from each of 6 pens of 52 pigs were challenged with an intratracheal inoculum of strain 232 lung homogenate. Within each pen, serum and nasal swabs were collected from 10 pigs (excluding seeder pigs), and an oral fluid[2] was collected from each pen: 4 weeks pre-challenge; at challenge; 2, 4, 8, and 12 weeks post-challenge (oral fluids were not collected at 4 weeks pre-challenge). Swabs and oral fluids were extracted by both a bead-based extraction method (Ambion Mag MAX-Viral Isolation Kit) and a spin-column extraction method (Qiagen QIAmp DNA mini kit). Extractions performed using the Ambion kit followed the oral fluid and total nucleic acid purification protocol. Extractions using the Qiagen kit followed the blood and body fluid protocol. DNA was tested by a previously assay[3] using two different master mixes (Ambion VetMAX-Plus qPCR master mix or AB TaqMan Universal PCR master mix) to further optimize the assay.

Results: The two extraction methods for oral fluids were compared and the spin-column kit resulted in greater sensitivity as compared to the bead-based extraction method. The opposite was true when extraction methods for nasal swabs were compared. Based on these results, oral fluids were extracted using the Qiagen QIAmp DNA mini kit and nasal swabs were extracted using Ambion MagMAX kit. When the two different PCR reagent mixes were compared the Ambion VetMAX-Plus qPCR master mix gave the most sensitive results regardless of sample type tested. Significant numbers of PCR positives were not detected until 8 and 12 weeks post-challenge for both oral fluids and nasal swabs. Positives attributed to maternal antibody transfer were detected by ELISA at the earliest time points and the post vaccination/challenge antibodies appeared at 8 to 12 weeks, similar to the PCR results. Due to the limited number of collection time points in this study all 3 sample types detected positives post challenge at the same time point (8 weeks post challenge). Therefore, it was not possible to determine which of the sample types was able to detect positives at the earliest time-point post-infection. However, differences in the sensitivities of these assays and sample types were observed. At eight weeks post challenge, 45% of serum, 72% of nasal swabs, and 100% of oral fluids were identified as positive. Stronger PCR positives were detected from oral fluid samples than from nasal swabs.

Discussion/Conclusion: In this study we optimized both the sample collection procedure and DNA extraction PCR protocols for nasal swabs and oral fluids and compared these results to serology. Future studies applying these techniques in the field will be needed to confirm these results, but demonstrate the potential for monitoring shedding of *M.hyo* through oral fluids or nasal swabs in the field.

Detection of *Mycoplasma suis* in pigs by real-time PCR

Erin Strait, Nyssa Levy, Cassandra Roe, Alejandro Ramirez
Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA

Introduction: *Mycoplasma suis* (formerly *Eperythrozoon suis*) is a blood-borne bacterial pathogen and causative agent of immune-mediated hemolytic anemia in pigs. Once infected, animals become life-long carriers, causing a variety of signs that have been reported to include reproductive failure, hypoglycemia, general unthriftiness, increased susceptibility to secondary infections, and even acute death. Good diagnostics for this agent are currently lacking, and because of this, the true prevalence and significance of this disease is unknown. This organism is not able to be cultured in the laboratory, and until recently, diagnosis of *M. suis* was primarily by acridine-orange staining of red blood cells; however, recent studies have shown this method to have limited sensitivity. Recently, an intracellular strain of *M. suis* was identified that was unable to be detected by this staining method. An ELISA test has been described, but is not commercially available. This highlights the need for a rapid and sensitive test such as real-time PCR. A real-time PCR assay targeting the msg1 gene has been recently described, but can only be performed on one instrument not commonly used, thus limiting its application. The main objective of this study was to develop a real-time PCR based on the msg1 assay that could be performed on a wide range of instruments and facilitate diagnosis.

Materials and Methods: Blood samples were collected in EDTA and/or heparin tubes from apparently healthy pigs in 4 age groups, each from different herds: 3-day old piglets (n=13), 12-18 week old pigs (n=17), 6-24 month old boars (n=23) and sows at time of slaughter (n=42). Additionally, meat juices (n=50) were obtained from muscle samples taken at the time of slaughter at 10 unique sites in 3 different states and processed at the Iowa State University Veterinary Diagnostic Laboratory- Meats Laboratory. DNA was extracted by spin column (Qiagen QiAmp DNA mini kit) and/or magnetic bead-based protocol (Ambion, Mag-Max kit).

Results: Extracting blood samples by the spin column method is the most sensitive protocol for positive detection of *M. suis* by real-time PCR. Positive samples were found within each sample group at various levels except for the 3-day old piglets which were all negative. EDTA-blood samples from PCR-positive (n=10) and PCR-negative (n=15) pigs were submitted for complete blood count analysis. While not statistically significant due to low sample number, a trend for lower clinical blood values in PCR-positive samples was observed for the parameters: hematocrit, hemoglobin and RBC count.

Discussion/Conclusion: Results of this study suggest that the presence and impact of *M. suis* in otherwise healthy pigs may be currently underappreciated. Further testing of both apparently healthy and clinically-affected animals will be necessary to determine the true prevalence and significance *M. suis*-induced disease in today's swine industry.

Development of nucleic acid preparation technologies for TaqMan® assay analysis of diverse animal sample matrices

Dan Kephart, Xingwang Fang, Quoc Hoang, Darcy Myers, Chris Willis, Mangkey Bounpheng
Life Technologies, Austin, TX

TaqMan® assay-based real-time analysis is a highly sensitive and specific technology for the amplification and analysis of target nucleic acid. While the design of TaqMan® assays and execution of real-time analysis is straightforward, the generation of accurate results is critically dependent on the quality of nucleic acids that are introduced into the reaction. For target nucleic acid amplification, this is further complicated by the size and physical/chemical makeup of the nucleic acids of interest, as well as the milieu of potential inhibitors that often accompany animal field samples. The coordinated development of nucleic acid preparation strategies that are optimized for downstream analysis as part of a field sample analysis workflow can have profound implications on the sensitive and accurate amplification of target nucleic acid. A perspective of the technical and workflow merits of current nucleic acid preparation strategies is presented. Data comparing the performance of representative methods clearly demonstrates a coordinated development of the entire workflow solution.

Comparison of silica columns and magnetic particle purification: Samples purified using the MagMAX® kit and a competitor silica column method were compared for sensitivity of detection. Published data (Amaresh et al.) is presented wherein macerated tissues (indicated) were extracted with TRIzol® LS Reagent and equivalent volumes (50 µl) of the resulting TRIzol® extracts were used for purification using the RNeasy® Mini Kit (Qiagen), Viral RNA Mini Kit (Qiagen), TRIzol® method (Invitrogen), EZ1® Viral RNA Mini Kit (Qiagen), and the MagMAX™-96 Kit (Applied Biosystems by Life Technologies). Recovered avian influenza RNA was amplified by qRT-PCR using primers and probes targeting the viral matrix gene.

Recovery of both RNA and DNA from sample matrices: Serial dilutions of HindIII-digested lambda DNA and Xeno™ RNA were spiked into the indicated matrices and purification was performed using the MagMAX™-96 Viral RNA Isolation Kit. Equivalent volumes (8 µl) of 50 µl purified samples were used for amplification of recovered DNA and RNA by qPCR or qRT-PCR, respectively.

Nucleic acid preparation must be developed as part of a workflow: we prepared equivalent amounts of representative pathogens present in diverse sample matrices by comparing several MagMAX™ kits and direct lysis approaches. Comparison of the performance of prepared samples in real-time PCR (DNA) and RT-PCR (RNA) analysis is presented. Performance of optimized sample-to-answer workflows was demonstrated by purification of a variety of pathogens from an array of sample matrices for subsequent amplification using specific primer-probe mixes.

As assessed by real-time analysis, we demonstrated that magnetic bead technology yields superior workflow and amplification effectiveness relative to column-based methods for the preparation of pathogen nucleic acids. Purification using MagMAX™ products is more reproducible and generates samples of substantially higher purity, which leads to increased sensitivity in subsequent real-time analysis. The chemistry is also highly amenable to automation, and has been implemented on the MagMAX™ Express instrument as well as a variety of open liquid handling platforms. Sample preparation is an integral component of the target nucleic acid amplification workflow. **Therefore, developing technologies with consideration of the sample matrix, nucleic acid of interest, as well as format of downstream analysis can have a profound impact on the successful amplification of nucleic acid from field samples.** We conclude by demonstrating the excellent performance of MagMAX™ purification technology as part of our workflow. **For Research Use Only. Not for use in diagnostic procedures.**

USAHA/AAVLD Joint Plenary Session

Monday, November 15, 2010
Salon ABCD

One Health: One-way street or are there opportunities for animal agriculture?

Moderator: **Lonnie King,** *DVM, MS, MPA, ACVPM, Dean, Ohio State University College of Veterinary Medicine*

07:50 AM **Welcome**
Steve Halstead, DVM, USAHA President-Elect - Program Co-Chair

08:00 AM **Keynote: One Medicine - It's all herd health**
Lisa Conti, DVM, MPH, DACVPM, CEHP, Director, Division of Environmental Health, Florida Dept. of Health and Co-Author (With Dr. Peter M. Rabinowitz), Human-Animal Medicine: Clinical Approaches to Zoonoses,Toxicants and Other Shared Health Risks – speaker sponsored by Pfizer. 167

08:30 AM **Emerging infectious diseases: The case for integrating science, medicine and public health**
Gary Simpson, MD, PhD, MPH, College Master and Professor of Internal Medicine and Infectious Disease, Departments of Medical Education and Internal Medicine, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center at El Paso, TX 168

09:00 AM **Producer perspective on One Health: What are the implications of being a One Health partner?**
Mark Engle, DVM, Director, Health and Transportation, PIC North America 169

09:30 AM **Break**

10:00 AM **One Health and the environment: Improving health in a wicked world**
Katey Pelican, DVM, PhD, Assistant Professor, University of Minnesota; Current principle investigator on a USAID One Health Grant 170

10:30 AM **Global prospective of One Health: Are we missing opportunities?**
Mo Salman, BVMS, MPVM, PhD, DACVPM, Professor of Epidemiology, Director, Animal Health Population Institute, Colorado State University. 171

11:00 AM **Emerging microbial threats: Challenges and opportunities at the human-animal-ecosystem interface**
James Hughes, MD, FACP, FIDSA, Professor of Medicine and Public Health, Emory University; and former Director of the National Center for Infectious Diseases at CDC . . .174

11:30 AM **One Health Discussion/Q&A Panel of speakers**
Lonnie King, Moderator

One Medicine: It's all herd health

Lisa Conti, DVM, MPH, DACVPM, CEHP Director, Division of Environmental Health, Florida Dept. of Health and Co-Author (With Dr. PeterM. Rabinowitz), Human-Animal Medicine, Clinical Approaches to Zoonoses,Toxicants and Other Shared Health Risks – speaker sponsored by Pfizer, Inc

Narrative:

The exciting concept of One Health, while not new, encourages systems thinking and implementation at addressing challenges to disease and injury prevention and control. By using the intersection of human, veterinary and environmental health, practitioners in these fields can manage a wide range of clinical and public health problems.

For most of us, a companion animal makes up part of our family structure and most people consume food of animal origin. Biologic, chemical and radiation hazards in our environment that impact these animals, also impact us. Our ability to attend to and mitigate these threats increase our community sustainability and our general health.

The task of identifying and controlling emerging pathogens and conditions benefits from an open communication and collaboration among human medical, veterinary medical and environmental health practitioners. The nation's response to the Gulf oil spill necessarily requires the input of multiple professions working together to address the impacts from occupational exposure, to wildlife and habitat threats, to harvesting food from these waters. Zoonotic influenza is an infectious disease that exemplifies the need for working across divides. Environmental changes including how we build our environments have considerable impact on human, animal and environmental health.

The growing awareness of the benefit of One Health linkages requires each of us in these professions to take initiative, starting as simply as knowing whom to contact in our communities and making those contacts.

Emerging infectious diseases: The case for integrating science, medicine and public health

Gary Simpson, MD, PhD, MPH
College Master and Professor of Internal Medicine and
Infectious Disease, Departments of Medical Education and Internal Medicine,
Paul L. Foster School of Medicine, Texas Tech University Health Sciences
Center at El Paso, TX

Narrative:

Emerging infectious diseases in the 21st Century have become increasingly complex and unpredictable. **Since 85% of emerging infectious diseases in recent decades are zoonotic in origin, the importance of understanding the dynamic interactions of the ecosystems of wildlife, domestic/agricultural animals, and humans has been demonstrated convincingly. Extensive experience with these infectious disease threats has taught that addressing them responsibly requires the collaborative and coordinated efforts of inter-disciplinary, multi-organizational working groups.** The example of the initial outbreak of hantavirus pulmonary syndrome will used to illustrate these concepts. The sustained collaborations that resulted from this event will be described.

Producer perspective on One Health: What are the implications of being a One Health partner?

Mark J. Engle, DVM, MS
Director, Health Assurance and Transportation, PIC North America

Narrative:

The One Health Initiative Task Force (OHITF) established by American Veterinary Medical Association (AVMA) articulated a definition for One Health: "One Health is the collaborative effort of multiple disciplines – working locally, nationally, and globally – to attain optimal health for people, animals and our environment."

OHITF also developed a Vision statement for One Health: "To promote and improve the health of humans, animals and our environment, individually and collectively, by encouraging and ensuring the acceptance and adoption of One Health and its associated activities."

The One Health concept is nothing new to veterinarians and producers. Veterinarians and producers practice One Health daily. The Veterinarian's Oath refers to the protection of animal health and the promotion of public health:

Veterinarian's Oath

Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health, the relief of animal suffering, the conservation of animal resources, the promotion of public health, and the advancement of medical knowledge.

I will practice my profession conscientiously, with dignity, and in keeping with the principles of veterinary medical ethics.

I accept as a lifelong obligation the continual improvement of my professional knowledge and competence.

Producers and veterinarians recognize the public health implications of the human/animal interface regarding food production; food safety, zoonosis, humans to animal transmission, and consumer perception. The One Health concept is prominent in both animal agricultural production and animal health.

A potential benefit of One Health would include funding for an enhanced disease surveillance, detection, and control infrastructure in developing countries. The integration of animal and human health infrastructure would hopefully provide access to funding and resources for much needed animal health research. In addition, common analysis of agriculture and human scientific studies could provide for an aligned interpretation of results with joint communication considering the impact on public perception, market access and trade implications

Producer concerns with One Health are rooted in a lack of communication, misinformation, limited interaction, and the "precautionary principle" to date. One Health appears to be a relatively new concept for Public Health. Human health is disease treatment centered not prevention oriented. One Health refers to more than just health care treatment; it is about disease prevention.

Today, there is clearly a lack of public understanding of animal health issues and modern animal agriculture. The level of awareness among public health regarding conventional animal agriculture and disease prevention efforts is bleak. Educational and outreach efforts need to be established so the public understands animal agriculture as well as the One Health concept. The partnership for One Health needs to be clearly defined. The objectives must emphasize issues relevant to both parties without slighting animal health.

The most pressing need for a transformation of this scope is leadership. For One Health to come to fruition, the One Health agriculture/public health leadership must be developed without biased agendas. Communication, outreach programs, and building trust will be keys to moving this concept forward.

One Health and the environment: Improving health in a wicked world

Katey Pelican, DVM, PhD
Department of Population Medicine, College of Veterinary Medicine, University of Minnesota

Narrative:

We live in a 'Wicked World' where complex crises are constantly challenging our ability to respond to them: climate change, the global food crisis, emerging infectious diseases.

All of these challenges pose a threat not just to human health, but to all the biological systems on which health depends. Unfortunately, traditional, discipline-driven science is not very good at understanding complexity and knows almost nothing about most of the species on Earth. Responding to these new threats, therefore, requires a new approach that teams excellent scientists from across many disciplines with a 'roll up your sleeves' practicality and commitment to global engagement. The new field of 'One Health' is working to understand and change how science, policy, and education work together to solve the wicked challenges of our generation that sit at the intersection of human health, animal health and the environment. However, questions of our changing environment and its relationship to health in animals and humans have often been under-represented in this emerging field in part due to challenges associated with the different cultures and languages of health and environmental disciplines.

Engaging the environment component of One Health in a real way will be critical to success as environmental and ecological sciences bring a whole-system perspective that is invaluable in understanding the complex challenges we face. We cannot separate food production from land use from environmental degradation from human nutrition from animal health. Ultimately it is the system that sustains us. The power of One Health will be to understand and strengthen that system, in all of its messiness, to improve health, but that potential has yet to be realized. Until One Health ensures the true involvement of all professions and sectors, it will not truly be One.

Global prospective of One Health: Are we missing opportunities?

Mo Salman
Institutions: Animal Population Health Institute – Colorado State University

Narrative:

The emergence of deadly zoonotic diseases during the last few decades, such as human immunodeficiency virus/acquired immune deficiency syndrome, severe acute respiratory syndrome, and West Nile virus, present an urgent need to renew and increase collaborative efforts between human and veterinary medicine. The concept that animal health and the environment influence human health has been addressed since the beginning of human history. The first charge of veterinary medicine was to benefit human health mainly through improving the safety of animal origin food. During the 20th century, however, cooperation between the two disciplines of human and veterinary medicine diminished due to several factors.

The interaction between human and veterinary medicine as two disciplines has been especially fruitful in the broad areas of patho-physiology and epidemiology. An exploration of this interaction using historical and contemporary examples in comparative medicine, zoonoses, zooprophylaxis, and the human-animal bond, reveals that a better understanding of animal and human disease, as well as societal changes such as interest in non-conventional medicine, are leading to a broader concept of an all inclusive medicine that includes animal and human medicine as well as social and other sciences.

The concept of "One Health/Medicine" has not been used to promote the veterinary profession in general and veterinary epidemiology specifically. This limitation is mainly due to an incomplete understanding during the last two centuries of the role of veterinary epidemiology in combating animal diseases, including zoonoses. Recently the major human and veterinary medical associations have enthusiastically embraced and endorsed the concept of One Medicine. The July 2007 resolution by the American Medical Association (AMA) resolved to promote collaboration between human and veterinary medicines, joint educational programs, efforts in clinical care, cross-species disease surveillance and control and new diagnostic methods, medicines and vaccines. The American Veterinary Medical Association (AVMA) passed a similar resolution at their July 2007 meeting. One can only hope that these initiatives are not lost in the proverbial subcommittee but will instead lead to definitive action. Other learned societies have endorsed this concept and numerous supportive essays have appeared but with limited action plans to demonstrate the integration or other valuable products. For the societies that include animals as sources for many of the potential zoonotic diseases, government agencies have started to indicate their willingness to work together toward one goal for better general public health. This motivation, which was supported by several meetings, conferences, publications, and speeches, has not changed the mode of operations of the various involved parties. Neither agencies nor institutions have changed their plans of action for coordination and engagement of the other side of the equation. For instance, the 2004-2009 avian influenza outbreaks have led to limited collaboration between the public health officers and animal health authorities. In many parts of the world it has been recognized that the lack of communication, insufficient appreciation of the duties of each actor, and the limited integration of plans of action between public health and animal health officers are the factors that contribute to the ineffective collaboration toward one goal.

Although we can decry, as did Schwabe in 1984 and Barthold in 2005, the shortcomings of our educational systems, assigning blame does little to solve the current dilemma. These systems have not produced a generation of comparative medicine specialists, including pathologists and epidemiologists, who are prepared for the current demands of linking the two medical disciplines under One Health, particularly in field operations.

For over the last fifty years modern epidemiology has possessed a unique approach to disease through preventive measures. The majority of these measures require dealing with the source of infection. Thus, in the case of zoonotic diseases, animals should be the focus. Epidemiologists as population-based scientists on both sides (human and animal) can collaborate to prevent zoonotic diseases. This type of collaboration should require the understanding of the entire ecological disease system including the social and culture environment, animal husbandry, animal production and the role of animals in the wellbeing of the society. It is necessary, therefore, to have a strong link in activities, especially in field operations, to demonstrate this type of collaboration.

Traditional academic or public institutions or divisional structures where the epidemiologists operate will prove ineffective because the few interested, capable epidemiologists are geographically dispersed and their duties are limited in order to satisfy the main mission of their corresponding departments or agencies. Therefore there is a need to take action in a prompt and effective manner without depending on institutional support.

The theme of "One Health/Medicine" is a valid approach in combating diseases that link animals and humans. Furthermore, diseases would require external factors to occur in addition to their causal agents. The theme, however, requires nourishment and action from medical and veterinary professions. Each of these professions should attempt to understand and appreciate the role of the other. The current logo of "One Health/Medicine" is missing the components of actual action toward the goal of unifying the approach to prevent diseases that have human and animal links. The lack of communication, appreciation, public health duties, animal health programs, and economic circumstances play a major role in the current limitation of progress.

As much as possible, veterinary professionals should attempt to integrate other related disciplines in their approaches for the wellbeing of animals, including preventive measures of animal diseases. Such professions as sociologists, economists, ecologists, wildlife biologists, and political scientists among others can support these approaches and enrich the profession of veterinary medicine. The discipline of veterinary epidemiology has demonstrated a good example in multidisciplinary approaches for preventive medicine. This effort should be expanded by including other disciplines and topics that are beyond the animal diseases but within animal wellbeing.

Medical professions can attempt to reach out to veterinary professions by expanding their horizon to understand that diseases are shared among all animal families. Animals other than human species can be used to understand and prevent these diseases in humans. This understanding would require comprehensive and comparative approaches to medicine. Veterinary medicine is unique in that it can reveal this comparative part of the medicine. It is therefore a major beneficial advantage to the medical profession to recognize the value of veterinary medicine. This recognition would require observation and integration of veterinary medicine in the preventive measures for diseases of public health interest.

Both medical and veterinary professions should emphasize preventive measures and place less emphasis on treatment. In the long term, the former can lead to less treatment and more effective approaches to diseases in a given population. This type of emphasis would require integration and synthesis of measures and other actions to reduce the disease impact in both animal and human populations. There is a need, therefore, to join forces to combat these diseases. These forces should not be emphasized at the top level. There is an urgent need to build these forces on a fundamental level to be effective at the local, national, and regional levels.

(boldface key findings or conclusions)

Global health should include the health status of animal populations. Animal protein is essential for our society wellbeing. A food production system requires comprehensive preparation plans to control the potential spread of infectious animal diseases. Food security and safety requires comprehensive preparation plans for controlling the spread of infectious animal diseases. Veterinary medicine, through reliable diagnostic tools and control measures, is the appropriate discipline to spearhead the effort to maintain good quality and quantity of animal protein for the community.

Emerging microbial threats: Challenges and opportunities at the human-animal-ecosystem interface

James Hughes, MD, FACP, FIDSA
Professor of Medicine and Public Health
Emory University; and former Director of the National Center for Infectious Diseases at CDC

Infectious disease mortality in humans decreased during the first half of the 20th century in the United States, leading to complacency among many public health officials and policymakers regarding their importance. **During the last 35 years, many new infectious diseases have been identified, and several other infectious diseases have increased in incidence in the United States.** In addition, many new diseases have emerged in other parts of the world, the majority of which were associated with cross-species transmission.

In 1992, the Institute of Medicine (IOM) of the National Academies issued a seminal report defining new, reemerging, or drug-resistant infections as those whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future. IOM identified **six factors** contributing to disease emergence: changes in human demographics and behaviors, advances in technology and industry, economic development and changes in land use, international travel and commerce, microbial adaptation and change, and breakdown of public health measures. In 2003, another IOM study added **seven more factors**: human susceptibility to infection, climate and weather, ecosystem changes, poverty, war and famine, lack of political will, and bioterrorism.

Alert frontline health care workers (veterinarians, physicians, laboratorians, pathologists, research scientists, and public health officials) **are critically important in emerging disease detection.** Examples include recognition of AIDS, hantavirus pulmonary syndrome, Ebola hemorrhagic fever outbreaks, West Nile encephalitis, SARS, and the anthrax attacks. The revised International Health Regulations, issued by WHO in 2005, highlight the need and provide one framework for strengthening biosurveillance capability for early disease detection and response.

The One Health Initiative reflects the convergence of human, animal, and ecosystem health and places emphasis on detection of microbial agents before cross-species transmission occurs, providing a second framework for detection and response. Areas of common interest to public health and animal health communities include foodborne diseases; antimicrobial resistance; infections associated with exotic and wildlife trade; avian, animal, and pandemic influenza; healthcare-associated infections; blood, organ, and tissue safety; biosafety and security; and bioterrorism, biodefense, and global health security.

Likely future challenges include another influenza pandemic, more antimicrobial resistance, more foodborne outbreaks, and unexpected events. **Vigilance, multidisciplinary partnerships, better predictive capability, strengthened human and animal health systems, enhanced diagnostic laboratory capacity, improved coordination and communication, transparency, and sustained political will are critically important nationally, regionally, and globally.**

AAVLD Poster Session

3 PM Friday, November 12 through
2 PM Sunday, November 14, 2010

Third Floor Foyer

***** Graduate student presentation

Evaluation of direct PCR and culture using spiked cattle, sheep and goat feces for different strains of *Mycobacterium avium* subspecies *paratuberculosis*

S. Robbe-Austerman[1], *Kevin Stokes*[1], *Kathyrn Fett*[1], *Darcy Myers*[2], *Beth Harris*[1]
[1]United States Department of Agriculture, National Veterinary Services Laboratories, Diagnostic Bacteriology Laboratory, 1920 Dayton Road, Ames, IA; [2]Life Technologies 2130Woodward Street Austin, TX

Introduction: In the United States there is little information published evaluating direct detection assays (fecal culture or PCR) for paratuberculosis in sheep and goats. Sheep are known to be infected with strains of *M. avium* subspecies *paratuberculosis* (MAP) that are particularly difficult to culture, and the commercially available assays and reagents are not USDA approved for use in these species. The objective of this study was to evaluate a commercially available and a homebrew direct PCR and liquid culture for sheep and cattle MAP strains, using fecal material from three ruminant species (cattle, goats and sheep).

