Proceedings of the

American Association of Veterinary Laboratory Diagnosticians

52nd Annual Conference

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San Diego, CA
October 7-14, 2009

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, October 10, 2009
Town and Country

Chair: Gary Anderson
Co-Chair: David Steffen
Sponsor: Bio-Rad Laboratories

"New and Emerging Diagnostic Technologies"

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San Diego

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Sponsor: Applied BioSystems Animal Health

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◊ USAHA paper

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Sponsor: TREK Diagnostic Systems

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Sponsor: Ventana Medical Systems, Inc.

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AAVLD/USAHA Joint Plenary Session

Monday, October 12, 2009
Atlas Ballroom

EMERGING VECTOR-BORNE DISEASES: WHAT IS THE RISK?

08:00 - 8:15 **Welcome and Announcements**
N. James MacLachlan, University of California-Davis

08:15 - 9:00 **Climate Change and Emerging Diseases: A Search for Patterns and Predictions in a Changing World -** E. Paul Gibbs, University of Florida

09:00 - 9:45 **Impact of Bluetongue in Europe - A Recent Example of an Emerging Disease -** Vincenzo Caporale, Istituto Zooprofilattico Sperimentale dell' Abruzzo, Italy

09:45 - 10:00 **Emergence of Bluetongue and Related Orbiviruses in the United States -** David Stallknecht, University of Georgia

10:00 - 10:30 **Break**

10:30 - 11:15 **Unique Challenges to North America Posed by Emerging Diseases -** Terry McElwain, Washington State University

11:15 - 11:45 **USDA Perspective -** John Clifford, Veterinary Services, USDA-APHIS

11:45 - Noon **Summation**

**** Please note: There are no corresponding abstracts for this session.**

AAVLD Plenary Session

Saturday, October 10, 2009
Town and Country

Chair: Gary Anderson
Sponsor: Bio-Rad Laboratories

"New and Emerging Diagnostic Technologies"

08:00 AM **Welcome –** *Gary Anderson*

08:05 AM **Microbe Hunting in the 21st Century**
Ian Lipkin 26

08:35 AM **New Laboratory Diagnostics for 21st Century Veterinary Medicine**
Chris Whitehouse 27

09:00 AM **Diagnosis of Microbial Diseases in the Genomic Era**
Craig Altier 28

09:25 AM **What Led to the Discovery of the Emerging Reston Ebolavirus in Pigs?**
Samia Metwally 29

09:50 AM Break

10:15 AM **Application of Toxicoproteomics in Diagnostic Veterinary Toxicology**
Christina Wilson 30

10:40 AM **Multiplexing of Serologic and Molecular Genetic Diagnostic Assays for Microbiologic Surveillance of Research Animals**
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11:05 AM **Portable Automated Non-PCR-Based Field Diagnostics – BVD as a Model**
Michael Connolly 32

Microbe Hunting in the 21st Century

W. I. Lipkin

Mailman School of Public Health and College of Physicians and Surgeons, Columbia University; Center for Infection and Immunity, Northeast Biodefense Center; The WHO Collaborating Centre on Diagnostics, Surveillance and Immunotherapeutics for Emerging Infectious and Zoonotic Diseases

Narrative: Differential diagnosis of infectious diseases is becoming increasingly important in clinical medicine and public health. Factors in raising global concern with respect to acute infectious diseases include burgeoning international travel and trade, political instability and bioterrorism, climate change and its effects on vector distribution, and the emergence and reemergence of zoonoses. The ability of agents rapidly to expand their geographic range and appear in unexpected locations is well illustrated by the global spread of the human immunodeficiency virus, the transfer of West Nile virus to the western hemisphere and its subsequent dissemination throughout North and South America, the recent emergence of chikungunya virus in Europe, and of multidrug resistant tuberculosis worldwide. Unexpected emergences have also occurred in the context of organ transplantation and immunosuppression. There is also a growing appreciation for a potential role for microbes as primary or co-factors in chronic cardiovascular, endocrine, neurodevelopmental and neoplastic disorders. Whereas the absence of effective therapies once made the diagnosis of viral infection primarily an academic exercise, the expanding armamentarium of countermeasures tailored to specific viruses, including small molecules, RNAi, therapeutic antibodies and vaccines, affords new opportunities to significantly reduce morbidity, mortality and health care costs due to viral infections.

Although culture and serology remain vital in diagnostic clinical microbiology and pathogen discovery, sequence-based methods have clear advantages with respect to speed, cost and portability. Furthermore, many are easier to implement because they require less investment in infrastructure and training than culture techniques. Lastly, sequence-based methods may succeed in instances where fastidious requirements confound cultivation.

Active collaboration between clinicians and laboratorians will be key to success in this new era. The most advanced technology will fail if samples are collected without attention to nucleic acid and protein lability. Data will be uninterpretable without accurate information on clinical course and sample provenance. In chronic diseases, wherein complex mechanisms such as early exposure and/or genetic susceptibility may contribute to pathogenesis, the most substantive advances in linking microbes to disease are likely to come from investments in prospective serial sample collections and an appreciation that many conditions reflect unfortunate host–microbe intersections.

In this lecture I will discuss mechanisms of microbial pathogenesis, routes to proving causation beyond Koch's postulates, and a staged strategy for microbial surveillance and discovery. In reviewing the strengths and limitations of various analytical platforms, I will provide examples that illustrate how each platform can be used to investigate clinical problems. I will also describe models of gene-environment-timing interactions and their implications for medicine and public health.

New Laboratory Diagnostics for 21st Century Veterinary Medicine

C. A. Whitehouse

Diagnostic Systems Division, U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), Fort Detrick, MD

Narrative: Current microbial detection strategies for pathogenic bacteria and/or viruses rely on either the ability to culture the infecting organism in the laboratory or require the use of agent-specific reagents (e.g., primers, probes, antibodies, etc.). These methods have the potential to miss pathogens that are as yet unculturable or those that are completely novel, for which their genomic DNA sequence has not been determined. Recently, new methods have been developed to overcome these limitations. The Ibis T5000 is an automated diagnostic system designed to rapidly identify microbes from virtually any sample type, without the need for lengthy culturing of the microorganisms or the knowledge of exactly which organism is potentially in the sample. The Ibis technology can accurately identify one or more microorganisms from a sample within 6-8 hours directly from a specimen. Importantly, it is not necessary to have specific *a priori* knowledge of the organisms being sought in a sample, thus it is an ideal technology for identification of new and emerging infectious agents. The Ibis T5000 process uses broad-range polymerase chain reaction (PCR) followed by mass spectrometry of the DNA to detect organisms in a quantitative and highly sensitive manner. As organisms are identified by the system, nucleotide base composition "fingerprints" are assigned to the microbes based upon their unique DNA or RNA sequences. The Ibis T5000 can process hundreds of samples in 24 hours, and this capability combined with its tremendous breadth makes it an ideal solution for high-throughput screening applications.

Another unbiased detection strategy makes use of high-throughput sequencing of cultured isolates or directly from clinical and/or environmental specimens without the need for prior culturing or cloning of DNA (termed, metagenomics"). As the cost of sequencing continues to decrease, this technology will begin to move into the routine clinical diagnostic laboratory. The talk will highlight the use of the Ibis T5000 system in several projects at USAMRIID and will also introduce the use of genomics and metagenomics as diagnostic tools in human and veterinary medicine and public health.

Diagnosis of Microbial Diseases in the Genomic Era

C. Altier

Animal Health Diagnostic Laboratory, Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University

Narrative: Bacterial and fungal diseases remain major health threats in both production and companion animal species.

To control and prevent microbial diseases, both the timely and accurate diagnosis of recognized diseases and the identification of novel or emerging pathogens is required. Networks of veterinary diagnostic laboratories across the country perform these functions admirably, but it is clear that the volume and scope of the samples they receive tax the capabilities of the technologies currently in use. Many of the identification schemes being utilized also lack the breadth required for the wide range of bacterial species found in veterinary clinical samples. Here we will discuss the adaptation and development of a technique used widely in research, genome comparison by DNA sequencing, as a primary method for the diagnosis of clinical disease in animals. In recent years, DNA sequence analysis of conserved microbial genes (primarily the 16S ribosomal RNA gene for bacteria) has become an important research method to identify microorganisms and to establish the phylogenetic relationships among them. This has led to the establishment of large databases harboring millions of sequence records, such as Genbank, that allow comparison to known organisms. At the same time, the cost of sequencing has decreased, while the speed and accuracy have increased, bringing this technique into the reach of clinical laboratories.

We will thus discuss the practical use of DNA sequencing as a diagnostic tool, comparing it to traditional phenotypic methods of microbe identification.

We will identify means by which DNA sequencing might be combined with traditional phenotypic methods of pathogen identification to produce rapid and accurate diagnostic algorithms. We will also discuss the opportunities and the pitfalls that exist in using large public databases for DNA comparison. We propose also that we have the opportunity to apply DNA sequencing to clinical isolates and to use the results to create a veterinary microbial DNA database that, in conjunction with existing identification schemes, can more accurately identify pathogens of animals. Such a database could become an asset to the veterinary diagnostic community and ultimately by other scientists as well.

What Led to the Discovery of The Emerging Reston Ebolavirus in Pigs?

S. Metwally

USDA, APHIS, Foreign Animal Disease Diagnostic Laboratory,
Plum Island Animal Disease Center, Greenport, NY

Narrative: In April-May 2008, the swine industry in the Philippines experienced outbreaks of a disease causing respiratory distress, abortion, high fever, unusually high mortality and close to 70% morbidity. This prompted the government of the Philippines to send samples to FADDL to provide confirmation on the presence of porcine reproductive and respiratory syndrome (PRRS) virus and to assist in choosing an effective vaccine for this disease. Similar outbreaks of severe swine disease, referred to as Porcine High Fever Disease, were reported in China in 2006. Laboratory Investigation into these cases consistently identified PRRS virus and thus PRRS virus had been implicated as the primary cause of these outbreaks. Tissues and sera collected from the outbreaks in the Philippines from five swine premises and inspection points were investigated at FADDL for the presence of classical swine fever, African swine fever and PRRS virus. Tissues were also cultured on five different cell lines to identify other viral pathogens that may have been associated with these outbreaks.

Three of the sites were positive for PRRS virus that was homologous to the Chinese isolates as determined by two unique deletions in the NSP2 gene, and were also positive for porcine circovirus type 2 (PCV-2). Cell cultures inoculated with organ samples (lung, spleen and LN) from PRRS-positive pigs showed cytopathic effects on cell lines that are not permissible to PRRS virus or PCV-2 suggesting the presence of unknown viral agents. The first attempt with electron microscopy examination on positive cultures failed to reveal virus particles, however; random amplification of cDNA followed by pan-viral microarray analysis indicated the presence of a virus similar to Ebola Reston virus (REOBV*)* in the vero cell culture and *Porcine teschovirus-1* (*PTV-1*, formerly known as *Porcine enterovirus-1*) in the SK6 (swine kidney cell line) culture. The teschovirus was confirmed to be *PTV-1* by diagnostic RT-PCR, while REBOV was verified by sequence analysis of a 3.7 kb portion of the viral RNA polymerase revealed by 28 positive REBOV sequence features from the microarray. Full genome sequencing performed on three *REBOV* from two farms revealed the isolates to be more divergent from each other than from the reference virus isolated in 1989. There was evidence to believe that two different virus genotypes were simultaneously circulating in swine on one of the two affected farms. The sequence divergence between the identified viruses and all other known REBOVs indicated polyphyletic origins of the infections in swine. The ebolaviruses are known to be extremely pathogenic in primates and humans and cause severe hemorrhagic fever leading to high fatality rate, while REOBV is thought to be pathogenic in the Asian monkey but not in African monkeys and humans. The chronology of human epidemics in non-human primates proves that filoviruses are prototypic of emerging and or reemerging pathogens. This recent discovery of the emergence of ERBOV in a new species of livestock generated concern from public health officials. Knowledge of basic mechanism of infection and pathogenesis of ERBOV in swine needs further research to develop strategies to prevent further infection of livestock and mitigate the risk of any spread to humans.

Reference:

[1] Barrette RW, Metwally SA, Rowland JM, Xu L, Zaki SR, Nichol ST, Rollin PE, Towner JS, Shieh WJ, Batten B, Sealy TK, Carrillo C, Moran KE, Bracht AJ, Mayr GA, Sirios-Cruz M, Catbagan DP, Lautner EA, Ksiazek TG, White WR, McIntosh MT. (2009) Discovery of swine as a host for the Reston ebolavirus. Science, Jul 10; 325 (5937):204-6.

Application of Toxicoproteomics in Diagnostic Veterinary Toxicology

C. Wilson, S. Hooser

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

Narrative: Toxicoproteomics encompasses the global characterization of protein complements that change in cells, tissues, and other biological samples resulting from exposure to toxins, chemicals, or pharmaceuticals. These investigations aid toxicologists in identifying key proteins and signature protein patterns involved in the biological processes that result from toxic insult. While this approach is more commonly used for biomarker profiling and elucidation of biological pathways in response to toxicity, these technologies can also be exploited for use in detecting specific protein toxins, such as ricin, for diagnostic veterinary toxicology. Due to the inherent sample complexity and proteinaceous nature of biological samples, sample preparation and fractionation are crucial when using proteomics techniques to isolate protein toxins from diagnostic samples. Therefore, the toxicoproteomics platform incorporated in this study uses a combination of affinity chromatography and size exclusion fractionation techniques to isolate intact ricin from castor beans and biological samples. This procedure briefly involves the following: 1) Size exclusion fractionation of the proteins in the sample, 2) lectin affinity selection of protein fraction, 3) trypsin digestion of isolated proteins into peptides, and 4) detection of the ricin peptides by MALDI/TOF/TOF mass spectrometry. This procedure affords optimal isolation and resolution of ricin and key peptides of the protein toxin for use in diagnosing suspect ricin exposure in diagnostic veterinary toxicology cases.

Multiplexing Serologic and Molecular Genetic Diagnostic Assays for Microbiologic Surveillance

W. R. Shek

Research Animal Diagnostic Services, Charles River Laboratories, Wilmington, MA

Narrative: The increasingly frequent importation of mutant animal models from many different research institutions with variable biosecurity and health surveillance programs poses a major risk to specific-pathogen-free (SPF) status of research colonies; this risk and the recent discoveries of indigenous lab animal pathogens that cause research interference highlight the need for laboratory test results that accurately represent the current health status of imported animals and their source colonies. Traditionally (i.e., since the 1960s), serology has been the principal diagnostic methodology by which laboratory animals are monitored for specific adventitious agents. More recently (starting in the mid 1990s), exquisitely sensitive molecular genetic assays utilizing the polymerase chain reaction (PCR) have also become a mainstay of laboratory animal diagnostics.

Advances in diagnostics and instrumentation along with the growing need of biomedical research for high throughput testing have lead to the development of multiplexing systems in which samples can be simultaneously tested for a panel of analytes. Mutliplexed assays are generally more efficient than singleplex tests as they require much less sample and reagent per assay and produce less waste; samples and reagents are added to fewer test wells, which reduces the need for complex and often unreliable automation. Moreover, useful sample and system suitability assay controls can be added without increasing the sample volume or number of test wells. The significance of control results for a sample is maximized because controls are internal, that is in the same well as the assays.

Using the Luminex xMAP® platform and the Biotrove OpenArray, we have developed multiplexed fluorogenic immunoassay and PCR test panels to monitor a variety of laboratory animal species for adventitious infections. The development and qualification of these assays will be discussed. As the principal objective of these tests is to determine whether infection has occurred (rather than to precisely establish the level of infection or identity of the etiologic agent), our assay qualification studies emphasized accurate and reproducible classification of known-positive and known-negative specimens, i.e., diagnostic sensitivity and specificity. Early detection of adventitious infections, which is especially important when monitoring SPF research animals, was assessed by testing specimens collected temporally from experimentally infected animals. In addition, limit of detection and selectivity were evaluated as these analytical characteristics can profoundly affect an assay's diagnostic accuracy. Results will be presented to show that the multiplexed serologic and PCR assays are suitable for lab animal health surveillance.

Portable Automated non-PCR-Based Field Diagnostics – BVD as a Model

M. Connolly

Integrated Nanotechnologies, Inc., Henrietta, NY

Narrative: Integrated Nano-Technologies (INT) of Henrietta, NY is developing a diagnostic method to identify virus and bacterial disease pathogens in the field. An ear tissue sample can be loaded into the self-contained, disposable assay cartridge for the INT Palladium system. The system lyses the cells, breaks down the DNA and RNA and cleans and concentrates the material for analysis. DNA or RNA from a target pathogen binds to sensors on a microchip and forms a hybridized nucleic acid bridge between inter-digitated wires printed on a silicon chip. A solution of metal clusters coats the bridge forming a nano-wire that can conduct an electronic current. Detection of the current over the wire confirms that the targeted material, and therefore the microbe/agent, is present. Absence of target nucleic acid will not result in a bridge and therefore no current is detected. The platform does not require a time-consuming, operator-involved PCR step as is needed with current technologies.

The Palladium system consists of a portable analyzer that is battery operated and weights less than five pounds. The second component is a small plastic cartridge containing the microchip sensor. Once the sample is loaded into the cartridge, the operator is able to push the operate button and wait for results onsite within five minutes. The microchip is capable of housing assays for multiple pathogens, allowing a cartridge that can simultaneously test a sample against a battery of diseases.

Microbiology Scientific Session

Saturday, October 10, 2009
San Diego

Moderators: Lindsay Oaks and Durda Slavic
Sponsor: Applied BioSystems Animal Health

01:00 PM **Comparison of TaqMan® PCR testing at Four Laboratories for *Tritrichomonas foetus* Detection in Bulls***
Hernan Montilla, Charles Estill, Lee Effinger, Mangkey Bounpheng, Darcy Myers, Gary Anderson, Richard Oberst, Jessie Trujillo, Tessa Guy 34

01:15 PM **Field Validation of a Real-Time PCR Detection Assay for *Tritrichomonas foetus* and Evaluation of Sample Transport and In-Pouch Pooling on Assay Sensitivity**
Jessie Trujillo, Tessa Guy, Kerry Rood, Bruce King 35

01:30 PM **Comparison of Assays for the Detection of *Tritrichomonas foetus* in Bovine Preputial Scrapings***
Laurie Baeten, Kristy Pabilonia, Christina Weller, Jeanette Bishop, Kristin Spencer, Lora R. Ballweber. 36

01:45 PM **Comparison of Smegma Sample Collection Sites and Diagnostic Methods for Improved *Tritrichomonas foetus* Detection in Bulls***
Hernan Montilla, Charles Estill, Lee Effinger, Mangkey Bounpheng, Darcy Myers 37

02:00 PM **Real time PCR *Tritrichomonas foetus* Diagnostic Performance Improves When Combined with Culture Results***
Jeff Ondrak, Jim Keen, Gary Rupp, Scott Reynolds, Jim Kennedy, Scott McVey, Bill Baker 38

02:15 PM **Development of Molecular Diagnostic Techniques to Detect and Distinguish Various *Eimeria* Species in North American Game Birds***
Richard Gerhold, Lori Lollis, Lorraine Fuller, Robert Beckstead, and Larry McDougald 39

02:30 PM **Host-Specific Differential Gene Expression From the 28 kDa Multigene Locus in *Ehrlichia chaffeensis*: Transcriptional Analysis and Promoter Characterization***
Lalitha Peddireddi, Chuanamin Cheng and Roman Ganta 40

02:45 PM **Detection and Speciation of *Leptospira* sp. in Clinical Samples Using Real-Time PCR Followed by Sequencing of Amplicons**
Natasha Novik, Simone Oliveira 41

* Graduate student presentation

Comparison of TaqMan® PCR Testing at Four Laboratories for *Tritrichomonas foetus* Detection in Bulls

H. J. Montilla[1], C. T. Estill[1], L. Effinger[2], M. Bounpheng[3], D Myers[3], G. Anderson[4], R. Oberst[4], J. D. Trujillo [5], T. Guy[5]

[1]Oregon State University College of Veterinary Medicine,158 Magruder Hall Corvallis, OR; [2]ODA-Animal Health Laboratory 635 Capitol St. NE Salem, OR; [3]Ambion, Inc., An Applied Biosystems Business 2130 Woodward Street Austin, TX ; [4]Kansas State Veterinary Diagnostic Lab, 1800 Denison Avenue Manhattan, KS; [5]Utah Veterinary Diagnostic Laboratory, Utah State University 950E 1400N Logan, UT

Narrative: *Tritrichomonas foetus (T. foetus)* is a protozoan that causes abortion in cattle. There is no effective treatment, thus prevention of infection is the best control method. Testing of bulls to confirm their negative status prior to the breeding season will prevent introduction of infected sires to the herd. The current gold standard of diagnostic testing consists on three serial cultures seven days apart of preputial smegma in culture media (InPouch TM®) followed by microscopic identification of *T. foetus* and species confirmation by Polymerase Chain Reaction (PCR); this process is error prone and takes up to 21 days. The objective of this study was to compare sensitivity and specificity of the TaqMan® PCR testing for *T. foetus* from smegma samples collected from bulls as performed by four different laboratories. Samples were collected from the glans of the penis and preputial crypts on two groups of bulls. The first group (n=11) was collected once weekly for three weeks, and the second group (n=10) was collected once weekly for six weeks. Bulls were diagnosed by InPouch TM® culture of three serial samples, seven days apart. All samples were evaluated under light microscopy every day for five days. A sample was classified as positive if motile trichomonads were seen on any of the five days. TaqMan® PCR was performed on these samples after 24 hours and day 5 of incubation at 37^{o}C. Each InPouch TM® culture was spun, the supernatant discarded, and the pellet resuspended in PBS. The pellet suspension was divided among four labs. Each lab then performed the Applied Biosystems DNA purification procedure using a modified version of the MagMAX™ -96 Viral RNA Isolation Kit. Xeno DNA (exogenous internal control) was added to the extraction procedure to provide an extraction/amplification control. Purified nucleic acid from the InPouch® DNA isolation was added to *T. foetus*/Xeno DNA TaqMan® PCR reaction wells. Results showed slightly differing Ct values for the *T. feotus* assay among the four laboratories, but the difference in variation did not alter the ability of the test to distinguish between *T. foetus* colonized and negative bulls. Inclusion of an endogenous control allowed for variation in testing results to be minimized. Sensitivity and specificity decreased when TaqMan® PCR was performed on day 5 of the culture compared to day 1. This study shows that **for improved reliability of TaqMan® PCR, smegma samples should be collected in TF media, incubated for 24 hours 37^{o}C prior to extraction. Sample collection site did not change inter laboratory diagnostic agreement.**

Field Validation of a Real-Time PCR Detection Assay for *Tritrichomonas foetus* and Evaluation of Sample Transport and In-Pouch Pooling on Assay Sensitivity

J. Trujillo[1], T. Guy[1], K. Rood[2], B. King[3]

[1]Utah Veterinary Diagnostic Laboratory, Utah State University, Department of Animal, Dairy and Veterinary Sciences, Logan Utah; [2]Utah State University, Department of Animal, Dairy and Veterinary Sciences, Logan Utah; [3]Utah Department of Agriculture and Food, Salt Lake City, Utah

Narrative: Previously, we validated a rapid, sensitive, cost effective, high throughput extraction and quantitative real-time PCR (qPCR) assay for the detection of bovine trichomoniasis. This detection assay can reliably detect 10 *Tritrichomonas foetus (T. foetus)*/ml with no false positive results detected.

The purpose of the current study was to answer the following questions so that science- based regulatory changes for *T. foetus* detection could be implemented in Utah. Questions addressed included 1) determine the sensitivity of the qPCR assay for detection of *T. foetus* in field samples as compared to the traditional In-pouch culture, 2) determine the effect of sample transport methods on assay sensitivity and 3) evaluate the effects of sample pooling on assay sensitivity.