Materials and Methods: Two MAP field isolates from cattle and three from sheep were used to spike fecal material from cattle, sheep and goats at roughly the following concentrations: $1.5x10^7$, $1.5x10^6$, $1.5x10^5$, $3.0x10^4$, $1.5x10^4$, $1.5x10^3$, $1.5x10^2$. Phosphate buffered saline was added as a negative control. Each dilution was replicated 3 times for each of the 3 fecal matrices, randomized and blinded for a total of 324 samples per set. One set of samples was provided to Life Technologies for nucleic acid extraction and amplification and 3 sets were tested at the National Veterinary Services Laboratories (NVSL) using 2 direct PCR methods: MagMAX™ Total Nucleic Acid Isolation Kit with TaqMan® MAP (Johne's) Reagents, and the Tetracore® MAP extraction kit with VetAlert™ Johne's Real-Time PCR. Culture was conducted at NVSL using TREK *para*-JEM® broth supplemented with the recommended amounts of *para*-JEM® EYS, *para*-JEM® AS, *para*-JEM® GS, and *para*-JEM® Blue for culturing cattle fecal samples.

Results: Using a cut-point of 37.0 C_T, both PCR methods detected 100% of samples spiked with $1.5x10^4$ or greater and detected 98% spiked with $1.5x10^3$. $1.5x10^2$ was near the detection limit and identified between 60- 52% of the samples. Both direct PCR methods identified sheep and cattle MAP strains equally well; there was no significant difference in C_T values between direct PCR methods. Furthermore, the performance of direct PCR was not affected by the type of fecal material. In contrast to direct PCR, culture results were affected by the strain of MAP. As expected, sheep strains were much less likely to be culture positive regardless of fecal matrix, with only the highest concentration ($1.5x10^7$) of spiked samples being positive for 100% of the samples. In contrast, 100% of cattle feces spiked with cattle MAP strains were detected down to the second lowest concentration ($1.5x\ 10^3$).

Conclusion: Both the commercially available and homebrew fecal direct PCR methods were equally able to detect sheep or cattle strains of MAP. Neither method was affected by the type of fecal material. While the current TREK liquid media formulation will detect fecal material heavily spiked with MAP sheep strains, modifications to the media (such as increasing egg yolk concentrations) will need to occur so that growth may be improved sufficiently for culture to be clinically useful in detecting MAP sheep strains.

Nystatin in BD BACTEC™ MGIT™ Para TB system liquid cultures to inhibit fungal contamination

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Introduction. To prevent the overgrowth of fungal contaminants, the polyene antifungal Amphotericin B is added to solid and liquid culture media for the recovery of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease in cattle. However, fungal overgrowth can still be problematic for culture from very highly contaminated fecal samples. This investigation assessed the effectiveness of another polyene, Nystatin (NYS), in preventing liquid culture overgrowth by problematic filamentous fungi, as well as the effect of Nystatin on MAP recovery.

Materials and Methods. Supplemented MGIT™ Para TB Medium tubes were seeded with eight organisms: two ATCC MAP strains at high or low inoculum levels, targets 10 or 100 cfu (N=3), and 500-1000 cfu of six fungi isolated as overgrown contaminants from cultured fecal samples (N=2). Antimicrobials in the culture medium were varied as test conditions. Experiment #1, seven conditions: drug-free, or NYS at 37.5, 75, 150, 300, 600 or 1200 units/ml of culture medium. Experiment #2, four conditions: medium with one of two antimicrobial formulations for samples highly contaminated by bacteria, 200 µg/ml nalidixic acid (NAL-200) or 75 µg/ml NAL plus 6 µg/ml ceftriaxone (NAL-75/CTR-6), alone or with 1200 units/ml NYS (NAL-200/NYS-1200 or NAL-75/CTR-6/NYS-1200) in addition to the standard 7 µg/ml amphotericin B and 19 µg/ml vancomycin. After 49 days in the MGIT™ 960 instrument, times-to-detection (TTDs) were analyzed by ANOVA-type general linear model and detection rates by binary logistic regression and Fisher's Exact Test using MINITAB ver. 15.1 statistical software.

Results. Experiment #1: 100% MAP ATCC 19698 and 700535 detection, and mean TTDs vs. drug-free tubes were significantly longer (0.75-1.5 days) with NYS-600 and NYS-1200 ($P < 0.05$). Only NYS-1200 prevented *Aspergillus fumigatus* detection and that of the *Mucor* and the *Scopulariopsis* as well. NYS-1200 significantly delayed detection vs. drug-free tubes of the *Monascus*, *Pseudallescheria boydii*, and *Aspergillus amstelodami*, with mean TTDs of 26.11 vs. 1.13, 4.98 vs. 1.38, and 31.03 vs. 4.48 days, respectively ($P < 0.05$). Experiment #2: 100% MAP detection and mean TTDs significantly delayed by 1-2 days in the presence of NYS-1200 ($P < 0.05$) for all conditions except strain ATCC 700535 with low inoculum and NAL-75/CTR-6/NYS-1200, for which mean TTDs were seven days longer than with NAL-75/CTR-6. *A. fumigatus* had 0% detection only in the presence of NYS-1200, and all *Mucor* cultures were negative for growth. *A. amstelodami* detected in three of four cultures without NYS-1200 but only one of four with NYS-1200. *Scopulariopsis* had 0% detection only with NAL-75/CTR-6/NYS-1200. The presence of NYS-1200 significantly delayed detection of the *Monascus* by 7.0-8.5 days and *P. boydii* by 1.25-1.75 days ($P < 0.05$).

Discussion/Conclusion. Nystatin at a growth medium concentration of 1200 units/ml was effective in preventing or delaying MGIT™ Para TB Medium liquid culture overgrowth by contaminant filamentous fungi, while MAP detection was delayed by two days or less under most conditions. However, a 7-day mean detection time delay for one MAP strain in the presence of NYS-1200 under one test condition indicates that further investigation is necessary into the use of Nystatin to control fungal contaminants in MAP liquid culture.

Seroprevalence of paratuberculosis in breeding cattle in Korea

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Introduction: Paratuberculosis (Johne's disease), a chronic and debilitating disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a major cause of economic loss in cattle. In Korea, national monitoring of breeding stock for MAP has been implemented. In this study, we report the results of serological test to determine the prevalence of MAP in breeding stock of beef and dairy cattle during 2008 and 2009.

Materials and Methods: For MAP, 3,927 serum samples [3,692 beef cattle (Hanwoo; Korean native cattle) and 235 dairy cattle] were submitted from Hanwoo Improvement Center and Dairy Cattle Improvement Center, respectively. They were classified into four different age groups of cattle for MAP; group 1 (≤2 year, *n*=1,509), group 2 (>2 years to ≤3 years, *n*=486), group 3 (>3 years to ≤4 years, *n*=441), group 4 (>4 years, *n*=1,491). We performed serological diagnosis of paratuberculosis by ELISA (Institut Pourquier, France) following manufacturer's instructions. None of the cattle was vaccinated against MAP. Also, the majority of seropositive cattle were used for MAP isolation with mesenteric lymph nodes. The lymph nodes were cultured in Herrold's egg yolk agar with mycobactin J for 12 weeks. Then, the suspected colonies were confirmed by IS 900 PCR.

Results: Overall seroprevalence of MAP was 0.5% (21/3,927) in this study. The seroprevalence was also determined by age groups: three (0.2%) of group 1, two (0.4%) of group 2, three (0.7%) of group 3, and 13 (0.9%) of group 4 were seropositive for MAP. Although positive samples were found in all age groups, the seroprevalence tended to increase with age. None of dairy cattle was positive in this study. The results of MAP isolation were positive for 11 (78.6%) of the 14 seropositive cattle tested.

Discussion/Conclusion: The seroprevalence of paratuberculosis in breeding cattle in Korea was 0.5%. This was much lower than in conventional cattle reported in previous studies in Korea (3.3 – 13.5%). However, our study showed that the MAP prevalence in dairy cattle (0%) was lower than that in beef cattle (0.6%) which is different from other studies. Although the exact reason was uncertain, we speculated that herd size and sanitary measures at farm level could be the main factors influencing MAP infection. **Also, the MAP-isolation (78.6%) of the seropositive cattle in this study was markedly higher compared with the other study (7.3%).** The differences in the specimens (mesenteric lymph nodes vs feces) cultured is the likely explanation for the observed differences in the culture results.

An efficient DNA extraction method for PCR-based detection of *Mycobacterium avium* subspecies *paratuberculosis* in bovine fecal samples

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Due to the lipid rich cell wall of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the complex nature of bovine feces, and intermittent organism shedding by the infected cattle, it is difficult to recover sufficient amount of high-quality MAP DNA from fecal samples which directly affects the sensitivity of downstream PCR tests. In this study, a DNA extraction method, designated the Mississippi Veterinary Research and Diagnostic Laboratory (MVRDL) method, was developed for PCR-based detection of MAP in bovine fecal samples. The MVRDL method combined multiple procedures, including chemical pretreatment, one-tube cell lysis and extraction, chelex resin absorption, and mini-column purification. The DNA yield and purity as measured by spectrophotometry were 3.36 fg/CFU MAP and A260/280 ratio of 2, respectively. This method was further evaluated by real-time polymerase chain reaction (RT-PCR). The detection limit of the RT-PCR assay was about 3 CFU in 1 ml MAP culture or 1 g MAP-spiked fecal material. A linear correlation was found between cycle-threshold (Ct) and log input CFU (ranging from 7.2 to 7.2 x 10^7 CFU/ml or CFU/g). In addition, the MVRDL method was validated by performing 7 Johne's direct fecal PCR proficiency tests administered by the National Veterinary Service Laboratories (NVSL). Based on culture results as the "gold standard", the specificity of MVRDL PCR was 100% and the sensitivity was 98.46% for samples containing more than 1.5 CFU/tube of fecal cultures. To the authors' knowledge, this is the most efficient MAP DNA extraction method in comparison with all previously published protocols.

Development of a bulk tank surveillance program for Johne's disease in New York dairy farms

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Introduction: Johne's disease in dairy cattle is caused by *Mycobacterium avium* subspp *paratuberculosis* (MAP). Diagnostic testing is an important tool to identify infected animals, however individual animal testing is expensive and labor intensive. A screening test to reliably identify herds with a significant MAP problem would be of great value to the industry. To evaluate the value of a bulk milk screening test, we collected data on MAP infection status based on 1) fecal culture of cows in the herd, and 2) contemporary environmental samples and compared these two estimates of MAP infection status to bulk milk MAP ELISA titer.

Materials and Methods: Between June 2007 and January 2009 we enrolled 99 farms in this study. These herds were selected from the New York State Cattle Health Assurance Program (NYSCHAP) Johne's Module Participants. Herds enrolled in this program are encouraged to participate in annual fecal culturing. The cost of testing is subsidized by the State of New York. Herds make their own decision in terms of the extent of fecal or serological testing for Johne's disease that is appropriate to support control in their herd. The fecal and serological testing results were used in this study to evaluate the ability of bulk milk MAP ELISA test to identify herds with important Johne's disease problems. Detection of antibodies against MAP, the causative agent of Johne's disease, was done using the IDEXX-Pourquier milk ELISA. To evaluate the usefulness of this milk ELISA for Johne's disease detection at herd level, ELISA results were compared to MAP herd status as determined by 1) culture of concurrently collected six environmental samples and 2) historical fecal or serological culture results of any animals in the herd during a one year period before sampling of the bulk tank.

Results: The relationship between bulk milk ELISA S/P and percent fecal positive cows that were lactating at the time of bulk tank sampling was determined. Regression results where ELISA S/P was used as a predictor for percent fecal positive showed a statistically significant association. Predicting fecal culture positive prevalence, bulk milk ELISA explained 45% of variation (R-squared) with a regression coefficient of 0.002 (SE .0003, P-value <.0001). In comparison, when the environmental MAP load was used as a predictor variable for the percent fecal positive cows, the percent explained variability was 10%, and the environmental load had a regression coefficient of 0.0036 (SE .0014, P=.011), indicating a prediction of the percentage of lactating cows that are MAP infected that is much less reliable compared to bulk tank ELISA S/P.

Significance: The value of bulk milk ELISA testing lies especially in predicting herd prevalence of MAP fecal shedders. The bulk milk ELISA results appear reliable in predicting whether a herd has a significant MAP prevalence or not. Herds with an estimated fecal MAP prevalence over 3% would be advised to perform further diagnostic evaluation of individual animals. Based on the animal testing results further management practices would be advised to reduce MAP transmission in herds. Subsequently, bulk milk ELISA testing may be used to monitor herd status over time.

Multi-level interpretation of the new IDEXX *M. paratuberculosis* ELISA on serum and milk based on likelihood ratio analysis

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Introduction: The new IDEXX ELISA for paratuberculosis measures the concentration of antibody in clinical samples, serum, plasma or milk. ELISA reader results, measured as optical density (OD) units, are transformed to S/P (sample/positive) ratios. Conventional ELISA interpretations employ a single cutoff for interpretation of S/P values as either negative (below the cutoff) or positive (above the cutoff). Prior studies demonstrated a strong correlation of S/P values with the probability animals are shedding *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in fecal samples collected at the same time as serum samples by likelihood ratio (LR) analysis highlighting the clinical value of knowing the magnitude of S/P or equivalent transformed ELISA OD values (Collins et al. 2005).

Methods: LR analysis was performed for data generated using the new IDEXX ELISA kit for paratuberculosis on both bovine serum or plasma samples and milk samples. Bovine serum/plasma samples originated from 221 non-infected and 331 fecal culture-positive dairy cattle. Bovine milk samples came from 649 non-infected and 248 fecal culture-positive dairy cattle. Roughly half of all samples originated from cattle in Europe and the others from cattle in the US.

Results: ELISA results on serum or plasma samples expressed as S/P values were divided into the following five ranges: 0.00 to 0.099, 0.10 to 0.199, 0.20 to 0.499, 0.50 to 0.999 and ≥ 1.00. The percentage of ELISA results in each range for the 321 MAP-infected cows was divided by the percentage of ELISA results in each range for the 221 non-infected cows to derive LRs. The resulting LRs for the IDEXX ELISA on serum/plasma were 0.3, 1.6, 2.4, 16.6, and 101.4, respectively.

ELISA results on milk samples expressed as S/P values were divided into the same five S/P ranges: 0.00 to 0.099, 0.10 to 0.199, 0.20 to 0.499, 0.50 to 0.999 and ≥ 1.00. The percentage of ELISA results in each range for 248 MAP-infected cows was divided by the percentage of ELISA results in each range for the 649 non-infected cows to derive LRs. The resulting LRs for the IDEXX ELISA on milk were 0.2, 0.4, 31.4, 89.0, and 361, respectively.

Conclusions: The magnitude or IDEXX ELISA S/P results were directly related to the LR. At the highest, so called high positive range, i.e. S/P ≥1.00, the odds such samples originated from MAP-infected dairy cattle was >100:1. These LR values can be used in combination with estimates of within herd prevalence of MAP infection (pre-test probability of infection) to significantly improve decision making based on ELISA results.

Comparison of isolation and PCR methods for detection of *Mycobacterium avium* spp. *paratuberculosis*

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The goal of this research was to evaluate the sensitivity and ease of use in detecting *Mycobacterium avium paratuberculosis* (MAP) DNA in feces using commercially available extraction and PCR detection kits. MAP is the etiological agent of Johne's disease in cattle. Feces of known status were obtained from the National Veterinary Services Laboratories from past Johne's disease fecal proficiency tests. The feces (n=21) representing high/moderate (n=11), low (n=4) and zero (n=5) shedding of MAP were included. Isolation protocols using both the Ambion MagMAX™ Total Nucleic Acid Kit and Tetracore® MAP extraction System were used to extract the DNA from fecal samples. In a checkerboard set-up including all possible combinations of extraction and mastermix assays, PCRs were performed using Applied Biosystems (AB)™ TaqMan® MAP (Johne's) Reagents and Tetracore® VetAlert™ Johne's Real-Time PCR DNA test kits. Although no statistical analysis was performed, our results indicate that extraction using the Tetracore® method could successfully be paired with either the AB™ TaqMan® MAP (Johne's) Reagents or the Tetracore® VetAlert™ Johne's Real-Time PCR DNA test kits, with the TaqMan® MAP assay providing greater sensitivity than the Tetracore® VetAlert™ Johne's Real-Time assay. Alternatively, the Ambion extraction could only be successfully paired with the AB™ TaqMan® MAP (Johne's) Reagents. The most sensitive method consisted of the Tetracore extraction and the AB reagents although, the Ambion extraction also produced results that correlated well to the samples of known status and was less labor-intensive. In a diagnostic setting, testing methods are influenced by assay sensitivity and specificity along with ease of performance. Our findings suggest that the protocol that provides the greatest sensitivity is also the more labor-intensive method. This benefit may be outweighed by an easier and faster protocol that yields slightly lower sensitivity.

Identification and speciation of *Mycobacterium tuberculosis* complex species in mixed cultures with non-*Mycobacterium tuberculosis* complex species using a differential PCR technique

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A diagnosis of *Mycobacterium tuberculosis* complex (MTBC) species (*M. tuberculosis*, *M. bovis*, *M. microti*, *M. africanum*, *M. caprae*, and *M. canettii*) in the U.S. can have devastating consequences to states, livestock producers, zoos and wildlife management teams. Previously, stakeholders have had to wait an average of 8-10 weeks to receive culture results. However, National Veterinary Services Laboratories (NVSL) has reduced the wait period for results to an average of 3-4 weeks by relying on molecular techniques, such as spoligotyping, directly from the primary liquid media cultures after a positive MTBC DNA probe result. Occasionally animals are co-infected with other atypical mycobacteria such as *M. avium,* confounding our ability to utilize spoligotyping. These mixed cultures can be very difficult to differentiate from a false positive MTBC DNA GenProbe result and can require weeks of sub-culturing and biochemical analysis for definitive identification. In an effort to improve efficiency in these cases, we investigated the use of a differential PCR assay to identify and discriminate between MTBC species in mixed cultures with atypical mycobacteria. Differentiation of MTBC is accomplished by amplifying and comparing PCR products from seven differentegions of difference in the genome. Sensitivity of this assay to detect MTBC was evaluated by combining cultures of *M. bovis* and *M. avium* at concentrations varying from 5% to 30% of *M. bovis*. ***Mycobacterium bovis* could be reliably detected at concentrations as little as 7.5% of the mixture. Validation of this method to identify MTBC organisms in mixed cultures allows NVSL to quickly and efficiently identify false positive DNA GenProbe results, speciate the MTBC organisms, and confirm the identification of *M. bovis*, which is required for the National Bovine Tuberculosis Eradication Program and in the past required weeks of culturing and biochemical testing.**

Development of a quantitative multiplex TaqMan RT-PCR to aid in the diagnosis of Epizootic bovine abortion (EBA)

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Introduction: Epizootic bovine abortion (EBA), or more commonly foothill abortion, is a late-term abortion/ weak calf disease in beef cattle and has significant economic impacts in California, Nevada and Oregon. The causative agent is a novel deltaproteobacterium vectored by the soft-tick *Ornithodoros coriaceus*. *In vitro* culture attempts have been unsuccessful. Historically, diagnosis has relied upon the history of the dam, and the presence of elevated fetal serum immunoglobulins combined with unique fetal pathology. A modified silver stain and pathogen-specific immunohistochemistry were developed to visualize the bacterial pathogen in fetal tissue. We report the development of an EBA-deltaproteobacterium specific quantitative real time PCR (qRT-PCR), for facilitating diagnosis in difficult cases and further delineating the pathogenesis of this unique disease.

Materials and Methods: *Multiplex TaqMan design:* Primers and a FAM-linked probe were designed to amplify a maximally unique 90bp portion of the EBA 16s gene in comparison to 15 other deltaproteobacterial 16s sequences. Primers and a CalFluorOrange-linked probe were also designed to amplify 88bp of the bovine beta-actin (BACTB) gene. The efficiency of the primers and probes were first determined individually for each reaction, and then as a multiplexed reaction. The resulting efficiency of the multiplexed TaqMan reaction was 93% for bovine beta-actin and 101% for EBA 16s.

Standard Curve Design: Primers were designed to amplify 442bp of the EBA 16s gene and 387bp of the bovine beta actin gene. The amplicons were produced using conventional PCR and bluntly cloned into TOPO4 (Invitrogen). Seven 10-fold dilutions of plasmids were created to serve as the standard curve. The results are reported as the ratio of the EBA 16s copy number to the bovine beta-actin copy number.

Experimental samples: Six cows were experimentally infected with EBA; two served as negative controls. The fetuses were recovered either at slaughter of the dam (n=6) or following abortion (n=2). Complete necropsies were performed. Eight tissues were taken for DNA analysis: tongue, skin, Peyer's patch, pre-scapular and mesenteric lymph nodes, spleen, thymus, and blood.

Results: Five of the six fetuses were diagnosed with EBA by histopathology, and immunohistochemistry (IHC). All eight tissues, from each of the five fetuses, were positive by qRT-PCR. The remaining case, recovered at slaughter, had minimal, non-diagnostic lesions, was negative by IHC and no serum Ig. However, qRT-PCR demonstrated six of the eight tissues to be EBA positive. All tissues from the negative control fetuses were EBA negative by all measures. Lymph nodes from infected fetuses carried the highest pathogen load, followed by the spleen. Other tissues carried lower and variable amounts of bacteria with the tongue routinely yielding the lowest. Comparison of pathogen load with EBA-diagnostic histopathology generated three groups, (1) mild to no lesions/low pathogen load, (2) diagnostic lesions/high pathogen load and (3) diagnostic lesions/low pathogen load.

Discussion/Conclusion: The development of a multiplexed quantitative real time PCR has enhanced our ability to diagnose EBA and to accurately quantify bacterial load in fetal tissue. The study suggests a pattern of bacterial growth followed by fetal clearance. The study also demonstrated that lymph nodes carry the highest pathogen load, though limited disease transmission studies suggest the thymus contained the highest levels of viable pathogen.

Management and leadership in the implementation of quality assurance in the diagnostic laboratory

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Introduction/Learning Objectives: (1) Identify common obstacles for the implementation of a quality assurance (QA) program in Latin America and the Caribbean (LAC) for veterinary and public health diagnostic labs. (2)Identify incentives for motivation and mobilization of resources toward QA adoption. (3)Be aware of both differences and similarities between animal and human labs, and of areas for potential cooperation on zoonotic diseases.

Background: As the public health partner on the United States Agency for International Development's (USAID) STOP AI project (Stamp out Pandemic and Avian Influenza [AI]), MSH and other partners are assisting countries improve local capacity to manage outbreaks of pandemic and/or AI. Several LAC countries requested STOP AI's assistance in QA implementation for animal and human labs. Difficulties have arisen from the improper alignment of resources, from resistance to change attitude and behavior, and the fact that human and animal labs had little opportunity for interaction or collaboration. From August 2008-May 2010 workshops were delivered to 24 organizations in 4 countries, resulting in a total of 30 teams and 156 participants being trained in management and leadership for QA implementation.

Design and Evaluation Methods: The method uses QA implementation as an instrument. The tools were adapted from MSH's Leadership Development Program (LDP), which has well documented success in improving the delivery of health services. LDP develops leading and managing skills by learning from doing. The content was delivered by workshops adapted to the needs, challenges and potentials identified by the teams in their work environment. Tutoring and coaching were available between workshops. Teams moved to identify their actual situation, and formulate objectives, time tables, action plans and evaluation parameters. Self evaluations were performed during and at end of the program. Teams presented progress and results to their senior management and stakeholders for commitment and support.

Results/Outcome and Challenges/Solutions: Learning by action encouraged participants to expand the LDP method to their work environment. After gaining an understanding of roles, self involvement, and moving from plan to action, teams delivered elements to build up their QA programs. Improved communication skills was cited by the participants as having had the most positive impact on their work climate, resulting in empowerment, commitment and motivation. Sustainability resulted from ownership, proactive participation, utilization of local resources, definition of goals and self-evaluation indicators, and recruiting senior management and stakeholders as active players. From the implementation in the field it was realized that the sooner senior management and stakeholders got involved with their teams, the better the chances the participants would reach their desired results and advance their QA programs.

Conclusions: LDP methodology creates changes in attitude and management, successfully improving implementation of QA. It develops human resources, creates conditions for building capacity, and enhances the capacity for rapid response for situations such as an outbreak of AI. The methodology generates support from senior management and stakeholders. Coordination and collaboration between animal and human sectors is required with zoonotic outbreaks. Implementation of QA will bring the laboratories closer to certifications, which will benefit both the human health and agricultural sectors.