For the field validation trial, 241 preputial washes were collected from slaughter bulls and tested with qPCR assay following 24 hrs incubation at 37^0C in In-pouch. Pouches were incubated at 37^0C and visually inspected on a daily basis for 5 days (conventional protocol). **38 pouches were detected positive by qPCR assay as compared to 24 which were culture positive (p=0.056). All the culture positive samples were detected by the qPCR assay.**

We then determined how sample transport methods (direct freezing vs refrigeration) affect qPCR assay sensitivity. For this study, we utilized serial dilutions of *T. foetus* (0, 10, 100, 1,000, and 10,000 cells/ml) spiked into negative field pouches (20 replicates per each dilution). For group one, spiked pouches were frozen at -20^0C. For group 2, pouches were held at 4^0C overnight. Samples (both groups) were place on ice packs and shipped overnight. Controls included spiked pouches where cell pellets were frozen at -20^0C and shipped on dry ice. Upon receipt of samples qPCR was performed. **No significant difference was observed between the frozen pouches as compared to controls at 10 cells/ml, however, a significant reduction in sensitivity for refrigerated pouches was detected with only 30% of replicates detected (p=0.011), resulting in nearly one log loss of sensitively.**

Lastly, we determined the affect of sample pooling on qPCR detection limits and efficiency. Treatment group 3 consisted of negative field pouches incubated for 24 hours and pooled (5 pouches per pool) then spiked with serial dilutions of *T. foetus*. A second group of negative field pouches (not incubated) were pooled and spiked with *T. foetus* dilutions (treatment group 4). Both groups evaluate the combinatory effects of potential PCR inhibitors (with and without the 24hr pouch incubation) and dilution effects of pooling on assay sensitivity. A 5th group included serial dilutions of *T. foetus* diluted 1:5 in pouch media (dilution effects of pooling). Control sets were not pooled. **A significant reduction in sensitivity for all pooled sample groups was observed with 15% of replicates detected (p=0.000002) at 10 cells/ml.** Most interesting, was the effect of concentrating inhibitors by pooling samples with highest level of inhibitors (group 3). Samples from this group were spiked with 3 logs difference in the concentration of *T. foetus*; however, no difference in *T. foetus* concentration was observed. **The additive effect of inhibitors following sample pooling resulted in a loss of the inherent quantitative nature of qPCR, supporting the observation that pooling of field samples greatly hinders the sensitivity of qPCR detection of *T. foetus*, which may lead to increased numbers of false negative samples with sample pooling.**

Comparison of Assays for the Detection of *Tritrichomonas foetus* in Bovine Preputial Scrapings

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Narrative: Multiple assays currently exist for the detection of *Tritrichomonas foetus* in cattle and significant differences in sensitivity and specificity have been reported. Here we provide data for the comparison of three different methods of detection for *T. foetus* in bovine preputial scrapings. A total of 99 samples were collected by veterinarians under field conditions for diagnostic testing which were subsequently used in this study. Culture using the commercially available In Pouch TF® (Biomed Diagnostics, San Jose, CA) was compared to conventional polymerase chain reaction (cPCR) and real-time polymerase chain reaction (rtPCR). Upon receipt of samples, the In Pouch TF® were incubated at 36.5°C for 72 hours then evaluated by microscopic examination. For cPCR, genomic DNA was extracted from the In Pouch TF® samples using the ZR Fecal DNA kit (Zymo Research, Orange, CA). For rtPCR, the Ambion MagMAX™-96 Viral RNA Isolation kit (Applied Biosystems, Foster City, CA) was used according to manufacturer's modifications for InPouch samples. The PCR reaction was carried out as previously described[1,2] for cPCR. For rtPCR, the newly developed VetMAX™ *T. foetus* test kit (Applied Biosystems, Foster City, CA) was used. This kit includes a Xeno™ DNA internal control which provides an evaluation of DNA degradation during the assay. The kappa statistics of correlation between culture and cPCR, culture and rtPCR, and cPCR and rtPCR were 0.845, 0.863 and 0.846 respectively (>0.845 very good correlation). **In conclusion, it appears that the three testing methods are similar in their ability to detect *T. foetus* in bovine preputial samples.**

A separate pilot study, designed to begin evaluation of the effect of In-Pouch® shipping conditions on cPCR results, was conducted. The number of organisms from a stock culture was determined and dilutions made in Diamond's media. These were then inoculated into In-Pouch TF® samples, collected by veterinarians under field conditions for diagnostic testing, to yield 12.5 and 25 organisms/ml (less than the 50 organisms/ml reported sensitivity[2] for this cPCR). Pouches were stored vertically for approximately 48 hours, at either room temperature (21–23°C), refrigerated (4°C) or frozen (-20°C). Genomic DNA was extracted and cPCR conducted, as above. Six replicates of 12.5 organisms/ml and 3 replicates of 25 organisms/ ml were evaluated[2]. For dilutions of 12.5 organisms/ml, cPCR detected *T. foetus* in 5/6 pouches held at each of the three temperatures. For dilutions of 25 organisms/ml, cPCR detected *T. foetus* in 2/3, 1/3 and 3/3 pouches held at room temperature, 4°C and -20°C respectively. **These early results indicate freezing may be the preferred shipment method for *T. foetus* when using cPCR, however additional data is needed.** Similar studies to determine proper shipping techniques would be useful for rtPCR.

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Comparison of Smegma Sample Collection Sites and Diagnostic Methods for Improved *Tritrichomonas foetus* Detection in Bulls

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Narrative: *Tritrichomonas foetus (T. foetus)* is a protozoal venereal disease in cattle causing abortion, and diminished calf crops with subsequent economic loss for livestock producers. This organism persistently and asymptomatically inhabits the epithelial lining of the bovine prepuce and is transmitted during coitus to the female reproductive tract. There is no approved treatment, thus culling is used to control the disease. In cows, self-cure/clearance of *T. foetus* is observed several weeks after infection. A more practical disease control method is prevention of infection by diagnostic testing of bulls to confirm their negative status prior to the breeding season. The current gold standard of diagnostic testing consists of three serial cultures at seven day intervals of preputial smegma in culture media (InPouch TM®) followed by microscopic identification of *T. foetus* and species confirmation by Polymerase Chain Reaction (PCR); this process is error prone and take up to 21 days. Diagnostic sensitivity for *T. foetus* is dependent on proper sample collection and testing method. The goal of this project was to compare diagnostic sensitivity of smegma collection sites (glans vs. preputial crypts) and evaluate TaqMan® PCR as the new standard for faster and more accurate T. foetus identification. Samples were collected from the glans of the penis and preputial crypts on two groups of bulls. The first group (n=11) was collected once weekly for 3 weeks, and the second group (n=10) was collected once weekly for six weeks. All samples were cultured via InPouch TM® and evaluated under light microscopy and by TaqMan® PCR. Results showed excellent agreement between cultures from samples acquired from the glans when compared to samples from the preputial crypts (n= 93). Decreasing agreement was noted during the following days of culture (n=93). These samples were also evaluated by TaqMan® PCR on day 1 and 5 during culture amplification. PCR results showed excellent agreement the first day (n= 93) but less agreement on day 5 (n= 93). When comparing culture results (days 1 thru 5) to those of TaqMan® PCR on day 1, for each bull, excellent agreement was found for samples from the glans (n=63) and also for the samples taken from the preputial crypts (n=63). Comparison on day 5 showed less agreement on the samples from the glans (n= 63) than on samples from the preputial crypts (n=63). When comparing one TaqMan® PCR test of samples incubated for 24 hrs (day 1) to three consecutive cultures seven days apart and evaluated each for five days the agreement was perfect for both samples acquired from the preputial crypts and samples collected from the glans of the penis. Based on these results we conclude that **samples collected from the glans penis are as diagnostic as those collected from the preputial crypts.** Additionally we conclude that **one TaqMan® PCR on a sample cultured for 24 hours (day 1) has the same diagnostic value as three consecutive cultures (InPouch TM®) seven days apart and evaluated under light microscopy.**

Real-Time PCR *Tritrichomonas foetus* Diagnostic Performance Improves When Combined with Culture Results

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Introduction: The recent epidemic of *Tritrichomonas foetus* (TF) infections in US cattle herds has increased demand for and utilization of TF diagnostic assays. Three consecutive TF negative cultures on preputial scrapings collected at least one week apart in sexually rested bulls is the recommended diagnostic standard to define TF-negative bulls. PCR using gel-based or real time amplicon detection are available as TF diagnostic options. Culture and gel PCR are established TF diagnostic assays that when used in combination with serial test interpretation improves bull TF diagnostic specificity. Real time (rt) PCR is rapidly replacing these two tests due to its higher throughput. The purpose of this study was to compare the performance of rt PCR to that of combined culture/ gel PCR for sample and bull TF classification in naturally infected herds.

Materials and Methods: Preputial scrapings were collected three times from non-virgin breeding bulls at approximately weekly intervals in three Nebraska TF-infected beef herds. Two herds (58 bulls) were sampled in Spring 2008 and a third herd (118 bulls) was sampled in Fall 2008. All ranches were experiencing reproductive losses due to TF infection. Testing was performed in order to identify and remove TF-infected carrier bulls from the herds. At each sampling, preputial scrapings were collected and immediately cultured for TF for 4 or 5 days at 37^0C using the In-Pouch^{TM} TF system with daily 100X microscopic inspection. Aliquots of each 4 or 5 day old culture were then subjected to gel-based and real-time PCR. Both the gel PCR and the rt PCR targeted the same TF-specific 5.8S ribosomal RNA and internal transcribed spacer (5.8S-ITS) region of the genome. A sample was classified as TF-positive if positive by both culture and gel PCR (serial test interpretation). A bull was classified as TF infected if at least one of three samples was culture/gel PCR positive (parallel interpretation). Samples were coded to blind analysis. Sample and bull TF status were analyzed separately. We compared culture/gel PCR findings to rt PCR alone and to combined culture/rt PCR results.

Results: Thirty three of 176 bulls (19%) were TF infected. Real time PCR detected 31 of the 33 infected bulls (6% false negative rate). Nineteen of 143 non-infected bulls were rt PCR false positive (13%). A total of 528 pouches were generated. Thirteen of 83 culture/gel PCR positive pouches were rt PCR false negative (84% sensitivity), and 21 of 445 culture/g PCR negative pouches were rt PCR false positive (95% specificity). In an attempt to correct these rt PCR accuracy problems we combined culture and rt PCR results using serial test interpretation of samples and parallel bull status interpretation. For bull status this method eliminated all 19 rt PCR false positive bull classifications; the two rt PCR false negative bull remained. For the 528 pouches all 21 rt PCR false positive pouches were eliminated, but the 13 rt PCR false negative pouches remained.

Conclusion: Real-time PCR when used as a standalone TF diagnostic test inaccurately classifies samples and bulls compared to combined culture and gel PCR. However, most of these rt PCR misclassifications can be eliminated if rt PCR is combined with culture using serial test interpretation and parallel interpretation of multiple tests in defining bull infection status.

Development of Molecular Diagnostic Techniques to Detect and Distinguish Various *Eimeria* Species in North American Game Birds

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Narrative: *Eimeria* species-associated coccidiosis is a major disease of captive-reared game birds released annually for hunting and restocking purposes. However, the *Eimeria* species infecting captive-reared game birds as well as the geographical distribution, host specificity, and pathogenicity of the parasites are incompletely known. Fecal, intestinal, or litter samples containing coccidia oocysts were collected from numerous captive-reared Northern Bobwhite (*Colinus virginianus*), Ring-necked Pheasant (*Phasianus colchicus*), and Chukar (*Alectoris chukar*) facilities from various locations throughout the United States. The samples were propagated and the oocysts were morphologically examined. DNA amplification by polymerase chain reaction (PCR) of the internal transcribed spacer (ITS) regions of the ribosomal RNA (rRNA) followed by cloning and nucleotide sequencing was performed to molecularly distinguish the separate *Eimeria* species. **To date, twenty-six Northern Bobwhite, twenty Ring-necked Pheasant, and fifteen Chukar *Eimeria*-positive samples have been collected from eight states. By PCR and sequence analysis, Northern Bobwhite, Ring-necked Pheasants, and Chukars contain at least four, five, and four *E*imeria species, respectively.** Species-specific PCR primers have been developed to distinguish the separate species and pure cultures of the various *Eimeria* species are being propagated in respective hosts to match the PCR primers with their respective *Eimeria* species. Comparison of the geographical distribution, host specificity, and pathogenicity of the various pure cultures will be performed to determine which species are most common, pathogenic, and can infect aberrant hosts.

Host-Specific Differential Gene Expression From the 28 kDa Multigene Locus in *Ehrlichia chaffeensis*: Transcriptional Analysis and Promoter Characterization.

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Narrative: *Ehrlichia chaffeensis* is a tick-borne, Gram negative, intracellular rickettsial pathogen, which is responsible for an emerging infectious disease in people, human monocytic ehrlichiosis. *E. chaffeensis* also causes infections in several vertebrate hosts, including dogs, goats, coyotes and white tailed deer. White-tailed deer are reported to serve as a reservoir host for this pathogen. The molecular strategies employed by *E. chaffeensis* for its dual host adaptation and persistence in its tick vector and vertebrate host are not known. One of the possible strategies employed by this pathogen in support of its adaptation and persistence, may be by altering its gene expression in response to its host cell environment. Our recent proteome analysis demonstrated that *E. chaffeensis* protein expression, including from a 28 kDa outer membrane protein multigene locus (p28-Omp), is influenced by the pathogen's growth in macrophage and tick cell environments. The pathogen expresses the p28-Omp gene 14 product predominantly when it is grown in the tick cell environment, whereas the expression is switched to p28-Omp gene 19 protein in macrophages. We tested our hypothesis that *E. chaffeensis* achieves its host cell-specific gene expression by employing transcriptional regulation by sensing the host cell signals. *E. chaffeensis* transcription in infected tick cells and vertebrate macrophages was analyzed by several independent RNA analysis methods. The RNA analysis supported our hypothesis and also aided in identifying transcription start sites and location of the promoter regions for the p28-Omp genes 14 and 19. Subsequent molecular mapping aided in defining promoter regions, location of RNA polymerase binding sequences and the putative regulatory elements to which *E. chaffeensis* that may influence the promoter activities in a host cell-specific manner. Electophoretic mobility shift assays demonstrated interaction of *E. chaffeensis* proteins with that of p28-Omp gene 14 and 19 promoter sequences. **The data from these studies provide important clues about molecular strategies employed by *E. chaffeensis* in its tick vector and vertebrate hosts.** The knowledge gained from these studies is important for carrying-out a more detailed investigation in order to precisely understand the gene regulatory mechanisms employed by *E. chaffeensis*. Such studies are critical for devising effective disease intervention strategies and for controlling tick-borne rickettsial infections in animals and people.

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Detection and Speciation of *Leptospira* sp. in Clinical Samples Using Real-Time PCR Followed by Sequencing of Amplicons

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Introduction: Leptospirosis is a zoonotic bacterial infection of worldwide distribution that affects wild and domestic animals. The disease is maintained in nature by chronic renal infection of carrier mammals, which excrete the organism in their urine.[1] Humans become infected through direct exposure to infected animals or their urine or through indirect contact via contaminated water or soil.[2] At least 200 Leptospira serovars have been identified as pathogenic and over 60 as non-pathogenic.[3] The main pathogenic species affecting swine and cattle in the United States are *Leptospira interrogans* serovars Hardjo, Pomona, Bratislava, Canicola, Icterohaemorrhagiae, and Grippotyphosa, whereas *L. biflexa* serovars Patoc I and Andamana are recognized as an ubiquitous non-pathogenic group. The objectives of this study were to develop and validate a real-time PCR test to detect *Leptospira* sp. in clinical samples in a more efficient manner and to further characterize positive samples by sequencing the obtained amplicons.

Materials and Methods: Primers described by Merien et al (1992) and a newly designed probe targeting the 16S rRNA gene of *Leptospira* sp. were used in a real-time format. A pure culture of *L. interrogans* serovar Hardjo was used as a positive control during test development and standardization. Once the real-time was standardized, specificity was tested using 10 different serovars of pathogenic *Leptospira* sp. and 2 non-pathogenic serovars. Specificity was also tested using 15 different bacterial species commonly isolated from swine and bovine tissues. Sensitivity was tested using ten-fold dilutions of a pure culture of *L. interrogans* serovar Hardjo siked in PBS, urine and tissue homogenate. Amplicons obtained following real-time PCR testing were sequenced and a dendrogram was used to group *Leptospira* sp. into pathogenic or non-pathogenic groups.

Results: The real-time PCR assay was positive for the 12 *Leptospira* serovars tested and negative for the 15 unrelated bacterial species. The test detected a minimum of 80 bacterial cells in PBS and 800 bacterial cells in urine and tissue homogenate. Two main clusters were identified following sequencing of the real-time PCR products. Cluster I included the pathogenic species *L. canicola, L. hardjo, L. pomona, L. icterohaemorrhagiae, L. bratislava, L. grippotyphosa, L. noguchii serovar panama, L. borgpetersenii serovar hardjo-bovis, L. borgpetersenii serovar tarassovi,* and *L. santarosai serovar shermani.* Cluster II included the non-pathogenic species *L. biflexa* serovars
Patoc 1 and Andamana.

Conclusion/Relevance: Real-time PCR followed by sequencing of amplicons proved to be a sensitive and specific method for detection and characterization of *Leptospira* sp. in clinical samples from swine and bovine.

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Pathology Scientific Session

Saturday, October 10, 2009
Golden West

Moderators: John Adaska and Kyathanaballi Janardhan

01:00 PM **Hypertrophic Osteopathy Associated with an Intra-Abdominal Neoplasm in a Cat** *+
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01:15 PM ***Phalaris* spp. Grass Staggers in Beef Cattle** *
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David *Steffen, Jon Beever, Brandy Marron* 45

01:45 PM **Cerebral Injury Following Hot-Iron Disbudding of Suckler Calves**
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02:00 PM ***Echinococcus granulosus* Infection in a Sheep**
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02:15 PM **Pathology of *Clostridium difficile* Infection in Horses: A Retrospective Study of 34 Cases**
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02:45 PM **Paraffin Section Tissue Aging and Immunohistochemistry: Myth or Reality?**
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03:00 PM **Swine Teschovirus Encephalomyelitis in Haiti** ◊
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Hypertrophic Osteopathy Associated With an Intra-Abdominal Neoplasm in a Cat

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Narrative: Hypertrophic osteopathy is a hyperostotic syndrome of the appendicular skeleton that is most commonly associated with intrathoracic neoplasia or inflammation. The condition is rarely associated with intra-abdominal lesions. The majority of cases have occurred in dogs and humans, but few have been reported in cats and horses. A 15-year-old, neutered male Domestic Shorthair cat presented for swollen limbs and difficulty in ambulation. Radiographs and gross postmortem examination revealed severe periosteal hyperostosis of the diaphysis and metaphysis of all four limbs, including the humerus, radius, ulna, carpi, metacarpi, femur, tibia, tarsi, metatarsi, and phalanges. The axial skeleton was spared. Hyperostotic lesions were characterized microscopically by lamellar bony trabeculae separated by adipocytes and scant hematopoietic tissue. In several areas, fibrous connective tissue and islands of cartilage were also present. A 2.5 cm x 2.5 cm peri-renal mass compressed the left kidney and adrenal gland. This mass consisted of well-differentiated tubules of cuboidal epithelial cells and was most consistent with a renal tubular adenoma, as mitotic figures were rare and no distant metastases were found. Thoracic pathology was absent. **Hyperostosis was most consistent with hypertrophic osteopathy secondary to the suspected renal neoplasm. The pathogenesis of hypertrophic osteopathy is uncertain, but predominant theories include neurogenic or humorally mediated increased circulation to the extremities**. Other possible mechanisms include increased levels of humoral or toxic factors that may be produced by neoplastic cells or are abnormally metabolized because of other pathology. Recent literature has also highlighted the potential role of vascular endothelial growth factor and circulating platelets. The mechanism by which this renal tumor caused hypertrophic osteopathy is unknown.

Phalaris spp. Grass Staggers in Beef Cattle

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Narrative: Four, adult, mixed-breed, beef cows presented to the Virginia-Maryland Regional College of Veterinary Medicine with weakness, ataxia and hind limb paresis that eventually progressed to lateral recumbency and death over a course of 2 to 3 days. The cows came from a beef cow-calf operation in West Virginia that had a total of 115 cows. Over a period of 2 months, 18 adult cows died with similar clinical signs. Cranial nerve problems affecting prehension or tongue movements were not evident; however, most of the affected animals were somewhat emaciated. No gross lesions were seen at necropsy in the brains or spinal cords of any of the cows. Histologically the cattle had extensive accumulation of light brown, granular pigment in the neurons of the ventral gray horns of the spinal cord, brain stem and pons. The pigment distorted the cell body, often resulting in bulging of the surface, and displacement of the Nissl substance. Necrotic neurons and spheroids in the white matter were common. The pigment accumulation was more severe in the thoracic and lumbar sections of the cord. Additionally, the most severely affected cow had dark green to brown pigment accumulation in renal tubular epithelial cells. Based on the histologic findings, a tentative diagnosis of *Phalaris* spp. grass toxicosis was made.

County agronomists involved in the field investigation identified new leaf blades and dried stems of reed canarygrass (*P. arundinacea*) in a pasture the cattle had grazed down 2-3 months prior to developing clinical problems. Representative samples were semi-quantitatively analyzed for tryptamine alkaloids. The new reed canarygrass leaf blades contained a tryptamine concentration of approximately 0.2% on a wet weight basis. The region had a moderate drought for the past two summers, which could have promoted the expansion of the drought-resistant reed canarygrass and higher concentrations of tryptamine alkaloids in the grass.

Delayed *Phalaris* toxicoses are associated with the tryptamine alkaloids present in the grass, which resemble serotonin structurally. These alkaloids are suspected serotonergic agonists in the central nervous system and eventually cause neurologic 'staggers' problems in ruminants. Delayed onset times of up to 4-5 months have been reported in sheep after removal from *Phalaris* pastures. Distribution of pigment in serotonergic tracts of the midbrain, brain stem and spinal cord with *Phalaris* toxicoses is rather distinct. Similar pigment (lipofuscin) is present with normal aging in cattle and other species. However, age-related lipofuscinosis has a different distribution than *Phalaris* toxicosis, does not accumulate to the extent seen in these cases, and is not associated with clinical signs in the animals. **From these findings, the diagnosis of delayed *Phalaris arundinacea* toxicosis was made. To the author's knowledge, this is the first description of canarygrass staggers in cattle in West Virginia.**

Neurodegenerative Hydrocephalus (NH) in Angus Calves

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Introduction: Hydrocephalus in calves is associated with viral, parasitic, nutritional and genetic etiologies. Genetic forms are reported in at least seven breeds (Aryshire, Friesian, Hereford, Jersey, Limousin, Marchae Shorthorn).[2-6]

Materials and Methods: Angus calves reported between September 2008 and June 2009 were included. Calves with NH received gross and histologic evaluation, extensive virology testing and evaluation of relationships. Samples were submitted to University of Illinois for genotypic analysis.[1]

Results: We observed a unique morphologic expression of hydrocephalus in 19 closely related Angus calves. Calves had severe hydrocephalus with a markedly enlarged cranium, cleft palate, muscle hypoplasia and variable kyphoscoliosis. The spinal canal was severely dilated in all calves. Most cases had no visible brain or spinal cord at birth. Calves were most often term but were decomposed suggesting in utero death. Rare cases had small amount of spinal cord or brainstem tissue and two cases lacked kyphoscoliosis. Calves had peripheral nerves and normal eyes although the optic nerves were shortened and did not extend into the optic foramina. Hydromyelia and tissue disorganization were seen in the remnant central nervous tissue available. Muscle was hypoplastic. No virus was detected by isolation or by PCR for Bluetongue or BVDV. No concurrent disease was noted in herds. All cases were related to a common ancestor present in maternal and paternal pedigrees.

Discussion/Conclusion: NH has a unique morphology and a familial pattern consistent with autosomal recessive inheritance. Morphologic features are dramatic craniomegaly, spinal dilation, cleft palate, and near total degeneration of central nervous tissue. Vertebrae are grossly abnormal and muscle hypoplasia is prominent. Kyphosis, scoliosis and twisted limbs are normally present but not requisite. Molecular genetic analyses indicate this phenotype is caused by homozygosity for a mutation in bovine patatin-like phospholipase domain containing 6 (PNPLA6), a gene demonstrated previously to have a role in neurodegeneration within other species .[7]

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Cerebral Injury Following Hot-Iron Disbudding of Suckler Calves

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Narrative: Following the disbudding of 30 calves with a hot-iron disbudder, three calves died over the next three days, one being found dead and the other two exhibiting profound dullness and terminal convulsions. Postmortem examination of these two calves revealed, in each case, a unilateral area of discolouration of the cerebral cortex and marked brain swelling immediately subjacent to the site of disbudding. The discoloured area corresponded to a zone of coagulative necrosis of all elements of the cerebral cortex, accompanied by meningeal necrosis and infiltration by inflammatory cell (mainly neutrophils) and mixed bacteria including numerous filamentous bacilli. Histological examination of calvaria revealed intact although necrotic bone, suggesting that bacteria may either have gained access via devitalized bone or, perhaps less likely, following bacteremic localization in necrotic parenchyma. The lesions described have marked similarities to those described in goat kids following the use of hot-iron disbudders. **The pathogenesis involves thermal injury to large meningeal blood vessels and consequent cerebral damage including infarction**. Several reports in the literature describe the condition in goat kids but the condition in calves has rarely been reported. All calves were disbudded on the same afternoon by the same operator, a person with twenty years experience of disbudding calves. Similar losses had not previously occurred on the farm and no convincing underlying predisposing factor could be identified.

Echinococcus granulosus Infection in a Sheep

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Narrative: At the request of a local meat inspector, an adult, female sheep was submitted for necropsy to the Utah Veterinary Diagnostic Laboratory (UVDL) in September 2008. Numerous thick-walled cysts varying from 1 to 4 cm in diameter replaced approximately 85% of the liver and were throughout lung lobes. Cysts contained abundant clear fluid. Histologically, **cysts were identified as mesacestodes consistent with a diagnosis of Echinococcosis due to infection with *E. granulosis.***

The sheep originated from Sanpete County in central Utah. In the early 1970s, a survey conducted in this county revealed a relatively high rate of human and canine *Echinococcus granulosus* infection: 25% of dogs tested had adult *Echinococcus* tapeworms and approximately 13% of adult sheep had hydatid cysts.

Echinococcosis or hydatid disease is an important disease in many parts of the world, and infection is by ingestion of *Echinococcus* sp. eggs, which develop subsequently into metacestodes or hydatid cysts. There are four species of *Echinococcus*: *E. granulosus*, *E. multilocularis*, *E. vogeli* and *E. oligarthus*; in temperate North America only *E. granulosus* is common. Adults inhabit the small intestine of carnivores, predominantly dogs, while the intermediate stage, known as a metacestode or hydatid cyst, occurs in herbivores or other accidental intermediate hosts, including human beings. Though other definitive and intermediate hosts exist, the dog/sheep cycle is the most widespread. Infected animals or human beings have variably sized but often large fluid-filled cysts that are most common in liver and lung.

In North America, ***E. granulosus still occurs in sheep-raising regions of Utah, California, Arizona, and New Mexico***. **Though infection is rare, the disease represents a serious zoonosis and results in carcass loss.**

Pathology of *Clostridium difficile* Infection in Horses: A Retrospective Study of 34 Cases

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Narrative: From January 1997 to August 2008, 34 cases of infection by *Clostridium difficile* were diagnosed in horses submitted for necropsy to the San Bernardino Branch of the California Animal Health and Food Safety Laboratory. During this period, a total of 220 horses with a history of digestive disease were submitted to our laboratory. The diagnosis of *C. difficile* infection was based on detection of toxins (A or B, or both), isolation of *C. difficile*, or both (toxin detection plus *C. difficile* isolation) in intestinal content of horses with a history of digestive disease (mostly diarrhea), coupled with pathological findings in one or more segments of the intestinal tract. In addition, other common causes of digestive system disease in horses were ruled out in these 34 cases (i.e. gastrointestinal parasites, *Salmonella spp*., *Lawsonia intracellularis*, *Clostridium perfringens*, *Clostridium piliformis*, gastrointestinal rupture and displacements, and enteroliths).

The age of the animals ranged from 3 days to 24 years, with 56% of the animals being between 1 and 5 years old. The group included mares (55.8%), geldings (29.4%) and stallions (14.7%). **The most common lesion observed was colitis (46.8%), followed by typhlocolitis (31.5%), enterocolitis (9.3%), enteritis (6.2%), jejunitis (3.1%) and others (3%). Interestingly, all the young foals less than 3 months old presented lesions in the small intestine only (enteritis, jejunitis and/or ileitis) without colitis and/or typhlitis.**

Histologically, the inflammatory lesions were similar in all segments of the intestinal tract and included mucosal, submucosal and/or serosal edema and necrotizing and pseudomembranous inflammation with predominance of neutrophilic leukocytic infiltration.

***C. difficile* is responsible for diarrhea and intestinal disease in horses. This infection seems to affect the small intestine in young foals, while older animals are more prone to develop colitis or typhlocolitis.**

Increased Number of Bovine Herpesvirus-1 (IBR) and Bovine Viral Diarrhea Virus (BVDV) Combined Abortions (2002 to 2009)

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Narrative: The number of bovine fetuses positive for bovine herpesvirus-1 (BHV-1 aka IBR) as determined by FA staining, virus isolation (VI) or PCR at Colorado State University-Veterinary Diagnostic Laboratory (CSU-VDL) has increased dramatically since the winter of 2002-2003. A total of 85 fetuses have tested positively for BHV-1 from January 13 2002 through May 14, 2009. In contrast, 1 IBR abortion was diagnosed in 1998 and none in the years of 1999-2001.

A total of 28 bovine fetuses were IBR FA + in the period of Jan 2002-March 2008. Fetal ages were stated by the submitter or estimated at necropsy to be 124 to 250 days of gestational age. Fetal tissues from these 21 cases plus the 1 case from 1998 were available for examination and 19 had autolysis plus lesions typical of IBR induced abortion including multifocal necrosis in the liver. Two of these also had multifocal necrosis in lung and another 2 also had multifocal necrosis in kidney. One case had suppurative placentitis. Viral inclusion bodies were found in one fetus. Two fetuses had no lesions. Eight IBR FA+ fetuses were tested for the presence of BHV1 DNA by PCR and all 8 were PCR positive. Only 2 BHV-1 viruses were isolated from fetal tissues. Vaccination histories are rarely given on the submission forms; however, in 8 IBR FA+ fetuses (from 4 herds), the vaccine history included using a MLV IBR containing vaccine in pregnant cows. In one case, the exact vaccination record of the heifers prior to breeding was unknown. Where the age of the cow was noted, all were 2 or 3 year old heifers.

This year, (March 7, 2008 until May 14, 2009), 57 IBR FA+ fetuses from several geographic areas were examined. Fetal ages were estimated to be 150 to 260 days of gestational age. Unlike the previous year's cases, 52 of 57 IBR FA+ fetuses were also FA+ for BVDV antigen. Fetal tissues from 55 of these cases were examined histologically. Seventeen cases had lesions typical of IBR, and one of these had intranuclear viral inclusion bodies. Eighteen cases had fetal pneumonia, which is not typical of BHV-1 infection. The severity of pneumonia varied; some cases were more suppurative, some more mononuclear, and some had many multinucleate giant cells. The fetuses with pneumonia did not have autolytic changes. One fetus had anomalies in the brain and skull but no other lesions. Thirteen fetuses had no histologic lesions and no autolysis. Eleven had low number of lymphocytes in spleen and/or thymus; 5 in conjunction with pneumonia, 3 in conjunction with necrosis, 1 in conjunction with pneumonia and palatoschisis, and 2 with no other lesions. One case of neosporosis and one case of salmonellosis were identified; in each case the fetus had pneumonia.

Eight IBR FA+ fetuses were also tested for BHV1 DNA by PCR and all 8 were PCR positive. Two noncytopathic BVDVs were isolated. To date, no BHV1 viruses have been isolated from the 2008-2009 abortions. For some cases from the current year (14 fetuses from 6 herds). there was a vaccine history of using a MLV IBR containing vaccine in pregnant cows However, for 11 fetuses from 9 herds, only inactivated IBR or no viral vaccines were administered to pregnant cows.

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Paraffin Section Tissue Aging and Immunohistochemistry: Myth or Reality?

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Narrative: Tissue ageing of paraffin sections has been sporadically reported in the human literature. Tissue ageing can be due to any environmental parameter (temperature, source of light or lack thereof, humidity, etc.) that may influence antigen immunoreactivity (antigen decay) in paraffin sections stored for prolonged periods in a laboratory setting. The literature indicates a tendency to antigen decay (reduced immunoreactivity) in paraffin sections stored for prolonged periods, but the degree of decay depends on the antigen in question and laboratory conditions (e.g., temperature, light, oxidants).

Markers for 27 cellular and infectious disease antigens were evaluated: Ki67, progesterone receptor (PR), multiple myeloma (MUM-1), thyroid transcription factor (TTF-1), GATA-4 (nuclear); neuron specific enolase (NSE), protein gene product (PGP) 9.5 (nuclear and cytoplasmic); melanoma marker PNL2, CD68, pancytokeratins, high molecular weight cytokeratins, vimentin, synaptophysin, CD117, muscle actin (cytoplasmic); CD3, CD79a, CD18, CD20 (cytoplasmic membrane); Bovine viral diarrhea virus (BVDV), *Lawsonia*, Bovine respiratory syncytial virus (BRSV), *Toxoplasma*, Porcine circovirus 2 (PCV2), Porcine reproductive and respiratory syndrome virus (PRRSV), *Listeria*, Bovine coronavirus. Four replicate, 5-µm-thick, paraffin sections were cut at 5 months, 3 months, 1 month, 3 days, or 1 day before immunohistochemistry was performed. Sets of slides were stored under 1 of the following 4 conditions: (1) room temperature in the dark; (2) 4 C in the dark; (3) room temperature and direct cool-white fluorescent light (4100-4200 Kelvin); (4) room temperature with window-sill exposure to both sunlight and ceiling-mounted fluorescent light. Simultaneous immunohistochemistry runs were performed in an automatic immunostainer using standard immunoperoxidase methods. Immunoreactivity of all slides was scored independently by 3 pathologists on a 5-tier scale (4 = highest; 3 = 10-15% reduction of reactivity; 2 = 16-60% reduction; 1 = >60% reduction; 0 = no staining).

Immunoreactivity declined within the first week of storage for some antigens (e.g., *Toxoplasma,* PCV2, PRRSV) or after several months (e.g., BRSV). Fluorescent light (4100 K) and sunlight were the first and second most adverse conditions, respectively. **The results indicate that some antigens (e.g., *Lawsonia*) are not affected by any test condition; some (e.g, BVDV, *Toxoplasma*) are affected mainly by exposure to sunlight or 4100K fluorescent light; others (e.g., Ki67, NSE) have slight reduction in reactivity in any test condition; and others (e.g., PCV2, PRRSV) have more severely decreased immunoreactivity in any test condition, particularly with sunlight or fluorescent light.**

We conclude that antigen decay occurs with exposure to light and may decrease immunoreactivity in paraffin sections. However, the deleterious effect of different light sources varies depending on the antigen in question. **Light-induced antigen decay (tissue ageing) should be considered in instances of unusual immunoreactivity with stored paraffin sections**.

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Swine Teschovirus Encephalomyelitis in Haiti

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Narrative: Teschovirus encephalomyelitis (previously Tesche/Talfan diseases, and later enterovirus encephalomyelitis) is an acute condition of pigs characterized by central nervous system (CNS) disorders. The causative agent of this disease is porcine teschovirus, genus *Teschovirus*, family *Picornaviridae*. Severe forms of teschovirus encephalomyelitis are now rare and are considered exotic to the United States.

In February and March 2009, approximately one thousand five hundred backyard pigs became sick and approximately seven hundred of them died or were sacrificed in the Lower Artibonite Valley of Haiti. Pigs of all ages were affected. The main clinical sign was posterior ataxia followed by paresis/paralysis on the second or third day of illness. Low or no fever was present. Few lesions were observed at postmortem examinations. The morbidity and mortality was about 60% and 40%, respectively.

Diagnostic samples (whole blood, brain, tonsil, lymph nodes, spleen, kidney, liver and lung) were submitted to Foreign Animal Disease Diagnostic Laboratory on Plum Island, New York, and the National Veterinary Services Laboratories in Ames, Iowa. These samples were negative for classical swine fever virus and African swine fever virus. **A porcine teschovirus was detected by reverse transcription – polymerase chain reactions (RT-PCR) in brain samples. Results of virus isolation, electronic microscopic observation of virus particles, histopathological analysis and nucleic acid sequencing supported a conclusion that the swine disease in Haiti is teschovirus encephalomyelitis.**

The Haitian government has started an information campaign on the cause of the disease and methods of prevention. There are currently no vaccines commercially available for this disease in the world. The disease outbreak is ongoing in Haiti.

Acknowledgments: The work of sample submission and diagnosis for teschovirus encephalomyelitis in Haiti was a result of collaboration of several groups including Haitian animal health authority, the Institute of International Cooperation in Agriculture, APHIS/IS Central America and Caribbean Area Office and the NVSL. The authors thank Dr. David Pyburn of USDA/APHIS for providing some photos for this presentation. The authors also thank Tami Beach, Annette Olson, Linda Cox, Heather Petrowski and many others in USDA/APHIS/NVSL and the National Veterinary Diagnostic Laboratory of Haiti who were involved in sample collection and diagnosis.

Toxicology Scientific Session

Saturday, October 10, 2009
California

Moderators: Catherine Barr and Patricia Talcott

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01:15 PM **Copper Deficiency in Kansas Bison**
Jerome Nietfeld, Deon van der Merwe, Daniel Fisher, Karma Loughney 54

01:30 PM **Ronidazole Toxicosis in Three Society Finches (*Lonchura striata*)**
Leslie Woods, Robert Higgins, Victoria Joseph, Mike Filigenzi, Birgit Puschner. 55

01:45 PM **Overview of Lead Isotope Ratios in Lead Sources and Exposed Raptors**
I*. Holser, L. Melton, L. Aston, R. Poppenga* 56

02:00 PM **Unusual Cases of *Nerium oleander* Toxicosis: A Dog and a Sea Lion**
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02:15 PM **Detection of Tetrodotoxin in GI and Kidney Samples by LC-MS/MS**
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02:45 PM **Fibrinosuppurative Pneumonitis Associated with Diesel Ingestion in a Herd of Beef Cattle ***
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03:00 PM **Nitrate Toxicity in a Cow-Calf Herd ***
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***** Graduate student presentation

Amanitin Intoxication in Dogs: 2005-2009

R. H. Poppenga, B. Puschner, A. Tiwary, M. Mukai, B. Chhetri

California Animal Health and Food Safety Laboratory System (CAHFS), West Health Sciences Drive, Davis, CA

Narrative: Amanitins (α-, β-, γ-, and ε-amanitin) are hepatotoxic cyclopeptides found in three genera of mushrooms: *Amanita*, *Galerina* and *Lepiota*. They are extremely toxic with oral LD_{50} dosages for a variety of animal species less than 0.5 mg/kg. The most commonly implicated amanitin-containing species in intoxications is *A. phalloides*, although other *Amanita* spp., such as *A. ocreata*, are also extremely toxic. In veterinary medicine, the confirmation of amanitin intoxication has historically been difficult in the absence of a history of ingestion of a mushroom and subsequent positive identification of the ingested mushroom as a species containing amanitin since assays for the detection of amanitins are not widely available. A rapid LC-MS/MS/MS method was developed by our laboratory for the detection of α- and β-amanitin in serum, urine, liver and kidneys. Since α-amanitin is one of the predominant amanitins in toxic mushrooms, its detection in antemortem or postmortem samples serves as a good biomarker of exposure.

A search of the CAHFS' database since the development of the amanitin assay in 2005 for cases positive for amanitin was conducted. A total of 30 cases were identified. All cases except one involved dogs; one case involved a human. Eighteen of the 30 cases originated in California, two cases originated in Mississippi, and one case each originated in Virginia, Kentucky, Massachusetts, Georgia and Ontario, Canada. Cases originating from California were primarily from central coastal and Sierra foothill counties where toxic *Amanita* spp.are commonly found. The majority of cases occurred between May and June, although fall and winter months were also represented. The largest percentage of affected dogs was less than one year of age (ranging from 3 weeks to 13 years). In many cases there was no known mushroom ingestion. In those cases in which a mushroom was known to have been ingested, clinical signs occurred as soon as 12 hours post-ingestion. The most common presenting signs were non-specific and included acute onset of lethargy, emesis and diarrhea. Consistent clinical pathologic changes included high ALT values (ranging from 542 to 20,213 U/L, hypoglycemia (as low as 19 mg/dl) and prolonged prothrombin and partial thromboplastin times. Fifteen of 21 dogs for which information was available died or were euthanized; six dogs recovered. The most consistent postmortem lesion in those dogs for which a necropsy was performed was panlobular hepatic necrosis, although significant gastrointestinal lesions were noted in a number of cases as well.

In people, early recognition of exposure and treatment improves survival. Amanitin can be detected in urine before the onset of clinical signs. Therefore, **urine is the preferred specimen for antemortem testing. Kidney is a preferred postmortem sample, since amanitins are found at higher concentrations and persist for longer periods in kidneys compared to liver. Interestingly, in one case involving the deaths of a bitch and her 3 week old puppy, amanitin was detected in a deparaffinized kidney tissue sample. Thus, retrospective assessment of exposure, in the absence of urine or fresh tissue samples, is possible.**

Copper Deficiency in Kansas Bison

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Narrative: Copper (Cu) deficiency occurs worldwide in many species, but is most important in young grazing ruminants. In domesticated ruminants poor weight gain or weight loss is the most important manifestation. Other signs can include poor hair coats, fading of the hair coat color, chronic diarrhea, lameness, anemia, congenital or neonatal ataxia, and sudden death. There is one published report from Canada of Cu deficiency in bison calves with diarrhea, weight loss, fading of the hair coat color, and osteochondrosis. We report Cu deficiency in two bison herds from Kansas.

The first herd consisted of 62 bison in eastern Kansas, in which 16 animals died or were euthanized for humane reasons from July through November, 2008. In all animals, there was chronic diarrhea that varied from mild to clear and watery. Terminally the animals became anorexic, isolated themselves from the herd and lost weight. Most, but not all, bison that died had watery diarrhea and became emaciated. Yearlings followed by 2 year-olds were most severely affected, but two mature cows also died. Multiple fecal samples from clinically affected animals and from four necropsied bison contained various combinations of low to moderate numbers of *Eimeria* spp., *Trichuris* spp., and strongyle-type ova and *Strongyloides* spp. The four necropsied animals were emaciated and had serous atrophy of fat and three had diarrhea. The only other significant findings were peritonitis in one animal and corona virus in a second. The herd was treated twice with fenbendazole and amprolium, and once with ivermectin, but the problem persisted. Liver from the fourth bison to be necropsied contained 1.72 ppm (wet weight) Cu. Subsequently, liver Cu in a yearling found terminally ill and 3 healthy cows (livers collected at slaughter) was 5.9, 38.0, 19.5, and 0.68 ppm. The low end of the adequate range for liver Cu in cattle (normal levels for bison are not available) is reported to be 25 ppm and the deficient range is reported as 0.5 to 10.0 ppm. **Based on these results, Cu deficiency was diagnosed.** In November, 2008 the owner began feeding loose mineral but consumption was inadequate so he began feeding 114 gm (4 oz)/head/day of a supplement composed of wheat middlings, alfalfa pellets and molasses with 2,000 ppm Cu, as well as other minerals and fat soluble vitamins. Death losses ceased and the bison appeared to the owner to be more active. Liver samples collected from seven healthy animals slaughtered between the end of January and June, 2009 contained 29 to 178 ppm Cu.

The second herd was from south-central Kansas and consisted of approximately 30 cows and 20 yearlings. In May, 2009 the herd veterinarian reported that during the previous five months ten yearlings had died. The only clinical signs were decreased feed consumption and emaciation, but not diarrhea. The only significant necropsy finding was serous atrophy of fat. Strongyle-type eggs were repeatedly found in the feces in spite of the herd having been treated with three different anthelmintics. **Liver from the last bison to die contained 4.7 ppm Cu, which is in the deficient range for cattle.**

Ronidazole Toxicosis in Three Society Finches (*Lonchura striata*)

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Narrative: Three finches were accidentally overdosed with ronidazole, a 5-nitroimidazole used for treatment of trichomoniasis. Greater than four times the recommended dose was administered orally as the only water source available. Finches developed neurologic signs or died 3 days into treatment and were submitted for necropsy. **Focal necrosis restricted to the cerebellar nucleus** characterized by neuronal necrosis, vacuolation of the neuropil, gemistocytic astrocytosis, occasional foci of hemorrhage and axonal swelling (spheroids) with demyelination was seen in all three birds. The liver from one finch was analyzed for ronidazole and it's metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) by high performance liquid chromatography-mass spectrometry HPLC-MS. Ronidazole was detected in the finch liver tissue at a concentration of 2700 ng/g and HMMNI was detected at 140 ng/g. **LC-MS analysis of liver is an effective method for detection and quantitation of ronidazole and the HMMNI metabolite.** Based on the kinetics and tissue residue studies of ronidazole, plasma, retina, muscle and liver samples should be obtained from animals with suspected ronidazole poisoning. The suspected medication or source material should also be retained for analysis to evaluate its true concentration. By application of the clinical, pathological and toxicologic procedures used in the birds of this report, a diagnosis of ronidazole toxicosis can be rapidly determined.

Overview of Lead Isotope Ratios in Lead Sources and Exposed Raptors

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Narrative: Lead is a toxic metal whose isotope ratios show great variation depending on the source of the metal. For example, lead from geographically distinct mining locations has different isotopic fingerprints. In theory, these unique fingerprints are retained by products, such as ammunition, derived from the mined lead. Isotope ratios can be determined using mass spectrometry techniques such as inductively coupled plasma mass spectrometry (ICP-MS) or thermal ionization mass spectrometry (TIMS). **The variability in lead isotope ratios can potentially be helpful in identifying the source of animal exposure or environmental contamination**. Isotope ratios have been used to implicate lead ammunition in the exposure and intoxication of California condors. This implication was a major factor in the ban on lead ammunition for hunting in California.

There are different instruments that provide varying degrees of accuracy as required by different applications of the data. Inductively coupled plasma (ICP) instruments commonly used for lead isotope analysis include (from most accurate to least): multiple collector magnetic sector (MC-MS-ICP-MS), high resolution (HR-ICP-MS) and standard quadrupole instruments. Data collected using an Agilent 7500ce ICP-MS in the toxicology lab of California Animal Health and Food Safety Laboratory System demonstrates the range of isotope ratios observed in both biological and inorganic samples. Instrumental analysis of the ratios is relatively straight forward. However, the interpretation of the data can be a challenge.

The application of isotopic ratios was used to explore potential sources of exposure to lead in turkey vultures. **The data suggest that higher levels in exposed animals indicate exposure to lead from multiple sources causing the isotope ratio to average within the birds.** Lead in miscellaneous bullets collected from a shooting range were also tested and showed lead from a large variety of mining sources similar to the spread seen for other inorganic sources of lead such as solder and sinkers.

Unusual Cases of *Nerium oleander* Toxicosis: A Dog and a Sea Lion

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Narrative: A 10-year-old German Shepherd male dog was seen ingesting oleander by the owner; vomiting and regurgitation of the plant followed. The dog was lethargic and showed signs of abdominal discomfort on palpation. The dog was treated with activated charcoal (AC), ampicillin, IV fluids, buprenorphine, metoclopramide, maropitant citrate and lidocaine. The heart rate and sinus rhythm were stable overnight. The next day, a digoxin test of serum was positive. An accelerated idioventricular rhythm, accelerated idiojunction rhythm, and intermittent complete AV-block were observed with ECG, which was partially controlled by atropine. A serum sample collected within 72 hours after exposure contained 0.78 ppb of oleandrin when analyzed by liquid chromatography/mass spectrometry. DigiFab® was administered and the dog was sent home with sotalol. The dog slowly recovered over the following two weeks.

Another case involved a 5-year-old female captive California sea lion at a resort. She was reported to be acting abnormally (with signs of GI pain) and refused to eat. There had been high winds during the night and there were many leaves in the pool, including oleander leaves. The only abnormalities noted on routine blood work were elevated PCV (68%) and BUN (70 mg/dl). She was treated with cefriaxone, ranitidine and SQ fluid. Although there was some clinical improvement over the next 3 to 6 days, she showed signs of anorexia again on the seventh morning. Oleander leaves were reported to be found again in the pool. She began having short seizures every 3-5 minutes that could not be controlled with diazepam and midazolam. Serum samples collected within 24 hrs after the suspect exposures of both incidents were both positive by digoxin assay. Despite treatment, the sea lion died and a necropsy was performed. Gastric content, serum and liver samples were positive for oleandrin by liquid chromatography/mass spectrometry.
Nerium oleander is an evergreen shrub which is commonly used as an ornamental plant. All parts of the plant, dried and fresh, contain cardiac glycosides that are highly toxic to animals and humans. All animal species appear to be susceptible to oleander poisoning and accidental exposure of livestock and camelids to *N. oleander* is common.

Several digoxin immunoassays can be used in clinical laboratories for rapid detection of oleander poisoning. Result interpretation must include careful evaluation of cross-reactivity and confirmatory testing. Because of its structural similarity, oleandrin can cross-react with certain digoxin immunoassays. The use of DigiFab® for the therapy of oleander poisoning is considered safe and effective in serious cases, but doses are empirical.

Detection of Tetrodotoxin in GI and Kidney Samples by LC-MS/MS

E. R. Tor, B. Puschner, M. S. Filigenzi, L. S. Aston, R. H. Poppenga

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Introduction: Tetrodotoxin, *Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethano-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol.* $C_{11}H_{17}N_3O_8$, CAS Number [4368-28-9], is one of the most potent neurotoxins, typically found in pufferfish (fugu), but also in other marine and terrestrial animals. Tetrodotoxin specifically blocks the voltage-gated sodium channels on the surface of the nerve membranes which results in respiratory paralysis and often death. With an intravenous LD_{50} in mammals of 2 – 10 μg/kg and an oral LD_{50} in rats of 20 μg/kg, tetrodotoxin is a very toxic compound. Analytical methods have been published for determination of tetrodotoxin in biological samples including the techniques of gas chromatography-mass spectrometry (GC/MS), TLC and ion-pairing liquid chromatography with fluorescence detection. In recent years, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has become the technique of choice for unambiguous determination of tetrodotoxin.