Inhibition of *Escherichia coli* K99 (F5) adhesion to calf intestinal villi by non-immunoglobulin proteins of bovine milk

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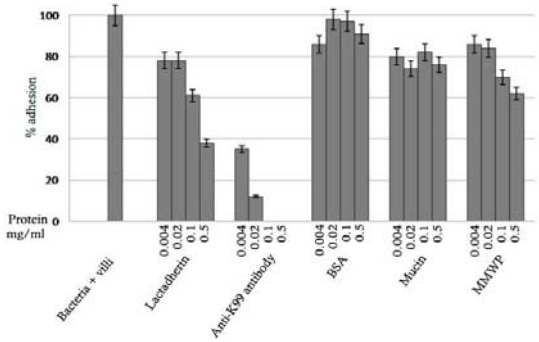
Introduction. *Escherichia coli* (*E. coli*) can cause diarrhea in calves as a result of enteric colibacillosis and/or septicemia. Those *E. coli* with K99 (F5) fimbriae can adhere to the intestinal villi and produce secretory diarrhea. This adhesion is mediated by fimbriae that recognize some glycoconjugates on the host cell surface. Infection can be prevented or controlled by inhibiting or reducing bacterial attachment to the intestinal surface. It is known that anti-adhesive components are present in bovine milk and they may contribute to local and systemic disease resistance in calves. The current experiment was designed to investigate the inhibition properties of non-immunoglobulin proteins of bovine milk: lactadherin (LAD), mucin (MUC1) and macromolecular whey proteins (MMWP) against *E. coli* K99 in new born calves.

Materials and Methods. Small intestinal villi were collected from 1 day old calves and subjected to 4×10^8 F5 positive *E. coli* which was pre-incubated for 1-hour with purified bovine milk LAD, MUC1 and MMWP, rabbit anti-F5 polyclonal antibody (positive control) and Bovine Serum Albumin (negative control) at 0.004, 0.02, 0.1 and 0.5 mg/ml, respectively. After 1 hour of incubation, the numbers of bacteria attached to the intestinal villi was determined by enumerating the bacterial cells along 50 μm villar lengths at 20 different places for each protein specimen.

Discussion. Results are summarized in table 1. Bovine LAD and MMWP had the most significant inhibition against *E. coli* K99 *in vitro* by 40% and 62% and by 30% and 38% at concentrations of 0.1 and 0.5 mg/ml, respectively. Bovine MUC1 did not inhibit the adhesion of *E. coli* K99 bacteria at any of the concentrations. BSA (negative control) did not inhibit the bacterial attachment; while anti-K99 polyclonal antibody (positive control) was very potent in inhibition of *E. coli* K99. Our experiment clearly demonstrated that lactadherin, a bovine milk fat globular membrane, significantly inhibited adhesion of *E. coli* K99 to the intestinal villi of calves *in vitro*. The amount of inhibition by MMWP was lower than lactadherin here; however it showed the presence of substances with inhibitory function in the bovine milk whey fraction. The mechanism of this interference with *E. coli* K99 bacterial attachment was not determined by our experiments. However, according to previous studies the inhibition is most likely mediated by carbohydrate residues on lactadherin protein.



Conclusion. Our experiment showed that non-immunoglobulin milk proteins may be involved in intestinal protection of newborn calves.

Detection of *Theileria (Babesia) equi* in the blood of sub-clinically infected horses with Piroplasmosis using real-time PCR

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Piroplasmosis, considered to be a foreign animal disease (FAD) in the United States, is a protozoan infection caused by *Theileria* (formerly Babesia) *equi* and *Babesia caballi.* Due to the recent outbreak of Piroplasmosis in horses in Texas, all horses being presented to New Mexico tracks for racing were required to have testing for *T. equi*. Since testing began in late 2009, 6, 908 horses have been tested through the New Mexico Department of Agriculture/Veterinary Diagnostic Services (either from NM or for admission to NM racetracks). Eighteen horses were found to be positive by cELISA. Seventeen of these horses were confirmed to be positive for *T. equi* infection by NVSL using nested PCR. The horse testing negative by PCR, but positive by cELISA was a foal born to a mare positive for *T. equi*. None of the horses had clinical signs of Piroplasmosis. Fourteen of the positive horses were euthanized and necropsied at the NMDA/VDS laboratory. Whole blood was taken from all fourteen horses prior to necropsy. Spleen and bone marrow were saved from eight of the positive horses for further analysis. Six of the positive horses were tested using real-time PCR to the 18S gene of *T. equi*. **Real-time PCR has the advantage over nested PCR in that it is a one step PCR procedure which limits the chance of template contamination and it can also be used for quantitative analysis.** Multiple copies of the 18S gene give equal or greater sensitivity of real-time PCR compared to the established nested PCR assay. PCR can be used in association to cELISA and CFT to detect an active, existing infection. PCR results are useful in interpreting the data when there are positive cELISA and negative CFT results. **Results of the real-time PCR assay show that *T. equi* can be detected in blood of sub-clinically infected horses with low numbers of parasitemia.**

Hepatic encephalopathy associated with hepatic lipidosis in Llamas

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Hepatic lipidosis is well described in llamas. Hepatic encephalopathy has been listed as a differential for llamas displaying neurologic signs, but the lesions have not been described. This report details the neurologic histopathologic findings associated with three cases of hepatic lipidosis with concurrent neurologic signs and compares these cases to three cases of hepatic lipidosis in the absence of neurologic signs and three cases without hepatic lipidosis. Llamas displaying neurologic signs ranged from 6 to 13 years of age and llamas in the control groups ranged from 5 months to 14 years of age. Neurologic signs included lethargy, depression, trembling, loss of pupillary light reflex, weakness, difficulty rising, recumbency, convulsions, seizure-like activity, and seizures. **Brain from all three llamas displaying neurologic signs had increased numbers of Alzheimer type II cells.** Alzheimer type II cells were not detected in llamas with hepatic lipidosis in the absence of neurologic signs, or in llamas without hepatic lipidosis. **Immunohistochemical staining for glial fibrillary acid protein displayed subjectively decreased astrocytic staining intensity in llamas with neurologic signs as compared to two out of three llamas with hepatic lipidosis in the absence of neurologic signs, and two out of three llamas without hepatic lipidosis.** Immunohistochemical staining for S100 did not vary between groups. **These findings are consistent with hepatic encephalopathy as reported in association with hepatic lipidosis in horses, and suggest that hepatic encephalopathy may be associated with hepatic lipidosis in llamas.**

Multiple testicular neoplasms in a canine

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An 11-year-old, male dog was presented to a local practitioner with a testicular tumor. The right testicle was approximately 5 times the size of the left testicle which appeared atrophied. The testicles were surgically removed. Two testicles in formalin were submitted to the SDSU-ADRDL for histologic examination.

Within the parenchyma of the right testicle was a slightly oval 35mm by 30mm mass. The mass elevated the capsule and occupied the seminiferous tissue. The cut surface of the mass revealed white, soft and coarsely lobulated tissue. In addition to this mass, there was another half circle mass in the right testicle measuring 20mm by 12mm. The cut surface showed a dark tan to beige, soft, well demarcated and lobulated tumor. The left testicle was diffusely white and firm and was interpreted to be severely atrophic. It contained two small tumors. The first was a crescent shaped mass measuring 15mm by 8mm. The cut surface of the crescent mass showed a soft, well demarcated, pale tan to white tissue. The second mass within the left testicle was a small tumor located adjacent to the crescent shaped tumor, measuring 9mm by 7mm. Its cut surface was tan to pale beige, lobulated and quite firm. Histologically the neoplasma were consistent with malignant seminoma (large right testicular tumor), Benign Interstitial Cell Tumor (Leydig Cell Tumor, medium-sized tumors in the right and left testicles) and Benign Seroli cell tumor (small left testicular tumor).

This case is quite interesting having three types of testicular neoplasms (Seminoma, Interstitial Cell Tumor and Sertoli Cell Tumor) simultaneously. Most neoplasms cause enlargement of the testis, but each neoplasm presents different gross and histologic lesions. According to the case history, the right testicle was enlarged and about five times the size of left testicle. This is considered a consequence of hormone production by the Sertoli Cell Tumor as indicated by atrophic seminiferous tubules. About a third of them produce estrogen (hyperestrogenism) which may also have other feminizing effects, such as gynecomastia. Other etiologies reported for testicular atrophy by direct or indirect (hormonal) pathways include heat stress, brucellosis, phytoestrogens, mycotoxins, vitamin and mineral deficiencies, high dose of heavy metals, organochlorine contamination, and genetic factors.

E-cadherin and multiple myeloma protein (MUM1) in the immunohistochemical characterization of canine cutaneous round cell tumors

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Immunohistochemistry for E-cadherin is used to distinguish canine cutaneous histiocytoma from other cutaneous round cell tumors. However, expression of E-cadherin is also reported in a proportion of 45 canine plasmacytomas. In the current study, we have expanded the number of plasmacytomas (n=72) examined, and compared E-cadherin and multiple myeloma protein 1 (MUM1) expression in plasmacytomas with that in 13 cutaneous histiocytomas, 12 epitheliotropic T-cell (CD3+) lymphomas, and 14 mast cell tumors.

Only plasmacytomas (100%) and 1 epitheliotropic lymphoma expressed MUM1. However, tumors of all 4 types had variable cytoplasmic (C), cell membrane (M), and/or paranuclear (P) E-cadherin expression. Of the 72 plasmacytomas, 54 (75%) were E-cadherin+ (12 C, 30 C/M, 4 C/P, 2 M, 5 M/C/P, 1 P). All 13 cutaneous histiocytomas were E-cadherin+ (7 M, 4 M/C, 2 M/C/P). All but 1 of 14 mast cell tumors expressed E-cadherin (1 M, 7 M/C, 5 M/C/P). Ten of 12 epitheliotropic lymphomas reacted with E-cadherin antibody (9 P, 1 P/C).

Among canine round cell tumors, MUM1 protein expression is typically limited to plasmacytomas and a few lymphomas. E-cadherin, a glycoprotein involved in intercellular adhesion, has been used as a marker of epithelial differentiation and, because of its consistent expression, of cutaneous histiocytomas. In this study, however, antibody to E-cadherin labeled neoplastic cells in 4 types of leukocytic neoplasm and, therefore, was not diagnostic for cutaneous histiocytoma.

Based on these results, we conclude that: 1) **Immunoreactivity for E-cadherin is not exclusive to leukocytes of cutaneous histiocytoma**; 2) **antibody to E-cadherin also labels neoplastic cells in most mast cell tumors, plasmacytomas, and epitheliotropic lymphomas**; 3) although histiocytomas usually have a membranous component to E-cadherin expression, the pattern of immunoreactivity among round cell tumors is variable and frequently concurrent in different cellular compartments; 4) **the distinctively paranuclear E-cadherin expression pattern in epitheliotropic lymphomas might distinguish them from other round cell tumors**; 5) **MUM1 is specific for plasmacytomas and can be used in combination with E-cadherin to discriminate among canine cutaneous round cell tumors.**

C-kit expression in canine non-cutaneous melanosarcomas

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Altered c-kit expression in canine mast cell tumors can be correlated to degree of malignancy and prognostic outcome. The c-kit receptor plays a critical role in melanocyte physiology, including melanogenesis, proliferation, migration, and survival. The activated c-kit receptor is responsible for transmission of pro-migration signals to melanocytes and hence may be involved in malignant progression of melanocytic neoplasms. All dogs with non-cutaneous melanosarcoma from the 2010 University of Tennessee College of Veterinary Medicine pathology archives were enrolled in a pilot study. Eleven cases of melanosarcoma were identified from the following anatomical sites: oral cavity (7), lingual (1), digital (1), conjunctival (1), and ocular (1). Melan A was expressed in 9/11 tumors (6 oral, 1 lingual, 1 conjunctival, and 1 ocular melanosarcoma). **C-kit expression was multifocal and of low intensity in only 1 oral melanosarcoma.** The other 10 neoplasms were negative for c-kit. Results from this pilot study suggest that **c-kit expression may be altered in a small percentage of dogs and thus plays some part in the pathogenesis of non-cutaneous melanosarcomas in the dog, as in people.** The study is being expanded to include PCR evaluation to determine whether c-kit mutations, specifically internal tandem duplications, might contribute to any alterations noted with c-kit immunohistochemistry. A greater understanding of molecular and genetic characteristics of these tumors may form the basis of selective drug therapy.

Ileocolic collision tumor in a cat: Anaplastic large cell lymphoma and a malignant globule leukocyte tumor

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A nine-year-old neutered male Domestic Shorthair cat was evaluated for weight loss, abdominal distension, and sporadic soft stool at the University of Wisconsin-Madison Veterinary Medical Teaching Hospital. Abdominal ultrasound revealed a colonic mural mass, abdominal lymphadenopathy, and abdominal effusion. The abdominal fluid was cytologically identified as a chylous effusion. Cytology of a fine needle aspirate of an enlarged ileocolic lymph node was suggestive of large cell lymphoma. Further ancillary testing was declined, and a single dose of L-aspariginase was administered as palliative treatment. After initial clinical improvement, abdominal distension worsened after 5 days, and the cat was humanely euthanized 7 days following treatment.

Gross necropsy revealed a multinodular, transmural ileocolic mass and severe lymphadenomegaly of ileocolic, mesenteric, and pancreatic lymph nodes. The spleen contained a single 0.7 cm diameter splenic mass and dozens of tan 1 mm diameter nodules. Microscopically, cells of the ileocolic mass and all affected lymph nodes were large anaplastic lymphocytes that were immunopositive for CD3; these cells also extended into the pancreas, mesentery, and mesocolon. On the basis of cell morphology and CD3 immunophenotype, the neoplasm was diagnosed as an anaplastic, large cell, T-cell lymphoma. The lymphoma focally abutted and intermingled with a morphologically distinct second round cell neoplasm in the wall of the colon. The cells of the second neoplasm had either distinct round brightly eosinophilic PAS-positive cytoplasmic globules or clear cytoplasmic vacuoles. The cells were immunonegative for CD3, CD20, CD79a, CD18, MHCII, cytokeratin, and synaptophysin, and were immunopositive for vimentin. This second population of cells was also metastatic to the spleen. Based upon morphology and immunophenotyping results the second neoplasm was diagnosed as a malignant globule leukocyte tumor. Microscopic examination also revealed, elsewhere throughout the alimentary tract, lymphoplasmacytic gastroenterocolitis with globule leukocyte hyperplasia.

Anaplastic large cell lymphoma and globule leukocyte tumor are both rare neoplasms. Collision tumor, the co-mingling of two independent neoplasms, is also a rare occurrence. Although collision tumor may represent random synchronous co-existence of two neoplasms, it may alternatively indicate a propensity for oncogenic transformation amidst the background of chronic inflammation that was present in this cat.

Heterobilharzia americana in a Mississippi dog: a parasitic disease likely underdiagnosed

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Heterobilharzia americana is a trematode blood fluke in the family Schistosomatidae that commonly infests raccoons and canines, but may naturally parasitize a wide variety of wild mammalian species including beavers, white-tailed deer, and bobcats. Raccoons serve as normal definitive hosts that shed fluke eggs in their feces. When in fresh water, eggs hatch and miracidia penetrate specific snail species that act as intermediate hosts. Parasitized snails shed cercarial stages that in turn penetrate and infest the definitive host. Thus, the disease is essentially waterborne and its occurrence is associated with the distribution of specific freshwater snail species found most commonly in swamps, bayous and other waterways; it is most commonly seen along the Gulf Coast and southeastern areas of the United States. Despite the small number of cases that have been reported, recent studies indicate that the disease is more widely distributed than previously suspected and may be underdiagnosed. This may reflect nonspecific clinical signs that may be confused with more common diseases producing gastrointestinal symptoms. *Heterobilharzia americana* may also produce dermatitis or "swimmers itch" in hosts including humans and non-human primates. We report the clinical and histopathological findings for one case of heterobilharziasis recently diagnosed in Mississippi in an 8-year-old male, neutered, mixed breed dog. The patient had a history of chronic (> 6 months) diarrhea, vomiting and weight loss. Initial bloodwork revealed eosinophilia and neutrophilia. Histological diagnosis was made from "punch" biopsies of the intestine. There were numerous pyogranulomatous nodules scattered throughout the mucosa, submucosa and muscularis of all intestinal sections and trematode ova that were consistent with *Heterobilharzia* were often present within the center of the granulomas. Diagnosis was confirmed by serologic detection of the parasite antigen CAA using an experimental lateral flow strip assay. Presence of CAA revealed an active infection with schistosomes. Treatment with praziquantel was initiated, and the patient has responded favorably since then, with weight gain and loss of associated signs. **The case represents the only documented case that has been diagnosed over a large number of years at the Mississippi State Diagnostic Laboratory in spite of being apparently widespread throughout the Gulf Coast states, and further underscores the probable underdiagnosis of the disease.**

Parvovirus associated erythema multiforme in a litter of English Setter dog

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Erythema multiforme (EM) was diagnosed in four of 9 puppies in a litter of English Setter dogs. The puppies developed erythematous skin lesions at the age of two weeks. The lesions worsened irrespective of treatment with antibiotics, antifungal and anti-inflammatory drugs. Microscopically, there was individual keratinocyte apoptosis associated with lymphocyte exocytosis in all layers of the epidermis. Intranuclear viral inclusions were seen in multiple tissues and organs. Several tissues were positive for canine parvovirus (CPV)-2 antigen and negative for canine distemper virus (CDV) antigen and canine herpes virus antigen (CHV) by direct fluorescent antibody assay. Negative stain electron microscopy detected parvovirus in the intestinal contents. The skin and intestine were positive for CPV-2b and negative for CDV by PCR. The muco-cutaneous junctions and small intestines had cells immunoreactive for CPV antigen by immunohistochemistry (IHC). **There is only one previous report on CPV-associated EM in a 2-month old Great Dane puppy. This report documents CPV-2b associated EM in a litter of English Setter dogs and substantiates the single previous report associating EM with CPV-2. The finding suggests that CPV should be considered as a possible cause of EM in dogs.**

Fatal pyogranulomatous myocarditis in ten Boxer puppies

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Between 2004 and 2009, ten pure-bred Boxer puppies with a history of sudden death were submitted for necropsy to the Minnesota Veterinary Diagnostic Laboratory (MVDL). The dogs were 9 to 16 weeks old and were submitted between April and October; three of the dogs were from the same litter. Original case records and archived materials were examined. Sections of paraffin-embedded heart tissue were stained with hematoxylin and eosin, Ziehl-Neelsen, Gram, Grocott's Methenamine Silver and Warthin-Starry. Immunohistochemistry was performed on paraffin-embedded heart tissue using antibodies for *Borrelia burgdorferi, Chlamydia sp., Toxoplasma gondii, Neospora caninum,* West Nile virus and Canine Parvovirus. PCR testing for *Borrelia burgdorferi* was performed on fresh heart tissue from five dogs. All dogs had marked myocarditis characterized by multifocal to coalescing separation and replacement of cardiomyocytes with an infiltrate that was predominantly composed of neutrophils and macrophages. In the cardiac tissue, six dogs had a few cells with positive immunoreactivity and four dogs had moderate immunoreactivity to anti-*Borrelia burgdorferi* antibodies. Of the five cases that were tested for *Borrelia burgdorferi* by PCR, one dog was positive. Although, the cause of the myocarditis remains elusive for these ten puppies, the similarities in the signalment of the affected puppies and the microscopic lesions suggest a common etiology. **There was at least a weak immunoreactivity for *Borrelia burgdorferi* in the heart tissue for all of the puppies, implicating this bacterium as a possible cause for the myocarditis.**

Necrobacillary transmural gastritis in a Red-necked wallaby (*Macropus rufogriseus*)

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A case of necrobacillary gastritis was diagnosed in a Red-necked wallaby (*Macropus rufogriseus*) from Potawatomi zoo in Indiana. A reportedly 3-year-old, female, Red-necked wallaby with a history of diarrhea and emaciation was found dead and necropsied. Gross lesions reported by the zoo veterinarian performing the necropsy included a stomach that was diffusely dark red to black and filled with black liquid, and gastric serosa adhered to the abdominal fat. Formalin-fixed tissues were submitted to the Indiana Animal disease Diagnostic Laboratory at Purdue University for histopathological examination. Microscopically, the stomach had multifocal necrosis in the mucosal lamina propria and submucosa accompanied by transmural hemorrhage and infiltration of mixed inflammatory cells, predominantly neutrophils and macrophages. Submucosal vessels were infiltrated by inflammatory cells and contained fibrin thrombi. Colonies of gram-negative, rod-shaped bacteria were scattered in the areas of necrosis and inflammation. The gastric serosa was thickened by granulation tissue that extended to the mesenteric adipose tissue (fibrous peritonitis). The heart had lymphoplasmacytic myocarditis, epicarditis and endocarditis with vasculitis, a lesion suggestive of septicemia. Due to lack of fresh tissues, DNA was extracted from formalin-fixed paraffin-embedded stomach and subjected to PCR amplification and direct DNA sequencing of 16S ribosomal RNA (rRNA) gene. Using GenBank database, the sequences of amplified product revealed 100% homology with *Fusobacterium necrophorum*. **Based on results of observed microscopic lesions, existence of gram-negative bacteria in lesions and gene analysis, we concluded that *F. necrophorum* caused severe transmural gastritis in the wallaby.**

Fusobacterium necrophorum, a gram-negative anaerobe, is a normal inhabitant of the alimentary tracts of humans and animals. In wallabies, mandibular osteomyelitis (lumpy jaw) caused by *F. necrophorum* infection has been reported, but necrobacillary gastritis has not been documented. To our knowledge, this is the first report describing *F. necrophorum* associated with necrobacillary transmural gastritis in a zoo wallaby.

Identification and genetic characterization of *Cytauxzoon felis* in asymptomatic domestic cats and bobcats

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The objectives of the current study were to assess the prevalence of *Cytauxzoon felis* infection among a population of domestic cats that were clinically healthy but at higher risk for parasite exposure and to determine if the strains present in these asymptomatically infected cats were genetically unique as compared to those present both in domestic cats that were fatally infected and in the natural reservoir host, the bobcat.

Using real-time PCR analysis targeting a portion of the parasite 18S rRNA gene specific for *C. felis*, **27/89 (30.3%) high-risk asymptomatic domestic cats from Arkansas and Georgia, and 34/133 (25.6%) bobcats from Arkansas, Georgia and Florida, were identified as positive for *C. felis* infection.**

Conventional PCR analysis was performed on all positive samples, targeting the *C. felis* ribosomal internal transcribed spacer regions 1 and 2 (ITS1, ITS2) in order to utilize the ITS sequences as markers to assess the genotype variability of the parasite population. Within the asymptomatically infected domestic cat samples, 3 genetically distinct parasite populations were identified. The *C. felis* ITS sequences from asymptomatic cats were identical to those previously reported from clinically ill infected cats, and 2 of the 3 sequence types were also present in infected bobcat samples.

While sequence diversity exists, evaluation of the ITS region does not appear to be useful to verify pathogenicity of *C. felis* strains within host species. However, the presence of asymptomatic *C. felis* infections in clinical healthy domestic cats warrants further investigation to determine if these cats can serve as a new reservoir for *C. felis* transmission.

Cerebral myiasis in a pet rabbit

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Aberrant migration of fly larvae into the brain occurs sporadically in animals and humans. Genera of flies whose larvae can cause intracerebral myiasis include *Cuterebra* (dogs, cats), *Hypoderma* (cattle, horses, humans), *Oestrus* (sheep), and *Dermatobia*, *Lucilia*, and *Phaenicia* (humans). The present report describes a case of cerebral myiasis in a pet rabbit caused by an unidentified fly larva. In early July 2008, two pet Dutch rabbits in South Dartmouth–MA developed a neurologic disease characterized by inability to ambulate, ataxia, vertical nystagmus, absent extensor thrust reflex, partial seizures, anisocoria, and lack of spinal reflexes. These rabbits were littermates, with similar husbandry. They were housed indoors at night and during the day, when the weather was appropriate (moderate temperatures and no precipitation), they were kept in a fenced-in outdoor area adjacent to a yard. The 2 sick rabbits were treated with prednisone, dexamethasone, enrofloxacin; fenbendazole, and metacam. The clinical course of the disease was 5 days. One rabbit was euthanized due to poor prognosis and submitted to the CAHFS Laboratory at Davis for necropsy while the other recovered. At necropsy, there were no significant gross lesions except for a markedly distended urinary bladder full of urine. No skin wounds were seen. **Histopathology examination revealed a single fly larva in the brain within the neuropil of one cerebellar peduncle.** The larva was surrounded by many gitter cells, few heterophils, and small numbers of erythrocytes (malacia, inflammation, and hemorrhage). Swollen axons, axonal spheroids, and pleocellular perivascular cuffing were also observed in the neuropil adjacent to the larva. Morphological features of larva included the presence of prominent, single-pointed, spike/thorn-like structures (chitinous spines/hooks) arranged in rows or distributed randomly along its cuticle, presence of oral hooks, and a trachea. Scattered throughout the neuropil of the brainstem, where no parasites could be found, there were areas of axonal swelling, presence of axonal spheroids, and infiltration by lymphocytes, monocytes, plasma cells and gitter cells. In the spinal cord, in the ventral and lateral areas of the cervical, thoracic, and lumbar white matter, there was axonal swelling with infiltration by myelinophages (wallerian degeneration/digestion chambers). Lesions were more severe and more extensive in the cervical and thoracic segments of the spinal cord. The ear canals were filled with large numbers of heterophils, mononuclear cells, fibrin, and numerous yeasts (bilateral otitis externa), and there was fibrinosuppurative inflammation of the surrounding soft tissues including the skeletal muscles of the head and adjacent salivary and ceruminous glands. **No fly larvae or any other lesions were found in the subcutis, nasal cavity, trachea, or middle/inner ears.** The genus of the fly larva present in the brain of this rabbit couldn't be identified by histopathology. Specimens of this maggot were not available for parasitological examination. The portal of entry of the fly larva into the brain was not evident from necropsy or microscopic examination. **Because the rabbit was kept outdoors during the day in summer months there was potential exposure to myiasis-causing flies. To our knowledge reports of cerebral myiasis in leporids is not published.**

Morphologic and molecular pathogenesis study of condemned kidneys in swine from Alberta

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Introduction: Kidney lesions are an important cause of tissue condemnation in slaughterhouses. In addition to the potential public health implications, organ condemnations have a significant economic impact on the food animal industry. Condemned pig kidneys were examined by bacterial culture, histochemistry, PCR, RT-PCR and specific molecular tests in order to identify the causative agents prevalent in the region.

Materials and Methods: Forty kidneys (30 condemned under the general category of "multifocal white spots" and 10 grossly normal) were collected at a slaughter house near Calgary (Alberta) and analyzed. Macroscopic abnormalities were classified using a 0-3 grade scale, based in the size and distribution of the lesions. Microscopic examination of the lesions included routine histology (H&E) and histochemistry. Matrix-assisted laser desorption/ ionization (MALDI) time of flight (TOF) mass spectrometry and specific biochemical tests were used to identify bacteria present in the tissues. For PCR, three small pieces of tissues were pooled from each kidney using separate instruments before being processed for nucleic acid extraction. The Qiagen Mini Kit was used to extract nucleic acid for Porcine Parvo virus (PPV) and Porcine Circovirus 2 (PCV-2) PCR while the Qiagen Viral Kit was used for extraction of Porcine Reproductive and Respiratory syndrome virus (PRRS) RT-PCR. The PCR for PPV PCR, PCV-2 PCR, and PRRS RT-PCR were performed using in-house optimized published protocols.

Results: Bacteria were isolated from culture media in six out of ten control samples (60%) and 21/30 (70%) condemned kidneys. Common swine pathogens were not found. *Streptococcus sp., Lactococcus garviae, Staphylococcus saprophyticus*, *Sphingobacterium multivorum, Acinetobacter spp.*, and *Stenotrophomonas maltophilia* were isolated from several cases displaying moderate to severe morphologic changes. PCV-2 was detected in 6/30 (20%) of condemned kidneys and 1 out of 10 (10%) control kidneys. PPV was detected by PCR in 14/30 (47%) of condemned kidneys and 6/10 (60%) control kidneys. All samples were negative for PPRS. PCV-2 was detected by PCR in 5 out of 6 condemned kidneys with bacteria isolated after culture. Bacteria were not isolated in the control kidney with positive PCV-2, but PPV was detected by PCR in half of the control kidneys that were positive for bacteria. Ten out of 14 (71%) condemned kidneys with PPV had bacteria.

Discussion/Conclusion: Renal lesions were associated with detection of both PCV-2 and PPV. There was a strong association between virus detection and isolation of bacterial organisms. The significance of the various environmental bacterial species isolated was not evident. Western blot analysis in serum samples will be performed to investigate a relationship between the isolated bacteria and sub-clinical disease in these pigs.

Abscesses in captive elk in Utah associated with *Corynebacterium pseudotuberculosis*

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A number of elk ranchers in Utah have reported discrete subcutaneous swellings on the chest, neck, legs, and head and diffuse swellings of the head in adult elk in the past 2 to 3 years. Lesions were grossly similar to those reported in cases of "pigeon fever" in horses. An affected young adult bull was submitted to the Central Utah Veterinary Diagnostic Laboratory for necropsy in October 2009. There was extensive swelling of the right side of the head with thickened skin and numerous dermal nodules. A large (7 x 4 cm) subcutaneous abscess containing brown fluid exudate was also on the right side of the head. There were no other significant gross lesions. Histologically, there was extensive cellulitis/panniculitis of the head. Plant foreign material was detected within inflamed areas of skin and subcutis. The plant material may have been the initiating cause of the abscessation. Multiple mixed bacteria were isolated from dermal nodules; however, *Corynebacterium pseudotuberculosis* was the primary isolate. Swabs of an abscess from another affected bull from the same ranch were taken in November 2009 and submitted to the CUVDL for culture. *Corynebacterium pseudotuberculosis* was also isolated from swabs submitted from abscesses of this bull.

The elk originated from a herd of approximately 150 captive Rocky Mountain Elk (*Cervus canadensis nelsoni*) in central Utah. Animals are used for meat and for seedstock. Subcutaneous swellings, particularly on the neck, have been noticed in several elk for the past 2 or 3 years, but more cases were seen in 2009. Seven or eight animals were affected out of approximately 150 and three of the affected animals died.

Corynebacterium pseudotuberculosis is associated with subcutaneous abscessation and disseminated disease in many different species worldwide and occasionally causes lymphadenitis in human beings. In horses, the bacteria causes an infection of the limbs called ulcerative lymphangitis and in the dry, western and southwestern states, it causes a condition known as pigeon fever or dryland distemper. In sheep and goats, *Corynebacterium pseudotuberculosis* causes caseous lymphadenitis, a disease characterized by abscessed superficial lymph nodes as well as internal abscesses in various organs.

Mononuclear subpopulations in captive Alaskan reindeer (*Rangifer tarandus*)

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Reindeer have evolved in high latitude ecosystems and their immune systems may differ from species that have evolved in more temperate climates. In order to understand the impact of new and emerging diseases on an individual species, it is important to characterize their immune systems. Monoclonal antibodies have been used to elucidate cellular components of the immune system of many mammals. However, only a limited number of monoclonal antibodies developed for ruminants have been shown to cross-react due to highly conserved epitopes on orthologous molecules present on white blood cells. Although these reagents exist, only a few studies have investigated the immune system of reindeer. The objective of this study was to verify cross reactivity of commercial monoclonal antibodies and to use these reagents for enumeration of lymphocyte and monocyte subpopulations in reindeer by multicolor flow cytometry.

Peripheral blood samples from adult reindeer (n=30) were obtained from the reindeer research program, University of Alaska, Fairbanks, Alaska. Peripheral blood mononuclear cells were isolated by density gradient centrifugation with ficol hypaque. Immunophenotyping of lymphocyte and monocyte subpopulations in mononuclear cell suspensions were performed by flow cytometry. Primary monoclonal antibodies to CD4 (IgG_1), CD8 (IgM), CD45 (IgG_{2a}) CD45RO (IgG_3), CD62L (IgG_1) and IgM (IgG_1) and fluorochrome conjugated secondary antibodies were used to identify mononuclear subpopulations. Labeled cells were analyzed on a multicolor flow cytometer and data were analyzed with multi-parameter software. Total mononuclear cells are first identified by CD45 labeling and then divided into lymphocytes with low side-scatter and monocytes with medium side-scatter. Lymphocytes are further divided into B-cells, CD4+ T helper cells and CD8+ T cytotoxic cells based on expression of specific surface proteins. Reference ranges were calculated as the central 95% confidence interval.

Lymphocytes comprise 83-89% of mononuclear cells. Between 48-60% of the lymphocytes are T-cells. The CD4+ helper T-cells are 29-36 % of lymphocytes, while the CD8+ cytotoxic T-cells are 17-23% of lymphocytes. Approximately half of the CD4+ cells co-expressed CD45RO and CD62L (45-52% lymphocytes), while a minority of CD8+ T-cells co-expressed CD45RO and CD62L (3-5% lymphocytes). Monocytes comprised 7-12% of mononuclear cells. We divided monocytes into three populations, based on the expression of CD62L and CD45RO. Between 16-40% of monocytes expressed only CD45RO, while 1-10% co-expressed CD45RO and CD62L. The remaining monocytes (50-66%) did not express either surface protein. We were unable to demonstrate cross reactivity for B-cells with the reagents tested and enumeration of these populations was not possible.

Our study shows that current commercial reagents are useful for identifying T-cells and monocytes in reindeer. Advanced cytometry allows for analysis of minor, functional subsets of cells that are significant in host defense. New reagents for B-cells identification in this species are needed, since B-cells are important for antibody production. Further studies are needed to assess other functional subpopulations in reindeer.

Development of a bead-based multiplex assay for simultaneous quantification of cytokines in horses

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Introduction: Cytokines are soluble indicators of cellular immune responses. They can be used to determine the host's immune status in numerous applications, such as infectious and inflammatory diseases, vaccine development, as prophylactic or prognostic markers or as indicators of treatment success. The detection and quantification of cytokines in farm and companion animals has been hampered by the lack of antibodies for many years. With the development of antibody pairs for equine cytokines during the past years, the quantification of these essential regulators of the immune response is possible.

Material and methods: A fluorescent bead-based system was used as matrix for the assay that allows the simultaneous detection of three cytokines in a single sample by a Luminex analyzer. The assay was developed using pairs of monoclonal antibodies to equine IL-4, IL-10 and IFN-α. Equine recombinant cytokine/IgG fusion proteins were validated as standards for quantification of the individual cytokines. The multiplex assay was compared to individual enzyme-linked immunosorbent assays (ELISA) using the same anti-cytokine reagents.

Results: The analytical sensitivities of the multiplex assay were found to be 40 pg/ml for IL-4 and 15 pg/ml for IL-10 and IFN-α. The sensitivity of cytokine detection by the multiplex assay was increased by 13 to 150-fold compared to the corresponding ELISA. The specificity of the multiplex assay was validated using cell culture supernatants from equine peripheral blood mononuclear cells (PBMC) stimulated with different mitogens or infected with equine herpesvirus type 1 (EHV-1). As predicted, supernatants from PBMC stimulated with different mitogens contained IL-4 and IL-10, but no IFN-α. EHV-1 infection of PBMC resulted in a dose-dependent secretion of IFN-α. IL-4 was not detectable in these samples. The resulting detection pattern for the multiplex analysis and assays performed with individual standard cytokines indicated that individual bead assays did not interfere or cross-react during simultaneous detection of equine IL-4, IL-10 and IFN-α.

Conclusion: The equine cytokine multiplex assay is a valuable and cost-effective tool for quantification of IL-4, IL-10 and IFN-α in horses and can be used for a wide variety of immunological and clinical applications. In the future, the assay can be expanded by adding bead assays for other equine cytokines and chemokines to the existing platform.

Monoclonal antibodies to *West Nile virus* (WNV) envelope glycoprotein increase the sensitivity of WNV-specific IgM antibody detection in equine serum

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Introduction: The quality of reagents is crucial for the development of specific and sensitive diagnostic assays. Here we developed monoclonal antibodies (mAbs) specific for the WNV envelope glycoprotein (E) to improve the sensitivity of a WNV-IgM capture ELISA previously performed using a mAb to St. Louis Encephalitis (SLE) E-protein that is cross-reactive with WNV.

Material and methods: The extracellular domain of WNV E-protein was expressed in a baculovirus system, purified using nickel-affinity chromatography, and used to immunize a BALB/c mouse. The mAbs were produced by conventional cell fusion and hybridoma technology. The resulting mAbs were tested for their specificity to WNV E-protein by different methods including ELISA and immunoblotting using the recombinant WNV E-protein, IFA using WNV infected Vero cells, their ability to neutralize WNV in an established neutralization assay, and their performance in a WNV IgM capture ELISA. Three selected anti-WNV E-protein mAbs were then compared to the anti-SLE conjugate previously used in the WNV-IgM capture ELISA.

Results: Five mAbs to WNV E-protein were obtained. All of them detected the recombinant WNV E-protein by ELISA and immunoblotting. They also detected WNV in infected Vero cells by IFA. Two of the mAbs neutralized WNV at titers of 1:1536, two mAbs did not neutralize and the fifth mAb had a low neutralizing activity of 1:12. Four of the mAbs correctly identified pre-tested positive and negative sera in a WNV-IgM capture ELISA. Three of the mAbs resulted in higher positive /negative (P/N) ratio than the existing assay using anti-SLE conjugate. Best results were obtained with clone 217 resulting in a 2-3-fold increase in the P/N ratios for the positive control without increasing the value of the negative control.

Conclusion: The increase in analytical sensitivity that can be obtained by using mAbs to WNV E-protein compared to cross-reactive anti-SLE conjugate can improve the detection of early stages of infection with WNV. The mAbs also provide useful tools for biomedical research or can be used as positive controls for the performance and quantification of WNV-specific serological assays.

Development of a microsphere-based assay for rapid detection and differentiation of four major serotypes of *Infectious bronchitis virus*

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Infectious bronchitis virus (IBV) is an avian coronavirus with major economic importance to commercial chicken producers worldwide. Economic losses from this highly infectious upper-respiratory disease are due to poor growth rate, decreased egg production and mortality. Vaccination with attenuated live virus is used to control the disease but due to multiple serotypes and antigenic variants of the virus that do not give cross protection, it is extremely important to choose right vaccine type to achieve protection. The conventional diagnostic method for detecting and typing IBV is virus isolation followed by the virus neutralization test in embryonated eggs. Common molecular diagnostic tests used for detecting and typing the virus include, reverse transcriptase-polymerase chain reaction (RT-PCR), and nucleotide sequencing. In an effort to improve on those diagnostic tests, we developed a high-throughput microsphere-based assay for detection and differentiation of the four most common IBV serotypes diagnosed in the USA; Arkansas (Ark), Connecticut (Conn), Delaware (DE) and Massachusetts (Mass). For the first step of this test, the hypervariable region of the spike glycoprotein was amplified by RT-PCR using biotinylated primers. Using serotype-specific probes for each of the four serotypes (Ark, Conn, Del, and Mass) hybridized to microspheres with different spectral addresses, we performed a hybridization reaction with the biotin labled amplicons. A fluorescent tag bound to the biotin labeled amplicons and the spectral addresses of the microspheres were measured in the Bio-Plex Suspension Array System (Bio-Rad, Hercules, CA). Specificity, sensitivity and cross-activity assays for the four serotype-specific probes bound to microspheres were performed. These probes were able to specifically detect their targeted serotypes with no cross activity each other. These results demonstrated the possibility that **this newly developed microsphere-based assay can be used for rapid detection and differentiation of different IBV serotypes**; however, validation of the assay with clinical samples needs to be conducted.

Equine methicillin-resistant *Staphylococcus aureus* incidences in Georgia and Kentucky from 1995 to 2003

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Ever since the first equine Methicillin-resistant *Staphylococcus aureus* (MRSA) infection was reported in Wisconsin in 1997, the importance of MRSA in horses has increased. However, knowledge about the epidemiology of MRSA in horses is still very limited. In this study, we investigated 152 equine *Staphylococcus aureus* isolates from Kentucky and Georgia, collected from 1995 to 2003 to observe the trend of MRSA in horses and compare the genetic relationship of these MRSA strains between the two states. All samples were obtained from sick animals at both University of Kentucky (Lexington) and Athens Veterinary Diagnostic Laboratories. All equine *S. aureus* isolates were confirmed to the species level with catalase test and *nuc* gene PCR, the presence of *mecA* gene and staphylococcal cassette chromosome *mec* (Scc *mec*) typing were also determined by PCR. Strain typing was achieved using repetitive-sequence based PCR (rep-PCR), and a PCR for the Panton-Valentine leukocidin gene detection were performed. The results showed that out of total 152 samples, **62 (40.8%) were MRSA and 48(64%) of Kentucky samples, and only 14(18.2%) of Georgia samples were MRSA strain**. More than 45% isolates obtained from 1996 to 2000 in Kentucky were MRSA stains. **Scc *mec* typing showed that type IVd was predominant (58 out of 62, 93.5%)** in our isolate collection and none of equine MRSA carried the Panton-Valentine leukocidin gene. More than 90% (56 isolates out of 62) of the MRSA strains were clustered in the group of rep-PCR pattern 11 with >95% similarity. Most of MRSA strains were isolated from reproductive or respiratory disease presentation. These data provide evidence for that, at least in some states, **MRSA already had a high prevalence as late as 1996 so this is not a recent phenomenon. Also it seems that one equine MRSA strain supposedly had a clonal expansion through two separate states and present with different clinical picture.**

A new ELISA kit (Swinecheck TGEV/PRCV recombinant) to detect and differentiate TGEV and PRCV antibodies in swine sera

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Transmissible gastroenteritis (TGE) is an important enteric disease of swine caused by a *Coronavirus*, the TGE virus (TGEV). Since the mid '80, the diagnosis of TGE has been greatly complicated by the emergence and spread of a distinct variant, the Porcine Respiratory *Coronavirus* (PRCV). Antibodies to both viruses cannot be distinguished by using classical serological methods such as the virus neutralization (VN) assay. Interestingly TGEV and PRCV demonstrate antigenic differences on their spike (S) protein. Monoclonal antibodies (MAbs) directed to common and distinct epitopes have been used to develop ELISA assays allowing differentiating TGEV and PRCV antibodies. These first ELISAs have used partly purified TGEV as antigen. However large scale production of such antigen is difficult and costly. More recently fragments of the TGEV S protein have been cloned into baculovirus transfer vectors. This allows the opportunity to use recombinant proteins as antigen instead of virus.

We have developed a differential ELISA using a truncated fragment of the TGEV S protein and 3 different MAbs. One of the MAbs is also used to purify the S protein fragment from the supernatant of recombinant baculovirus-infected cell cultures and to immobilize it onto polystyrene microtiter plates. The two other MAbs which recognize epitopes common to both TGEV and PRCV or specific for TGEV (absent on PRCV) were conjugated to horseradish peroxidase (HRPO). The assay is simple and rapid to perform. Briefly, serum samples were first incubated for 1 hour at 37^0C. After washing, the plates were incubated for 30 minutes at 37^0C with either conjugated MAbs. After further washings, the substrate (TMB) was added and incubated for 10 minutes at room temperature.

The test was evaluated using a panel of 106 negative (15 herds), 87 PRCV positive (16 herds), and 99 TGEV positive (9 herds) sera originating from well characterized herds regarding TGEV and PRCV. Sera were also examined using the VN assay and another commercial differential ELISA kit as reference tests. The kit appeared to be highly sensitive (C.I. 95%: 96.26% to 100%) and specific (C.I.: 95%: 96.50% to 100%) to detect and differentiate TGEV and PRCV antibodies.

The major advantages of the Swinecheck TGEV/PRCV recombinant kit compared to the other commercial kit are its simplicity and its rapidity (eg. 2 hours instead of 4). **Swinecheck TGEV/PRCV recombinant kit appears to be an effective and convenient tool to detect and differentiate TGEV and PRCV antibodies in swine sera.**

A new ELISA kit (Swinecheck mix APP) to detect antibodies to several serotypes of *Actinobacillus pleuropneumoniae* antibodies in swine sera

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Actinobacillus pleuropneumoniae (APP) remains an important swine respiratory pathogen in many countries worldwide. However the virulence of APP isolates greatly varies. Interestingly virulence is correlated with the capsular type. Among the 15 different APP capsular types identified so far, serotypes 1, 5, 7, and 9 appear to be the most virulent. By contrast serotypes 4 and 10 are poorly virulent. Moreover virulence of serotypes 2, 3-6-8, 12 and 15 varies depending on the isolates and virulence of serotypes 13 and 14 is poorly characterized. Due to this diversity in virulence, measures to prevent or control APP infections are mainly focussing on the most virulent serotypes.

Serological testing is routinely used to monitor swine populations for APP, mainly to prevent introduction of infected animals into naïve herds. For economical reasons testing is usually restricted to the most virulent serotypes. Numerous tests for detecting APP antibodies have been developed. One of the most effective is an indirect ELISA using highly purified long-chain polysaccharides (LC-LPS) as antigen. LC-LPS antigens for capsular types 2, 5, 10, 12, 13, and 14 are unique. By contrast LC-LPS from capsular types 1, 9 and 11, 3-6-8 and 15, and 4 and 7 are similar. Presently, seven ELISA kits using different LC-LPS antigens are commercially available (Swinecheck APP, Biovet). They allow detecting antibodies against APP serotypes 1-9-11, 2, 3-6-8-15, 4-7, 5, 10, or 12.

In order to reduce the cost of APP serological testing, we have tried to develop a test using a combination of LC-LPS antigens from the most important serotypes. However we have observed that combining several antigens was difficult due to differences between antigens in their affinity for polystyrene. Finally, we have succeeded in combining antigens for serotypes 1-9-11 and 2 as well as antigens for serotypes 4-7 and 5. The resulting mix-ELISAs for serotypes 1-9-11-2 and 4-7-5 demonstrate sensitivity and specificity similar to those of the respective individual tests.

Biovet is now offering a new ELISA kit with three antigen combinations i.e. 1-9-11-2, 3-6-8-15 and 4-7-5 (Swinecheck mix APP). This kit represents an interesting tool to screen for antibodies against the most important APP serotypes. **Positive results may be confirmed using the respective individual kits.**

Prevalence of serotype and biofilm formation of *Actinobacillus pleuropneumoniae* isolated from diseased pigs in Korea

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Introduction: Biofilms are microbial communities that exist on biotic and abiotic surfaces, encased in a self-synthesized extracellular polysaccharide matrix. This is an important virulence trait of many pathogenic bacteria because of the increased resistance to antibiotics and host defense. *Actinobacillus pleuropneumoniae* has been reported that some strains have the ability to form biofilm. Especially, it was notable that some of the serotypes of these strains have important implication to biofilm formation. The purpose of this study was to investigate the prevalence of serotype and biofilm formation of *A. pleuropneumoniae* isolated from diseased pigs in Korea.

Materials and Methods: Field isolates (n=34) of *A. pleuropneumoniae* were originated from diseased pigs submitted to the Animal Disease Diagnostic Center at National Veterinary Research & Quarantine Service from 2008 to 2009 in Korea. Serotyping was performed using combination with toxin profiling PCR[4] and other PCR. Reference strains (serotype 1 to 15 including 5a and 5b) were used as positive control of PCR and biofilm assay. Quantitative biofilm assay in microtiter plates was performed as previously described. The quantities of biofilm formation were measured at 590 nm using a spectrophotometer.

Results: Field isolates were identified to serotype 5 (n=28), serotype 1 (n=4) and serotype 2 (n=2). On the other hand, biofilm formation (OD_{590nm} > 0.2) was observed in 22 (64.7%) of the 34 field isolates : 19 (67.8%) in serotype 5 and 3 (75.0%) in serotype 1, respectively.

Discussion/Conclusion: The predominant serotype of *A. pleuropneumoniae* was serotype 5 which was consistent with the previous Korean studies. Interestingly, isolation of serotype 1 in Korean pig industry was first reported in this study. Our data indicate that majority of *A. pleuropneumoniae* isolates have the ability to form biofilms, especially in serotype 5. Although biofilm formation of *A. pleuropneumoniae* was not investigated in Korea, the result of this study was partially consistent with studies in other countries.[3] **Our study showed that *A. pleuropneumoniae* serotype 5 was the most frequently detected in Korean pig industry. And, 22 (64.7%) of 34 field isolates exhibited biofilm positive phenotype.** An ongoing study was examining the pathogenicity of *A. pleuropneumoniae*, depending on the biofilm formation.

Improved method for *Bacillus anthracis* isolation from animal hides

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Introduction: Recent cases of cutaneous and gastrointestinal anthrax in humans in the United States and Europe caused by handling drums made from contaminated animal skins have exemplified the risk of animal skins as a potential reservoir of *Bacillus anthracis*. Due to this risk, sensitive diagnostic testing of skins for import or export or in connection with a suspect human exposure is crucial. Traditional isolation methods for *B. anthracis* from animal hides have an unknown sensitivity and may not be well suited for samples with high levels of background contamination. The objective of this work was to evaluate the ability of different treatments and media to increase the sensitivity of *B. anthracis* detection in lamb skins.

Materials and Methods: Various combinations of methods to inactivate vegetative bacteria and media for the recovery of *B. anthracis* spores were evaluated. These combinations were compared to the current NVSL method which uses no method of inactivation, enrichment of skins in trypticase soy broth with polymyxin B (TSP) followed by plating to heart infusion agar with 5% bovine blood (HIAB) and three selective media, phenylethanol alcohol with 5% sheep blood (PEA), mannitol egg yolk polymyxin B (MYP) and polymyxin B lysozyme EDTA thallous acetate agar (PLET). The spore extraction method described by Levi et al was used followed by one of three different methods of spore shock to reduce vegetative bacteria; heat shock at 65°C for 20 minutes, ethanol treatment, [3] and treatment with 2% phenol. [6] Several broth enrichment methods were also evaluated prior to plating including TSP, two formulas for PLET broth, and PLET-TMS broth. Aliquots (100 ul) of treated and untreated samples were plated to HIAB, PLET, and PLET agar with trimethoprim sulfamethoxazole (PLET-TMS). [4] The plates were incubated overnight at 37°C for PLET and HIAB and 30°C for PLET-TMS and read daily for three days for presence of *B. anthracis* and amount of contaminant overgrowth. *Bacillus anthracis* suspect colonies were confirmed via colony morphology, hemolysis, and gamma phage susceptibility.

Results: The sensitivity of the isolation method was increased when treatment to reduce vegetative cells was used. *Bacillus anthracis* was undetectable in TSP broth at 4×10^4 spores per 2 gram square due to overgrowth of other bacteria. *Bacillus anthracis* was consistently detectable at 4×10^2 spores per square using heat shock and ethanol treatment, showing at least a two log improvement in sensitivity over the current NVSL method. PLET-TMS plates showed the greatest selectivity of the media, with no overgrowth on any plate, and detection at 4×10^2 spores per square. Broth enrichment of samples proved ineffective and showed either inhibition of growth for *B. anthracis*, or inadequate inhibition of other bacteria resulting in overgrowth.

Conclusion/Relevance: The results from this study support an improved protocol for *B. anthracis* isolation from spiked lamb skins allowing for increased confidence in laboratory testing results. A protocol consisting of spore extraction, heat shock or ethanol treatment, followed by plating to blood agar and PLET-TMS increased the sensitivity of the detection of *B. anthracis* spores in animal hides. Further work is needed to evaluate this methodology using other strains of *B. anthracis*.