Materials and Methods: A selective analytical method for detection of tetrodotoxin in kidney and GI content samples using liquid chromatography coupled with triple quadrupole/linear ion trap mass spectrometry was developed. Samples were extracted with acetonitrile:1% aqueous acetic acid (70:30, v/v). The extracts were washed with methylene chloride followed by SPE clean-up using a 500 mg STRATA X-C^R cartridges (Phenomenex Corp., Torrance, CA). The purified extracts were evaporated to dryness, using a nitrogen evaporator (Analytical Evaporator, Organomation Assoc. Inc., Berlin, MA) set at 70^{o}C. The extracts were re-dissolved in 500 μL of methanol:water (80:20, v/v), loaded into the YM-30 centrifree ultrafiltration device (Amicon Bioseparations, Tullagreen, Ireland) and centrifuged for 30 minutes. Samples were then filtered through a 0.45 μm HPLC filter (Millipore Corp., Milford, MA) into a small volume autosampler vials. An Agilent Model 1100 high performance liquid chromatograph coupled with a hybrid triple quadrupole/linear ion trap mass spectrometer, model 4000 Q TRAP (Applied Biosystems/MDS SCIEX, Concord, Canada) was used in all analyses. The analytical column was a 150 x 2.1 mm x 5 μ ZIC-HILIC (Merck KGaA, Darmstadt, Germany). The mobile phase consisted of: (A) 0.01 M ammonium acetate in 0.1% formic acid in water; (B) 0.01 M ammonium acetate in 0.1% formic acid in methanol at a flow rate of 100 μL/min under a linear gradient of 70% (A) to 90% (A) over 12 min. The injection volume was 2 μL. Mass spectral data were acquired in positive ion electrospray ionization (ESI) mode, using the multiple reaction monitoring (MRM) scan function. The precursor ion for tetrodotoxin was the $[M+H]^+$ ion of *m/z* 320. The product ions of *m/z* 302, 284, 256, 162 were obtained using collision energy (CE) = 46, declustering potential (DP) = 80, collision exit potential (CXP) = 15, and entrance potential (EP) = 10. Each set of samples contained a reagent blank, control and fortified samples.

Discussion: This LC-MS/MS method was proven suitable for positive identification of tetrodotoxin in tissues (kidney) and GI contents. The method was successfully used in this laboratory for the diagnosis of tetrodotoxin poisoning in a marine mammal. Diagnostic samples positive for tetrodotoxin require special handling due to "select agent" regulations.

Conclusion: This method offers significant advantages over other methods, providing unambiguous results needed for reliable diagnosis of tetrodotoxin poisoning.

Selenium Poisoning in Swine

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Narrative: The Washington State Department of Agriculture received four complaints in December 2008 and January 2009, involving illness and/or deaths in swine. All four complainants lived in the same town and purchased their swine feed from the same location. All complainants reported observing problems in their pigs within 1 ½ to 2 weeks after feeding a commercial medicated swine feed. More than 18 pigs were affected in total. Feed refusal and weight loss were the most common initial problems reported.

One complainant in particular reported losing four of 8 weaner Hampshire × Duroc pigs. These pigs were purchased in mid December when they were approximately 2 months of age. The pigs began eating the suspect feed at this time and by the end of the month weight loss was noted. By January 22nd, four pigs had died and two others were continuing to exhibit feed refusal and weight loss. Prior to death, the pigs seemed to be starving, but would eat little of the suspect feed. All four developed paresis and paralysis, indicated by an inability to rise, and died shortly thereafter. No fever, diarrhea or respiratory difficulties were noted prior to death.

An 18.6-kg pig was presented to the Washington Animal Disease Diagnostic Laboratory on January 23rd for necropsy. The cadaver was in good postmortem condition. The body condition was poor with decreased muscling and minimal adipose tissue stores. Gross abnormalities consisted of moderate, diffuse, chronic serous atrophy of fat and moderate, diffuse, subacute lymphadenomegaly. **Histopathological evaluation revealed focal, bilateral acute neuronal degeneration of the cranial cervical spinal cord, consistent with lesions caused by excess selenium.** Affected neurons were distributed bilaterally in the ventral most aspect of the gray matter of the ventral horns. Liver [3.8 ppm], kidney [4.1 ppm] and hair [3.4 ppm] samples all contained excessive selenium concentrations, within the reported toxic range. Serum and hair samples collected from a clinically affected pig from the same premise also contained significantly elevated selenium concentrations [2.2 ppm and 5.9 ppm, respectively].

Subsequent investigations revealed that the suspect feed was a medicated complete feed to be fed free choice to growing swine weighing 45 to 120 pounds, with a label indicating selenium was to be present at 0.25 ppm. **The Washington State Department of Agriculture investigator collected eight samples of the recalled swine feed from four different sites and selenium was found to be present at levels ranging from 26.8 to 47.8 ppm.** The feed was manufactured on November 25, 2008 and distributed to 10 feed retailers located in Washington, Oregon and Idaho. The feed recall was initiated on January 29, 2009. Evidence from the investigation suggests that a 0.25% selenium premix that was made immediately before the suspect feed likely contributed to the elevated selenium levels found in the contaminated feed.

Fibrinosuppurative Pneumonitis Associated with Diesel Ingestion in a Herd of Beef Cattle

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Narrative: A herd of 1600 crossbred Angus beef cattle were moved into a new paddock that contained diesel fuel tanks surrounded by a low concrete barrier filled with rainwater. Over the weekend, animals reaching for the water broke a half-inch fuel line allowing diesel fuel to leak into the water. Eighteen animals were found dead on Monday morning within 300 yards of the tanks. On gross necropsy the referring veterinarian described a strong diesel odor, froth exuding from the nose and mouth and severe and diffuse pulmonary hemorrhage. Fixed tissues along with fresh samples including lung, rumen contents and a water sample from the concrete barrier were submitted to the Washington Animal Disease Diagnostic Laboratory for histopathology and toxicology examination. Histologic examination revealed a severe fibrinosuppurative pneumonia consisting of alveoli diffusely filled with edema fluid, fibrin and large numbers of neutrophils, eosinophils and foamy macrophages admixed with occasional clumps of wispy basophilic material. Rare foci of necrosis disrupted alveolar septa. A few areas of mild to moderate periportal hemorrhage were in the liver. No lesions were detected in myocardium, kidney, spleen or adipose tissue. The water sample settled into two layers of approximately equal volume which were tested separately using dichloromethane extraction and gas chromatography. The upper layer was 92% diesel and the lower layer was 0.47% diesel. The liquid and solid portions of the rumen contents were 78% and 21% diesel, respectively and lung tissue was 2.3% diesel. **The final etiologic diagnosis was diesel fuel ingestion with inhalation/aspiration pneumonia**.

Pneumonia is the most common cause of death associated with petroleum hydrocarbon ingestion in ruminants. Pulmonary lesions range from pneumonitis and bronchiolitis, to severe, necrotizing bronchopneumonia and pleuropneumonia. The pulmonary lesions in some of the milder natural exposure cases in ruminants and in experimental exposure in dogs given intratracheal kerosene are similar to a well described condition in humans that occurs after accidental hydrocarbon aspiration known as fire-eaters pneumonitis. The condition involves an acute, intense, fibrinous inflammatory response that resolves over a period of a few weeks. Differences among human and animal cases may be due to dose, duration of exposure or the specific toxic substances present in the hydrocarbon mixtures. The unique physiology of eructation in ruminants and consequent repeated inhalation of volatile hydrocarbons may also contribute to more severe lesions seen in some animals. **Ingestion of petroleum hydrocarbons should be considered as a differential diagnosis in cases of ruminants with fibrinous pneumonia and history of possible exposure**.

The cattle in this case apparently preferentially drank the contaminated water despite the presence of troughs containing uncontaminated water which were up a hill in the paddock. There are multiple reports in the literature of cattle, sheep and goats voluntarily ingesting diesel fuel and other hydrocarbon mixtures. **This case and others demonstrate that even short or limited exposure to petroleum hydrocarbon contaminated water poses a threat to ruminants and therefore fuel tanks in pastures and paddocks should be well fenced and carefully maintained.**

Nitrate Toxicity in a Cow-Calf Herd

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Narrative: In December 2008, 4 adult cows and three 600-lb calves were found dead in the afternoon in a herd of 80 crossbred cows housed in north central Iowa on pasture and fed in a barn lot. The animals were randomly spread throughout the lot and adjacent pasture. Cows were vaccinated yearly with a killed 10 way vaccine, and the feed consisted of millet hay, pasture, mineral tubs and grass hay, with water from a free flowing creek. The farmer fed the cattle twice a day by grinding up 3 to 4 round bales of millet hay, which had been fed for 2 months already. Postmortem examination of one cow at the ISU VDL revealed the cow was in good condition with no gross lesions and normal colored blood. The rumen was full of dark brown to black roughage and abundant sandy soil with minimal grain present. The abomasum and intestine contained muddy material which was firmer in the colon. A midterm fetus (crown rump length: 33cm) was present. The attending veterinarian performed 3 necropsies on the farm with similar findings. Histopathologic lesions were absent in the brain, lung, heart, liver, spleen and kidney tissues.

Nitrate ocular fluid was 110 ppm. Six different bales of millet from 2 separate farms were tested for nitrate levels, and on a dry weight basis, 3 were found to be greater than 20,000 ppm nitrate (20,600 to 22,300 ppm), 1 was at 19,900 ppm and 2 were at 1,500 and 5,300 ppm. The 4 bales with the most elevated nitrate concentration were from the same farm. **Based on high nitrate levels in the ocular fluid and in 5 of 6 bales of millet hay, a diagnosis of nitrate toxicosis was determined**. Due to the amount of high nitrate feed left, it was decided to dilute that feed with the hay containing lower levels of nitrate. High nitrate bales were fed at ¼ of the total ration until it was gone around early April. No more cows died, no abortions occurred in the herd, and the calves born were of normal size. The bulls recently underwent breeding soundness evaluations, and were deemed adequate for breeding.

These cows were potentially exposed to a significant dose of nitrate in feed. If a cow received all her forage nutrition from the millet and assuming consumption at 3% of body weight, a 545-kg cow may have had exposure levels of 19,853 to 22,059 ppm on a dry matter basis. Exposure to greater than 9,000 ppm has been associated with acute death. If the highest concentrated nitrate bales were fed at ¼ the ration, the exposure dose would be up to 5,500 ppm.

With a significantly high exposure dose of nitrate, the lack of abortions or additional clinical animals is surprising. It has been proposed fetal hypoxia initiates abortion, but whether it is a result of fetal cortisol release in response to the hypoxic event or whether there is fetal death first is speculative. These cattle were all in the 5 month range of gestation. It is possible nitrate abortion would be more significant in late term gestation when there is greater oxygen demand by the fetus and dam for rapid fetal growth. Reports of high fetal ocular, amniotic fluid, thoracic fluid and stomach content nitrate concentrations have been associated with abortion, premature births, stillborns, and weak newborn calves, although the levels are variable and interpretation is difficult without additional diagnostics.

Virology Scientific Session

Saturday, October 10, 2009
Town and Country

Moderators: Beate Crossley and Marie Gramer

01:00 PM **Development and Evaluation of One-Step TaqMan® Real-Time Reverse Transcription-PCR Assays Targeting NP, M and HA Genes of Equine Influenza Virus***
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03:00 PM **Diagnostic Laboratory-Based Retrospective Survey of Midwestern US Swine for the Novel H1N1 Influenza A Virus Identified During Flu Outbreaks in Human Population by Matrix Gene Sequencing**
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***** Graduate student presentation

Development and Evaluation of One-Step TaqMan® Real-Time Reverse Transcription-PCR Assays Targeting NP, M and HA Genes of Equine Influenza Virus

Z. Lu[1], T. M. Chambers[1], S. Boliar[1], P. J. Timoney[1], A. J. Branscum[2], S. E. Reedy[1], L. R. Tudor[1], E. J. Dubovi[3], M. L. Vickers[4], S. Sells[4], U. B. R. Balasuriya[1]

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Narrative: Equine influenza is an acute and highly contagious respiratory disease of equids caused by infection with equine influenza virus (EIV). The objective of this study was to develop novel TaqMan® real time RT-PCR (rRT-PCR) assays to detect a wide range of EIV strains including both subtypes of the virus (H7N7 and H3N8). We developed eight rRT-PCR assays targeting the nucleoprotein, matrix, H3 and H7 hemagglutinin genes of EIV. The assays were evaluated with EIV prototype strains of both subtypes and recent EIV isolates from the United States. None of the eight assays cross-reacted with any other tested equine respiratory viruses. Subsequently, three assays which can detect circulating H3N8 subtypes (EqFlu NP, M and HA3 rRT-PCR assays) were validated using nasal swabs collected from both experimentally inoculated horses and from field cases. The sensitivity of these assays was determined by comparison with traditional virus isolation in embryonated hens' eggs. All three rRT-PCR assays have high specificity and sensitivity as compared to virus isolation (93%, 89% and 87% sensitivity for EqFlu NP, EqFlu M and EqFlu HA3 rRT-PCR assays, respectively). Both the EqFlu NP and EqFlu M rRT-PCR assays are at least 100-fold more sensitive than virus isolation and the Directigen Flu A® assay based on testing serial decimal dilutions of prototype viruses of both subtypes. These two assays had analytical sensitivities of ≥10 molecules of EIV RNA. **In summary, the developed assays provide a fast and reliable system for the diagnosis and subtype identification of EIV isolates**.

Identification and Characterization of H2N3 Avian Influenza Virus from Backyard Poultry and Comparison to Novel H2N3 Swine Influenza Virus

M. Lea Killian[1], Y. Zhang[2], B. Panigrahy[1], D. Trampel[3], K. Yoon[3]

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Narrative: In early 2007, H2N3 influenza virus was isolated from a duck and a chicken in two separate backyard poultry flocks in Ohio. The same subtype influenza virus with hemagglutinin (HA) and neuraminidase (NA) genes of avian lineage was identified in a swine herd in Missouri in 2006. The objective of the study was to compare genetic, antigenic and biological properties of the avian influenza virus H2N3 to the swine influenza virus.

Avian isolates were low pathogenic by *in vivo* chicken pathogenicity testing. **Sequencing and phylogenetic analyses revealed that all genes of the avian isolates were comprised of avian lineages whereas the swine isolates had contemporary swine influenza virus internal genes, showing that the avian H2N3 viruses did not originate from the swine virus.** Sequence comparisons of the HA and NA genes demonstrated that the two avian viruses were similar, but not identical, to the swine viruses. The avian and swine isolates were antigenically related as determined by hemagglutination-inhibition (HI) and virus neutralization (VN) assays.

The findings suggest that the avian and swine viruses originated from the same group of H2N3 avian influenza viruses. Although serological surveys using the HI assay on poultry flocks and swine herds in Ohio did not reveal further spreading of H2 virus from the index flocks, continuous surveillance would be necessary to ensure that transmission had not occurred. **Contemporary H2N3 avian influenza viruses appear to be easily adaptable to poultry and swine, raising concern for inter-species transmission of avian viruses to humans.**

RT-PCR Detection of H3N2 Influenza Virus in Oral Fluid Samples from Experimentally Infected Pigs

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Introduction: The utility of oral fluid sampling for the detection of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV) 2 in pigs has been recently demonstrated[1, 2]. The objective of this study was to evaluate the detection of influenza virus by RT-PCR in oral fluid samples collected from experimentally inoculated pigs.

Materials and Methods: Forty weaned pigs were obtained from a PRRSV negative and influenza vaccinated sow herd. Piglets were randomly assigned to one of 4 pens containing 10 animals each. Pens were randomly assigned to one of 4 vaccine treatments, received 3 and 5 weeks pre-inoculation, and all pigs were intratracheally challenged with 10ml of 10^7 $TCID_{50}$ /ml of a homologous swine virulent cluster 4, H3N2 on DPI 0. Pen 1 served as negative controls and received sham vaccinations. Pens 2 through 4 received a novel vaccination and booster combination, homologous to the challenge virus. One oral fluid sample was collected from each pen prior to challenge and daily for 4 days post-inoculation using a previously described protocol[1]. Briefly, a length of cotton rope (5/8" diameter) was hung at shoulder height to the pigs in each pen for 15-20 minutes. After which, an oral fluid sample was extracted from each rope, centrifuged, and frozen until assayed. Ropes were hung once daily, at the same time each morning. For comparison, nasal swabs were obtained on day 2 and day 4 post challenge using sterile dacron swabs and RNA was extracted using the RNeasy Minikit (Qiagen Inc.) procedure[3]. All oral fluid samples were extracted using an automated 96-well plate extraction procedure (Ambion MagMax-96 AI/ND #AM1835). Nasal swabs and oral fluids were assayed by real time RT- PCR for the detection of the influenza matrix protein gene. Pigs were necropsied on DPI 4, and lung tissue and broncho-alveolar lavage (BAL) were submitted from each pig to confirm influenza infection by histology and qRT-PCR respectively. Real-time qRT-PCR for the BAL was performed in the same manner and with nasal swabs.

Results: Table 1 shows the oral fluid, nasal swab and BAL RT-PCR results. In oral fluid, influenza was detected in 1 of 4 samples by DPI 1, 3 of 4 samples by DPI 2, and 4 of 4 samples on DPI's 3 and 4.
Influenza virus was detected in oral fluid samples post-inoculation by real-time RT-PCR. The utility of oral fluids for influenza detection is worth looking at more closely.

Table 1. Oral fluid and nasal swab PCR results

	Day 1		Day 2		Day 3		Day 4		
	OF	NS	OF	NS	OF	NS	OF	NS	BAL
Pen 1	0	ND	+*	7/10	+	ND	+	10/10	10/10
Pen 2	+*	ND	+	10/10	+	ND	+	10/10	10/10
Pen 3	0	ND	0	8/10	+	ND	+	10/10	10/10
Pen 4	0	ND	+	8/9	+	ND	+	9/9	9/9

+*: samples had cycle threshold (Ct) levels between 40-45, indicating low amounts of viral RNA.

References:

1 Prickett et al., 2008. J Swine Health Prod 16(2) 86-91.
2 Prickett et al., 2008. J Vet Diagn Invest 20:156-163.
3 Richt et al, 2004. J Vet Diagn Invest 16:367-373.

Preliminary Validation of Real-Time RT-PCR Assays for the Detection of Novel H1N1 Influenza A Virus

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Narrative: The 2009 outbreak influenza A H1N1 (nH1N1) is a unique virus that was first detected in April 2009. The virus contains a unique combination of gene segments not previously reported in the United States or elsewhere. Each of the eight gene segments is of swine origin, and two of the gene segments (matrix and neuraminidase) belong to a Eurasian lineage. The virus was initially detected in humans in the United States in mid-April 2009 and has since been identified in humans worldwide, with the suspected epicenter of the outbreak located in Mexico. Although the virus was thought to be of swine origin, the first isolation from swine was not reported until early May in Canada. The source of the infection was thought to be a worker recently returned from Mexico, but it was later determined that the worker was not the source of the infection.

The nH1N1 virus was detected with less sensitivity and did not type readily with the existing PCR protocols. In order to monitor and to prepare for an outbreak of nH1N1 in U.S. swine herds, the United States Department of Agriculture, Agriculture Research Services (USDA ARS) Virus and Prion Diseases of Livestock (VPDL) and Southeast Poultry Research Laboratory (SEPRL) and National Veterinary Research Laboratory (NVSL) worked together to create, modify, and evaluate diagnostic tests for the detection of nH1N1. Multiple real-time RT-PCR assays were compared and evaluated, including currently available assays- as well as assays developed by the Canadian Food Inspection Agency, Centers for Disease Control and Prevention, and USDA ARS. Each assay was run on the same panel of archived swine influenza isolates as well as equine influenza and other swine respiratory viruses. Due to the extreme urgency for a set of surveillance tests to identify the nH1N1, only a cursory comparison of existing and modified protocols was conducted at the NVSL. In addition to the archived viruses, the assays were run on swab samples from swine experimentally infected with a human isolate from the CDC. With the limited data available, the matrix assays were ranked on sensitivity, ability to detect all type A influenza, availability of reagents for NAHLN labs, and ease of deployment. The N1 assay was evaluated for specificity and sensitivity. **Two assays were deployed to the NAHLN laboratories for use in surveying swine population on June 1, 2009. These assays will continue to be monitored, tested, and improved, and the data used for further validation of these assays**.

Rapid Detection of the New Swine-Origin H1N1 Virus by Real-Time and Gel-Based RT-PCR Assays

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Narrative: To date, the new swine-origin influenza A H1N1 (S-OIV) virus has infected more than 35,000 people and caused the deaths of 163 persons in 76 countries. The WHO raised the alert level for the S-OIV to the highest possible level. This entirely novel H1N1 virus has not circulated previously in humans. The genome of the S-OIV contains 6 genes (PB1, PB2, PA, HA, NP and NS) from current circulating swine influenza viruses in North America and 2 genes (NA and M) from Eurasian swine influenza viruses; this most likely indicates that pigs may have played a central role in the generation of this virus. So far the virus has been found in one swine farm in Canada, indicating the potential for the infection of pigs. In order to detect and differentiate the S-OIV from the current circulating swine influenza viruses in North America, an accurate and rapid molecular–based assay needs to be developed. For this purpose, we designed a TaqMan-based real-time RT-PCR assay targeting the matrix protein. This assay was able to specifically detect the S-OIV and differentiate it from swine influenza viruses circulating in North America, including the classical, human-like and triple and double reassortant swine influenza viruses. Gel-based specific RT-PCR assays were also developed to detect the S-OIV based on the neuraminidase and matrix genes. These assays were sensitive and specific for the SOIV. **These results demonstrate that the newly developed real-time and gel-based RT-PCR assays can be used to detect and differentiate the human H1N1 virus from current circulating swine influenza viruses**.

Cross Hemagglutination Inhibition Activity Against the Novel Influenza A/H1N1 Virus in Pigs Vaccinated with a Regional Autogenous Trivalent Swine Influenza Virus Vaccine

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Narrative: In 2007, endemic swine influenza virus (SIV) infection became a recurring, persistent and increasingly serious disease in northeast Kansas herds. SIV-associated illness during the immediate post-weaning period (when pigs were transitioning to solid diets and adapting to a new environment) resulted in high mortality and morbidity. Long-term consequences in survivors were suppressed growth, increased culling for non-performance, and a decreased percentage of ideal weight-market animals. Five separate herds located in the same region consistently experienced this pattern of SIV- associated disease in weaned pigs. Hemagglutin and neuraminidase sequences of circulating isolates resulted in the preparation of a "regional autogenous" SIV vaccine, which contained H1N1, H1N2 and H3N2 influenza subtypes. The vaccine was administered to pregnant females pre-farrowing with a goal of enhanced passive maternal protection for weaned piglets.

Hemagglutination inhibition (HI) testing as part of a routine "herd profile" to monitor vaccine-induced SIV antibody levels in vaccinated dams and their offspring was conducted. Indicator strains for the HI test included the homologous vaccine strains, heterologous subtype reference strains and the novel influenza A/H1N1 pandemic strain.

HI results indicated that the trivalent regional autogenous vaccine was effective in generating high titer antibody responses that were passively transferred to the offspring of vaccinated dams. **At ~one week of age, pigs from regional farms where the vaccine was used had HI geometric mean titers ranging from 471 to 894 for the H3N2 viruses and 804 to 1870 for H1N2 and H1N1 viruses. Sera from these pigs were also screened for HI activity against the novel influenza A/H1N1 pandemic strain; interestingly, cross-reactive antibodies to the pandemic H1N1 strain were found in a significant number of sera.** In view of the unexpected HI activity against the novel H1N1 virus, additional screening of sera from production pigs from across the country may provide useful information about herd immunity and the possible use of the trivalent vaccine to protect pigs from A/H1N1.

Incorporation of Multiple Primer and Probe Sequences to Improve Swine Influenza Virus Hemagglutinin Subtyping by Real-Time RT-PCR

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Narrative: Subtyping of the hemagglutinin (H) gene of the Swine Influenza Virus (SIV) by real-time RT-PCR was first implemented in the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) in 2003. In recent years there have been an increasing percentage of H untypeable strains from clinical specimens, presumably due to genetic changes in field strains resulting in mismatches with the primers and/ or probes used in the assay. An updated real-time RT-PCR assay was developed to replace the existing assay in use at the ISU-VDL for detecting and differentiating H1 and H3 subtypes of SIV. The new assay has multiple primers and probes to expand the subtyping capabilities. The reagent mix contains a total of 13 primers and 3 probes. In addition to updating the primers and probes, the assay also uses a mastermix that can be run under "fast" conditions, decreasing the instrument run time from about 2 ½ hours to one hour and 10 minutes.

A total of 203 specimens were run with both the existing and updated assays. Qualitative results were in agreement on 180 of the samples, with the newer assay yielding a lower Ct (i.e. greater sensitivity) in almost all cases. For 20 of the samples, a subtype could not be determined by the old assay, but could be by the updated assay. Two of the samples subtyped as only H1 by the old assay but H1 and H3 by the new assay. Only one sample subtyped with the old assay (with a Ct of 39.21) but not with the updated assay. Sequencing was performed on 45 of the samples, with subtype determination confirmed by sequencing on 42 samples. For the 3 samples that were not in agreement, 2 tested as both H1 and H3 by the assay but sequenced as only one of the subtypes. The other tested as only H1 by the assay but sequencing revealed the presence of both subtypes.

Molecular assays for RNA viruses are generally problematic because of the tendency of these agents to mutate, resulting in mismatches between the target and the primers and probes used in the assay. To maintain optimum detection of these agents, it is critical to monitor the performance of the assays and update as necessary with new primers and probes. Incorporation of multiple sets of primers and probes may be of value for enhanced assay performance.