Optimization of an in vitro assay to detect *Streptococcus equi* subsp *equi* in 0.9% NaCl

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Introduction: Strangles is a highly infectious upper respiratory disease with a high morbidity rate and a high financial burden for the equine industry. It has become necessary to test both symptomatic animals and potential carrier animals for the etiological agent: *S. equi*. Bacterial culture and polymerase chain reaction (PCR) of nasopharyngeal washes and guttural pouch lavages have been used for both diagnostic testing and for the detection of *S. equi* in carrier animals, but currently have low sensitivities. Currently, there is no standard laboratory method being used to perform either bacterial culture or PCR on nasopharyngeal wash and guttural pouch lavage samples. We hypothesized that flocked swabs (Copan Diagnostic) submerged in standardized cell suspensions of *S. equi* prepared in 0.9% NaCl would detect more colony forming units (CFU) than BBL™ CultureSwab™ submersion; that centrifugation of a 1 ml aliquot of the sample would improve the detection limit compared with swab submersion samples for both bacterial culture and DNA amplification; and that PCR amplification of the centrifuged sample would be more sensitive than aerobic culture.

Material and Methods: Using 7 known serial dilutions of *S. equi* in 0.9% NaCl, the sensitivities and thresholds of colony detection for 4 methods of processing samples for bacterial culture were compared (BBL™ CultureSwab™, flocked SWAB, centrifugation and sampling with a loop, and centrifugation and sampling with BBL™ CultureSwab™) and for 3 methods of processing samples prior to DNA amplification (BBL™CultureSwab™ immersed in 1 ml NaCl, centrifugation of a 1 ml aliquot, and flocked SWAB immersed in 1 ml NaCl).

Results: Bacterial culture using a flocked swab submerged in the fluid was statistically superior to all methods ($P<0.001$) when data was examined using Poisson regression. Both centrifugation of a 1 ml aliquot prior to PCR and the flocked swab/PCR offered compelling rates of identification with only a 30% lower chance than the flocked swab culture.

Discussion/Conclusion: Flocked swab culture, flocked swab/PCR, and 1 ml aliquot PCR methods will be compared in a future field trial using strangles animals and outwardly healthy animals screened for strangles carrier status. The final method will be submitted to the Clinical and Laboratory Standards Institute for consideration as a national standard operating procedure for the collection and processing of these samples from horses.

Culture and real-time PCR based diagnosis of bovine tritrichomoniasis

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Tritrichomoniasis is a venereally transmitted protozoal disease of naturally bred cattle and is caused by *Tritrichomonas foetus*. It is responsible for great economic losses to the beef cattle industry through reduced fertility and abortions. In this study we have compared the sensitivities of the two detection methodologies: culture and Real-time PCR on clinical specimens submitted to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo during the calendar year 2008. The clinical specimens were preputial washes from individual bulls collected from multiple herds by the Veterinarians and inoculated into Inpouch media (Biomed Inc.)

For the culture method, the specimens were incubated at 37°C and microscopically examined for presence of live motile Trichomonad organism(s) for six days. The TaqMan Real-time PCR testing was carried out on specimens after two days of incubation @ 37°C in the lab as per the McMillen *et.al.* protocol (Vet. Parasitol. 2006).

Of the 781 specimens tested by both the methods, there were 74 and 89 specimens positive by culture and Real-time PCR respectively. Sixteen of the Real-time PCR positive specimens were culture negative, whereas only one specimen was detected to be culture positive but Real-time PCR negative. The latter single specimen was Tritrichomonas spp. (not *T. foetus*) and assumed to be the non-pathogenic intestinal Tritrichomonad. Statistical analysis of the results of these 781 specimens revealed an overall 97.95% agreement between the two test methods. This % agreement refers to results of those specimens which were found to match for the positive and negative results by both the methods. Interestingly, if only the positive results are taken into consideration, culture identified 83.14% [i.e. (73/89)100] of the Real-time PCR positive specimens.

It can be inferred that Real-time PCR minimizes the possibility of obtaining false negative and false positive results that occur with culture. Thus, the rapid and accurate Real-time PCR has greater sensitivity and specificity and is therefore recommended as the method of choice for diagnosis of *T. foetus* infection.

Identification of enhanced pathogenicity of *Brachyspira* species associated with clinical disease in swine

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Porcine spirochetes cause economically important diseases in growing pigs. Swine dysentery (SD), a severe mucohemorrhagic diarrhea with high morbidity, is caused by *Brachyspira hyodysenteriae.* Porcine intestinal spirochetosis (PIS), caused by *B. pilosicoli*, is characterized by a mucoid, cement-gray diarrhea and depressed growth. While the incidence of these conditions appeared to decline in the late 1990's, possibly due to the changes in swine production and the transition to high-health operations, clinical cases have been increasing recently. Colony characteristics of *B. hyodysenteriae* are strong beta-hemolysis and ring-phenomenon positive while *B. pilosicoli* is weakly hemolytic and ring-phenomenon negative, with PCR providing a more definitive confirmation of species. From 2001-2007, the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) performed an average of 1400 tests for *Brachyspira* per year. In 2009, that number jumped to over 3600 cultures for this pathogen. Recent isolates have demonstrated altered colony characteristics and frequently cannot be identified by PCR testing based on 16SrRNA and *nox* gene targets. Results of 16S rRNA sequencing have identified Brachyspira species previously described as non-pathogenic, including *B. murdochii* and *B. intermedia*, isolated from pigs with clinical and histopathologic signs of SD and PIS. These findings may indicate an expansion of virulence into previously non-pathogenic species or the transfer of or the exchange of inducible phages between *Brachyspira* species. Further characterization of the pathogen is needed to combat the resurgence of this economically severe disease.

Mycoplasma bovis real time PCR validation and diagnostic performance

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Mycoplasma bovis is an important bacterial pathogen in cattle producing a variety of clinical diseases including pneumonia, polyarthritis, otitis, mastitis, conjunctivitis, vaginitis, and abortion. *M. bovis,* which requires specialized culture conditions to diagnose, is frequently associated with other bacterial pathogens which are more easily identified. Real time PCR is a valuable diagnostic technique that can rapidly identify infectious agents in clinical specimens. A real time PCR assay was designed based on the *uvr*C gene to identify *M. bovis* in diagnostic samples. Using culture as the gold standard test, this assay performed will in a variety of diagnostic matricies. Initial validation testing was conducted on 122 milk samples (sensitivity 88.9% [95% CI 68.4-100%], specificity 100%]); 154 lung tissues (sensitivity 89.0% [95% CI 83.1-94.9%], specificity 97.8% [95% CI 93.5-100%]); 70 joint tissue/fluid specimens (sensitivity 92.3% [95% CI 82.1-100%], specificity 95.5% [95% CI 89.3-100%]); 26 nasal swabs (sensitivity 75.0% [95% CI 45.0-100%], specificity 83.3% [95% CI 66.1-100%]); 8 BAL fluids (sensitivity 80.0% [95% CI 44.9-100%, specificity 100%]); 4 ear swabs (sensitivity 100%, specificity 100%); and one tracheal wash (100% agreement). A review of clinical cases from 2009 revealed PCR was used much more frequently than culture. Comparisons between PCR results and clinical disease (clinical signs, histopathology, signalment) using kappa levels of agreement demonstrated very good to near perfect agreement. **Diagnostic performance of this testing method indicates that it is a rapid, accurate assay that is adaptable to a variety of PCR platforms and can provide reliable results on an array of clinical samples.**

Development of a real-time PCR assay to detect *Mycoplasma bovis* in milk and tissues

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Mycoplasma bovis is a significant pathogen of cattle causing diseases such as mastitis, pneumonia, otitis, and arthritis. Diagnosis of the agent is usually performed using culture followed by identification of the species using biochemical testing, species-specific antibodies or sequence analysis. One of the major disadvantages of culture is that it is time consuming, taking 10 to 14 days for identification. Development of a rapid, sensitive, and specific PCR assay could significantly improve treatment and culling programs. We describe a real-time PCR for the identification of *M. bovis* based on the RNA polymerase (*rpoB*) gene. To demonstrate assay specificity, we showed that the *rpoB* real-time PCR assay did not react with a variety of common Gram negative and Gram positive bacteria. We also tested the assay using *Mycoplasma* positive cultures that were speciated using 16S sequencing. The assay identified *M. bovis* but not *Mycoplasma agalactiae*, *Mycoplasma arginini, Mycoplasma alkalescens, Mycoplasma edwardii, Mycoplasma bovigenitalium, M.ycoplasma bovirhinis, Mycoplasma bovoculi, Mycoplasma canis, Mycoplasma spumans,* or *Mycoplasma felis.* We then used this real-time assay to test clinical samples and compared the results to culture. As an additional confirmation, all specimens that gave a positive using the real-time PCR were also amplified using primers specific for *Mycoplasma* and the product sequenced. To date we have completed results for 402 milk, 35 lungs tissue and 5 synovial fluids using culture, real-time PCR and sequencing. Of the 35 lung tissues tested to date, 5 were culture positive, 2 *M. bovis*, 1 *M. bovirhinis* and 2 *M. alkalescens*. Only the two lungs that were positive for *M. bovis* by culture were positive using real-time PCR. Of the 5synovial fluids tested, 1 was culture positive for *M. bovis* and it was also positive using the real-time PCR. Of the 402 milk samples tested, 1 was positive for *M. bovis* by culture; however, 2 samples were positive for *M. bovis* by real-time PCR. The discordant result was confirmed by sequence analysis. **Compared to culture, the real-time PCR assay described here had an overall sensitivity of 100% and specificity of 99%. The real-time PCR described here compares well to culture and will assist producers and veterinarians in making timely treatment and culling decisions**.

In vitro antimicrobial inhibition of _Mycoplasma bovis_ isolates submitted to the Pennsylvania Animal Diagnostic Laboratory using flow cytometry and a broth microdilution method

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Introduction. *Mycoplasma bovis* is an economically important pathogen of cattle in the United States, leading to cases of mastitis, pneumonia, conjunctivitis, otitis, and arthritis. Appropriate selection of antimicrobial therapy is a means of reducing *M. bovis.* *Mycoplasma bovis* isolates submitted to the Pennsylvania Animal Diagnostic Laboratory between December 2007 and December 2008 (n=192) were tested for antimicrobial sensitivity to enrofloxacin, erythromycin, florfenicol, spectinomycin, ceftiofur, tetracycline, and oxytetracycline using a broth micodilution testing method.

Materials and Methods. Early log phase suspensions of *M. bovis (*~$4.5x10^5$ cfu/ml) grown at 37° C in 5% CO_2 were exposed to doubling concentrations of enrofloxacin, spectinomycin, erythromycin, tetracycline, oxytetracycline, ceftiofur, and florfenicol starting at 0.0125µg/mL, 1µg/mL, 0.0125µg/mL, 0.05µg/mL, 0.05µg/mL, 0.49µg/mL, and 2µg/mL, respectively, in the presence of the redox reagent alamarBlue. The minimum inhibitory concentration (MIC) for each antimicrobial was determined at 24 hours based upon the color shift from blue to red. A subset of the 192 total *M. bovis* samples were selected in order to determine the MIC values of isolates from dairy cows with mastitis (n=6 isolates) and calves with pneumonia (n=6 isolates) using a standardized flow cytometric technique. Flow cytometry is based on fluorescent single cell counting system, which is simple to use, provides results in minutes, and has high throughput capability that allows capture of real-time data. Flow cytometry data can be used to determine minimum inhibitory concentrations of antimicrobials by determining the living and dead cell populations on a four-decade logarithmic scale. Suspensions of *M. bovis (*$5x10^3$ - $5x10^6$ cfu/ml) were exposed to different concentrations of antimicrobials, and examined for living and injured/dead mycoplasma at 0, 3, 6, 12, and 24 hours by flow cytometry. Propidium iodide, a stain for cell membrane damage, and SYBR green I, a double-stranded DNA stain, were used at 4mg/mL and 1:10,000 vol/vol concentrations, respectively. Analyses were performed on an XL-MCL flow cytometer (Beckman-Coulter) using side scatter (SSC) dot plots to count 20,000 cells based upon the physical properties of *M. bovis.* Data were further analyzed using FCS Express software (v.3).

Results. As previously reported, erythromycin and ceftiofur showed no ability to inhibit growth of *M. bovis* when using both the broth microdilution and flow cytometric methods. The antimicrobials which were most inhibitory for *M. bovis* using the broth microdilution method were florfenicol and enrofloxacin with a MIC range of 2 - 32 µg/mL and 0.1 – 3.2 µg/mL, respectively. Spectinomycin, oxytetracycline, and tetracycline showed a wide ranging level of efficacy in isolate inhibition with MIC ranges of 4 - >256 µg/mL, <0.025 – >12.8 µg/mL, and <0.025 – >12.8 µg/mL, respectively. Using flow cytometry, the MIC ranges of enrofloxacin, spectinomycin, ceftiofur, erythromycin, tetracycline, oxytetracycline, and florfenicol ranges were 0.1 – 0.4 µg/mL, 4 - >256 µg/mL, >125 µg/mL, >3.2 µg/mL, <0.025 - >6.4 µg/mL, 0.8 - >12.8 µg/mL, and <2 - 4 µg/mL, respectively.

Conclusions. There was a high level of congruence between the broth microdilution method using alamarBlue reagent and the flow cytometric methods. The data suggest that both methods may be appropriate for use in sensitivity testing of *M. bovis* isolates. The flow cytometry method offers the ability for a laboratory to have a high-throughput assay that allows for data in real time while the broth microdilution method with alamarBlue allows for easy to interpret and cost-effective testing. Both methods suggest that enrofloxacin and florfenicol are most effective against *M. bovis.*

News Digger Project

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Introduction: The News Digger project aims to provide only high-quality information that is relevant to the argument and whose accuracy, completeness, importance and currency has been ascertained. This service was initially created to support the Bluetongue site developed by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (I.Z.S. A.e M.), and from the beginning was designed and developed as an autonomous application to enable its use in other websites on different topics for which the Institute is a Centre of Excellence and thus provide an expert profile that evaluates and selects news items released over the Internet for publication on its own sites.

Materials and Methods: News Digger consists of two independent modules: the client module and the server module. The client module consists of a standalone application for a generic personal computer, currently available in Visual Basic but also being developed in Java, which is connected to the Internet in order to carry out targeted searches using engines such as Google or Yahoo, or even an ad hoc list of addresses (Uniform Resource Locator - URL). The application enables the specification of filters with the words that must be contained in the text as well as those that must be excluded during the search.
The results, complete with their metadata, are saved in a database, enabling the expert to view and select the information considered most interesting through the use of simple forms. The database can be remote or local, depending on the chosen configuration. The server module is a web application based on services that interface with the database and enable the list of news items selected by the expert to be displayed and managed, in order of publication date. These web services can be used by any web-based application to display the list of news items in the site's own graphical format. Another two Web services enable users to subscribe to and unsubscribe from a newsletter containing the latest validated news items, which the server system sends automatically by e-mail. In addition, a Java library interfacing with the Web services has been developed which provides methods for the selection of news items without the need to develop a web services client. This can be used for sites developed in Java; in other cases, a specific web services client must be developed in the same language as the site. This operation is in any case simplified, as the services have been developed using the SOAP (Simple Object Access Protocol) standard, a light protocol for the exchange of messages among software components.

Results: The "News Digger" module can be seen in use at the OIE Bluetongue Reference Laboratories Network, at http://oiebtnet.izs.it/btlabnet/. The application was developed under the auspices of the OIE in order to construct a global network of OIE Bluetongue Reference Laboratories with the aim of improving knowledge of the epidemiological situation of this disease and thus facilitate international trade in live ruminants. The web-based system, developed by the Institute in J2EE, collects epidemiological and laboratory data, including data on bluetongue virus (BTV) and characterisation of the genetic strain (sequences), if available. At the top of the page is a link to the latest news, where items are listed as OFFICIAL or NOT OFFICIAL and ordered by date. It is also possible to subscribe to the newsletter in order to receive an e-mail containing the latest news selected by the expert as soon as it is published on the site. Each mail contains a link to unsubscribe from the newsletter (as required by Italian legislation), making this operation simple, fast and secure. News is also distributed using a third method, through the site EUBTNET (http://eubtnet.izs.it/btnet/), through subscription to an RSS Feed. **Discussion/Conclusion:** The availability of high-quality, up-to-date data checked by an expert before publication undoubtedly has an enormous added value and is the project's qualifying point. The hidden but constant and highly specific input of the expert is essential. This is the reason that not all the Institute's sites offer this service, even though the information technology is available. A further benefit is the ability to receive information in different, coexisting ways, enabling the user to use whichever is most convenient at any time. E-mail notification of the latest news avoids continually having to check the reference site, while use of the RSS Feed has the further benefit that an e-mail address is not necessary and it is possible to unsubscribe by simply removing the feed address from the list.

Center for Veterinary Medicine Diagnostic Network

Renate Reimschuessel
Center for Veterinary Medicine

CVM will develop a network for coordinating federal and state veterinary diagnostic laboratories to respond to high priority chemical and microbial feed contamination events. This network will coordinate the facilities, equipment and professional expertise of U.S. veterinary diagnostic laboratories along with those of the federal government. This network will provide the means for quick identification of report of animal injury associated with animal feed contamination, and to establish protocols for immediate of veterinary diagnostic reporting to FDA.

CVM will provide grants/contracts/cooperative agreements to state veterinary diagnostic laboratories in a effort to further FDA capacity and rapid response to adverse feed contamination events; initially enroll two state veterinary diagnostic laboratories with toxicology, microbiology, and pathology capabilities for both companion and food animal diagnostics;

Participating laboratories will investigate, assay and follow up on reports of animal injury associated with chemical and microbial animal feed contaminants of companion and food animals;

This network is currently being developed and insight from the AAVLD community would be of value following the presentation.

Sulfur levels in bovine liver

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Diagnosis of sulfur toxicosis in cattle is currently based on clinical signs, confirmed exposure to excessive concentrations of sulfur in feed and water, and evidence of polioencephalomalacia (PEM) on post mortem examination. Sulfur toxicosis is also associated with feed or water refusal and digestive incapacity resulting in decreased weight gain and respiratory disease. Sulfur levels determined in bovine liver are not interpreted with respect to sulfur toxicosis due to the lack of published normal values in bovine tissue. The purpose of this study was to establish a normal range of sulfur concentrations in cattle.

Inductively coupled plasma (ICP) atomic emission spectroscopy analysis was used to determine the concentrations of sulfur in thirty-one liver samples from cattle submitted for necropsy to the Oklahoma Animal Disease Diagnostic Laboratory. Cattle used in the study died from causes unrelated to neurologic disease and/or sulfur toxicosis. ICP analysis for sulfur was carried out by the Department of Population Medicine and Diagnostic Sciences at Cornell University.

Sulfur wet weight concentrations in thirty-one bovine liver samples ranged from 1788 mg/kg to 2051 mg/kg with the mean 1919 mg/kg (95% confidence interval). Sulfur dry weight concentrations in the same bovine liver sample pool ranged from 7803 mg/kg to 8611 mg/kg with the mean 8207 mg/kg (95% confidence interval).

Evaluation of a rapid immunoassay detection kit in comparison to the gold standard direct fluorescent antibody test for the diagnosis of rabies

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Introduction: Rabies continues to be a disease of major significance within the United States. The direct fluorescent antibody (dFA) technique is the Gold Standard for primary rabies diagnosis and is performed on fresh brain material from rabies-suspect animals. Through the application of this technique, there are between 6,000 and 10,000 animal rabies cases diagnosed every year in the US. Fluorescence microscopy requires relatively expensive equipment and specially trained, experienced laboratorians.

Materials and Methods: A rapid immunoassay detection kit (RIDT) is under development for the detection of rabies virus antigen in animals from BioNote, Inc (Korea). The kit inset describes the capability of being able to detect virus in concentrations of $\geq 10^{2.0}$ LD_{50} /0.03ml in mice in both brain and saliva samples. In view of the need for diagnostics where fluorescence microscopy may be too costly or impractical, we evaluated the experimental test kits on brain tissues received at the Kansas State University Rabies Laboratory. Currently the product insert instructs that testing be performed on a highly diluted brain homogenate.

Results: Our initial findings indicate that sensitivity of this testing platform is significantly improved if testing is completed on a 10% brain suspension, with no further dilution, as directed by the instructions of the experimental test kit. Among 200 samples examined in this pilot study, there was concordance in results by the dFA and immunoassay in 87 positive samples and 111 negative samples. Test results were discordant in 3 samples which were positive by dFA, albeit with weakly staining antigen and sparse distribution, but negative by RIDT. Thus the sensitivity of the RIDT is 97.7% and the specificity in this pilot evaluation was 100%.

Discussion/Conclusion: The public health infrastructure of the US should, in theory, be in support of optimal rabies prevention including community-based knowledge and response to rabies-suspect animals, support for humane management of such animals including euthanasia, as needed, and packaging of specimens, facilitated access to diagnostic services and the preservation of expertise. This defense system is necessary to mitigate wildlife rabies incursions into the domestic animal and human population, and also to prevent the re-introduction of canine-rabies-virus-variants (i.e., dog-to-dog-transmitted rabies virus variants). Nevertheless, the cost of rabies diagnostic testing in some US States is borne by the potentially exposed human or the owner of a potentially exposed domestic animal. Moreover, from a global perspective, a field test for rabies would be advantageous, compared to no diagnostic data at all. A field or bench-top diagnostic method could provide rapid results to regions of the world or to wildlife biologic surveys for which currently there are few to no diagnostic options. Although it is clear that much more comparative analysis will be necessary towards the validation of this new method for specific purposes, the implementation of some diagnostic method is better than no diagnostic method or a "shoot-and-bury" mentality when it comes to a disease of such public health impact as rabies.

Molecular epizootiology of skunk-associated rabies in the Mid-western United States

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Introduction: Historic records, descriptive epizootioology, antigenic analyses with monoclonal antibodies and sequence analyses all support two distinct origins of skunk-associated rabies in the Midwestern United States. Records implicate both the spotted skunk (Spilogale putorius) and striped skunk (Mephitus mephitis) in transmission, but the spotted skunks have been nearly-extirpated from most of their historical range. The South-Central rabies virus variant appears to have evolved from an insectivorous bat virus while the North-Central variant is most closely related to "Cosmopolitan" or globally distributed canine rabies virus variants. The South-Central variant occurs in Arkansas, Oklahoma, Missouri, Kansas and Nebraska. The North Central variant occurs in the Dakotas with historical extension south to Missouri. The geographic convergence of North-Central and South-Central variants occurs near the Kansas-Nebraska border.

Materials and Methods: In this study, full length nucleoprotein (N) and glycoprotein (G) gene sequences were generated on 78 rabies-positive isolates from 64 striped skunks, 3 cows, 3 cats, 2 dogs, 2 raccoons, 2 red foxes, 1 horse and 1 bobcat. The sequences of isolates were aligned and edited using Bioedit and phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4. Pair-wise comparisons were conducted to generate ratios of non-synonymous to synonymous mutations.

Results: There was no significant difference in the nucleotide diversity between the South-Central and North-Central variants for either the N or G gene. However, neighbor-joining analysis shows considerable diversity within the South-Central group with four sub-groups represented by samples differing at three amino acid sites within the N coding region and eight sites with G. Based upon diversity and neutrality statistics, both variants are subject to purifying selection but the values for the N gene suggest twice as much pressure is at play on the South-Central variant than on the North-Central variant. In contrast, there is positive selection pressure on the ectodomain of the G gene of both variants but overall, there is more purifying pressure on the G of the North-Central variant in comparison to the South-Central variant.

Discussion/Conclusion: The North-Central and South-Central skunk rabies virus variants present a unique opportunity to investigate how viral, host, and landscape characteristics influence the emergence and expansion or contraction and possible extinction of viral variants. The South-Central variant originally emerged from a bat variant and was sustained by skunk-to-skunk transmission most likely primarily in spotted skunks. With the shift to striped skunks, the successful variant appears to be subject to intense purifying selection on the N gene and there is positive selection pressure on the ectodomain of the G gene. In contrast, the North-Central variant is a result of more recent emergence from Cosmopolitan dog variants. Moreover, the North-Central variant occurs where skunk population densities are generally lower than those present in the South-Central area. Thus, the differences in genetic variation and estimates of evolutionary pressures may reflect the effect of incubation period (longer being more favorable when susceptible hosts are farther apart), viral dose necessary for infection, average viral shedding and the role of G in viral attachment and internalization versus the role of the nucleoprotein in the structural formation of new virions. These findings raise intriguing questions about rabies virus genomic variation and the characteristics of "permissive" host species. Continued investigation of host ecology, landscape features, and characteristics of the two rabies virus variants is warranted to understand the spatiotemporal occurrence, intensity and public health impact of skunk-associated rabies in the Central Great Plains.

Detection of *Koi herpes virus* DNA in healthy koi tissue and blood

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Koi Herpes Virus (KHV) has recently been classified as a member of the *Herpesvirales* order. Although one of the unique features of *herpesviridae* from *Herpesvirales* is latent infection, it has not been consistently demonstrated that KHV can cause latent infection and be reactivated from latency. A latent infection is characterized by the presence of the viral genome in infected host cells but an absence of infectious virus in the latently infected host.

To investigate if latent infection is a feature of KHV, ten clinically healthy fish were tested from a koi population with a history of a KHV outbreak. Blood, plasma and tissues were cultured on KF1 cells for virus isolation. DNA was isolated from tissues and white blood cells using a spin column method as well as a magnetic bead protocol that can be automated allowing for high throughput testing.