Antigenic Characterization of Contemporary H3N2 Swine Influenza Virus Isolates Using a Novel High Throughput Serum Neutralization Assay

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Narrative: Swine influenza virus (SIV) surveillance programs rely on RT-PCR and partial hemagglutinin gene sequencing to subtype and genetically characterize influenza strains. While reference strains are well characterized, antigenic properties of new isolates are inferred based on sequence homology.

Traditional serum neutralization assays measure the ability of antibodies to prevent influenza infection in cell culture. Problems with the serum neutralization assay include its labor intensiveness, variability and subjectivity. To overcome these limitations, a high throughput serum neutralization assay (HTSN) has been developed and used to antigenically characterize contemporary SIV isolates. Key assay features include the use of a swine cell line, a single serum dilution, use of reference antisera and quantification of neutralizing antibodies using the cell viability dye Alamar Blue in conjunction with a fluorescent plate reader. Viruses are run against a panel of antisera representing SIV subtypes and cross-reactive neutralizing antibodies expressed as a serum neutralization ratio.

To validate this new assay, reference viruses and the corresponding antisera were evaluated using hemagglutination inhibition (HI) and HTSN assays. A good correlation was observed between these two assays. To further apply this assay for antigenically characterizing recent viral isolates, antisera against contemporary isolates representing common SIV subtypes (H3N2 clusters I, III, IV; H1N1 reassortants, H1N1/H1N2 human-like) were generated in pigs using whole killed virus preparations. Cross-reactivity of approximately 40 recent H3N2 SIV isolates was assessed using the HTSN assay.

The analysis indicated that H3N2 SIV isolates with amino acid similarity >97% to the commonly used H3N2 cluster IV reference strain/commercial vaccine strain A/Swine/ON/33853/2005 (ON05) showed strong reactivity with cluster IV antisera and antisera generated using a newly updated commercial SIV vaccine. Isolates with <97% similarity to ON05 showed moderate reactivity with cluster IV antisera and poor reactivity with commercial vaccine antisera. Additionally, 20% of isolates failed to react with any antisera.

Based on genetic and antigenic analysis, significant antigenic drift has occurred in the dominant sub-type of H3N2 (cluster IV). HTSN results suggest an inability of commercial vaccine to protect against isolates <97% similar to ON05 (40% of the isolates used in this study). Additionally, a potential emerging cluster of H3N2 with moderate genetic similarity to cluster II H3N2 (92% similarity) was identified by lack of reactivity with antisera used in the HTSN.

Diagnostic Laboratory-Based Retrospective Survey of Midwestern US Swine for the Novel H1N1 Influenza A Virus Identified During Flu Outbreaks in Human Population by Matrix Gene Sequencing

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Narrative: In April of 2009, a novel H1N1 strain of influenza A was identified in the human population. The virus contained a unique blend of genetic reassortment among influenza viruses of swine, avian and human in origin. In particular, neuraminidase (N) and matrix (M) genes of the virus are closely related to those of Eurasian lineage of swine influenza viruses (SIVs). Although the virus has been identified only in human population without any epidemiological link to pigs, the question still remained as for whether or not this novel strain had been present in the US swine since the genetic profile of the virus is of SIV not human influenza viruses.

Due to the high conservation rate of the M gene sequence and the fact that the virus contains the unique M gene which has not been reported in North American swine, it can be hypothesized that the M gene would be a better target for sequencing to detect and differentiate the novel H1N1 virus from endemic H1N1 SIV. The current study was conducted to retrospectively look for the novel H1N1 influenza virus from SIV cases identified at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) to see if this virus has been circulating in swine population. In addition, the degree of homology of the M gene among confirmed cases of H1N1 SIV was also assessed.

Over 100 H1N1 virus isolates or H1N1-positive lung samples were obtained from the ISU VDL's archive from the year 2008 to May 2009, originating in herds in the midwestern United States. An isolate of the novel H1N1 human virus (A/California/04/2009) was obtained and used as the positive control in sequencing. Viral RNA was extracted and amplified for M gene using reverse transcription PCR, and primers complementary to the 5' and 3' terminal conserved regions were used to achieve full-length sequence. Utilizing Lasergene software (DNAstar), a consensus sequence was created for each case, and all sequences were aligned using the Clustal V method. Matrix gene sequences of various novel H1N1 viruses, SIV, avian influenza viruses (AIVs) and human influenza viruses which were available in GenBank were also employed in sequence comparison.

All of the M genes sequenced from H1N1 SIV cases shared less than 87% homology with that of the novel H1N1 virus while over 90% homology was observed among the M genes sequenced from H1N SIV cases, resulting in two distinctly separate clades, SIV H1N1 (North American) and novel H1N1, on a phylogenetic tree. Both clades were distinct from clades made of AIVs or human influenza viruses. These results indicated that **the novel H1N1 virus has not been introduced into the swine population in the midwestern United States at least up to the time that this study was conducted.** However, relatively high rate of sequence variation was unexpectedly observed among M genes sequenced from H1N1 SIV cases despite of the general perception that M gene is highly stable. It remains to be further studied what role such a variation of the M gene plays in pathogenesis and immunobiology.

Epidemiology Scientific Session

Sunday, October 11, 2009
Town & Country

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09:45 AM **Implications of an Optimal Livestock Tracing System Using a Simulation Model for Foot-and-Mouth Disease ***
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10:00 AM Break

10:30 AM **Meeting the Requirements for Drug Safety & Efficacy in Minor Use Species ***
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***** Graduate student presentation
◊ USAHA paper

A BioPortal System for Global Surveillance of Animal Diseases

A. Perez, P. Willeberg, M. Ascher, Z. Whedbee, M. Thurmond

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Narrative: Establishment of a global animal disease surveillance program will be critically important before success can be achieved toward world-wide control and eradication of infectious animal diseases with high social and economic impact, such as foot-and-mouth disease (FMD) or avian influenza (AI). A key prerequisite for formal, purposeful global surveillance will be an operational IT system capable of capturing surveillance-related data and information and of routing it in real time to decision makers for assessment and analysis.

The objective of this paper is to describe some of the attributes of a currently operational web-based information system for global surveillance of animal diseases, referred to as the BioPortal (https://fmdbioportal.ucdavis.edu). The BioPortal is a web-based system, developed as part of a multi-agency effort, that makes available in near real-time via the web animal disease-related global data. There is no fee or charge for its use. The BioPortal can integrate data in disparate formats with various analytical tools. Users can operate the program at different levels of security, in cases where restricted data are being considered.

Currently, the databases available in unrestricted access to the BioPortal have been made available for public use by various organizations and through public websites. Databases available at the FMD BioPortal include the FMD News database, FMD serotype data for samples submitted since 1957 to the World Reference Laboratory in Pirbright, England; the OIE WAHID FMD database, and GenBank FMDV sequence submissions. Since becoming operational in January 2007, 370 users from 46 countries or international organizations have subscribed to the FMD BioPortal. For the highly pathogenic AI (HPAI) BioPortal, surveillance data collected by Denmark between 2005 and 2007 and HPAI sequences from the GenBank are available. For users with the required permits that allow restricted access, selected databases are available. Users can search multiple databases, create tables and apply graphics, download selected records to Excel files, analyze data, align virus sequences, build and compare phylogenetic trees of virus isolates, and display temporal, spatial, and phylogenetic relationships among isolates.

Current initiatives involve the development of BioPortal prototypes for animal diseases other than FMD and HPAI. BioPortal prototypes were developed for vesicular stomatitis virus (VSV), low pathogenic AI (LPAI), and OIE-listed diseases through agreements with USDA-ARS, with the international reference laboratory for AI in Padova, Italy, and with the Food and Agriculture Organization (FAO) of the United Nations. .

This presentation will demonstrate operation of the BioPortal system using data collected from the VSV epidemic in the United States (2004-2006), from the LPAI epidemic in Italy in 2007, and by the OIE and the FAO on a global scale for >15 animal infectious diseases. **Use of the BioPortal will enhance the ability of countries to prepare for and respond to animal disease epidemics.**

Over-Wintering of Vesicular Stomatitis Virus (VSV) in 2004-2006

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Narrative: From 2004 through 2006, 751 vesicular stomatitis (VS) outbreaks caused by vesicular stomatitis virus serotype New Jersey (VSNJV) were reported in nine states of the southwestern United States. The normal model of the space scan statistic and phylogenetic techniques were used to test the hypothesis that the 2005 and 2006 VSNJV outbreaks were likely to be associated to over-wintering of VSNJV in specific geographic regions of the southwestern United States infected in 2004 and 2005, respectively.

Use of the space scan statistic led to the identification of two clusters of outbreaks for which the Euclidean distance (*d*) to the nearest outbreak reported during the previous or posterior year, whichever was shorter, was significantly ($P<0.01$) shorter than the mean value of *d* estimated for the entire epidemic. Clusters were centered in Colorado and Wyoming and included, respectively, 375 and 21 outbreaks. The phylogenetic analysis of 49 VSV samples collected from 2004 through 2006 in the United States and of 10 VSV samples originated from Mexico indicated that the Colorado and Wyoming clusters were caused by two different sublineages of VSNJV, derived from the lineage identified at early stages of the epidemic and distinct from other viral genetic lineages circulating in endemic areas of Mexico. Findings were displayed using a publicly-accessible web-based system (after appropriate registration) referred to as the FMD BioPortal (https://fmdbioportal.ucdavis.edu/) and a video demonstration of the results is publicly available at (http://fmdbioportal.ucdavis.edu/vsv/demo.avi).

Results presented here support the hypothesis that specific sub-lineages of VSNJV over-wintered in a limited geographical region of the United States affected by VS in 2005 and 2006. These finds will help to identify areas in which the virus is most likely to over-winter in the event of a VS epidemic and, ultimately, to design and implement prevention and control strategies to prevent or limit the impact of future VS epidemics in the United States.

Bovine Viral Diarrhea Virus in Calves in US Beef Cow-Calf Herds

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Introduction: One of the goals of the Beef 2007-08 study was to take an in-depth look at persistent infection (PI) with bovine viral diarrhea virus (BVDV) on U.S. beef cow-calf operations, since this agent can cause a variety of disease conditions affecting animal health (respiratory and digestive disease) and reproductive efficiency. The primary means that BVDV sustains itself on operations is through the creation of animals that are persistently infected (PI). These animals shed massive amounts of BVDV into their environment their entire lives. When other animals come in contact with the PI animals they can become infected with BVDV and develop clinical disease or, if they are pregnant animals, their calves can become the next generation of PI calves provided the exposure occurs at the right stage of gestation. Vaccination can increase resistance to BVDV infection and thus lower the frequency of BVDV PI calves. However, testing and elimination of PI animals is often necessary to effectively eliminate the risk of PI calves.

Materials and Methods: The U.S. Department of Agriculture's National Animal Health Monitoring System (NAHMS) conducted the Beef 2007-08 study, which focused on beef cow-calf health and management practices in 24 States. These major beef cow-calf producing States represented 79.6 percent of U.S. operations with beef cows and 87.8 percent of U.S. beef cows. During the study, beef producers were offered the opportunity to collect ear notch samples to be tested for persistent infection (PI) with BVDV. Producers participating were encouraged to collect ear notches from their entire 2008 calf crop for testing.

Notches were collected and frozen dry until they were submitted for testing. At the laboratory the samples were tested using an antigen capture enzyme-linked immunosorbent assay (ELISA) test according to the manufacturer's instructions.

Results: Overall, 205 operations collected ear notches from calves born between November 2007 and June 2008. The number of notches collected per operation ranged from 3 to more than 500. A total of 44,150 notches were collected and tested. The prevalence of positive samples among the tested notches was 0.12 percent (53/44,150). Within herds the prevalence ranged from 0 to 16 percent. Among the operations that submitted ear notches 18 operations had 1 or more positive samples for a herd prevalence of 8.8 percent. Ten herds had only 1 positive sample and one herd had 10 positive samples. Prevalence of positive operations was similar across herd size, region of the U.S., and calf age. Approximately 1 in 7 producers believed that testing and removing BVDV PI calves from the herd would increase the value of the remaining calves. Almost one-half of producers were uncertain if the value of the remaining calves would change.

Discussion/Conclusion: The low prevalence of BVDV PI in beef calves from this study is consistent with other studies. Despite the low prevalence at the animal level, approximately 1 in 12 herds had at least 1 BVDV positive animal, suggesting that many herds are likely to have BVDV circulating in their herds and potentially causing adverse health and reproductive effects. Many operations are uncertain about the value of testing their calves for persistent infection with BVDV. More work is needed to document the economic effects of testing for and controlling persistent infection with BVDV on cow-calf operations.

Novel H1N1 Influenza A Virus in an Alberta Swine Herd

J. Clark

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Narrative: In March 2009, the world became aware of the existence of a novel H1N1 virus that was circulating in the human populations in Mexico and the southern USA. The genetics of that virus were determined to be those historically associated with a triple reassortant H1N1 swine influenza A virus that has occurred in the North American swine population since the late 1990`s with the addition of Eurasian swine genetics on the matrix and neuraminidase genes.

Questions related to the risks this novel virus represented for animal populations lead to widespread communication to the veterinary and swine production communities in Canada for the need for enhanced awareness and reporting in the swine industry. The Canadian Food Inspection Agency was advised in late April 2009 of a swine herd in Alberta with a history of influenza-like illness and contact with an individual with a travel history to Mexico and subsequent influenza like illness following his return to Canada. CFIA imposed a precautionary quarantine and investigated the herd. Initial testing of nasal swabs using rtPCR with a standard primer for the matrix gene produced negative results. Subsequent testing using conventional PCR primers obtained from the National Microbiology Laboratory indicated the presence of an influenza A. Sequencing methods demonstrated a H1 subtype with 99% homology to the matrix gene in the novel H1N1 strain. Sequencing of the neuraminidase gene indicated homology with the neuraminidase gene of the novel H1N1 virus. A novel H1N1 influenza A virus was isolated from the samples submitted from the swine herd. On May 15th, the CFIA reported that the full sequence of the virus indicated that the virus found in the pigs was the same as the virus causing illness in humans around the world.

The CFIA developed a strategy/approach/plan to resolve the animal health issues associated with this farm, in line with the public health concerns. Public health and animal health authorities, nationally and internationally, were engaged in discussion. All groups and organizations supported the controlled marketing with no cull approach which the CFIA advocated. Crowding conditions in the barn forced a limited cull of approximately 500 mature hogs to alleviate animal welfare concerns and to allow a period of time to do testing in the herd. The hogs were euthanized using penetrating captive bolt pistols and transported to a rendering establishment. The rendered material was buried in landfill due to concerns about negative public perception of incorporating the end product into animal feeds.

Tests on samples collected on May 14 and May 25 showed evidence of continued virus presence.
The preliminary results of research to determine the virulence of the novel H1N1 virus for animals and the associated risk indicates the novel virus produces clinical signs similar to the seasonal swine influenza A viruses. **The initial risk management decisions in this herd were precautionary due to the lack of information to determine the risk to the swine and human populations of North America and suggested a virus negative test on the entire herd was needed to release movement restrictions. As additional information became available that provided insight to the risk this virus posed for the human and animal community, it became difficult to modify the initial precautionary approach for *several reasons including low risk tolerance by public health authorities*.** Slaughter facilities were unwilling to take the hogs from this location and therefore the producer was able to convince government of the need to provide him with financial assistance to destroy the herd and allow him to resume operation with a replacement herd.

Space-Time Clustering, Reproductive Number Estimation, and Visualization of Highly Pathogenic Avian Influenza (AI) in Wild Birds of Denmark in 2006 Using A Prototype AI Bioportal

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Narrative: In 2006, H5N1 highly pathogenic avian influenza virus (HPAIV) cases occurred in Denmark as part of the European epidemic that affected mainly migrating wild birds. A total of 45 Danish wild bird cases were diagnosed, of which only one was found through active surveillance using fecal sampling from resting areas for migrating species, whereas passive surveillance of dead wild birds provided 44 cases. One backyard, mixed poultry flock also became infected late in the epidemic.

This study found spatial and temporal clustering of cases associated with the distribution of tufted ducks. The estimated reproductive number (R) suggests that there were limited conditions for disease spread, ultimately resulting in the extinction of the epidemic. As a tool for visualizing the spatial and temporal spread of the epidemic, a prototype AI BioPortal was established to provide on-line web-based access to the data.

AI BioPortal tools include mapping, graphing, phylogenetic trees construction, play-back scenarios, and visualization of results of temporal-spatial cluster analyses. **Features of the AI BioPortal compare favorably to the design of existing national and international surveillance information systems, and the system may become a useful tool for disease surveillance and for decision support in the event of future AI epidemics both at national and international levels.**

Epidemiology of Bluetongue Virus and Infection Among Ruminants in California

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Narrative: Bluetongue virus (BTV) is an economically important, emerging disease of ruminants transmitted by various species of *Culicoides* midges. Twenty-four (likely 25) serotypes of BTV are recognized globally, four (10, 11, 13 and 17) of which are endemic in much of the United States whereas 11 other serotypes have been described only in the southeastern states (serotypes 1,2,3,5,6,9,12,14,19,22,24). Although all ruminants are susceptible to BTV infection, bluetongue disease is most common in certain breeds of sheep and species of wild ungulates. Affected animals exhibit hemorrhage and ulceration of the mucous membranes in the upper portion of the gastrointestinal tract, coronitis, laminitis, and facial edema. Cattle rarely have clinical signs. In contrast, the invasion and rapid spread of BTV 8 within the European Union has resulted in severe disease of many species of domestic and wild ruminants, and marked reproductive effects as a consequence of vertical transmission of the virus especially among cattle. Furthermore, multiple serotypes of BTV are spread in Europe by new vector species, which have always been present but not previously incriminated in the transmission of BTV. The economic consequences within the member countries of the European Union have been considerable from direct losses attributable to illness, animal movement restrictions, and the cost of vaccination programs.

In an effort to proactively address the potentially increased risk of incursion of novel serotypes or strains of BTV into the United States, the objectives of this study were to establish the incidence of BTV infection among California ruminants, to determine the seasonality of infection, to identify the serotypes and virus strains that are currently present, and to identify potential environmental risk factors that contribute to infection of susceptible livestock. A total of 123 calves from 10 dairy herds were enrolled from December, 2008 to March, 2009 due to the fact that BTV infection typically occurs in California between July-November. These dairies represent four geographically distinct regions of the state where monthly collections of serum and whole blood from each calf were analyzed respectively by BTV-specific cELISA and qRT-PCR assays.

Preliminary results indicate a low cumulative incidence of BTV infection (as determined by viral RNA detection in blood) amongst the study cohort, and a high rate of seropositivity for BTV antibodies**.** BTV was identified by qRT-PCR in the blood of a calf located in Stanislaus County; this calf was qRT-PCR positive for vial nucleic acid from birth until three months of age. BTV was isolated from blood collected at the initial sampling, and this virus was identified as BTV serotype 11 via serum neutralization assays. **The epidemiological significance of this apparent instance of congenital or perinatal BTV infection is unknown but will be discussed further. The lack of infection of the other calves through May of 2009 reflects lack of exposure to the bites of BTV-infected *Culicoides* during the winter months, whereas the high incidence of BTV antibodies likely reflects the ingestion of colostrum from seropositive dams**.

Bluetongue Virus Control in California Sheep Using PCR-Based Detection of the Virus Predicted by a Vector-Borne Disease Model

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Narrative: Bluetongue virus (BTV) is the non-contagious, insect-borne causative agent of Bluetongue infection in ruminant species. Bluetongue is considered endemic in the southwestern region of the United States, and is primarily transmitted in the United States by the biting midge, *Culicoides sonorensis*. Typically, a single Bluetongue virus serotype predominates annually in a geographic area, though the circulating serotype may not remain the same from year to year. Bluetongue virus vaccines are serotype specific and do not cross-react sufficiently to protect the host from clinical disease, so currently vaccination strategies are reliant on annual and regional identification of circulating Bluetongue virus serotypes. Bluetongue virus surveillance in the vector, *Culicoides* spp. using real-time PCR methodology could provide early seasonal information regarding the current circulating Bluetongue virus serotype, which would more effectively focus vaccination strategies for sheep producers. Additionally, through early detection of Bluetongue virus in the insect vector, the transmission cycle could be interrupted using various *Culicoides*-directed control strategies. Strategies for vector-control may include the cost effective, seasonal placement of "mosquito-fish" in water sources to feed on *Culicoides* larvae, biorational pesticide vector-control approaches, as well as the use of traditional pesticides.

A vector-borne disease model using vector-host transmission and basic reproduction number (Ro) as the measurement of risk outcome was used to assess the impact of different Bluetongue virus intervention strategies. The Ro was determined using a stochastic approach; parameters and distributions used to estimate Ro were obtained utilizing expert opinion and data retrieved from published literature. Different strategies including vaccination and vector control were evaluated. The model evaluated BTV transmission intervention by focusing on the different stages of virus and vector dynamics. Based on average flock size of 1000 sheep, BTV serotype-specific vaccination alone provided a maximum benefit to cost ratio of 3.13, compared to "mosquito-fish"-based vector-control alone which provided an average 4.05 benefit to cost ratio. Combination of these two strategies resulted in a significant ($p<0.05$) improvement in the benefit to cost ratio of 21.33. **The model demonstrates economic justification for pursuing field validation for early BTV detection and larvae-based vector control strategies for Bluetongue virus**.

Implications of an Optimal Livestock Tracing System Using a Simulation Model for Foot-and-Mouth Disease (FMD)

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Narrative: Foot-and-mouth disease (FMD) is one of the most economically important diseases of livestock. Simulation modeling has been used to predict its spread and identify cost-effective FMD-control strategies.

Using information from a survey designed to estimate time to trace livestock shipments, we simulated four different scenarios to better understand the value of an optimal tracing system in California compared with the current system. FMD epidemics were simulated with the index herd being either a large (> 2,000 head) or a small (<1,000 head) dairy herd and assuming either the current tracing system or a new electronically-based (optimal) system. We simulated 100 epidemics for each of the four different scenarios evaluating the outcome over the first 200 days of the epidemic.

Results showed that if FMD were introduced into a large dairy, it would result in on average **772 infected premises (IPs) and 85,432 animals, compared with 350 IPs and 18,659 animals with an optimal tracing system in place**. When FMD started in a small dairy herd, it was estimated that an **average of 1,092 IPs with 136,831 animals** would be infected under the current tracing system vs. **166 IPs with 8,400 animals**, if the optimal system were in place. **Evaluation of the tracing system efficiency showed that epidemic size could be reduced 30-40% if tracing efficiency were improved by even 1 or 2 days**.

Results demonstrate the importance of an effective livestock-tracing system for a highly contagious disease such as FMD.

Meeting the Requirements for Drug Safety and Efficacy in Minor Use Species

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Narrative: Approved medications available for use in minor species, or to treat less common conditions in major species, are very limited. Pharmaceutical companies can rarely justify the costs associated with meeting the stringent criteria necessary for drug approval for limited-demand animal drugs. While veterinarians have traditionally had the option to prescribe medications that they have deemed medically appropriate, the FDA CVM is applying more stringent requirements for use of drugs in an "off-label" manner. Specific requirements for food-producing animals have been published in the CFR (21 CFR Part 530) to ensure that extra-label use in animals providing products that will enter the human food chain does not present a risk to public health. Of particular interest in the food safety arena is the need to establish an adequate withdrawal, withholding, or discard time for meat, milk, eggs, or any other food that may be derived from the treated animals. Currently, the prescribing veterinarian must establish these guidelines, often without the benefit of controlled trials and adequate published data.

The "Minor Use and Minor Species Animal Health Act of 2004" was intended to streamline the process in order to make more medications legally available to veterinarians to treat species or conditions that would otherwise have no legal treatment options. Phase I involves establishing the safety of the individual drug in the target minor species or for the target condition. Animals are treated with therapeutic doses along with higher doses in an attempt to determine if the drug produces toxicity in any target tissues. In Phase II, tissue residue studies are performed in order to determine the location and duration of drug persistence in animal tissues. This is necessary to both prevent drug toxicity and establish a safe drug withdrawal period in the treated species that may enter the human food chain. Phase III entails establishing efficacy of the drug in the species being studied. In the case of an antimicrobial, evaluation of the Minimum Inhibitory Concentration (MIC) values of that drug against pathogenic bacterial isolates collected through diagnostic laboratories can provide information on the potential therapeutic value of the drug in the target species. Establishing the Area Under the Elimination Curve (AUC) in that species facilitates calculation of AUC/MIC ratios needed to confirm efficacy against certain bacterial pathogens with a particular antimicrobial.

Current projects include evaluation of Therapeutic Compounds for Bacterial Gill Disease in Fish, CIDR estrus synchronization implants in goats, lasalocid in pheasants, tulathromycin in goats, ivermectin in rabbits, fenbendazole in gamebirds, and lincomycin in honeybees**. The process for approval often utilizes services of veterinary diagnostic laboratories, and may provide future options for collaboration between diagnosticians and researchers in facilitating legal approval of beneficial medications**. Public concerns over increasing antimicrobial resistance of bacteria of human origin, possible associations between human-isolated bacterial resistance and animal antimicrobial use, and the public's expectation of a secure food supply will require definitive research and scientific results in order to ensure the safety of our food sources, and extra-label use of medications may not be an option in the future.