No gross lesions or microscopic changes were observed via necropsy and histological examination. No infectious virus was isolated from the blood, plasma or tissues of the fish that tested positive for KHV antibody; however, **KHV DNA was detected in the brain, eye, spleen, gills, hematopoietic kidney, trunk kidney, intestine and white blood cells from 9 of the 10 koi. KHV DNA was also detected in intestinal contents from 7 of the 10 koi. Our study demonstrated that KHV DNA can be detected in clinically healthy koi previously exposed to KHV and suggests KHV becomes latent in fish.**

Detection of *Canine distemper virus* in shelter dogs in New Mexico using real time RTPCR

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Canine Distemper Virus (CDV) is a contagious disease of canines throughout the world. To aid in clinical diagnosis, NMDA/VDS has implemented a published real-time RTPCR assay to be performed on specimens from live and dead canines. The dual labeled 3' 6-FAM probe with a 5' TAMRA quencher and primers were designed to detect an 83-bp fragment of the nucleocapsid (N) protein-encoding gene of CDV. Samples of conjunctival and pharyngeal swabs and whole blood were taken from 88 dogs at a local NM animal shelter to be screened for CDV. All incoming dogs were vaccinated upon arrival with a live attenuated vaccine.

Pharyngeal and conjunctival swabs were processed for nucleic acid extraction using the 96 well RNeasy Kit for high-throughput testing (Qiagen). Whole blood samples were processed using the RNeasy mini kit (Qiagen). Following nucleic acid extraction, real-time RTPCR was performed on the ABI7500 Fast machine (Applied Biosystems). The PCR was performed using the AgPathID one-step rtPCR Kit (Applied Biosystems). High-throughput testing was completed within 24 hours of sample collection.

Multiple sample types from each dog were tested. 20.4% of the dogs tested were determined to be positive by real-time RTPCR. Sixteen of the eighteen positive dogs had positive conjunctival samples. It was also noted that all positive dogs had been vaccinated within sixteen days of testing. Two of the positive dogs had clinical signs consistent with CDV infection. This rapid method of detection helps to confirm the causative agent and keep animal groups free of infection.

Even though RTPCR is a rapid, sensitive, and specific method of detection of CDV, due to possible amplification of a live attenuated vaccine, it is important to consider vaccination history of animals, as well as clinical signs, and histopathological findings in association with PCR results.

Novel variants of *Canine parvovirus* isolated from raccoons in the southeastern United States

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Viruses of the feline parvovirus subgroup (*Parvovirus, Parvoviridae*) include a number of genetically-related viruses of carnivores that are antigenically and biologically distinguishable, including feline panleukopenia virus (FPV), canine parvovirus (CPV), mink enteritis virus (MEV), and raccoon parvovirus (RPV). The emergence of novel parvovirus variants with newly acquired host range properties, as exemplified by the evolution of CPV from FPV or a FPV-like virus, is driven by mutations in the VP2 gene which encodes the major viral capsid protein. In nature, although the spill-over of these carnivore parvoviruses into aberrant hosts is presumably not uncommon, the recognition of a virus jumping from one vertebrate species to another followed by its adaptation and establishment in the new host species appears to be a rare event.

Our existing knowledge on the parvoviruses that spill-over into or are maintained in wild carnivores is incomplete. Outbreaks of gastroenteritis in raccoons have historically been associated with FPV or RPV infection in captive raccoons at rehabilitation centers or shelters. Experimentally, raccoons were clinically susceptible to infection with FPV, RPV, and MEV, but resistant to CPV-2. Based on these limited field and experimental data, raccoons were generally considered to not be a competent host for CPV, but over the last few years this paradigm appears to have changed. Since 2006, outbreaks of gastroenteritis associated with novel CPV variants have been diagnosed at rehabilitation centers in Virginia, Kentucky, Tennessee, Georgia, and Florida. The pathology observed in affected raccoons was consistent with parvoviral enteritis in other species, including severe enteritis affecting the majority of the small intestine, with blunting and fusion of intestinal villi and dilation of crypts. Parvovirus antigen was detected by immunohistochemistry in mesenteric lymph nodes and affected sections of intestine in all raccoons. Parvoviral antigen was also detected in Purkinje cells in the cerebellum of many affected raccoons. Virus was isolated in Crandell-Rees feline kidney cells from samples of small intestine, lymph node, and/or brain collected from affected raccoons and the complete coding region of the genome was determined for eight representative isolates. All raccoon isolates appear to be host range variants of CPV-2a, as they contain the diagnostic asparagine at position 426. At least two amino acid substitutions, at VP2 positions 300 and 305, appear to be under positive selection, with both residues believed to be contained in antibody neutralization sites and/or involved in host cell receptor binding (i.e., transferrin receptor). Representative raccoon CPV isolates did not react with a panel of monoclonal antibodies specific for CPV, FPV, and RPV in hemagglutination-inhibition tests, confirming the antigenic novelty of these genetic variants. The impact that these novel CPV strains will have on wild or domestic animal health are not known; however, the detection of CPV infection in wild and released rehabilitated raccoons highlights the potential for viral spill-over and introduction into free-ranging animal populations.

Keeping PRRSV PCR assays current – a continual challenge

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded positive-sense RNA virus characterized by reproductive failure in sows and respiratory problems in piglets and growing pigs. In light of the high economic impact of this agent, rapid and accurate diagnosis is critical. RNA viruses such as PRRSV are notorious for their high mutation rate, and consequent genetic variability, which provides a significant challenge with regard to real-time RT-PCR (rRT-PCR) testing. **With rRT-PCR testing, even minor discrepancies between primer or probe sequences and viral target sequence can diminish the ability of the assay to detect the virus.**

Our laboratory utilizes Applied Biosystems™ TaqMan® NA and EU PRRSV Reagents for detection of PRRSV from clinical samples by PCR. This product contains multiple primers and probes for both the North American and European subtypes of PRRSV, so it has enhanced ability to detect a wide range of field strains compared to traditional real-time PCR assays containing one primer/probe set for each target. Even with this improved detection capability instances have occurred in which this product failed to detect or only weakly detected North American PRRS virus in clinical specimens. In these instances, communication between the client and diagnostician(s) involved alerted us to the potentially false negative results. These samples were confirmed as positive using alternate real time and gel based PCR assays. The target areas of these discrepant samples were sequenced, and this information and corresponding samples were forwarded to Applied Biosystems™ for further analysis. The bioinformatics group at Applied Biosystems™ confirmed the presence of mismatches between the virus in the specimens and the sequences of the primers and/or probe for the North American PRRSV target in the PCR assay. We were provided with sequences for two additional primers and an additional probe to add to the existing PRRSV PCR reagents to allow for detection of these variant North American viruses without diminishing the sensitivity of the assay. Our lab performed an internal comparison and confirmed that the addition of the primer/probe set does result in detection of these new strains with no overall decrease in assay sensitivity.

This situation emphasizes the importance of open communication between the diagnostic laboratory and its clients. Educating clients about tests used in the lab, including assay limitations, and encouraging feedback when test outcomes do not correlate with expected results, allows for appropriate additional investigation to determine the true status of the client's samples. Also of great importance when utilizing commercial reagents is ongoing interaction with the manufacturer's technical support personnel and bidirectional transfer of information and samples for the continual monitoring and improvement of the assay. In the case of this particular assay, this communication and ensuing action resulted in an improvement of the assay, which benefits the manufacturer of the assay, the testing laboratory, and the clients served.

Porcine circoviral associated disease (PCVAD) in a New Jersey swine production unit

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Porcine circovirus-2 (PCV-2) is a non-enveloped single stranded DNA virus of family *Circoviridae* and genus *Circovirus*. It causes porcine circoviral-2 associated disease (PCVAD) in young pigs. PCVAD is an economically important swine disease that has emerged globally in last two decades. It can be manifested in different clinical forms such as systemic, respiratory, enteric, reproductive etc. or can be subclinical. In July 2009, a swine production unit reported deaths in a number of littermates with history of ill thrift, diarrhea and respiratory difficulty before death. The litter was farrowed 10 weeks previously with 18 total births including 4 mummified fetuses. Three dead pigs and tissues from two pigs were submitted to the laboratory for diagnostic testing. At necropsy, pigs had scant fat and were icteric. Peripheral and visceral lymphadenopathy was seen. Lungs were mottled and failed to collapse. Other organs involved included liver, intestine, heart, and kidneys. Gross lesions revealed systemic involvement. Microscopically, there was lymphoid depletion and histiocytic replacement of follicles in lymphoid tissues. Intracytoplasmic inclusions were present throughout the lymphoid tissue. Granulomatous inflammation was present in liver, kidney, lung, heart, and intestine. Tissues were tested for PCV-2, PRRS virus, influenza virus, and *Mycoplasma hyopneumoniae*. **Lesions and detection of nucleic acid confirmed the PCVAD caused by PCV-2. *Mycoplasma hyopneumoniae*, PRRS virus, and influenza virus were not detected. *E. coli*, *Moraxella*, and *Pasteurella multocida* were isolated from various tissues indicating bacterial septicemia. To our knowledge, this is the first report of PCVAD in NJ swine. Our report supports recent case trend analysis indicating the rise of PCVAD in the USA.**

Development of *Swine influenza virus* nucleic acid purification and amplification workflow for improved surveillance

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Swine Influenza Virus (SIV) is a (-) ssRNA, enveloped, Influenza A virus in the family *Orthomyxoviridae.* It is a continuously evolving virus that causes swine respiratory disease with fever, reduced activity and food intake, conjunctivitis, sneezing and coughing and nasal discharge. SIV results in poor performance of market and breeding animals, thus causing major economic losses to swine producers. Respiratory disease can be caused by multiple SIV genotypes continuously undergoing changes by genetic drift and shift. SIV genotype is determined by the antigenic characteristics of two surface glycoproteins (hemagglutinin and neuraminidase). The three main genotypes currently present in the swine population are H1N1, H1N2, and H3N2. Monitoring/surveillance and genetic characterization of SIV in swine herds are critical for successful to control of the disease and are essential for selection of vaccine treatment and novel vaccine development.

The following data is under review to support a product license application filed with the USDA Center for Veterinary Biologics.

We have developed an integrated workflow consisting of high throughput nucleic acid purification, TaqMan® RT-PCR to screen for Swine Influenza Virus, Type A (SIV-A), and reagents for genotyping. Nucleic acid is purified using the MagMAX™ Nucleic Isolation technology and purified viral RNA is detected using the VetMAX™ Swine Influenza Virus Detection Kit. The Swine Influenza Virus Detection Kit consist of a Swine Influenza Virus, Type A (SIV-A) TaqMan assay. TaqMan® SIV-A genotyping assays were developed to differentiate (SIV-H1/H3 and SIV-N1/N2).subtypes. The SIV-A assay targets multiple genetic regions of the Influenza A genome decreasing the likelihood of false negatives due to viral mutation. The assay is multiplexed with an internal control RNA, Xeno™RNA, to monitor nucleic acid purification efficiency, detect the presence of reaction inhibitors, and determine assay reagents functionality. The Swine Influenza A genotyping assays are two independent duplex assays, H1/H3, and N1/N2. The analytical sensitivity is 50 copies of SIV-A transcript RNA, 25 copies of H1 and H3, and 50 copies of N1 and N2, per 25ul TaqMan PCR.

The sensitivity of the VetMAX™- SIV assay was compared to a published SIV screening protocol (Richt, et. al. 2004). Nucleic acid from an SIV vaccine (Novartis PneumoSTAR™ vaccine) was purified using the MagMAX™ -96 Viral RNA isolation kit. The purified nucleic acid was then serially diluted to extinction and tested using both the VetMAX™ SIV screening reagents and the published assay protocol. SIV Cts using the VetMAX™ SIV screening reagents were 4-5Cts earlier than the published protocol and sensitivity was approximately 64-fold better. To ensure the specificity of the SIV screening assay, RNA from field samples containing other common porcine pathogens (PCV2, *M.Hyopneumoniae*, PRRSV) were purified using MagMAX™ -96 Viral RNA Isolation kit and tested with the VetMAX™ SIV screening reagents (n=3). No false positives were detected in development studies using the SIV assay.

The performance of the workflow was evaluated using field samples (n=60) of known SIV status (54 SIV positive and 6 SIV negative); diverse reference SIV genotypes were included to ensure functionality of genotyping assays. RNA was isolated from swine nasal swabs, tonsil and lung tissue homogenates, virus, and cell lysate supernatant using MagMAX™ -96 Viral RNA Isolation kit. The purified RNA was utilized for TaqMan® RT-PCR employing the SIV screening assay on the Applied Biosystems 7500 Fast Real-Time PCR System. SIV-positive field samples were then tested with the genotyping assays. The SIV screening and genotyping results showed 100% concordance with secondary laboratory PCR results. All SIV reference genotypes (H1N1, H1N2, and H3N2) were correctly identified. These results as well as the superior analytical sensitivity demonstrate that this method provides an economical and rapid solution for SIV identification.

The Iowa FAST Eggs Plan – A model for protecting poultry health, human health, and business continuity during a high path avian influenza outbreak

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The Iowa FAST Eggs Plan is a voluntary plan for commercial table egg producers intended to facilitate business continuity following an outbreak of high pathogenicity avian influenza virus (HPAI). Participation in the Iowa FAST Eggs Plan will reduce the time required for regulatory officials to determine that it is safe for eggs and egg products from non-infected premises located within a Control Area to move into market channels located outside the Control Area. The Iowa FAST Eggs Plan has 4 components. 1) A Biosecurity Checklist for Egg Production Premises which consists of a list of important biosecurity measures that will help prevent introduction of avian influenza virus onto an egg production premises. 2) Egg production premises must provide global positioning system coordinates to the state to verify the premises location. 3) Epidemiology data will allow investigators to determine if an egg farm has been exposed directly or indirectly to birds and other animals, products, materials, people, or aerosol from an infected premises. 4) Absence of HPAI virus on a FAST Eggs Plan premises will be documented by requiring a minimum of five dead chickens from daily mortality from each house on the farm to be tested each day and found to be negative by the RRT-PCR test.

A high level of biosecurity is necessary before approval to move eggs and egg products can be given, but biosecurity alone does not guarantee approval. Before Incident Commanders approve such movement, results of a) active and passive surveillance, b) geographic proximity to infected premises, and c) other pertinent factors will be considered. The Iowa FAST Eggs Plan Biosecurity Checklist for Egg Production Premises and Auditors consists of a list of important biosecurity measures that were selected on the basis of extensive input from egg producers, state and federal epidemiologists, and veterinarians employed by the egg industry, universities, and federal regulatory agencies. To participate in the FAST Eggs Plan, an egg production premises must utilize all biosecurity measures on the checklist.

Auditors are tasked with confirming the validity of biosecurity programs and submitting a written report of their findings to the State Veterinarian, federal Area Veterinarian in Charge (AVIC), and to the manager of the egg premises. The State Veterinarian and the AVIC will use this information to determine if the level of biosecurity is sufficient to qualify the premises for participation in the FAST Eggs Plan. An approved audit, no more than 6 months old, must be on file with the State Veterinarian and AVIC for an egg premises to participate in the Iowa FAST Eggs Plan. If a premise fails a biosecurity audit, the reason(s) for failure will be provided in writing to the farm manager. Farm managers then have the option of taking corrective action and requesting another audit.

As of April 1, the Iowa FAST Eggs Plan became operational. The goal of the Iowa FAST Eggs Plan is to allow non-infected premises to obtain permits to move eggs and egg products into market channels after eggs are held for 48 hours.

Isolation of *Avian influenza virus* from PCR negative cloacal samples of waterfowl

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Infection with influenza A viruses is a major concern of human and animal health officials. In particular, avian influenza virus (AIV) has become one of the most important zoonotic pathogens because of its potential to cause severe disease outbreaks in avian and human hosts. Since waterfowl are considered an important reservoir of AIV, studies are ongoing in several countries on AIV surveillance in wild birds. Virus isolation in specific-pathogen-free (SPF) embryonated chicken eggs (ECE) remains a gold standard technique for AIV detection. However, some laboratories prefer molecular methods such as real time reverse transcription-polymerase chain reaction (RRT-PCR) for initial sample screening because of their high throughput sample processing and rapid results. Samples found positive on RRT-PCR are then inoculated in ECE for virus isolation and characterization. This approach is based on the premise that RRT-PCR will detect all AIV-positive samples. We conducted this study to determine if AIV can be isolated from cloacal samples of waterfowl that were initially found to be negative by screening with RRT-PCR. We tested **1,369 RRT-PCR negative cloacal samples** for virus isolation in **commercial non-SPF eggs** and found **82 samples to be AIV positive** when infected allantoic fluids were tested by RRT-PCR. Of these, ten isolates were subtyped using gel-based RT-PCR methods. **These isolates included H7N2 (n=7), H7N1, H1N2, and H2N2. These results highlight the fact that isolation screening by RRT-PCR may result in some samples to be false negative for AIV.**

Reverse zoonotic transmission of pandemic *H1N1 influenza virus* infection in cats: Clinical disease and diagnostic approaches

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Background: Influenza viruses remain one of the most significant infectious diseases of One Health concern. The unknown host susceptibility range of the novel 2009 pandemic H1N1 virus (pH1N1) remains problematic. Moreover, atypical hosts can provide an opportunity for viral evolution or the development of a new reservoir. We recently reported the first confirmed case of pH1N1 influenza virus infection in an indoor domestic cat following presumptive human-to-cat transmission where influenza-like illness (ILI) had first occurred in two of three family members. This feline index case raised awareness of pH1N1 infection in household pets. To gain insight into reverse zoonosis of pH1N1 in companion animals, we employ a sero-molecular epidemiological study to better characterize clinical disease, facilitate diagnosis, monitor infection rates and evaluate viral evolution at the human-animal contact interface to advance the understanding of influenza virus evolution in populations.

Methods: Study populations include 1) clinical cases of companion animals experiencing upper and lower respiratory illness following ILI in human household contacts and 2) sera archived from companion animals visiting the ISU Veterinary Medical Center. In suspected clinical cases, broncho-alveolar lavage fluid or oro-pharyngeal swabs are tested with real time PCR and viral isolation. Viral genome sequencing is confirmatory for pH1N1 infection. Additionally, serum is tested to assess antibody responses with Influenza NP ELISA and specific hemagglutinin inhibition assays.

Results: Our epidemiological study has confirmed pH1N1 infection in four cats following apparent reverse zoonosis from humans. Clinical signs include lower and/or upper respiratory disease, lethargy and inappetance, 3-5 days following influenza like illness in humans. Several cats developed severe lower respiratory disease characterized by a bilateral dorsal alveolar pattern on radiography and had lymphopenia. Detection of pH1N1 influenza virus by real-time RT-PCR in upper airway secretions and bronchoalveolar lavage fluid indicated that these samples were diagnostically useful particularly in acute cases. Appropriate samples for antemortem diagnosis depend on the clinical signs (upper vs. lower respiratory disease), severity of signs and stage of disease. In cases where infection with pH1N1 is retrospectively suspected, serology is preferred over real-time RT-PCR as the period of virus shedding appears to correlate with apparent acute clinical disease. In two cats that died from pH1N1 infection, a virus was isolated from fresh lung tissue in the absence of other know respiratory pathogens. In a multicat household and in our retrospective study, serology aided in the presumptive diagnosis of H1N1 in seven cats. Importantly, radiographic and pathologic features of cats with pH1N1 viral pneumonia were consistent with small airway disease (bronchointerstitial pneumonia with severe alveolitis), and are very similar to the typical findings in humans with severe pH1N1 viral infection (Mauad et al, 2010). In two cats that died of bronchointerstitial pneumonia due to pH1N1 (from which virus was isolated), a severe bronchointerstitial pneumonia with extensive alveolitis was confirmed with histopathology.

Conclusion: Here we report on the clinical manifestations and diagnostic approaches for SpH1N1 infection in the atypical host, the domestic cat. Clinical disease in cats was associated with significant lower respiratory disease similar to findings in recent human cases, suggesting the discovery of a relevant animal model.

Pandemic 2009 (*H1N1) influenza virus* infection in commercial turkeys

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Pandemic (H1N1) 2009 influenza A virus (pH1N1) was initially detected in the United States in April 2009 in humans. In the subsequent year, the virus had been detected in multiple animal species including ferrets, cats and swine. This is a case report of the first confirmed case of pH1N1 influenza virus infection in a commercial turkey breeder flock in the United States following presumptive human to turkey transmission.

In November 2009, personnel with flu-like symptoms arrived to work at a commercial turkey farm in Virginia. Turkeys on the farm were inseminated, sampled for routine surveillance programs, and treated with antibiotics. Within a 2 week period of time, egg production within the turkey flock decreased by over 90%. There was no apparent clinical disease in the flock aside from the drastic decrease in egg production. Samples (swabs and serum) were collected and sent to the Virginia Department of Agriculture and Consumer Services in Harrisonburg, VA. Serum specimens were screened by the agar gel immunodiffusion assay and tracheal/oropharyngeal swabs were screened by the avian influenza matrix real-time RT-PCR assay. Presumptive positives subsequently were forwarded to the NVSL for confirmation of influenza. Real-time RT-PCR, virus isolation, and serology confirmed the presence of pH1N1 in the flock. **Of particular interest is the fact that the virus isolates did not hemagglutinate chicken red blood cells.** Phylogenetic analysis of all eight gene segments was performed to determine the virus lineage was pandemic origin. In February 2010, a commercial turkey flock in California was also infected with pH1N1. **These isolations emphasize the importance of biocontainment and biosecurity for susceptible commercial poultry in order to reduce the possibility of a reassortment event between avian influenza and pH1N1 viruses.**

Technological innovation in analytical departments

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Introduction: Since the early 90s, the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" has used an information system to support its diagnostic activities (SILAB), tracing samples from receipt to the final test report without reference to the specific test conditions. In 2009, analysis began of all the specific phases in the conduct of a given test with the aim of completely eliminating the use of paper (lab books, worksheets, work charts), on which the raw data is currently reported. This took place as part of a larger process of document dematerialisation, now one of the most significant elements in reforming the management of administrative activities in the digital age and one of the most important ways of reducing public expenditure.

Materials and Methods: The Institute's laboratories have been ISO/IEC 17025-accredited since 1995. The Institute currently carries out around 1600 different tests (bacteriological, anatomopathological, molecular biological, chemical, clinical, histological, physical, parasitological, virological and serological) in line with standard operating procedures (SOPs). As an individual analysis would be somewhat complex, the work charts were divided into 2 main groups: graphic and non-graphic. Each combination of test and method is matched to a single chart which itself refers to a methodology and an SOP. A single chart may be used for more tests. The chart is controlled through a revision number, a supervisor and a history of changes. Each chart not only gathers and saves the raw data but must also enable entry of the final result directly into SILAB, in such a way as to avoid duplicate entries and errors.

Graphic charts (such as 96-well microplate charts for ELISA or charts for AGID/gel diffusion) attribute a significance to position, making them ideal for transfer to touch screens, where the corresponding cell can be selected (positive/negative) through the pressure of a finger or pen.
Each chart is now filled in by the department technician by reading the barcode affixed to the sample.

Non-graphic charts show a high degree of variability. It was therefore decided to develop an application providing an area to design the chart as well as enabling data entry. In practice, the chart can be designed directly through the composition of previously defined base elements: a header, itself consisting of a fixed part, reporting the test - method, and a variable part, containing the sample reference/s; various operational phases, constructed using the predetermined data structures; entry of the final results (common to all charts) and any other fields to be reported in SILAB.

Results: A new graphic charts application was developed and integrated directly into SILAB; it is currently used for about 80% of tests carried out by the Serology Department at Head Office. Where the workbenches are arranged in groups in the center of the lab, touch screens have been installed which drop down from beams running across the ceiling. For most other departments using non-graphic charts, an extension of SILAB called SISCHEMI has been developed which is currently used in parallel at Head Office and is being tested at all other departments, albeit at different stages. By April 2010, 70 charts managing 103 non-serological tests had been produced.

Discussion/Conclusion: **The paper chart is generally used as the basic outline to which any necessary modifications can be added manually to adapt it to specific cases. In contrast, the computer charts, although flexible and parametric (being constructed automatically according to the number of samples and the specific assays making up the test), check respect of formal rules established in the design phase. For this reason a number of issues which could not be predicted on the basis of the paper model were reported; these were resolved through modification of SILAB as well as of the chart application. It was necessary to overcome an initial diffidence with regard to the new procedures, and above all provide the necessary hardware. It was decided to use monitors fixed to the wall, to avoid taking up workspace, with wireless and silicone IP-65 compliant keyboards to provide protection against wash down. Departments are also connected to a Wi-Fi network, enabling Internet cables to be avoided.**

Encephalitis and coronary arteritis in calves experimentally infected with an isolate of *Bovine enterovirus*-1

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The pathogenesis and virulence of *Bovine enterovirus*-1 (BEV-1) in cattle is largely unknown. Reports concerning its virulence suggest that there might be an association between BEV-1 infections and a range of diseases in cattle that vary from respiratory to enteric to reproductive disease and infertility. In this study, we characterized the pathologic changes associated with acute infection of BEV-1 in calves experimentally infected with the Oklahoma isolate of BEV-1. The virus localized to the terminal ileum, ileocecal and cecocolonic junctions, and spiral colon, and ileocecal lymph nodes; virus was detected in the cytoplasm of enterocytes, lamina propria macrophages, endothelium, neurons of the submucosal and myenteric plexi, and lymphocytes of the submucosal lymphoid tissue. Although no clinical signs were noted following acute infection, BEV-1 was localized in the cerebellar white matter of a calf with encephalitis and in the heart of another calf with coronary arteritis. **This study establishes that BEV-1 is infectious to young calves and that the virus produces lesions similar to enterovirus infections in other species.**

Infertility and abortion in a captive bison herd associated with *Bovine viral diarrhea virus*

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Bovine viral diarrhea virus (**BVDV**) is a common cause of infertility, abortions, stillbirths and weak calves in domestic cattle herds. Here we report infertility, abortions and neonatal calf deaths in a captive **bison** (*Bison bison*) herd associated with BVDV infection and proximity to domestic cattle. A captive bison herd had a history of reduced pregnancy rates (63 to 89%) for several years. Bison cows were vaccinated with 1 dose of an inactivated viral (IBR, BVD1/2, BRSV, PI3) vaccine, a 7-way *Clostridium spp*. toxoid and a *Pasteurella spp.* bacterin as weaned calves and at pregnancy examination. A serosurvey of 34 bison cows in Dec. 2008 indicated BVDV 1 serum neutralizing (SN) titers of <4 to 32 in 31 bison cows, 1 titer of >8192 and 2 titers of 2048. Since the herd history included the use of a MLV viral vaccine in replacement heifers in 2000, the high BVDV 1 SN titers in 3 bison cows were attributed to prior vaccination. However, potential sources of BVDV infection included a herd of domestic cattle on the same ranch as well as neighboring premises. In the summer of 2008, all bison and cattle were tested for BVDV PI by RT-PCR on pooled ear notches and were test negative. In 2009, bison bulls were tested for *Tritrichomonas fetus* and were test negative.

In Nov. 2009, 16 of 74 bison cows and 0 of 41 bison calves surveyed had BVDV 1 SN titers of >=512. An aborted bison fetus, of approximately 230 days of gestational age, from the main bison cow herd (which included the seropositive cows detected in Nov. 2009) was submitted in April 2010. The fetus was moderately autolyzed, exhibited mandibular brachygnathia, cortical thickening of the long bones with reduced medullary cavity size and moderate thymic atrophy. Fetal lung cells were detected positive for BVDV by FA and nitrate levels in the aqueous humor were <10 ppm. On histologic examination, there was mild lymphoplasmacytic epicarditis, and pneumonia with marked lymphocytolysis in the thymus. Two 1 week old bison calves from the heifer bison herd were found dead. On necropsy, one calf showed marked thymic atrophy while the other calf presented moderate erosive abomasitis with multifocal mucosal hemorrhages. Necrotizing enteritis with moderate to marked lymphoid depletion of the Peyer's patches was found in both calves. BVDV FA positive cells were detected in the thymus of one calf and the lymph node of the second calf. *Salmonella spp.* or other significant enteric bacteria were not cultured; BVD viruses were not isolated. In the same time period, the domestic cattle herd lost 30 calves due to diarrhea. This domestic cattle herd had fence contact with the bison heifer herd. An 8 day old calf was submitted for necropsy. The calf had reduced body condition and reddened, heavy lungs. The thoracic thymus was reduced in size and histologically there was marked cortical atrophy. Intestinal contents were rotavirus positive (ELISA); BVDV FA positive cells were seen in thymus and spleen. Culture of the abomasum yielded *Clostridium perfringens*.

Previous reports of seropositive bison in farmed and free-ranging bison herds, and BVDV isolations indicate that bison are susceptible to infection with BVDV. It is likely that BVDV infection of bison have similar clinical manifestations as in domestic cattle including reproductive and early calf losses. In theory, diagnostic tests for BVDV in cattle should be of utility in bison; however, validation of these tests has not been done for this species.

Bovine viral diarrhea virus (BVDV) in the United States, France, Spain and the United Kingdom: Vaccination and testing practices, cost, satisfaction and impact

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Introduction: IDEXX Laboratories manufactures and sells diagnostic tests used to detect bovine viral diarrhea virus (BVDV) in cattle. The purpose of this study was to better understand BVDV vaccination and testing practices among veterinarians in the United States (US), France (FR), Spain (ES) and the United Kingdom (UK). The study examined similarities and differences in the four countries in several areas: use, cost, satisfaction and turnaround time for current of BVDV tests and estimated impact of BVDV on cattle productivity.

Material and methods: Telephone interviews were conducted with 402 cattle veterinarians in the US, FR, ES and the UK by a third-party market research agency between January and March 2010. Respondents were selected from a random list and screened for eligibility based on a minimum number of cattle serviced by the veterinarian (1,000 head in the US and 500 in Europe).

The survey addressed the following topics: Percentage of veterinarians recommending or administering vaccines for BVDV, Percentage of cattle where the veterinarian recommends BVDV diagnostic testing, Percentage of cattle tested for BVDV, Amount paid by producers per BVDV test, Turnaround time for BVDV testing, Perceived cost per head of BVDV infection

Results: 98% of US veterinarians administer or recommend BVDV vaccinations (86% FR, 86% ES, 97% UK). US veterinarians estimate that 77% of cattle serviced in their practice are vaccinated for BVDV versus much lower percentages estimated by European veterinarians (11% FR, 38% ES, 37% UK). Among US veterinarians, 78% administer or recommend BVDV diagnostic tests (76% FR, 87% ES, 98% UK). US veterinarians estimate that 15% of cattle serviced in a year in their practice are tested for BVDV as compared to a wider range for Europe (4% FR, 17% ES, 14% UK). When asked the cost per head that a producer pays for a BVDV test, including the entire cost of the test and any labor, administration and shipping costs, US veterinarians estimate that producers pay $11.10 per test ($22.56 FR, $8.55 ES, $20.56 UK). Veterinarians in the US and Europe are generally satisfied with the BVDV tests they currently use. In total 37% of US veterinarians are “very satisfied” (50% FR, 20% ES, 36% UK) and an additional 50% are “somewhat satisfied” (40% FR, 69% ES, 55% UK) with current BVDV tests. However, veterinarians in the US and Europe are less satisfied with turnaround time for BVDV test results. Only 30% of US veterinarians are “very satisfied” (23% FR, 14% ES, 13% UK) and an additional 36% are “somewhat satisfied” (34% FR, 53% ES, 41% UK) with turnaround time for BVDV test results. Among US veterinarians, 74% report a turnaround time of five days or more from when a sample is collected until they receive BVDV test results back from the laboratory (86% FR, 85% ES, 78% UK). Veterinarians believe there is a significant financial impact of BVDV even in vaccinated cattle. US veterinarians estimate that BVDV costs an operation $30.55 per head for all animals across the entire herd in the form of lower productivity or mortality compared to a much wider range of estimated impact in Europe ($13.74 FR, $39.36, ES, $52.68 UK).

Significance: Even though a high proportion of veterinarians in all geographies recommend BVDV testing (76-98%), veterinarians estimate that only 4-15% of cattle serviced in their practice are actually tested. Producers in FR and the UK pay significantly more for a BVDV test than producers in the US and ES. Veterinarians in the US and Europe are generally satisfied with the BVDV tests. However, veterinarians are less satisfied with the turnaround time for BVDV test results. Most veterinarians (74-86%) reported an average turnaround time of five days or more from when a sample is collected until they receive BVDV test results. Even in vaccinated herds, veterinarians believe BVDV has a significant economic impact, ranging from $13.74 in FR to $52.68 in the UK per head across the entire herd.

Failed detection of *Bovine viral diarrhea virus* type II infection by direct FA

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There were two recent cases in which direct FA (FA) failed to detect BVDV II antigen in fresh tissues. In the first case, an adult beef breed cow in good body condition was found dead and presented for necropsy to Mississippi Veterinary Research and Diagnostic Laboratory (MVRDL). Multifocal shallow erosions and ulcers were appreciated in the oral cavity predominantly along the soft palate and tongue. Numerous erosions and shallow ulcers were also present along the entire length of the esophagus. The abomasal mucosa was multifocally reddened (hyperemic). Histopathology revealed multifocal ulcerative and lymphocytic stomatitis, multifocal ulcerative esophagitis, moderate eosinophilic and lymphocytic enteritis, and multifocal moderate to severe nonsuppurative perivascular encephalitis. Immunohistochemistry (IHC) revealed the presence of BVDV antigen in the epithleial cells lining tubuloacinar mucous and mixed glands of the oral mucosa. Rare peyer's patches in the small intestine also contained occasional positive staining macrophages. However, FA with proper positive and negative controls failed to detect BVDV antigen in these tissues. BVDV was isolated from the tissue pool and genotyped as BVDV II by a real time reverse transcriptase-polymerase chain reaction (RRT-PCR). In the second case, the lung tissue of a feedlot calf that had died following an episode of bloody diarrhea was submitted to MVRDL. BVDV II was isolated, but direct FA again failed to detect BVDV antigen in the tissue specimen. In conclusion, the results of these cases suggest a need for reevaluating direct FA-based detection of BVDV II.

High throughput PCR testing strategies for BVDV

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Reliable detection of bovine viral diarrhea virus (BVDV) is critical for maintaining the health of a herd. Assays used for detection of persistent infection (PI) include antigen capture ELISA, immunohistochemistry, and reverse transcriptase (RT) PCR. In this paper, data is presented to support the exclusive use of real- time RT PCR for detection of persistently infected animals from pooled as well as individual samples.

Sample types used to identify PI animals include serum, whole blood, and ear notch. Ear notch samples rapidly gained wide acceptance as the optimal sample for detection of persistent infection because these are easily obtained samples and because of presumed minimal amounts of maternal derived antibody. Our BVDV testing strategy was validated using a pool size of 24 for either ear notches, serum or whole blood. This pool size maximized sensitivity and cost reduction when using magnetic bead extraction and a highly optimized real-time RT PCR. Individual samples within a positive pool were tested by both antigen capture ELISA and real-time RT PCR. Veterinarians were requested to re-test positive animals and submit whole blood and ear notches no earlier than 4 weeks after the original sample collection. Individual testing by ELISA revealed that ear notch samples from young animals may have significant amounts of antibody eluting from the ear which can mask detection of viral antigen. Individual PCR reactions eliminate this problem due to removal of protein in the extraction step. Results also show that correlation of pool CT with prediction of a persistently infected animal within the pool was not reliable. However individual real-time PCR CT results were predictive of persistently infected animals. Those samples with CT levels of < 24 were usually confirmed to be persistently infected. Individual samples with CT levels 30 or greater usually cleared by 4 weeks indicating either environmental contamination or acute infection. Some clients had difficulty taking clean samples with an ear notcher as evidenced by a high percentage of positive pools ultimately ascribed to environmental contamination. Whole blood sampling was instituted for these farms and this significantly reduced the “false” positive rate. Continual client education plays a critical role in a successful PCR testing program.

Bovine viral diarrhea virus modulation of monocyte derived macrophage

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Bovine Viral Diarrhea Virus (BVDV) is a single stranded, positive sense virus of the *Flaviviridae* family and is the causative agent of the disease known as Bovine Viral Diarrhea (BVD). Disease can range from persistently infected (PI) animals displaying no clinical symptoms of disease to an acute, severe sickness, with bloody diarrhea, high fever, mouth ulcers, and pneumonia. PI animals are the result of infection with a noncytopathic (NCP) BVDV in utero, which results in immune tolerance, and lifelong infection. Superinfection of PI animals with cytopathic (CP) virus, may lead to a fatal syndrome called mucosal disease. The role of BVDV as primarily a respiratory pathogen remains controversial. Experimental studies have indicated mild clinical respiratory disease and interstitial pneumonia induced in 4- to 6-month-old calves inoculated with BVDV. However, most studies have focused on BVDV1 role in immunosuppression. Limited studies to date have focused on the macrophage cytokine response to BVDV2. Therefore, we have compared macrophage cytokine responses to four strains of BVDV2 of differing biotype and virulence. MDMs displayed an altered cytokine response after inoculation with the four strains of BVDV2. With the cytopathic and high virulence BVDV2 strains, we observed a greater initial induction (2 hours post inoculation) of pro-inflammatory cytokines such as IL-1ß, IL-12p40 and TNF-α. However, this response is not sustained, and a significant decrease in transcription between 4-6 hours post inoculation was observed. Furthermore, results of others have indicated that gene transcription of Major Histocompability Complex (MHC) II and CD80/86 of monocytes inoculated with BVDV1 were down regulated by 24 hours post inoculation. MDMs are professional antigen presenting cells (APC) with the ability to activate an adaptive response via MHC class II expression and proper co-stimulation to T cells. Our results and those of others, suggest that **BVDV may alter the ability of antigen presenting cells to prime a productive T cell response by modulating cytokine, MHC class II and CD80/86 expression**. Loss of function in APCs is one means by which BVDV could modulate the immune response to other pathogens. These studies indicate that infection of MDM with BVDV can be used as an in vitro model to study alterations in immunoregulatory pathways that leads to immunosuppression.

AAVLD 2010 EXHIBITORS

Minneapolis grand ballroom

Third floor
Salons E,F,G

Exhibit Hall Times

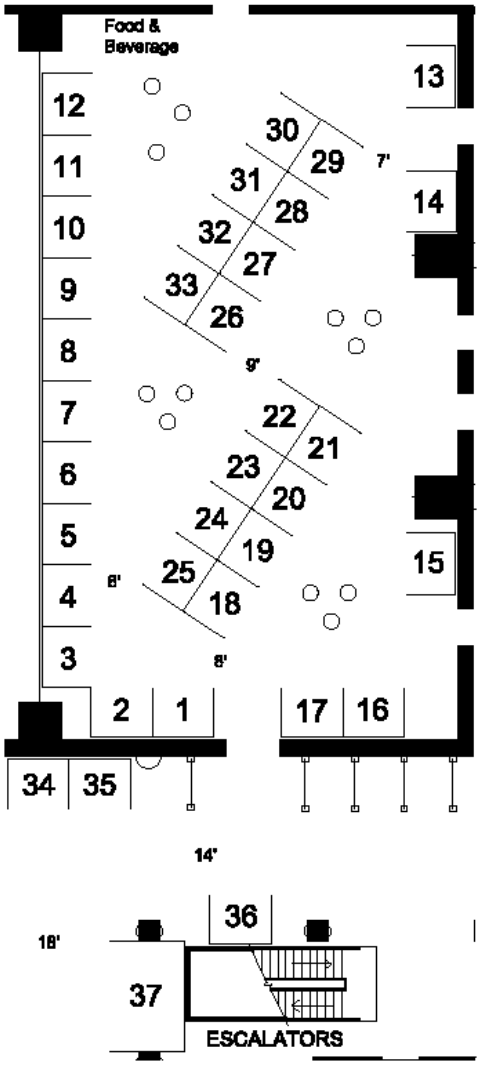
Saturday, November 13

9:30 am – 6:00 pm

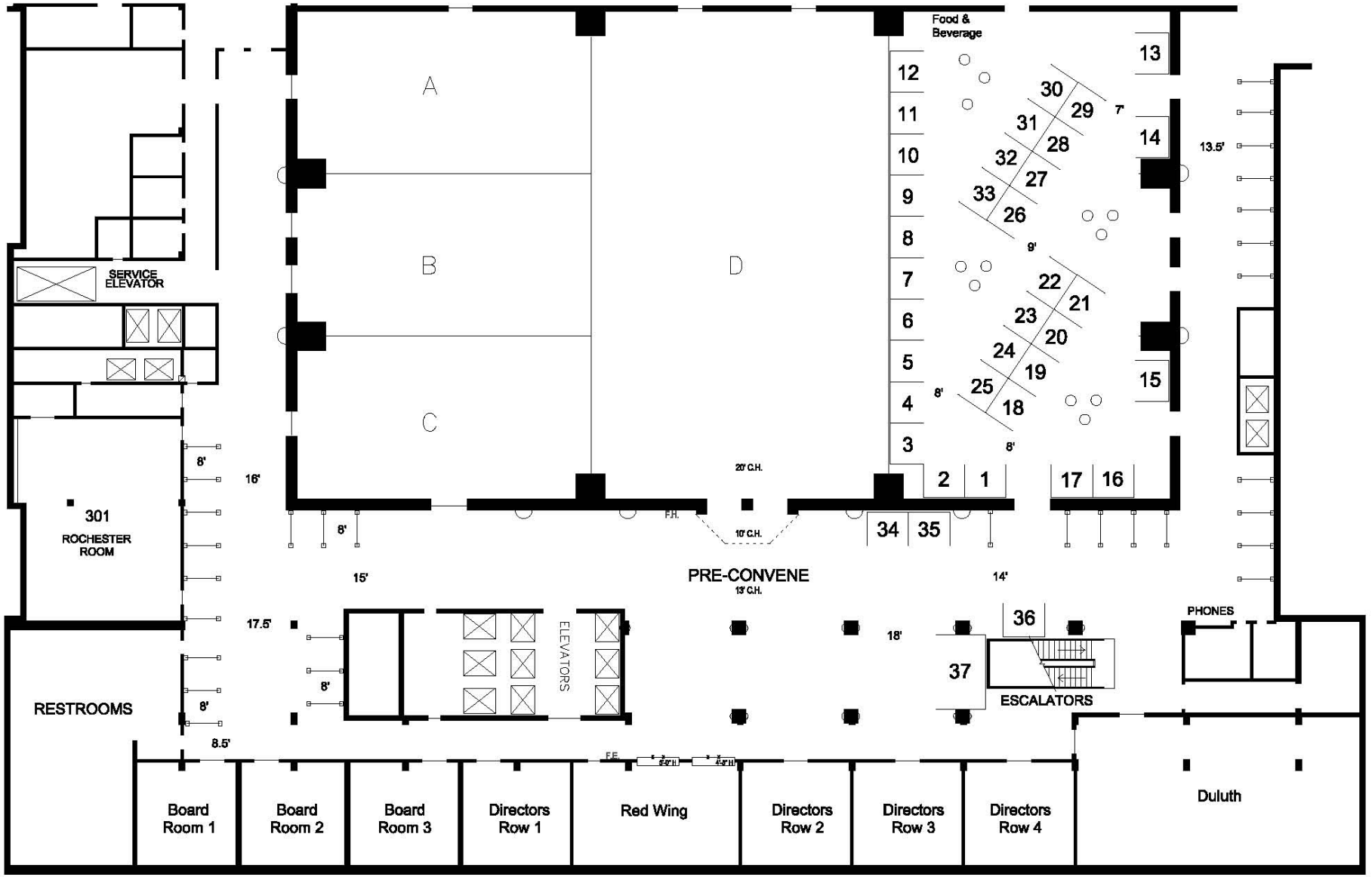
Sunday, November 14

7:00 am – 2:00 pm

Booth	Exhibitor
1	Advanced Technology Corp. VADDS
2	Anaerobe Systems
3	Prionics, USA, Inc.
4	Advanced Animal Diagnostics (Entira)
5	Progressive Recovery, Inc. (PRI)
6	Abaxis, Inc. - Animal Health
7	ID Vet
9	Elsevier/Saunders/Mosby
10	CDC Epi-X
11	VertiQ Software LLC
12	Thermo Fisher Scientific, Inc
14	Automated Technologies Inc. (ATI)
15	Centaur, Inc.
16	Merrick and Company
17	Luminex Corporation
18	Ventana Medical Systems, Inc.
19-20	Computer Aid, Inc.
21	Tetracore, Inc.
22	National Institute for Animal Agriculture (NIAA)
23	Bruker Daltonics
24	Biovet, Inc.
25	Global Vet Link
26	Synbiotics Corporation
27-28	Qiagen, Inc.
29	TREK Diagnostic Systems
30	VMRD, Inc.
31	UltraPath Imaging Clinic, Inc.
32	Hardy Diagnostics
33	Biosearch Technologies, Inc.
34-35	IDEXX
36	NCE-Crawford-Emcotek
37	Applied Biosystems Animal Health



American Association of Veterinary Laboratory Diagnosticians
November 12-14, 2010



Minneapolis Hilton Hotel
Minneapolis, Minnesota

Drawing History		
Initial Date:	1-20-10	Joe Conn
Revision:	6-23-09	Joe Conn
Revision:	8-26-10	Joe Conn
Revision:		

Scale

36 - 8' x 10' Booths
1 - 12' x 17.5' Booth
31 - 4' x 8' Posterboards

Exhibit Services, Inc.
1814 Tappan Blvd.
Tampa, Florida 33619
Ph. 813.623.1163
Fax 813.623.5913

American Association of
Veterinary Laboratory Diagnosticians

Exhibit Directory

Hilton, Minneapolis, MN
November 12-14, 2010

Abaxis, Inc

Booth **6**

3240 Whipple Rd
Union City, CA 94587
www.abaxis.com
Contact: 800.822.2947
vetscansales@abaxis.com

Abaxis offers a comprehensive suite of VetScan in-office laboratory equipment for the animal health market. And all of these products deliver the universal VetScan promise of speed, accuracy, simplicity, ergonomics and economics. Your lab could be Better. Actually.

Advanced Animal Diagnostics (Entira)

Booth **4**

1912 Hwy 54 East, Suite 205
Durham, NC 27713
www.advancedanimaldiagnostics.com
Contact: Joy Parr Drach
919-544-0089
joy@aadiagnostics.com

Advanced Animal Diagnostics (AAD) is an innovative R&D company focused on bringing highly accurate, rapid animal diagnostic tests to the farm. Our initial focus is on the early diagnosis and detection of mastitis in dairy cows. AAD's technology uses the differential inflammatory cell count that relies on the body's own immune system to detect mastitis at a subclinical level earlier and more reliably than traditional methods even in colostrum. AAD has introduced QuickSmear™, a revolutionary rapid differential slide that replaces manual milk differential smears in research settings. QuickSmear delivers reliable, easy-to-read results in less than a minute compared to the time-consuming, labor intensive manual smear preparation. QuickSmear slide technology will be demonstrated at the company's AAVLD booth.

Advanced Technology Corp. VADDS

Booth **1**

79 North Franklin Turnpike, Suite 103
Ramsey, NJ 07446
www.vetstar.com
Contact: Joseph Bove
201.934.7127
jbove@vetstar.com

VADDS – the value adding LIMS system

Advanced Technology Corp is pleased to invite you to visit our booth. We specialize in providing a comprehensive veterinary LIMS system for an affordable price.

VADDS is the number one LIMS system among public veterinary labs in North America.
VADDS is the most widely used LIMS system among AAVLD accredited labs.
There are now nearly 30 laboratories using **VADDS.**
New in 2010:

- Streamlined Instrument Interfaces
- Rapid Implementation Time
- Improved Instrument Interfaces

We look forward to continuing to work with the veterinary community in order to offer the best value LIMS system for many years to come.

Please don't spend more to end up with less!

EXHIBIT HOURS

Saturday, November 13
9:30am – 6:00pm

Sunday, November 14
7:00am – 2:00pm

Anaerobe Systems

Booth **2**

15906 Concord Circle
Morgan Hill, CA 95037
www.anaerobesystems.com
Contact: Steve Cox
408.782.7557
stevecox@anaerobesystems.com

Anaerobe Systems produces true Pre-Reduced Anaerobically Sterilized (PRAS) plated and tubed media. Benefits of using PRAS media include reduced time handling cultures, substantially increased recovery, and rapid diagnosis. Anaerobe Systems will display our new AS-580 Gloveless Anaerobic Chamber, PRAS culture media, and educational materials for anaerobic bacteriology.

Applied Biosystems by Life Technologies

Booth **37**

2130 Woodward Street
Austin, TX 78744
www.appliedbiosystems.com/animalhealth
Contact: John El-Attrache
512.651.0200
john.el-attrache@lifetech.com

We know in the production animal health industry, the results you report can have a profound impact on your customer's business and on your reputation. False positives can cause problems for the producer; false negatives can be catastrophic for the industry. That's why Applied Biosystems is focused on providing products that help to instill confidence. Committed to the animal health industry, we provide products and services designed to help you adapt and succeed in an evolving environment. Our next-generation molecular testing tools include automated sample prep, superior molecular reagents and master mixes, and easy-to use instruments for PCR analysis. Find the confidence you're looking for—explore molecular technology for animal health at booth 37.

Automated Technologies, Inc. (ATI)

Booth **14**

1663 South Atherton Street, Suite 3
State College, PA 16801
www.limspro.com
www.atiinternational.com
Contact: Joseph Akl
814.237.3001
jrakl@atiinternational.com

ATI LIMS PRO continues to raise the bar with an innovative Ver 10.0 of our versatile Laboratory Information Management System (LIMS) in use at nine laboratories. Experience the power of a multi-tasking, multi-threaded LIMS. Marvel at the ease of generating professional looking reports, the power of data mining, the simplicity of conducting ad-hoc queries, the customized worklists and worksheets. You can also associate photos, voice recordings, even video with your cases and warehouse, track and print photographs along with your reports. All of this plus daily /weekly /monthly /yearly workload reporting, quality assurance reporting, turn-around time metrics, and much more. And let's not forget the customized billing, invoicing, and accounts receivables. We provide you with customized LIMS for your laboratory preconfigured on a server for a set fee. Please stop by our booth for a demo and speak with one of our representatives about how you can take advantage of our all inclusive (Server, Software, Customizations & Training) one price offering.

Biosearch Technologies, Inc.

Booth **33**

81 Digital Drive
Novato, CA 94949-5728
www.biosearchtech.com
info@biosearchtech.com
Contact: Marc Beal
800.436.6631 (U.S. and Canada)
+1.415.883.8400 (World wide)
Marcb@biosearchtech.com

Biosearch Technologies, Inc., located in northern California, is an ISO 9001:2008 certified manufacturer of sophisticated oligonucleotides for real-time qPCR applications. Since its founding in 1993, Biosearch has been at the forefront of advancing nucleic acid technology with the invention of CAL Fluor® and Quasar® fluorophores, and the Black Hole Quencher® (BHQ®) series of dyes – the industry standard in full spectrum quenchers for real time qPCR.