Eliciting Expert Opinion to Model Time-to-Trace Livestock Shipments Between Premises in California

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Narrative: Current animal identification and data collection systems in the US are not integrated, may not be electronic and may fall short of USDA's long term 48 hour objective. Speed with which animal movements can be traced under the current system should be assessed.

50 USDA and CDFA experts involved in a recent tuberculosis (TB) outbreak in California were surveyed to determine the number of days needed to trace all animal shipments to and from a given livestock premises in California during the 4 weeks preceding hypothetical diagnosis of FMD on that premises. A variety of different sizes of dairies, calf/heifer ranches, and salesyards were considered.

Response rate was 22%. Time to trace shipments to or from a given premises were not significantly different ($P>0.05$). **Large dairy herds (>10,000 animals) were estimated to require 18 days** to trace 4 weeks of animal shipments compared with 10 days for dairies with less than 5,000 animals ($P<0.001$). It was estimated to take an additional 5 days to trace animal shipments into a dairy that occurred in the 4^{th} vs. 3^{rd} week ($P<0.001$). Shipments into and out of **calf/heifer ranches and salesyards would require about 7 and 11 days, respectively**, and varied by number of animals on the premises.

Results presented here, show that the current system does not meet USDA's 48 hour objective and would likely result in extensive losses in the face of an FMD epidemic.

Effect of Movement Controls and Biosecurity on Transmission of Disease by Indirect Contact in the Control of Foot-and-Mouth Disease in Livestock Production Systems in the Central United States

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Introduction: The potential impact of an introduction of Foot and Mouth Disease (FMD) in Kansas was assessed and the effects of control measures were compared by use of simulation modeling. Kansas has a large livestock population including cattle, swine, sheep and goats. Simulation models were developed using the North American Animal Disease Spread Model (NAADSM), a spatially explicit, stochastic model developed for evaluation of control measures for infectious foreign animal diseases.

Materials and Methods: Based on data from the U.S. Department of Agriculture's National Agricultural Statistic Service and Kansas Confined Animal Feeding Permit data, a simulated population of livestock operations was generated. The population included 60,778 herds defined by latitude and longitude, production type (Cow-calf, Large Feedlot, Small Feedlot, Dairy, Swine, Sheep, and Goats), and herd size. For simulation purposes, a single 242 head cow-calf herd in central Kansas was selected as the initial latently infected herd in an otherwise susceptible population.

Direct and indirect contact rates were estimated between each production type pair based on expert opinion. Direct contacts included the shipment of livestock between herds in either a latent, subclinical or clinical state. Herds detected as positive for FMD in the model, were quarantined preventing further direct transmission. Indirect contacts included veterinarians, feed truck deliveries, milk truck pick-ups, salesmen, nutritionists, AI technicians, hoof trimmers, employee contact, and neighbors. Three levels of reduction in indirect contact were modeled by implementing movement controls following the first detection (10%, 20% and 30% of baseline level) and three levels of probability of disease transmission following indirect contact were modeled (10%, 15% and 20%), along with either no vaccination or a 10 kilometer vaccination ring around infected premises.

Results: The majority of FMD transmission was due to indirect contact between herds. Given the assumptions made in developing these simulations, vaccination had little effect. Increasing the effectiveness of movement controls to decrease indirect contact and decreasing the probability of disease transmission following contact decreased the median number of herds and animals infected and destroyed, as well as the length of the outbreak.

Discussion/Conclusion: These results highlight the importance of biosecurity and movement restrictions and the need for further research in order to assess their proper role as well as the role of vaccination in an FMD outbreak affecting U.S. production systems. Movement controls may be disruptive to animal welfare and continued farm production, making optimal implementation essential during an outbreak. Accurate estimates of the probability of transmission following indirect contact and the effect of specific biosecurity practices in decreasing the probability of transmission in U.S. production systems are also needed. Effective biosecurity practices may control transmission and mitigate animal welfare concerns associated with increased movement controls, allowing continued production on non-affected farms**.** Because most disease spread was the result of indirect transmission over a distance, local vaccination around an infected premise did not decrease simulated disease spread.

Use of Random Effects Linear Models in the Herd-Level Evaluation of Bovine Serum Selenium, Copper and Zinc Concentrations

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Introduction: Little attention has been given to statistical means of herd-level evaluation of clinical chemistry data. Random effects linear modeling is a statistical technique that may have utility in evaluating clinicopathological variables for use in herd-level testing, and may also offer a means to interpret results. The technique appears particularly well suited to this application because it is insensitive to heterogeneous variation among herds and assumes only a normal distribution of errors. Output from the models includes within and among herd variance components, an intercept which is a measure of central tendency in the population, and Best Linear Unbiased Predictions (BLUPs) of the effect of each herd in the model. Standard errors for each of these parameters can be calculated.

In the following example serum concentrations of selenium, copper, and zinc from herd samples submitted to the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University were evaluated by the use of a random effects linear model. These are the nutritional elements most likely to be deficient in the diets of cattle in North America.

Materials and Methods: Data analyzed were from diagnostic submissions to DCPAH between January 2007 and September 2008 for which serum analysis for nutritional trace elements was requested. Criteria for inclusion included bovine species, adult age, and three or more samples submitted from the same herd on the same day. No distinction was made among breeds. The dataset consisted of 165 herds with 1585 animals. The number of animals per herd ranged between 3 and 100 with a median value of 6. Data were analyzed using the Mixed procedure of the SAS* system. The model included no fixed effects and only herd as a random effect.

Results:

Element	Intercept	Variation Attributable to Herd	Sample Size for >80% Power
Selenium (ng/mL)	85.2±4.9	82%	3
Copper (ug/mL)	0.78±0.013	49%	10
Zinc (ug/mL)	1.24±0.035	65%	5

The proportion of variation attributable to herd effect was significant ($p<.01$) for each element, but varied substantially among the three. This difference was reflected in the differences among elements in the sample sizes necessary to detect herd effects as large as or larger than a deviation of 25 percentile units above or below the intercept with 95% confidence more than 80% of the time.

Discussion/Conclusion: Random effects linear models have utility in identifying clinicopathological tests with high proportions of among herd variability. The models may be used to estimate necessary sample sizes for herd-level testing. For diagnostic investigation, individual herds could be included in a model solution to examine for the significance and magnitude of herd effects. Further investigations combining random effects linear models with epidemiological evaluations are necessary to determine the magnitude of herd effects that are of diagnostic significance.

* SAS Institute Inc. Cary NC

Management of Bacterial Kidney Disease in Chinook Salmon Hatcheries Based on Broodstock Testing by Enzyme-Linked Immunosorbent Assay (ELISA): A Multi-Year Study

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Narrative: During the mid-1980's through the 1990's, bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* continued in Chinook salmon *Oncorhynchus tshawytscha* in Idaho Department of Fish and Game (IDFG) hatcheries despite control methods of: (1) injection of returning adult fish with erythromycin to reduce pre-spawning mortality and vertical transmission of *R. salmoninarum*, (2) iodophor topical disinfection of eggs, and (3) treatments of juvenile fish with erythromycin-medicated feed. Programs to manage BKD through measurement of *R. salmoninarum* antigen levels in kidney tissues from spawning female Chinook salmon by enzyme-linked immunosorbent assay (ELISA) were tested over 13 to 15 brood years at three IDFG hatcheries. The ELISA results were used for either: (1) segregated rearing of progeny from females with high ELISA optical density (OD) values (usually OD {greater than or equal to} 0.25) indicative of high *R. salmoninarum* antigen levels, or (2) culling of eggs from females with high ELISA OD values. The ELISA-based culling program had the most positive effects on the study populations. Mortality during rearing was significantly lower ($P < 0.0001$) at each hatchery for brood years that culling was used in comparison to brood years that culling was not practiced. The prevalence of *R. salmoninarum* in juvenile fish, as evidenced by detection of the bacterium by the direct fluorescent antibody test, also decreased significantly (P {less than or equal to} 0.0005) at each hatchery. In addition, the proportions of returning adult females with ELISA OD values {greater than or equal to} 0.25 decreased 56% to 85% for fish reared in brood years during which culling was practiced, whereas proportions of ELISA negative adults increased 55% to 58%. This management strategy allows IDFG Chinook salmon hatcheries to reduce or eliminate prophylactic erythromycin-medicated feed treatments**. We recommend using ELISA-based management of BKD in Chinook salmon hatcheries where it is a concern.**

Porcine Circovirus Type 2 (PCV2) Enzyme-Linked Immunosorbent Seroprofiles of Pigs in PCV-Associated Disease Affected and Non-Affected Farms

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Introduction: Clinical expression of post-weaning multisystemic wasting syndrome (PMWS) has evolved in Europe during the last few years. Nowadays, pigs still show typical PMWS, but PCV2 is also responsible for non-specific elevated mortality, growth retardation and size variability in batches of growing pigs that are described as PCV2 diseases (PCVD). The objective of this study was to evaluate a possible relationship between PCV2 ELISA seroprofiles and the status of the herds regarding PCVD.

Materials and Methods: *Farm inclusion and definition*. Three types of farm were identified: farms where sows and gilts were vaccinated with CIRCOVAC® to the label recommendations for at least 1 year (Group V); farms not vaccinated and PCVD-free (NV) with acceptable growth performances and no PCVD clinical signs; farms not vaccinated and PCVD-affected (NV+) showing PCVD signs, as growth retardation, high weaning-to-slaughter mortality, wasting, heterogeneity, respiratory or digestive disorders that could not be cured with antibiotic treatments. PCVD diagnostic was confirmed using the current method.

Blood Sampling. The same day, 5 pigs were bled at about 3, 6, 9, 12, 15, 18, 21 and 24 weeks of age. *PCV2 antibody titres.* PCV2 antibody titres were measured using the SERELISA PCV2 Ab (Synbiotics) kit according to a quantitative method as previously described. Mean antibody titres and standard deviation in each group were tested using a Student T-test between two groups for each defined age. *Active seroconversion*. Slopes of the second part of the curves were described using the following formula: (Max. log10titer – Min. log10titre)/(Time in weeks separating max and min titres)

Comparison of adjustment curves. A non-linear regression was applied to fit each curve and a mathematical transformation allowing V-shape adjustment was chosen to modelize the central part of each curve accounting for exposure to PCV2 and seroconversion of the population. The shape of the model depended on only one parameter which allowed a simple comparison of the curves. Model parameters were considered as significantly different if their confidence intervals were not overlapping.

Results: The average titre in piglets in V farms was significantly higher up to 6-8 and 12-14 weeks of age than in NV and NV+ farms, respectively ($p < 0.05$). Typically, the 3 profiles showed first a decrease followed by an increase up to the end of the economical life of the pigs. However, active seroconversion started at the age of 6-8 weeks in NV farms, at 12-14 weeks in NV+ farms and V farms. The averaged slope of the active seroconversion in the NV+ farms was almost 3 times sharper than for the NV and V farms. Analysis of the modelized curves confirmed that seroprofile in NV+ farms was significantly different than in the two other groups . Shape of seroprofiles in NV and V farms were not found different.

Discussion/Conclusion: Vaccination of the gilts and sows with CIRCOVAC induces high antibody levels in the sows and in their piglets (3) as was confirmed here with lower initial mean titres observed in the non-vaccinated farms. In this study, seroconversion profiles in affected farms appeared sharp, with an abrupt rise. This aspect may indicate that PCV2 replication in diseased farms was not well under control. In contrast seroconversion profiles in the non affected farms, vaccinated or non–vaccinated, both showed a soft, progressive increase occurring later in life in vaccinated farms. This type of seroprofile is compatible with a firm control of PCV2 replication and shedding within the pig population and the induction of an active immunity in the most favourable conditions, especially for pigs born to vaccinated herds.

Microbiology Scientific Session

Sunday, October 11, 2009
Golden West

Co-Moderators: Deepanker Tewari and Kristy Pabilonia
Sponsor: TREK Diagnostic Systems

08:00 AM **Evaluation of a Second Generation BOVIGAM® Interferon Gamma (IFN-γ) Assay with Alternative Antigens for Stimulation of Whole Blood Cultures ◊**
Roland Hardegger, Mario Pürro, Björn Schröder, Jean-Louis Moyen, Eamonn Gormley, Ray Waters, Mitchell Palmer, Tyler Thacker, Adam Whelan, Martin Vordermeier, Alex Raeber. 91

08:15 AM **Response of Sensitized Elk to Single Cervical Tuberculin (SCT) and Comparative Cervical Tuberculin (CCT) Tests ◊**
Shylo Johnson, Pauline Nol, Robert Meyer, Mike Dunbar, Jack Rhyan 92

08:30 AM **Seroepidemiology to Determine Parvovirus Prevalence in US Poultry**
Laszlo Zsak, Keith Strother, Michael Day 93

08:45 AM **Mycoplasma Found in Bedding of Dairy Herds with Associated Mycoplasma Mastitis in Dairy Cattle**
David Wilson, Jessie Trujillo, Anne Justice-Allen, Greg Goodell. 94

09:00 AM **Determination of the Sample Type, Serologic Data and Culture Methods for the Detection of Canine Brucellosis**
William Fales, Gayle Johnson, Irene Ganjam, Audrey Rottinghaus, Thomas Reilly 95

09:15 AM **Development of Real-Time RT-PCR for Detection of Viral Hemorrhagic Septicemia Virus**
Yan Zhang, J Cui, K Ott, Beverly Byrum. 96

09:30 AM **SYBR Green Real-time PCR Detection and Differentiation Assay for *Mycoplasma* Species in Biological Samples**
Jessie Trujillo, Anne Justice-Allen, Timothy Morley 97

09:45 AM **Reduction in Mycoplasma Mastitis in Utah Dairy Herds Participating in a Follow-Up Project**
David Wilson, Anne Justice-Allen, Jennifer Maddox. 98

10:00 AM Break

10:30 AM **Application of the IDEXX MAP ELISA for the Diagnosis of Both Johne's Disease and Caseous Lymphadenitis Disease in Sheep and Goats ◊**
Beth Mamer, Wayne Ayers, Marie Bulgin 99

10:45 AM **Turkey Cellulitis: Descriptive Epidemiology and Molecular Characterization of Potential Etiological Agents *◊**
Matheus Costa, Simone Oliveira, Scott Wells, Morgan Hennessey, Rob Porter, Andre Ziegler, Srinand Sreevatsan 100

11:00 AM **Differential Gene Expression Study of bTB-Positive Cattle and bTB Test-False Positive Cattle in Michigan***
Ailam Lim, Steve Bolin 101

11:15 AM **Alternative Assays and Testing Algorithm for Confirmation of Suspect False Positives in a Commercial ELISA for Porcine Respiratory and Respiratory Syndrome Virus***
Siyuan Liu, Won-Il Kim, Sheela Ramamoorthy, Kyoung-Jin Yoon 102

11:30 AM **Epidemiology of Infectious Salmon Anemia Virus in Farmed Salmon***
Fernando Mardones, Andres Perez, Tim Carpenter 103

11:45 AM **Experiences after Eighteen Months of Bovine Viral Diarrhea Eradication Program in Switzerland**
Dieter Brunner 104

***** Graduate student presentation
◊ USAHA paper

Evaluation of a Second Generation BOVIGAM® Interferon Gamma (IFN-γ) Assay with Alternative Antigens for Stimulation of Whole Blood Cultures

R. Hardegger[1], M. Pürro[1], B. Schröder[1], J. L. Moyen[2], E. Gormley[3], W. R. Waters[4], M. Palmer[4], T. Thacker[4], A. Whelan[5], H. M. Vordermeier[5], A. J. Raeber[1]

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Narrative: BOVIGAM®, a rapid laboratory assay, measures gamma interferon (IFN-γ) production in whole blood samples after induction of a cell-mediated immune response (CMI) with *Mycobacterium bovis* antigens. The test is widely used in the field and its excellent performance in bovine tuberculosis (bTB) eradication programs in many countries worldwide is well documented. Traditional use of Bovigam is based on measuring the difference in IFN-γ production between stimulation with bovine and avian tuberculin (PPD). Recent advances in the use of Bovigam include the application of alternative antigens such as ESAT-6 and CFP-10 for stimulation. We have analysed the performance of BOVIGAM® in field studies using an antigen cocktail and compared it with BOVIGAM® using bovine and avian PPD for stimulation. In the absence of a true gold standard, diagnostic test specificities and sensitivities were estimated through a Bayesian modeling approach based on data obtained from a bTB free herd (63 cattle) in Switzerland and a herd (49 cattle) with high bTB prevalence in France, respectively. Using the single intradermal comparative cervical tuberculin test, 32 of the 49 animals in the French herd were found to be reactors with the bovine PPD. Diagnostic sensitivities estimated in the French herd for BOVIGAM® with PPD and antigen cocktails were 90% with a 95% confidence interval (CI) of 80-96%, and 84% (95% CI 70-95%), respectively. Diagnostic specificities in the Swiss herd were 92% (95% CI 85-96%) and 97% (95% CI 92-99%), respectively. **These results show that in combination with antigen cocktails used for stimulation of the cell mediated immune response, a higher specificity but slightly lower sensitivity in comparison with PPDs could be achieved.** With the use of antigen cocktails, absolute levels of IFN-γ are measured with the BOVIGAM assay and therefore the test can benefit from a higher range of the IFN-γ detection part. We have made several improvements of the BOVIGAM resulting in a lower detection limit for IFN-γ, more flexibility with regard to incorporation of different reagents for stimulation (PPDs, alternative antigens) and improved ease-of-use. Inter- and intraplate variances could be reduced to < 5%, < 10 respectively. Furthermore, the second generation BOVIGAM assay uses a one component substrate and less washing steps and thus reduces time and cost and allows full automation for high throughput testing needs. In conclusion, the new IFN-γ assay can be used as in improved tool for the detection of TB infected cattle.

Response of Sensitized Elk to Single Cervical Tuberculin (SCT) and Comparative Cervical Tuberculin (CCT) Tests

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Narrative: Elk, *Cervus elaphus,* are subject to the regulations concerning intradermal tuberculin testing under the USDA's uniform methods and rules for the eradication of bovine tuberculosis. Though the single cervical tuberculin (SCT) and comparative cervical tuberculin (CCT) tests are approved methods of anti-mortem detection of *Mycobacterium bovis* infection, few studies quantify the response of elk to these tests. Furthermore, results are acquired after the injection sites are palpated and measured at 72 hours post injection requiring re-handling of the animals. Infrared thermography, the remote measure of surface temperature, may be able to reduce the time to results and eliminate the second handling of the animals by measuring temperature changes associated with inflammation at injection sites. Our objective was to examine the response of sensitized and non-sensitized elk to the tests by palpation, skin thickness measurement and IRT.

To this end, 10 elk were sensitized to *M. bovis*, 9 elk were sensitized to *M. avium* and 19 elk were not sensitized. The sensitized elk were tested 119 days after injection of 0.1 ml derivatives of the selected bacterium. The animals from the three different groups were randomly divided into two blocks; block 1 received 0.1 ml of 2 mg/ml of the purified protein derivative (PPD) and block 2 received 0.1 ml of 1 mg/ml of the PPD for the SCT test. Testing of block 1 was offset by one day from block 2 testing. The SCT and the CCT were conducted concurrently on each animal on the right side and left side of the neck, respectively. In addition to the PPD injections sites which were measured for skin thickness and palpated, two additional sites for the SCT and CCT were measure and palpated, a saline injection and a control site. IRT images were taken at 0, 10, 24, 48, and 72 hrs post injection of all sites.

No significant difference by palpation (x^2=1.09. P=0.78) for detecting a response occurred between the two different concentrations of the PPD for the SCT. Increase in skin thickness for the SCT ranged from 0.0 mm to 8.5 mm and the mean for sensitized animals at the PPD injection site was 3.0 mm (± 0.5 SE). Based on palpation results, 68.4% of the sensitized elk and 36.8% of the control elk had a response to the PPD injection on the SCT. For the CCT, skin thickness increased from 0.0 mm up to 10.0 mm. The mean at the bovine PPD site was 4.8 mm (± 1.3 SE) for *M. bovis* sensitized, 2.8 mm (± 0.1 SE) for *M. avium* sensitized, and 0.7 mm (± 0.2 SE) for the control elk. **Ninety percent (9 of 10) of *M. bovis* sensitized were suspects or reactors.** Of the 9 elk that had *M. avium* senstinogen and of the 19 elk that were controls, 26 plotted in the negative zone for *M. bovis* and 2 of the control elk plotted in the suspect zone for 92.9% specificity. Preliminary IRT analysis has not indicated any significant temperature changes associated with the different sites.

The changes due to the PPD injections are often small and **changes in the concentration of the PPD for the SCT did not result in significant changes in detecting a response.** The small changes, however, may mean less inflammation that could be masked by ambient conditions making IRT difficult to use on elk.

Seroepidemiology to Determine Parvovirus Prevalence in US Poultry

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Narrative: Recently we described a molecular screening method for the detection of unknown viruses in intestinal samples of chickens and turkeys exhibiting characteristic signs of enteric disease. The technique is based on random amplification of particle-associated nucleic acids in clinical samples. Using this method, we successfully identified novel chicken and turkey parvoviruses in intestinal homogenates from affected birds. Sequence analysis of these viruses demonstrated that the chicken and turkey parvoviruses were closely related to each other and representative of a novel group within the *Parvoviridae* family.

A polymerase chain reaction (PCR) assay with primers targeting conserved nonstructural gene sequences proved to be highly specific and sensitive to detecting parvoviruses in experimentally infected chickens. In a nationwide survey, a total of 138 field enteric samples from poultry flocks were tested by PCR for parvovirus presence. Of the tested chicken samples that were collected in 54 farms, 77% showed the presence of parvovirus, while 78% of the turkey samples that were received from 29 farms were parvovirus positive. **For the first time, our data clearly demonstrate that parvoviruses are widely distributed in commercial poultry flocks in the United States.**

To detect chicken parvovirus (ChPV)-specific antibodies in sera, an enzyme-linked immunosorbent assay (ELISA) was developed using the structural VP2 antigen that was expressed from a baculovirus recombinant. The ELISA was 93.3% sensitive and 100% specific in detecting ChPV-infected birds. Subsequent assays identified IgG type ChPV-specific maternally-acquired antibodies in day-old chickens and demonstrated the production of both IgM- and IgG-type virus-specific antibodies in young birds following infection with ChPV. **The availability of an ELISA for detection of virus-specific antibodies and its ability to differentiate between maternally acquired antibodies and antibodies produced following acute infection could prove to be a valuable tool to study epidemiology and biology of chicken and turkey parvoviruses.**

Mycoplasma Found in Bedding of Dairy Herds with Associated Mycoplasma Mastitis in Dairy Cattle

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Narrative: *Mycoplasma bovis* is an important bovine pathogen causing mastitis, pneumonia, metritis and arthritis. Mycoplasma infections are considered contagious, with transmission from cow to cow as a contagious mastitis spread at milking time and as a respiratory disease. This study investigated the possibility of mycoplasmas as environmental pathogens on dairy farms.

In a previous study, *Mycoplasma* spp. were detected in bulk milk from 16/222 dairy farms in Utah (7%). Follow up was conducted on positive farms. One dairy, milking 4,500 cows in dry lot and freestall housing, experienced an outbreak of clinical mastitis caused by *Mycoplasma* spp.; both samples of the farm's recycled bedding sand that were tested by PCR were positive for *M. bovis*. Amplicon sequencing showed 99% homology with *M. bovis*. Subsequently, bedding samples from 20 cow-housing pens on the farm were cultured for *Mycoplasma* spp.; 14/20 samples (70%) were culture-positive.

In total, 48 bedding samples from 3 dairy farms with mycoplasma mastitis were cultured for *Mycoplasma* spp.; 27/48 samples (56%) were culture-positive. Three bedding samples (1 from one farm, 2 from another farm) were also tested with PCR; all 3 verified presence of *M. bovis*. Except for the identification of mycoplasmas in the straw bedding of one pen, 26/27 positive samples (96%) were sand bedding.

A pile of recycled bedding sand from the farm experiencing the mycoplasma clinical mastitis outbreak was tested weekly. *Mycoplasma* spp. confirmed by PCR as *M. bovis* was isolated multiple times from samples of the sand over a period of 8 months. Daily high temperature appeared to be most associated with changes in mycoplasmal growth in bedding sand. Sand became culture-negative in hot weather and culture-positive again when the pile was moved twice during cooler fall weather. **Weather data and controlled temperature experiments suggested that mycoplasmas survived best in sand when temperatures were between 4° C and 10° C (420,000 – 2,130,000 cfu/g, 67% survival after 24 hr), and that frozen (13% survival after 24 hr) or warm conditions (greater than approximately 20° C, or especially greater than 30° C) (0 – 33,400 cfu/g), did not support mycoplasmal growth in sand as well.** Mycoplasma also grew deep (15-18 cm) within the sand pile, but were not recovered on the surface (1-2 cm) for more than two weeks after the pile was moved to a given place. *M. bovis* survived in sand at 37° C only when kept wet in an incubator. *Mycoplasma* spp. were cultured from two of four prolonged refrigeration-experiment sand samples after 80 days at 4°C.

The sand pile used in the study had already been subjected to a recycling process on the farm but still contained mycoplasma organisms. Of disinfectants tested, 0.5% sodium hypochlorite and 2% chlorhexidine solution killed all mycoplasmas after 30 minutes, but weaker dilutions did not kill all of the organisms. Whether a 1:10 solution of bleach or other chlorine based cleaners could be used to wash sand effectively on farms, especially those with bedding recycling systems in place, should be investigated further. **The results suggested that the most practical method of eliminating *M. bovis* from recycled bedding sand might be to turn and/or spread out the pile allowing more of it to dry out, especially in dry and arid climates such as in Utah.** The possibility of mycoplasma infections being transmitted from contaminated bedding to dairy cattle requires further investigation.

Determination of the Sample Type, Serologic Data and Culture Methods for the Detection of Canine Brucellosis

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Narrative: Abortion and infertility are the principle clinical signs associated with canine brucellosis. Since the etiological agent, *Brucella canis*, is an intracellular pathogen, its recovery from individual animals suspected of being infected is difficult. Therefore a retrospective study to evaluate the probability of success and hence the ability to establish a diagnosis was conducted by evaluating swab and tissue isolations, blood cultures and serologic techniques.

Four hundred (400) samples from (14) different culture sites and (525) blood cultures were submitted to the UMC-VMDL from 1997-2008 for the detection of *Brucella canis* using standard methods of isolation, and/or serologic techniques.

Of the (525) blood cultures using the Oxoid® Signal System, 90 were positive for *Brucella canis*, 352 were negative and 83 resulted in the isolation of other organisms.

Fourteen (14) different types of samples, other than blood, were submitted for culture in order to detect *Brucella canis*. Of the 400 samples submitted 8 produced positive B*rucella. canis* cultures: cervical swab (1/1), fetal tissues (5/27), testicular tissue (1/5), uterine swab (1/1). Vaginal swabs produced no positive results in attempts to recover *Brucella canis* (0/342). However, (437) other microbial isolates were found which consisted of *Actionomyces* spp, alpha, beta and gamma hemolytic streptococci, *Bacillus spp*, *Corynebacterium spp*, enterics, *Pasteurella spp*, *Pseudomonas spp*, *Moraxella spp*, *Acinetobacter spp*, and *Staphylococcus spp*. Hemolytic and non-hemolytic *E. coli*, *Pasteurella spp* and streptococcal isolates were most commonly found.

Blood culture is useful in confirming *Brucella canis* infections as (90/525) samples from 290 dogs were culture positive. On an individual patient basis, multiple blood cultures were not more successful than a single culture in detecting positive animals. Blood culture was successful in confirming infection in 20 known seropositive dogs (IgM, IgG or the TAT (tube agglutination test >1:100), 30 seronegative dogs and 11 dogs where serologic status was unknown. Blood culture failed to detect organisms in blood of 35 seropositive dogs, and 194 seronegative or unknown status dogs.

These data indicate that to isolate *Brucella canis*, cultures of fetal tissue, testicular tissue, and cervical or uterine swabs are sometimes useful, whereas the culture of vaginal swabs is non-productive. **Blood culture is the most helpful bacteriologic test, and detects seronegative as well as seropositive dogs.** Serologic pre-screening of serum for the detection of antibodies to *Brucella canis* is helpful in determining which animals to test with blood culture.

Development of a Real-Time RT-PCR for Detection of Viral Hemorrhagic Septicemia Virus

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Narrative: Viral hemorrhagic septicemia (VHS) is the most feared disease for farmed fish and causes significant economic loss in European countries. Since 2005, VHS has occurred in several locations in Great Lakes and caused massive die-offs in multiple native fish population. It continues to threaten the fish industry, tourism, and economy in the Great Lakes regions. Currently, viral isolation using cell culture is the standard method for diagnosis of VHS. However, this method is time consuming, labor intensive, and expensive. The conventional RT-PCR method used for confirmation of viral isolation is not sensitive for the IVb strain of Viral hemorrhagic septicemia virus (VHSV), which is prevalent in the Great Lakes. Here we report development of a real-time RT-PCR (RRT-PCR) method for VHSV. **The RRT-PCR is designed to amplify the nucleoprotein gene of all four genotypes of VHSV. This RRT-PCR is more sensitive (>100 time) than the conventional PCR.** Further evaluation will determine if this method can be used for detection of VHSV from clinical samples.

SYBR Green Real-time PCR Detection and Differentiation Assay for *Mycoplasma* Species in Biological Samples

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Narrative: Some mycoplasma species are considered significant pathogens in human and animal medicine, while others are considered opportunistic pathogens depending on their biological location within the host and their association with pathology. Additionally, mycoplasmas have fastidious growth requirements and thus different molecular based detection methods have been developed to detect common mycoplasma species in clinical samples. Here, we present the development and validation of a rapid, sensitive, cost effective real-time PCR (qPCR) detection assay for this genus utilizing SYBR dye. PCR primers utilized in SYBR PCR are based on those previously utilized in the conventional nested PCR (cNPCR) assay (Baird, S. C., et. al. 1999, J Vet Diagn Invest 11:432–435) with minor sequence modifications for increased specificity in qPCR. One set of PCR primers are utilized to amplify the conserved 16s-23s rRNA intergenic spacer region of the mycoplasma genome resulting in amplicons of various lengths depending on the specific mycoplasma(s) present. Various sizes of PCR amplicons and the sequence variations within those amplicons affect the determined melt temperature (TM), thus allowing differentiation of various species within the genus.

A side by side comparison of the relative sensitivity of SYBR qPCR compared to the cNPCR assay was performed by utilizing serial dilutions of *Mycoplasma bovis (M. bovis,* ATCC strain 25025) in negative bulk tank milk. Extracted DNA (Qiagen DNeasy) was run concurrently utilizing both methods. For cNPCR amplicon detection, capillary electrophoresis was performed on the Agilent Bioanalyzer. **Both assays were able to detect *M. bovis* equally and the SYBR qPCR had a linear range of detection over 6 logs and a PCR efficiency of approximately 86-90%.** We then set out to determine the actual limits of detection of SYBR qPCR by determination of colony forming units (CFU) of *M. bovis* detectable. Serial dilution of glycerol stocks of *M. bovis* were plated for CFU determination by standard microbial techniques. DNA extracted from volume equivalents of serially diluted *M. bovis* were utilized in SYBR qPCR**. SYBR qPCR was able to detect the 1 CFU *M. bovis* within the linear range of detection with a detection limit of 0.1 CFU.**

We utilized purified stocks of various pathogenic mycoplasma species for the determination of amplicon size and TM profiles for mycoplasma speciation. Mycoplasma species initially characterized included *M. bovis*, *M. dispar*, *M. agalactiae, M. alkalescens, M. arginini, M. bovigenitalium, M. californicum, M. canadense, M. conjunctivae, M. synoviae* and *M. ovipneumoniae* and the common environmental contaminant *Acholeplasma laidlawii*. Although many different mycoplasma species amplicons have overlapping TMs, mycoplasma species present are generally discernable based on host species and in some samples more than one mycoplasma species could be identified. Moreover, when uncharacterized amplicons are detected via SYBR qPCR, direct DNA sequencing of the amplicon allows for identification of novel pathogen/host interactions which constitutes the power of discovery of SYBR qPCR.

We analyzed 180 bulk tank milk samples utilizing conventional culture and subsequently SYBR qPCR. **Sixty-two of the bulk tank milk samples cultured positive for *Mycoplasma* spp. Of these, 51 samples were also PCR positive. Twenty eight of the culture-negative samples were *Mycoplasma* spp., positive utilizing SYBR qPCR protocol.** Of the qPCR positive bulk tank samples, 38 samples were confirmed to contain *M. bovis* alone (confirmed through DNA sequencing of the PCR amplicons), 20 samples had PCR amplicons of different size than *M. bovis*, and seven of these samples contained two different amplicons, one being *M. bovis* and others were identified as *M. alkalescens, M. arginini, M. bovigenitalium* and *M. gateae*.

Reduction in Mycoplasma Mastitis in Utah Dairy Herds Participating in a Follow-Up Project

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Narrative: Mycoplasma infections are an important disease complex in dairy cattle and calves. Progress in control of *Mycoplasma* spp. mastitis in previously positive Utah dairy herds and possible management changes were evaluated approximately one year after the disease was detected by a surveillance project.

Mycoplasma spp. had been detected in bulk milk from 16/222 dairy farms (7%) in a previous study in Utah. Follow up was conducted on 10 positive farms using a questionnaire developed at Utah State University. Seven positive farms (70%) had cultured bulk tank milk for mycoplasma; testing was still being done monthly on 4 farms (40%). Individual cow milks were cultured for mycoplasma on 8 farms (80%); 3 farms (30%) had cultured milk of all lactating cows for mycoplasma approximately one month after detection by the surveillance project and 5 farms (50%) cultured milk from all cases of clinical mastitis. Four of the latter 5 farms also cultured milk from cows after calving, and were continuing to culture both clinically mastitic and post-calving cows' milk. The one farm that cultured only clinical mastitis cases had stopped "after several months", after selling all positive cows and finding no more.

During the previous 3 months, 2 farms (20%) had found no mycoplasma from cultures of all clinically mastitic or fresh cows, and the farm that had previously sold all positive cows after culturing clinical mastitis cases had found no bulk tank milk cultures positive for *Mycoplasma* spp. **Therefore 3 farms (30%) had no further evidence of mycoplasmal mastitis during the previous 3 months**. Four (40%) farms continued to have mycoplasma mastitis cases diagnosed (2 had individual positive cows, one had one positive bulk tank, and one had not sold all known mycoplasma-positive cows). Three farms (30%) were of unknown mycoplasma status because they had no current mycoplasma testing program or did not know the results of bulk tank testing that was being done by their herd health veterinarian.

Of the 7 producers who had cultured individual cows and found some positive for mycoplasma, 5 (71%) had sold all positive cows during the previous year and 2 (29%) had not; 4 (57%) milked all mycoplasma-positive cows last and 3 (43%) did not, but the latter 3 stated that they sold mycoplasma-positive cows "right away".

All of the farms with no further culture evidence of mycoplasma in cows, and farms with very low prevalence remaining, were among the farms that cultured milk from all clinical mastitis cases for mycoplasma. Of **farms that had used only bulk tank monitoring for mycoplasma, all were still positive or had unknown status**. All herds increased lactating cow numbers, and 6 had decreased BTSCC. Milking practices were virtually unchanged since mycoplasma was diagnosed; management changes if any focused on testing and handling of mycoplasmal cows. The most successful practice associated with elimination of myocplasma mastitis was culture of milk from all clinical mastitis cases and post-calving cows followed by culling of all mycoplasma-positive animals found.

Mycoplasma mastitis persisting in herds was associated with observing adult cows with droopy ears, clinical mastitis moving from one mammary gland quarter to another, and nonresponsive respiratory disease in calves. An important benefit of reducing or eliminating mycoplasma mastitis from dairy herds was an association with reduced clinical mastitis, and an even greater decrease in rate of treatment for clinical mastitis.

Application of the IDEXX MAP ELISA for the Diagnosis of Both Johne's Disease and Caseous Lymphadenitis Disease in Sheep and Goats

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Narrative: Johne's disease is caused by infection of mesenteric lymph nodes and intestines of ruminant species with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP bacteria are transmitted from adult to their young *in utero* or via colostrum, milk and feces. Caseous lymphadenitis disease (CLA) is caused by abscess formation in skin, lymph nodes and internal organs due to infection with the bacteria *Corynebacterium pseudotuberculosis.* CLA bacteria are transmitted to animals or the environment by draining external abscesses from infected animals. Both of these cell-mediated bacterial diseases cause chronic infections that are difficult to eradicate from positive herds or flocks because infected animals are difficult to identify, and, infected animals will shed these bacteria into the environment where they are viable for at least a year. Because animals infected with these bacteria cannot be cured, control depends on detection and removal of positive animals.

This diagnostic testing to identify CLA positive animals is part of a larger study to identify Johne's-positive small ruminates. The majority of the flocks/herds we are testing for Johne's are also infected with CLA. A serology ELISA test is not available to detect CLA positive animals.

Two bovine serology ELISA tests for detecting MAP positive animals are compared with culture to detect CLA-positive animals:

- MAP **ELISA** tests: IDEXX Herdchek using 0.250 S/P cutoff on sheep and goat serum/plasma/ milk samples; and, IDEXX Pourquier using 0.300 S/P cutoff.
- **Culture** of feces and tissues for MAP using increased sediment inoculum and time in culture with liquid culture media: BACTEC™ MGIT™ para TB liquid medium.
- **Culture** of abscesses/tissues for CLA on Columbia Blood Agar.

We have assayed serum and milk samples from three producers that have both CLA and MAP positive animals with the two ELISA tests. One producer vaccinates all animals for CLA. Of 137 serum samples tested with both ELISA tests, IDEXX Herdchek identified 51 MAP antibody positive and IDEXX Pourquier identified 33, with 29 samples positive with both tests. The Herdchek S/P results were all 1.00 or higher for these 29 serum samples both tests identified as positive. Eighteen serum samples assayed had positive results (S/P: 0.250-0.700) on the Herdchek test and were negative on the Pourquier test. Twenty-seven of these animals were MAP fecal culture positive. Ten animals were CLA and MAP culture positive. If animals were sampled January-June, and serum samples positive on both ELISA tests, the fecal samples from these animals (21) were MAP culture positive. Of 44 milk samples tested with both ELISA tests, Herdchek identified 14 Johne's antibody positive milk samples, and Pourquier 11 positive milk samples, with agreement on seven samples. Ten animals tested by milk ELISA were fecal culture positive. Of the 40 serum samples tested from the CLA vaccinated flock, six were positive with the Herdchek ELISA (S/P: 0.515- 2.259), two were positive with the Pourquier ELISA (S/P: 0.778-1.501). From this flock, three were CLA culture positive and six were MAP culture positive.

Thin animals with a positive Herdchek S/P are considered suspect for either CLA or MAP. These animals are euthanized and cultured. If the initial serum and milk samples from these animals are positive with an S/P is greater than 0.700, the animal is MAP positive. If only the serum has a positive S/P reaction of 0.250-0.700, this animal can have either bacterial infection. We have identified a sheep flock that is CLA infected and vaccinated, but is MAP negative. So far animals from this flock that have internal CLA lesions are ELISA positive. **The IDEXX Herdchek MAP ELISA will detect either CLA or MAP positive animals using the 0.250 S/P cutoff on serum samples.**

Turkey Cellulitis: Descriptive Epidemiology and Molecular Characterization of Potential Etiological Agents

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Narrative: Turkey cellulitis is a major disease across all geographic regions of the US. In 2006 and 2007 it ranked among the top five disease concerns. The infection shows a relatively low prevalence, although it can be devastating in the individual farms affected. It appears with high prevalence and with high mortality in heavy market-age birds, and is more common in males than females. Dead birds usually show "bubbly tail", fluid filled blisters associated with root-broken feathers. In addition, there are cases where individuals will have an accumulation of gelatinous fluid under the skin, usually along the thighs (inguinal area) and breast. Currently, the agent associated with the development of cellulitis in turkeys is unknown, with clostridia being the main suspect. This study aims to (1) characterize the descriptive epidemiology of turkey cellulitis, including evaluation of the time, place, and host characteristics of this disease in turkeys and (2) identity of the molecular characteristics of clostridia associated with turkey cellulitis. To achieve these goals, farms with high and low risk of having the disease were identified and are now being monitored. Live and dead birds showing clinical signs and/or lesions characteristic of cellulitis are submitted weekly to the University of Minnesota Veterinary Laboratory for testing. In the absence of clinical signs and lesions, randomly selected birds at the ages of 6, 8, 16 and 20 weeks from flock involved in the study are sent for diagnostic testing. Samples collected from each bird includes: liver and sub-cutis swabs, stool and litter from growing facilities. Samples are cultured and isolation of *Clostridium* sp. is attempted. *Clostridium* sp. isolates are further characterized by sequencing of the 16s rRNA gene, allowing the identification to the species level. *Clostridium perfringens* and *Clostridium septicum* isolates are further characterized for the presence of toxin genes using a multiplex PCR and by multilocus sequence typing (MLST) to infer relatedness. Quantitative real-time assays are used to define the number of *C. perfringens* and *C. septicum* in fecal and litter samples. The first samples from the turkey flocks involved in this study were received in November of 2008. We have cultured samples from 159 healthy birds and 59 birds from cellulitis outbreaks. Our preliminary data indicates that ***Clostridium septicum* is consistently isolated from clinical samples (subcutaneous swabs and liver) of birds affected by cellulitis**. **High numbers of *C. perfringens* and *C. septicum* are detected in the fecal samples of outbreak birds, but not in healthy birds**. **High numbers in the fecal samples is not translated into high numbers in the environment (litter samples)**. Preliminary MLST data suggests that *C. septicum* isolates recovered from clinical cases are fairly clonal.

Relevance: This is the first study to comprehensively characterize the etiological agent of turkey cellulitis. New diagnostic techniques for detection and typing of *C. septicum* were developed and validated and are now available for routine diagnostics of Clostridial dermatitis.

Differential Gene Expression Study of bTB-Positive Cattle and bTB Test-False Positive Cattle in Michigan

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Narrative: Bovine Tuberculosis (bTB) is a disease caused by *Mycobacterium bovis* infection. Under current federal regulations, cattle that test as reactors on two successive federally approved diagnostic tests are examined post mortem for bTB. Currently, in Michigan, <1% of cattle that test as reactors on two successive tests are confirmed as positive for bTB.

We conducted a microarray-based comparative genomic hybridization study to examine the altered gene expression patterns in three groups of cattle. Those groups included cattle that had bTB (n = 4), cattle that did not have bTB but tested positive for bTB by two successive tests (double reactors, n = 4), and cattle that did not have bTB but tested positive for bTB by the caudal fold skin test only (single reactors, n = 7). Cellular RNA from peripheral blood mononuclear cells (PBMCs) was harvested at four hours post-stimulation with purified protein derivative made from *Mycobacterium bovis* (bovine PPD). The RNA from individual cattle was co-hybridized with a pooled control RNA from 12 healthy cattle that were non-reactors on both the CFT and on the whole blood gamma interferon assay for bTB. Hybridization was done using a mononuclear leukocyte derived bovine cDNA microarray (BOTL5) with duplicated spot features representing 1,391 genes.

At a 2 fold or greater change in expression level compared with healthy cattle ($P \leq 0.01$), we detected 2 genes differentially expressed in single reactor cattle, 7 genes in double reactor cattle, and 4 genes in the true bTB positive cattle. All of these genes are unique to each of the groups of cattle. Of the 2 genes in single reactor cattle, one was up-regulated (2.7 fold) and the other was down-regulated (-5.7 fold). In double reactor cattle, most of the differentially expressed genes were up- regulated (2.1 to 5.1 fold), whereas, in the bTB positive group, most were down-regulated (-2.1 to -4.0 fold). **The unique differences in gene expression between cattle that are bTB positive and cattle that test-false positive for bTB might be exploited to develop a rapid quantitative PCR assay to differentiate false test-positive cattle from cattle that have bTB.**

We have conducted additional experiments, using similar groups of cattle and overnight stimulation of whole blood with bovine PPD. The cellular RNA from those animals was hybridized to a bovine long-oligo microarray (BLO Plus) representing 10,219 bovine genes. Preliminary data from those experiments supports the hypothesis that differential gene expression patterns will provide a sufficient number of uniquely regulated genes to develop a real-time quantitative PCR assay that can be used to reduce the expense of indemnification and needless slaughter of healthy cattle.

Alternative Assays and Testing Algorithm for Confirmation of Suspect False Positives in a Commercial Enzyme-Linked Immunosorbent Assay for Porcine Reproductive and Respiratory Syndrome Virus

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Narrative: Porcine reproductive and respiratory syndrome (PRRS) cost the US swine industry approximately $600M annually. Accurate and timely diagnosis of virus infection is necessary for effectively monitoring infection status and controlling the disease. Serology provides a great assistance in that endeavor along with PCR assay. The commercial ELISA kit (IDEXX HerdCheck) has been the main serological tool for PRRS virus (PRRSV) in North America and many pig producing regions throughout the world. Although the kit has been mostly a reliable test for detecting PRRSV exposure at the herd level, the specificity of the kit has been frequently challenged by the occurrence of unexpected false positive result at varying rates. An indirect fluorescent antibody (IFA) test is commonly employed to confirm or disprove the suspect false positive reactivity in the ELISA. However, the reliability of test result has been often questioned due to the subjectivity in determination of test result and perceived less sensitivity. The following study was conducted to develop alternative assays and establish a confirmatory testing algorithm when suspect false positive results occur.

The ORF7 genes, encoding the nucleocapsid (N) protein of both Lelystad and VR2332 strain of PRRSV, were cloned into *Autographa clifornica* multiple nuclear polyhedrosis virus (baculovirus) at downstream of the polyhedron promoter followed with 6×His tag. The recombinant baculoviruses, designated as bac-6×His -ORF7EU and bac-6×His -ORF7NA, were obtained by transfection into Sf-9 insect cells with cationic lipids. The 17.5-kDa and 17.3-kDa N fusion proteins were expressed in Sf-9 cells respectively. Each recombinant protein was purified by immobilized metal affinity chromatography. The proteins were then applied to Western immunoblotting analysis (WIA) and a microsphere immunoassay (MIA) to detect antibodies specific for PRRSV in comparison to the standard IFA test and ELISA. In addition, the IDEXX ELISA kit was employed in a blocking assay format using monoclonal antibodies specific for the N protein of PRRSV. All assays were validated using sera from pigs with known infection status. Diagnostic performance of the assays was assessed on field samples with various statuses of PRRS as determined by ELISA, including suspect false positives.

In WIA each antigen demonstrated specific reactivity with reference hyperimmune sera raised against a strain of North American (NA) or European (EU) PRRS viruses and also showed the cross reactivity for both antisera as expected. The WIA using these antigens correctly identified positive and negative sera collected from experimental pigs, demonstrating the immunoreactive nature of the antigens. MIA using beads coupled with these antigens on the sera from experimental pigs yielded quantitative results in concordance with known status of the samples. When field samples were tested, MIA clearly classified suspect false positive samples in ELISA as negative, which was in agreement with results of WIA and blocking ELISA. These results indicate that **the ORF7 fusion proteins produced in this study can be utilized as specific antigen with high quality in alternative diagnostic immunoassays for PRRSV**.

Epidemiology of Infectious Salmon Anemia Virus in Farmed Salmon

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Narrative: Infectious salmon anemia (ISA) is a World Animal Health Organization (OIE)-listed disease that is considered one of the most important diseases of salmon because of its highly contagious nature and the economic consequences that it inflicts to affected regions. ISA is caused by an *Orthomyxovirus* referred to as Infectious salmon anemia virus (ISAV). The infection affects predominantly farmed Atlantic salmon (*Salmo salar*) during the marine grow-out phase. One of the largest farmed salmon regions in the world, located in southern Chile, has recently experienced the reemergence of the disease, which has affected 113 farms of Atlantic salmon since June 2007. The spatio-temporal pattern of the epidemic was assessed using techniques for the detection of clusters in time and in space and taking into account the commercial compartmentalization of the farms. In addition, using different indirect approaches, we estimated the farm-level basic reproductive number (R_h), which was defined as the expected number of secondary infections that resulted from a single infected farm during its period of infectiousness.

Results suggested that **cases clustered around the index case (IC) in a propagated epidemic mode**. The **estimated attack rate of the epidemic was 38%.** Results suggested that **delayed depopulation may have been a key factor in the spread and persistence of ISAV**. Clustering of cases supported the hypothesis that **seawater transmission from ISAV-infected farms is a critical factor in controlling disease**. The value of R_h during the first two years of the epidemic ranged from **1.8 to 2.0**. The overall mean distances between infected farms (using Euclidean and shortest seaway estimations) were about **11-12 km**, which is consistent with the size of the clusters identified in this study.

Exploratory analyses presented here characterized the size, duration, and dynamics of the ISAV epidemic in one of the most important regions for salmon farming in the world. Through the estimation of the most important epidemiologic parameters during an outbreak, this study generates new hypotheses and provides useful information for spatial disease control planning in salmon farming areas.

Experiences After Eighteen Months of Bovine Viral Diarrhea Eradication Program in Switzerland

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Narrative: In 2006 the officials decided to eradicate Bovine Viral Diarrhea (BVD) in Switzerland. It took many months of discussion to convince all parties involved on how to start a BVD eradication program.

When it started in early 2008, five tests, four of them commercially available, were validated and accepted by the Swiss reference laboratory (Institute of Veterinary Virology, University Bern), to detect BVD-Antigen in ear notches (alternatively blood samples were also being used in small programs and studies) for use in the Swiss BVD Eradication Program.