Biosearch supplies businesses with custom oligos for all stages of product development from design through commercialization. A vertically integrated structure allows Biosearch to precisely control the raw materials and manufacturing process, reducing costs and turnaround time.

Biosearch produces a variety of probe formats, including Dual-Labeled BHQ® Probes, BHQplus™ Probes, Molecular Beacons, and Scorpions® Primers. Picking the right probes and primers is made easy through Biosearch's free RealTimeDesign™ software, a web-based qPCR assay design application for gene expression analysis, SNP genotyping, and multiplexed qPCR.

Biovet

Booth **24**

9025 Penn Avenue South
Minneapolis, MN 55431
www.biovet-inc.com
Contact: Stephanie Unverferth or Sheila Braun
877,824.6838
biovetusa@biovet-inc.com

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

Bruker Daltonics

Booth **23**

40 Manning Road
Billerica, MA 01821
www.bdal.com
Contact: Nancy Salt
978.663.3660 x1492
nrs@bdal.com

Bruker Daltonics is a leading provider of analytical systems for key emerging molecular diagnostic applications. Driven by our innovative, easy-to-use and cost-effective systems for microbial identification, molecular histology and toxicity assessment, we provide the latest in high performance and high value systems for the clinical research laboratory.

CDC/EPI-X

Booth **10**

1600 Clifton Road NE M/S D-40
Atlanta, GA 30333
https://expi.cdc.gov
Contact: Amanda Evanson
404.639.5049
aevanson@cdc.govm

Epi-X is CDC's secure, web-based communications and alerting system that keeps frontline public health officials informed about developing health threats. The system rapidly establishes secure communication channels between its users, providing a safe way to exchange confidential information. *Epi-X* helps public health officials stay informed, inform others, and share expertise.

Centaur, Inc.

Booth **15**

PO Box 25667
Overland Park, KS 66225-5667
www.centaurunavet.com
Contact: Mark Metrokotsas
913.390.6184
centaurunavet@aol.com

Please stop by Centaur, Inc.'s booth to see what new products we are able to introduce of those that are currently awaiting USDA approval. We intend to add to our existing participation in the equine market with our equine infectious anemia and foal IgG tests, the TB market for non-human primates, the small animal market with our heartworm test, and our multi-species blood analyte tests.

Centaur continues to produce a number of pharmaceutical products under FDA guidelines in categories that include sanitizers and disinfectants (one of which is our DEA approved 7% iodine formulation), skin and wound products, bulk liquids to treat metabolic disorders, to serve as laxatives, to preserve tissues and to serve as delivery vehicles for custom compounded medicinals. In addition, Centaur has expanded to include contract manufacturing of FDA and non-regulated medicinals for select customers.

Computer Aid, Inc.

Booth **19-20**

470 Friendship Road
Harrisburg, PA 17111
www.compaid.com
Contact: John Kucek
717.856.8294
john_kucek@compaid.com

Computer Aid, Inc. (CAI) is a $275 million information technology (IT) firm based in Pennsylvania. We have supported public-sector clients since 1991; today support State agencies from coast to coast, as well as Federal government. In a time of dwindling IT dollars and staff, we have teamed with our public-sector clients to redeploy software across similar agencies, resulting in lowered costs for all. Specifically in the field of agriculture information, in 2006 the Pennsylvania Department of Agriculture (PDA) and other agriculture agencies formed the Animal Health Information Management Consortium (AHIMC) specifically to share and expand on the capabilities of PDA's state-of-the-art Microsoft .NET database system. USALIMS is a web-based smart client tool that features automated client report generation, distribution and auditing, advanced search options, NAHLN interface, and client account and billing management. USAHERDS is a web-based system for complete program management, animal traceability, LIMS integration, radius reporting, and much more.

GlobalVetLink

Booth **25**

2520 N Loop Drive, Suite 7100
Ames, IA 50010
www.globalvetlink.com
Contact: Kaylen Henry
515.296.0860
khenry@globalvetlink.com

Company Representatives: Kevin Maher and Kaylen Henry

GlobalVetLink (GVL®), the innovator in electronic animal health documentation, is connected with diagnostic laboratories across the US. GVL offers a system for online diagnostic laboratory test submittal and resulting that replaces the paper intensive EIA process. GlobalVetLink offers veterinarians, state animal health authorities, and diagnostic laboratories a solution to collaborate within a secure, web-based platform. Our applications include certificates of veterinary inspection (approved by all 50 states), online EIA system, Equine Passport Permits (in specified regions), animal movement data, veterinary feed directives, and more…all by the click of a mouse. Fast, secure, online....we make paperwork, paperless.

Hardy Diagnostics

Booth **32**

1430 West McCoy Lane
Santa Maria, CA 93455
Contact: Christopher A Catani, (ASCP), Rm(ACM)
800.266.2222
sales@HardyDiagnostics.com

If you are doing any microbiology in your laboratory, please stop by our booth and learn how Hardy Diagnostics can help you with the microbiology supply needs, and pick up a free veterinary microbiology catalog.

Hardy Diagnostics offers over 10,000 microbiology supplies in total and has been in business for over 30 years. Visit our website www.HardyDiagnostics.com and become a customer today, and experience the Hardy Diagnostics difference. “A Culture of Service”

IDEXX Laboratories

Booths **34-35**

One Idexx Drive
Westbrook, ME 04092
www.idexx.com/production
Contact: Mary Spear
207.856.8059 or 207.749.4117
mary-spear@idexx.com

About IDEXX Livestock and Poultry Diagnostics
IDEXX Livestock and Poultry Diagnostics is the worldwide leader in diagnostic tests for livestock and poultry. For over 25 years, animal health professionals have turned to IDEXX for proven diagnostics, information-technology and equipment solutions. The IDEXX portfolio addresses more than 50 pathologies effecting bovine, small ruminant, porcine, poultry and equine populations. For more information, please contact us at phone: (800) 548-9997 or (207) 556-4300; email: LPDweb@idexx.com; or visit our website at www.idexx.com/production.

ID Vet

Booth **7**

France
www.id-vet.com
Contact: idvet.info@id-vet.com

ID VET develops and produces ELISA kits for the detection of infectious veterinary diseases. Created in 2004 by Philippe Pourquier, IDVET is now a leader in veterinary diagnostics in Europe. http://www.id.-vet.com/http://www.id.-vet.com/

In addition to a full range of ELISAs for classical livestock diseases, IDVET proposes innovative ELISAs for emerging pathologies, including one-of-a-kind tests for Equine Virale Arteritis, West Nile Fever, APP (all serotypes), Rift Valley Fever, and Influenza H5, H7, N1, and N2.

These kits are not USDA registered but may be obtained with import permits.

IDVET will consider any proposal to introduce itself into the American market in upcoming years.

Please feel free to contact us by email - mailto:idvet.info@id-vet.commailto:idvet.info@id-vet.comidvet.info@id-vet.com - or visit us in the Exhibit Hall – Booth 7.

AAVLD/USAHA Upcoming Meetings

2011: September 28–October 5
Buffalo, New York

2012: October 17-24
Greensboro, North Carolina

Luminex Corporation

Booth **17**

12212 Technology Blvd
Austin, TX 78727
www.luminexcorp.com
Contact: Dawn Fredericy
512.219.8020
dawn@luminexcorp.com

Luminex Corporation develops, manufactures and markets proprietary biological testing technologies with applications throughout the diagnostic and life sciences industries. The Company's xMAP® multiplex solutions include an open-architecture, multi-analyte technology platform that delivers fast, accurate and cost-effective bioassay results to markets as diverse as pharmaceutical drug discovery, clinical diagnostics and biomedical research, including the genomics and proteomics markets. The Company's xMAP® Technology is sold worldwide and is already in use in leading clinical laboratories as well as major pharmaceutical, diagnostic and biotechnology companies. Further information on Luminex Corporation or xMAP® can be obtained at www.luminexcorp.com.

Merrick and Company

Booth **16**

2450 S Peoria Street
Aurora, CO 80014-5475
www.merrick.com
Contact: Ross Graham, DVM, PhD
703.680.6086
ross.graham@merrick.com

Merrick and Company is an employee-owned, national architectural and engineering (A/E) design firm with over 400 employees headquartered in Colorado with offices in New Mexico, Georgia and Canada. Founded in 1955, Merrick provides full service architectural and engineering, construction, project management, laboratory commissioning services to federal clients including the USDA, DOD, DOE and DHS as well as universities and institutions, international and private clients. In addition, Merrick provides consulting services on laboratory setup and operations, biosafety, facility/program risk assessments, laboratory facility operation, including biowaste disposal.

We have been a single-source provider of services for animal, human and plant health analytic, research laboratories and high containment facilities for over 20 years. We are committed to sustainable green design practices and our design firm has consistently implemented sustainable design principles in not only energy conservation, but pollution prevention, waste reduction and recycled materials on all laboratory designs.

National Institute for Animal Agriculture

Booth **22**

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 source for individuals, organizations, and the entire animal agriculture industry to receive information, education and solutions for animal agriculture challenges. NIAA accomplishes this by coordinating and promoting industry programs and materials that assist animal agriculture professionals in addressing emerging and current 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.

NCE-Crawford-Emcotek

Booth **36**

6250 Hazeltine National Drive, Suite 116
Orlando, FL 32822
www.animal-cremation.com
Contact: Brian Gamage
800.228.0884 x1891
bgamage@nce-crawford.com

Since 1974, NCE-Crawford-Emcotek has equipped thousands of animal care, research, and control facilities with incineration/cremation systems which meet their unique operational demands and professional standards. Everyone of our incineration systems meets the most stringent Federal EPA and State environmental requirements. Underwriters Laboratories (UL) has thoroughly tested and approvedmany of our products for your peace of mind and safety. We offer both "random feed" and "batch loading" incineration systems, ideally designed to perform large carcass and communal disposals as well as individual cremations. Visit our booth and speak with our representative to receive your free brochure and product literature.

Prionics USA, Inc.

Booth **3**

9810 Hupp Drive
La Vista, NE 68128
www.prionics.com
Contact: Tom Kellner
402.212.5126
thomas.kellner@prionics.com

Based in Zurich, Switzerland, Prionics is one of the world's leading providers of farm animal diagnostic solutions and is a recognized center of expertise in BSE and prion diseases. Founded in 1997, Prionics researches and markets innovative diagnostic solutions for major farm animal diseases; thereby making a major contribution to the protection of consumer health.

In 2005, Prionics acquired Pfizer Animal Health's diagnostic portfolio and, in 2006, entered into a strategic partnership with the Animal Science Group of the University of Wageningen (Netherlands). With the acquisition in 2009 of the tuberculin business of former Lelystad Biologicals, Prionics is one of largest providers of bovine TB diagnostic solutions worldwide. Winner of the Swiss Economic Award for "Company of the year" (2002) and the European Biotech Award for "Excellence in Biotech Business" (2004), in 2006 Prionics was nominated as the world's best animal health company.

The Company operates R&D facilities in Switzerland and the Netherlands and has regional hubs in key markets such as Germany, Italy, the Netherlands, and the USA. Prionics is also represented by distribution partners around the world.

Progressive Recovery, Inc. (PRI)

Booth **5**

700 Industrial Drive
Dupo, IL 62239
www.pri-bio.com
www.progressive-recovery.com
Contact: Shanon Jones
618.286.5000
sjones@progressiverecovery.com

Progressive Recovery, Inc. is a global company, providing your single source for engineering and manufacturing of Biowaste / Effluent Decontamination Systems and Caustic Digester Units for carcass disposal. PRI's systems represent the final boundary in sterilization treatment of liquid and solid wastes before discharge to the environment.

Located in Dupo, Illinois, PRI has more than 25 years experience in modular equipment design and fluid processing. Our unique EDS designs have been used in BSL-4, BSL-3 AG, and BSL-3 Enhanced and BSL-3 facilities since 1999.
PRI is even more responsive to client needs since the completion of an 18,000 sq ft addition to the manufacturing facility. The new shop is capable of manufacturing individual units up to 30,000 gallon capacity. This addition improves material handling and reduces manpower to allow a more efficient and faster production schedule; meeting clients' deadlines.

Quality equipment, manufacturing excellence, and engineering support are recognized with PRI's name and history. www.pri-bio.com

Qiagen, Inc.

Booths **27-28**

19300 Germantown Road
Germantown, MD 20874
www.qiagen.com
Contact: Pam Daniels
240.686.7688
pam.daniels@qiagen.com

QIAGEN Inc., offering over 500 products, is the worldwide leader of sample and assay technologies for research in life sciences, applied testing and molecular diagnostics.

Saunders/Mosby

Booth **9**

2020 77 Street
Somerset, WI 54025-7447
Contact: Darrin Swanson
888.874.5874
d.swanson@elsevier.com

Providing books, media and journals for veterinary professionals.

Synbiotics Corporation

Booth **26**

12200 NW Ambassador, Suite 101
Kansas City, MO 64163
www.synbiotics.com
Contact: John Trabucco
800.228.4305
johnt@synbiotics.com

Synbiotics Corporation is a leading developer, manufacturer, and marketer of veterinary diagnostics and services for veterinary practices, reference laboratories, and animal breeders world-wide. Our core competencies include immunology, immunodiagnostics, and reproduction.
We manufacture at two sites: San Diego, California and Lyon, France. Both facilities are permitted by the United States Department of Agriculture (USDA) and are ISO 9001:2000 certified.

Our product range covers multiple-species including canine, feline, equine, swine, bovine, avian and non-human primate. Synbiotics product lines provide excellent sensitivity, specificity, repeatability and ease of use; many are considered to be gold standards in the industry.

Our most popular companion animal product lines are DiroCHECK®, WITNESS®, ViraCHEK®, TiterCHEK®, LAB-EZ®, ASSURE®, D-TEC® CB and FUNGASSAY®. For production animals, ProFLOCK® and SERELISA® kits are widely used in more than 70 countries.

We strive to be your preferred diagnostic resource by providing you with scientific excellence, innovative products and customer-centric service.

Tetracore, Inc.

Booth **21**

9901 Belward Campus Drive, #300
Rockville, MD 20850
www.tetracore.com
Contact: Brian K. Kijowski
240.268.5417
bkijowski@tetracore.com

Company Representatives: Dr. William Nelson, Dr. Beverly Mangold, Tracy Fecteau, John Kelly, and Brian Kijowski
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 *Classical swine fever virus* (CSFV), in addition tospecific detection reagents for *Porcine reproductive and respiratory syndrome virus* (PRRSV), *Foot-and-mouth disease virus* (FMDV), *West Nile virus*, *African swine fever virus* (ASFV) and others. We are also proud to announce the TCOR4 – an all-new real-time PCR thermocycler. Featuring four sample wells and multiplex capability, it is small, highly portable, completely self contained, and has an 8-hour (rechargeable) battery life.

Thermo Fisher Scientific, Microbiology

Booth **12**

12150 Santa Fe Trail Drive
Lenexa, KS 66215
www.thermofisher.com
www.remel.com
Contact: Rich St. Clair
913.895.4256
rich.stclair@thermofisher.com
Thermo Fisher Scientific Microbiology Division, is a customer-oriented manufacturer and distributor of quality microbiology products and now offers the PathoProof™ Bovine Mastitis Compete-12 and PathoProof™ Bovine Mastitis Major-3 kits. Results are available within 4 hours for mastitis-causing organisms.From sample collection through identification, we provide a complete line of Remel and Oxoid branded products routinely available for next-day delivery from local distribution centers across the U.S. In addition to the PathoProof™ assays, we feature dilution bottles, Prepared and Dehydrated Culture Media and Quality Control organisms

TREK Diagnostic Systems

Booth **29**

982 Keynote Circle, Suite 6
Cleveland, OH 44131
www.trekds.com
Contact: 1.800.871.8909
info@trekds.com

TREK Diagnostic Systems is a dedicated microbiology company, providing innovative, automated products to the microbiology laboratory. TREK's products provide cost-effective improvements in workflow efficiencies, ergonomics, and test result performance. Signature products include the VersaTREK® Automated Microbial Detection System, Sensititre ARIS® 2X Microbiology System, and the Vizion® Digital Imaging System.

UltraPath Imaging Clinic, Inc

Booth **31**

2228 Page Road, Suite 106, Brassfield Center 1
Durham, NC 27703
www.ultrapathimaging.com
Contact: Connie Cummings
919.598.7500
connie@ultrapathimaging.com

UltraPath Imaging Clinic, Inc. is located in Research Triangle Park, North Carolina. We accept specimens shipped from anywhere around the globe. We offer Transmission electron microscopy and scanning electron microscopy to pathologists and veterinarians for those hard to diagnose cases or unique cases that come along that regular light microscopy just can't diagnose.

Ventana Medical Systems, Inc.

Booth **18**

1910 Innovation Park Drive
Tucson, AZ 85755
www.ventantamed.com
Contact: Tom DesRoches
520.730.2739
tom.desroches@ventanamed.com

Innovations in Science and Medicine That Improve the Quality of Life

Ventana Medical is one of the world's leading developers and manufacturers of medical diagnostic instrument and reagent systems providing leading-edge automation technology for use in slide-based diagnosis of cancer and infectious disease. Our products are found in hospital-based histology laboratories, independent reference laboratories, and the drug discovery laboratories of some of the world's largest pharmaceutical and biotechnology companies, government labs, veterinary labs, and medical research centers. We serve our customers through wholly owned subsidiaries in the USA, Europe, Japan and Australia, and our instruments are installed in no fewer than 55 countries.

VertiQ Software LLC

Booth **11**

18525 Sutter Blvd, Suite 280
Morgan Hill, CA 95037
www.vertiq.com
Contact: Paula Lomanto
408.778.0608
paula@vertiq.com

VertiQ (the leader in Medical Examiner case management software) has developed a special LIMS system for animal disease and diagnostic laboratories. Designed and in use by San Diego County ADDL, the system is based on the same toolkit that has been used successfully for many years for Medical Examiners and forensic laboratories.

A key component of the system is the ease of customization. Every laboratory works in a different fashion from other agencies in their field so customizing a system to fit the specific internal needs of the laboratory is very important.
VQ-LIMS-ADDL includes the following modules: Administrative (including Billing and Accounts Receivable), Necropsy and Laboratory Functions. It also includes a tickler system, internal communications, task management, reports, barcode chain of custody, image and document linking. Pricing upon request.

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www.vmrd.com
Contact: Luke Brown
509.334.5815
luke@vmrd.com

VMRD, Inc., a family business that was started in Pullman, Washington, in 1980, develops and manufactures high-quality, USDA-licensed diagnostic test kits for infectious disease agents such as *Anaplasma*, BLV, BTV, CAEV, EIAV, MCFV, *Babesia caballi* and *Babesia equi* and *Neospora*; research reagents such as FA conjugates, IFA slides and controls, as well as a wide selection of monoclonal and polyclonal antibodies against infectious agents, immunoglobulins, CD markers, MHC antigens, cytokines, and hormones. VMRD also produces an array of RID kits for quantitative analysis of immunoglobulin concentrations in many species, and other immunological assays such as Coombs' and FPT kits. VMRD's commitment to high-quality products, friendly customer service, and excellent technical support has contributed to our continual growth. Visit www.vmrd.com for more information.

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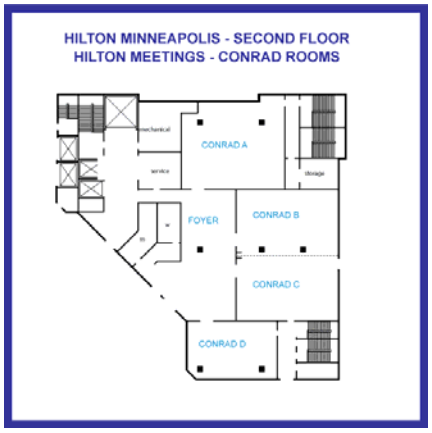
Minneapolis
Grand Ballroom
Salons A,B,C
Salon D
Salons E,F,G

1. Rochester
2. Board Room 1
3. Board Room 2
4. Board Room 3
5. Director's Row 1
6. Red Wing Room
7. Director's Row 2
8. Director's Row 3
9. Director's Row 4

1. Marquette Ballroom
1a. Marquette I
1b. Marquette II
1c. Marquette III
1d. Marquette IV
1e. Marquette V
1f. Marquette VI
1g. Marquette VII
1h. Marquette VIII
1i. Marquette IX

2. Symphony Ballroom
2a. Symphony Ballroom I
2b. Symphony Ballroom II
2c. Symphony Ballroom III
2d. Symphony Ballroom IV

Conrad Foyer
Conrad A,B,C,D



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Saturday – November 13, 2010

Computer Aid, Inc.	6:00-7:00 pm	Salon A	National Agribusiness Technology Center (NATC) - The Complete Animal Health Surveillance Solution; AGRAGuard featuring USALims and USAHerds
Applied Biosystems, Inc.	6:00-7:00 pm	Salon B	*Things That Make You Go "Hmmm"* Presented by Applied Biosystems by Life Technologies
IDEXX	6:00-6:30 pm	Rochester	*Driving Laboratory Diagnostic Testing by Engaging Practicing Veterinarians, a Companion Animal Example* Presented by William Goodspeed—Corporate Vice President and Water Livestock, Poultry & Dairy
VADDS	6:00-6:15 pm	Salon C	*LIMS Options for Frozen Budgets* Presented by Joseph J. Bove, President, Advanced Technology Corp
Biovet, Inc.	6:15-6:30 pm	Salon C	*New Developments in Diagnostic Kits* Presented by Dr André Broes, DMV, Ph.D, Director, R&D & Technical Services Manager
Newport Laboratories	6:30-6:45 pm	Salon C	*Technology, Growth, and Leadership* Presented by Dr. Randy Simonson, General Manager & Chief Operating Officer
SDIX	6:45-7:00 pm	Salon C	*Rapid and Cost-Efficient Salmonella Enteritidis Testing* Presented by Tim Lawruk

Break Refreshments – Exhibit Hall

Saturday, November 13	*Sunday, November 14*
9:30 am – 10:00 am	7:00 am – 8:00 am
11:30 am – 1:00 pm	9:30 am – 10:30 am
3:00 pm – 6:00 pm	12:00 pm – 2:00 pm

Upcoming AAVLD/USAHA meetings:

2011:	September 28–October 5	Buffalo, New York
2012:	October 17-24	Greensboro, NC

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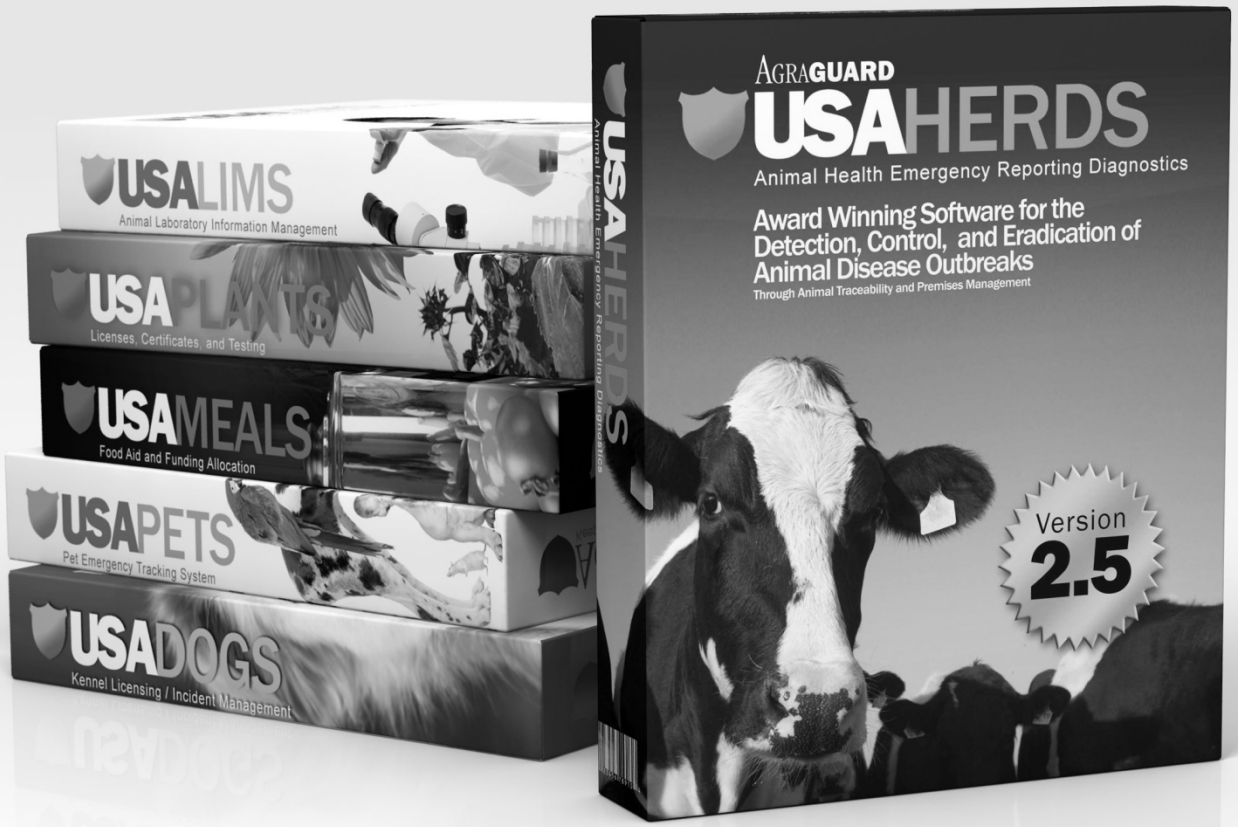

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Refer to the abstracts by Dr. Chinta Lamichhane for additional details.

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