1. HerdCheck BVD AG/Serum plus – EU Protocol (Idexx Laboratories Inc.)
2. PrioCHECK BVD AG PI focus ELISA (Prionics AG)
3. BoVIR-SL *real time* PCR (AnDiatec GmbH)
4. cador BVDV *real time* PCR (Qiagen GmbH)
5. Immunochemistry (in house method by the Institute of Pathology, University Zürich)

To decide which test would be suitable for our laboratory we participated in a national interlaboratory ring test, which provided blinded panel ear notch samples as well as blinded blood samples for the commercial methods mentioned above, which were approved for the respective sample materials.

Based on our experiences with the assays, testing protocols, support and the results obtained, we decided for the IKMI lab to start the eradication program with two of these tests, the *real time* PCR of AnDiaTec (test automated by Corbett) for cattle younger than 2 month and the Prionics ELISA (test performed on Beckman Biomek) for all the rest of the samples. Occasionally we also used the Idexx ELISA for blood samples, when cattle destine for exportation had to be tested.

The official BVD eradication program in Switzerland started in February 2008 and throughout the past 18 months we tested over 300,000 samples, which highlighted additional aspects of the overall program that were not evident in the initial ring trial evaluation.

The first phase (Alp-phase) of the Swiss national BVD eradication program consisted in testing all cattle destined to spend the summer in the Alps, a factor unique to central Europe. In general, these cattle are older than 6 months and our lab used mainly the Prionics ELISA for BVD testing. After false negative results were observed we adjusted the cut off (means adjust the sensitivity), raised the age for PCR testing to 6 month and intensified retesting critical results with our *real time* PCR method. Through June 2009 we have detected 15 false negative animals (tested with ELISA). For some this might only seem like a few, but in the Swiss epidemiological context, we considered it considerably high. Our lab concluded the false negative results were a consequence of the limited sensitivity of ELISA tests when used with routine field samples and not perfectly standardized samples used for approval.

At this time the so called "Alp-phase" has been completed. All cattle in Switzerland were tested and those identified as positive were slaughtered. Now the "Calf-phase" is currently underway and only approved *real time* PCR diagnostic tests and the Idexx ELISA EU Protocol are approved for further testing. If a positive is detected the farmer can request the Swiss reference lab retest to verify the original results, as it was in the "Alp-phase" of the program, using *real time* PCR.

If our lab could restart the program, we would not test with anything but our *real time* PCR assay. The method is reliable, easy to perform and we had no false negative results reported so far. Since the sensitivity or specificity is, as in no test, 100 percent a few samples have to be retested. But if eradication of BVD is the purpose, *real time* PCR should be the method of choice.

Pathology Scientific Session

Sunday, October 11, 2009
San Diego

Moderators: Jerome Nietfeld and Juergen Richt
Sponsor: Ventana Medical Systems, Inc.

08:00 AM **Host Predilection of Epidemic Vesicular Stomatitis New Jersey Virus Strains in Cattle ***
Paul Smith, Daniel Mead, Elizabeth Howerth, David Stallknecht, Deborah Carter, Raymond Noblet 107

08:15 AM **The Early Pathogenesis of Foot-and-Mouth Disease in Cattle after Aerosol Inoculation***
Jonathan Arzt, Juan Pacheco, Luis Rodriguez 108

08:30 AM **CD8+/perforin+/WC1- gammadelta T, CD4+/perforin- alphabeta T, and B Lymphocytes Infiltrate Vasculitis Lesions of American bison (*Bison bison*) with Experimentally-Induced Sheep-Associated Malignant Catarrhal Fever ***
Danielle Nelson, William Davis, Wendy Brown, Hong Li, Donal O'Toole, Lindsay Oaks 109

08:45 AM **Median Infectious Dose (ID50) of Porcine Reproductive and Respiratory Syndrome Virus Isolate MN-184 for Young Pigs via Aerosol Exposure ***
Timothy Cutler, Apisit Kittawornrat, Steven Hoff, Chong Wang, Jeffrey Zimmerman 110

09:00 AM ***In situ* Kinetics and Distribution of the Vesicular Stomatitis Virus During Early Infection in Cattle Inoculated via Scarification and Black Flies ***
Janildo Reis, Luis Rodriguez, Danny Mead, George Smoliga, Paul Smith, Corrie Brown 111

09:15 AM **Systemic Distribution of Lesions and Viral Antigen in Alpacas Acutely Infected with Type 1b Bovine Viral Diarrhea Virus (BVDV) Isolated from Crias with Naturally-Occurring Persistent BVDV Infections**
David Steffen, Christina Topliff, John Schmitz, John Kammerman, Jamie Henningson, Clayton Kelling 112

09:30 AM **Outbreak of Malignant Catarrhal Fever in Cattle Associated with a State Fair**
Dale Moore, Paul Kohrs, Timothy Baszler, Cynthia Faux, Peter Sathre, John Wenz, Leonard Eldridge, Hong Li 113

09:45 AM **Permissiveness of Swine to Infection with H5N1 Highly Pathogenic Avian Influenza Viruses**
Bruce Janke, Richard Webby, Kelly Lager, Jason Buehler 114

10:00 AM Break

10:30 AM **Development of a Forensic Pathology Course for Veterinary Students**
Doris Miller, Melinda Merck 115

10:45 AM **Foot-and-Mouth Disease in Feral Swine: Susceptibility and Transmission**
Fawzi Mohamed, Seth Swafford, Heather Petrowski, Alexa Bracht, Brandon Schmit Andrew Fabian, Juan Pacheco-Tobin, Ethan Hartwig, Mary Lou Berninger, Consuelo Carrillo, Gregory Mayr, Karen Moran. 116

11:00 AM **Evaluation of Commercial Monoclonal Antibodies for Use in Immunohistochemical Staining for Porcine and Bovine Group A Rotaviruses**
Joe Brodie, Kyoung-Jin Yoon. 117

11:15 AM **Perianal Squamous Cell Carcinoma Including a New Variant of Giant Cell Carcinoma in Boer Goats**
Tawfik Aboellail, Brian Porter, Karen Fox. 118

11:30 AM **Cattle as Potential Amplifying Hosts of Vesicular Stomatitis New Jersey Virus ***
Paul Smith, Daniel Mead, Elizabeth Howerth, Deborah Carter, Raymond Noble, Luis Rodriguez. 119

11:45 AM **Progressive Accumulation of PrPCWD and Spongiform Encephalopathy in the Obex of Rocky Mountain Elk (*Cervus elaphus nelsoni*) with Chronic Wasting Disease and Use as a Means of Scoring the Stage of Disease and Predicting Accumulation of Prion in Peripheral Tissues**
Terry Spraker, Tom Gidlewski, Jenny Powers, Scott Wright, Aru Balachandren, Margaret Wild, Katherine O'Rourk 120

***** Graduate student presentation

Host Predilection of Epidemic Vesicular Stomatitis New Jersey Virus Strains in Cattle

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Introduction: Vesicular stomatitis (VS) is extremely important to animal health authorities because the clinical symptoms in cattle, swine, and other cloven-hoofed animals mimic those of foot-and-mouth disease. Epidemiological data collected during epidemics in the western United States combined with limited experimental studies using swine and cattle suggest that host predilection of epidemic vesicular stomatitis New Jersey virus (VSNJV) strains result in variations in clinical response, extent and duration of virus shedding and transmissibility following infection in different hosts. Laboratory challenge of cattle and horses with heterologous VSNJV strains to investigate a potential viral predilection for these hosts has not been thoroughly investigated. Our goal was to determine the extent to which clinical outcome and extent and source of virus shedding in VSNJV infected cattle are dependent on virus strain and route of inoculation.

Materials and Methods: In separate trials, homologous VSNJV strains (82-CO-Bovine and 82-AZ-Bovine), and heterologous strains (06-WY-Equine and OSS-122[Ossabaw Island, sand fly]) were inoculated into cattle via infected black fly bite. For each trial, 3 cows were inoculated on the lower lip, 2 on the coronary bands, and 2 on the neck. Animals were observed and swabbed daily to determine extent and duration of viral shedding. Clinical scores, based on size of lesion, and overall health of clinically affected animals, were analyzed using a nonparametric, Kruskal-Wallis test of rank.

Results and Discussion: Clinical scores among the viruses were significantly different ($p=0.0408$). A pair-wise comparison showed that animals inoculated with 82-AZ-Bovnie had more severe disease symptoms than those inoculated with 82-CO-Bovine and OSS-122. Lameness and behavioral changes were only observed in animals inoculated with 82-CO-Bovine and 82-AZ-Bovine. Cattle inoculated on the lip or coronary band with 82-AZ-B shed virus for an average of 5.2 days. Cattle inoculated with 82-CO-B, 06-WY-E and OSS-122 shed for an average of 2.1, 3.0, and 2.6 days respectively. Maximum virus titers were not significantly different among the virus strains, but were numerical higher for animals inoculated with 82-AZ-B. **Clinical outcome, and extent and duration of shedding in cattle appear dependent upon both virus strain and inoculation site. Clinical outcome in which obvious discomfort and behavioral changes were observed only in animals inoculated with heterologous strains of VSNJV**. Additional trials to be conducted on heterologous VSNJV strain inoculations of horses and swine will add to a greater understanding of VSNJV epidemiology, and a greater predictive ability of disease progression during VSNJV outbreaks.

The Early Pathogenesis of Foot-and-Mouth Disease in Cattle After Aerosol Inoculation

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Narrative: Sixteen steers were inoculated with foot-and-mouth disease virus (FMDV) strain O1-Manisa via an aerogenous inoculation system which closely simulates natural infection for the purpose of characterizing the early (previremic) events of infection. Antemortem samples collected from cattle were serum, nasal swabs, and oral swabs; euthanasia at predetermined time – points spanning 0.1 – 240 hours post aerosolization (hpa) were followed by necropsy with collection of up to 46 distinct tissue samples per steer. All samples were screened for FMDV by virus isolation and for FMDV RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR). Tissues were additionally examined by immunohistochemistry and multi – channel immunofluorescence microscopy. Antemortem disease was characterized by earliest onsets of detection of de novo replicated FMDV RNA from nasal swabs at 6 hpa, oral swabs at 24 hpa, and serum at 22 hpa. Vesicles were first observed at 48 hpa. De novo replicated FMDV RNA was detected in nasopharyngeal and pulmonary tissues from 3 – 240 hpa. **Prevalence of FMDV – positivity of tissues from previremic steers indicated that nasopharyngeal tissues are the most frequent primary site of viral replication followed by lungs. The follicle associated epithelium of mucosa – associated lymphoid tissue of the nasopharynx was the earliest site of microscopic localization of FMDV antigens.** Onset of viremia coincided with a markedly increased quantity of viral antigens in pulmonary samples.

CD8+/perforin+/WC1- gammadelta T, CD4+/perforin- alphabeta T, and B Lymphocytes Infiltrate Vasculitis Lesions of American Bison (*Bison bison)* with Experimentally-Induced Sheep-Associated Malignant Catarrhal Fever

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Introduction: Sheep-associated malignant catarrhal fever (SA-MCF) is a fatal disease associated with lymphoproliferation, lymphocytic vasculitis, and mucosal ulceration in susceptible species. SA-MCF is an important threat to American bison (*Bison bison)* due to their high susceptibility to infection and disease caused by *ovine herpesvirus*-2 (OvHV-2). The pathogenesis of MCF is poorly understood.

Materials and Methods: Captive bison were screened for antibody against the MCF group viruses, for OvHV-2 by semi-nested PCR, and for bovine viral diarrhea virus by reverse transcriptase PCR. Six OvHV-2 negative bison were infected by nasal aerosolization with OvHV-2 from pooled nasal secretions of sheep during OvHV-2 shedding peaks. Once OvHV-2 was detected by PCR in the peripheral blood and the bison developed clinical signs, the animals were euthanized. Full necropsies were performed.

Antibodies reactive with bovine immune cell markers were proven to cross-react with bison markers using indirect immunofluorescence (IF). Lymphocyte subsets in vascular lesions from bison with SA-MCF were phenotyped by concurrent detection of up to three immune cell markers on ethanol-fixed cryosections of urinary bladder, kidney, and liver using indirect polychromatic IF. Vascular lesions in the urinary bladder of bison with SA-MCF were localized using DAPI, a fluorescent nuclear stain identifying cell nuclei, polyclonal antibodies specific for von Willebrand's factor (vWF), an endothelial cell marker, and a single leukocyte marker. Single leukocyte markers (CD2, CD3, CD4, CD8α, CD25, CD79a, CD335/NKp46, WC1, CD14, and the TCR1-N24 δ chain) were used to determine which immune cell subtypes were consistently present. Lymphocytes were further phenotyped by concurrent detection of three lymphoid cell markers in vascular lesions of the urinary bladder, kidney, and liver.

Results: CD8+/perforin+ γδ T cells, CD4+/perforin- αβ T cells, and B cells were consistently present in vascular lesions in the urinary bladder, kidney, and liver of all six bison with SA-MCF. CD8+ αβ T cells, NK cells (NKp46 +), macrophages (CD14+ or calprotectin+ cells), and WC1+ γδ T cell cells (WC1+) were not consistently identified in vascular lesions.

Discussion: In previous reports, single-color immunohistochemical detection of CD8+ cells in the vascular lesions of cattle and bison was interpreted as cytotoxic αβ T cells since WC1+ cells were not identified. However, this study identified CD8+/perforin+/WC1- γδ T cells and CD4+/perforin- αβ T cells within the vascular lesions of bison. γδ and CD4 T cells have the potential for cytotoxicity and/or regulatory function, and both may contribute to the pathogenesis of MCF.

Conclusion: Since there is evidence of infection in CD8+ (presumably γδ T) lymphocytes and no current evidence of infection in cells that compose the vascular walls, we hypothesize that lymphocyte infection causes immune dysregulation and immune-mediated vasculitis in bison with SA-MCF. Improving understanding of immunopathological mechanisms of MCF will assist in developing appropriate vaccine strategies to prevent disease in susceptible animals.

Median Infectious Dose (ID50) of Porcine Reproductive and Respiratory Syndrome Virus Isolate MN-184 for Young Pigs via Aerosol Exposure

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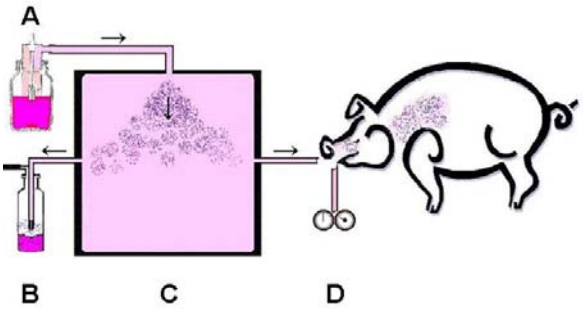
Introduction: The objective of this research was to quantify the likelihood of porcine reproductive and respiratory syndrome (PRRSV) transmission via aerosols as a function of exposure dose. This data is critical to understanding of airborne transmission of PRRSV between pigs, within herds, and between herds.

Materials and Methods: The study used PRRSV isolate MN-184 (kindly provided by Dr. Scott Dee, UMN). All procedures were approved by the Iowa State University IACUC committee (#9-07-6429-S). All pigs were confirmed PRRSV negative prior to commencement of the experiment and were housed in HEPA-filtered isolation units throughout the experiment to avoid inadvertent transmission of pathogens.

The study was conducted in 10 replicates, 10 pigs per replicate, with pigs randomly assigned to treatment. One negative control pig and one positive control pig were included in each replicate. The negative control pig was used to validate biosecurity procedures, i.e., was present in the laboratory during the aerosol exposure treatments, was housed in the same room as pigs exposed to PRRSV aerosols, and was monitored throughout the observation period. The positive control pig was inoculated intramuscularly with 10 ml of fluid from the last air sample in each run to prove that infectious, airborne PRRSV was in the air to which pigs were exposed throughout the experiment.



To conduct the experiment (Fig 1), PRRSV MN-184 was aerosolized [A] into a dynamic aerosol toroid [C]. Pigs to be exposed to the PRRSV aerosol were anesthetized, fitted with a canine surgical mask attached to a pediatric spirometer [D]. Each pig respired 10 liters of virus aerosol. Air samples [B] were collected before and after each pig were used to estimate the exposure dose.

Figure 1. **[A]** Nebulizer, [**B]** Impinger, [**C]** Aerosol reservoir, [**D]** Spirometer

Serum samples collected 5 and 10 days post-exposure (DPE) were tested for the presence of PRRSV to determine whether exposure resulted in infection. The dose-response curve for exposure to airborne PRRSV was derived from the proportion of pigs infected by dose.

Discussion/Conclusion: Three replicates were disqualified due to failure to meet quality criteria; therefore, the infectious dose 50 (ID50) estimate was based on 7 replicates. Initial analysis showed that the infective dose 50 (ID50) of MN-184 under the parameters of this study (pig body size and age, exposure dose and time) was $<1 \times 10^{1}$ TCID50. Under comparable conditions, this ID50 estimate is much lower than a previous estimate based on PRRSV isolate VR-2332 (Hermann et al., 2009). Thus, the data suggest that the ID50 for airborne PRRSV may vary among isolates.

In situ Kinetics and Distribution of the Vesicular Stomatitis Virus During Early Infection in Cattle Inoculated via Scarification and Black Flies

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Introduction: Vesicular stomatitis virus (VSV) affects cattle, horses and pigs. Primarily spread by biting flies, it is a disease of significant economic impact in horses. In cattle and pigs VSV exists as a major confounder for foot-and-mouth disease. The pathogenesis of the disease has not been well elucidated. Although animals are systemically ill, no viremia has been reported. There are studies documenting the late stages of the VSV infection, but there are no studies regarding the *in situ* temporal distribution of the virus during the early infection in livestock.

Materials and Methods: Ten Holstein steers were inoculated on the right coronary bands (CBs) with VS New Jersey virus - six received the virus via scarification (SC) with 10^7 plaque forming units (PFU) and four were inoculated by VSV-infected black fly (*Simulium vittatum*) bite (FB), representing an estimated dose of 10^2 to 10^3 PFU. Euthanasia was at 12, 24, 48, 72, 96 and 120 hours post inoculation (HPI) for SC, and 24, 48, 72 and 96 HPI for FB. All animals were mock inoculated on the left side with the same technique that was used on the right. Two additional steers, euthanized at 24 HPI, were mock inoculated on the right side while the left was untouched. The CBs, axillary, prescapular, popliteal and prefemoral lymph nodes (LN) from all limbs, as well as spleen and blood were collected. Samples were processed for virus isolation (VI) and reverse Real Time PCR (rRT-PCR) for detection of viral RNA. *In situ* hybridization (ISH) for detection of viral RNA and immunohistochemistry (IHC) for detection of viral protein were done on formalin-fixed paraffin-embedded tissues.

Results: Grossly there were swelling and hyperemia present at the right CBs as early as 24 HPI in both FB and SC groups, with progression to vesicles at 48 and 96 HPI. Microscopically at 12 HPI lesions were present only in small focal areas of the CB epidermis whereas at 24 HPI, there was widespread and coalescing intra- and inter-epithelial edema predominantly in the midzone of the stratum spinosum. From 72 to 120 HPI there was extensive epithelial necrosis with cleft formation and loss of the epidermis. *In situ* hybridization revealed intense cytoplasmic signal in keratinocytes from 12 until 48 HPI in both FB and SC groups, with faint staining at 72 and 96 and no staining at 120 HPI. In the lymph nodes, ISH highlighted scattered cells at cortical and subcapsular areas at 24 HPI only. Immunohistochemistry in coronary band showed intense cytoplasmic labeling of keratinocytes until 72 HPI, with faint staining at 96 and 120 HPI. In the lymph nodes, in contrast to what was observed with ISH, there was positivity until 120 HPI, with positive cells stained in the subcapsular regions at 24 HPI and at perifollicular regions after 48 HPI. Positive VI and rRT-PCR were restricted to the site of inoculation and draining LNs.

Discussion: Inoculation by SC or FB produced very similar disease, although the FB inoculum dose was logarithmically lower, suggesting that there may be factors facilitating infection provided by the fly bite. Abundant virus was produced at the site of inoculation, in keratinocytes. There was limited viral spread to local lymph nodes with ISH demonstrating that some virus was replicating there for only a very short period after infection. Presence of small amounts viral protein, as detected by IHC, in the lymph nodes at 96-120 HPI, when there was no evidence of viral nucleic acid, likely represents viral antigen presentation rather than active viral replication. VI and rRT-PCR of blood and spleen were consistently negative, which confirms the lack of viremia reported in previous studies. Taken together, the results suggest that there is a strong local response preventing systemic spread.

Conclusion: Vesicular stomatitis virus replicates to high copy numbers at the inoculation sites, whether by scarification or fly bite, with limited spread to local lymph nodes and no viremia. Much lower inoculation doses are needed with FB. IHC consistently detected the presence of the virus and will be useful for diagnostic purposes.

Systemic Distribution of Lesions and Viral Antigen in Alpacas Acutely Infected with Type 1b Bovine Viral Diarrhea Virus (BVDV) Isolated from Crias with Naturally-Occurring Persistent BVDV Infections

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Narrative: Natural occurrence of persistent bovine viral diarrhea virus (BVDV) infections in alpacas, as in cattle, is widely recognized. [1] We hypothesized that acute BVDV infection would lead to lymphodepletion of gut-associated lymphoid tissues, similar to acute disease in calves.[2] The objectives of the present study were to characterize systemic distribution of lesions and viral antigen deposition in acutely infected alpacas following experimental infection with type 1b noncytopathic BVDV (BVDV C006) isolated previously from a cria with naturally occurring persistent BVDV infection.

Lymphopenia with 50% reduction in cell counts developed in inoculated alpacas and the timing of lymphopenia was similar to that reported in calves. Lymphodepletion of target gut-associated lymphoid tissues was evident. BVDV antigen was deposited most consistently in aggregated lymphoid tissues of stomach compartment 3 (C3) as well as ileal Peyers patches. Staining was also common in aggregated lymphoid tissues of the colon and in lymphoid follicles at the rectal anal junction. Viral antigen was more variably evident in thymus, mesenteric lymph nodes, prescapular lymph nodes, parotid lymph nodes, nasopharyngeal tonsil and pharyngeal tonsil. Antigen distribution was most often diffuse in dendritic cells and lymphocytes of affected follicles. Occasionally staining was concentrated along the periphery of follicles opposite the mucosa. Rare individual macrophages stained in the lamina propria of the gut. Most gut associated lymphoid tissues, if adequately sampled, were antigen positive. Sparse distribution of follicles, perhaps due to age related lymphoid atrophy, created inefficiency in consistently identifying mucosal lymphoid patches in sites other than C3 and ileum. Mild lymphocytolysis and varied, generally moderate lymphodepletion due to BVDV infection was associated with antigen deposition. Lymphodepletion in alpaca tissues was modest compared to lymphodepletion of target lymphoid organs of acutely-infected calves. Restricted pathological effects of BVDV in lymphoid organs of alpacas compared to calves may be strain related or could be associated with elevated interferon responses as observed in cultured alpaca cells associated with limited in vitro permissiveness of alpaca cells to infection with noncytopathic BVDV.[3]

In summary, acute noncytopathic BVDV infection of alpacas is characterized by lymphopenia and lymphodepletion of mucosal associated lymphoid tissues suggesting infection may be immunosuppressive and likely enhances susceptibility to infections with respiratory tract or enteric pathogens.

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Outbreak of Malignant Catarrhal Fever in Cattle Associated with a State Fair

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Narrative: Malignant Catarrhal Fever (MCF) is an infectious disease of domestic cattle, wild ruminants, and pigs. The disease is caused by ruminant rhadinoviruses (subfamily gamma herpesvirinae), namely alcelaphine herpesvirus-1 carried by wildebeest, ovine herpesvirus (OvHV)-*2* endemic in domestic sheep, caprine herpesvirus-2, or white-tailed deer MCF virus. MCF in cattle is usually a sporadic disease of low morbidity and high mortality. **This case report describes a severe disease outbreak and death in cattle exhibited at a state fair and naturally infected with OvHV-2.**

Twenty-four of the 121 head of cattle co-housed with domestic sheep in the fair barn died due to malignant catarrhal fever (MCF). The attack rate was about 20% (N=24), the duration of clinical signs was 5 days, with a range of 1-26 days, and the average number of days to death was 70 days, with a range of 46 to 139 days. The epidemic curve demonstrated that the peak of the cases (cattle deaths) occurred about 56 days after exposure. The most common clinical signs were anorexia, depression, fever, diarrhea, and dyspnea. Gross lesions identified from field necropsies included oculonasal discharge, keratoconjunctivitis with corneal opacity (classic "head and eye" form of MCF), mucosal erosions in the mouth, enlarged mesenteric lymph nodes, and gastroenteritis. Histological examination was done on 6 cases and all showed lymphocytic and/or necrotizing vasculitis, most commonly in the lung, although the range of tissues submitted for examination was very limited. All cases were confirmed as MCF by detecting viral DNA using a previously validated OvHV-2 specific Real-Time PCR assay. An analysis of the spatial distribution of the MCF cases showed the attack rate in different areas of the fair barn was associated with distance from the center of the barn but not with distance from the center of the sheep pens. The ventilation system of the show barn directed air from the periphery of the building toward the center of the building and was the most likely mode of apparent enhanced viral transmission.

Outbreaks of MCF in cattle are unusual, particularly those associated with livestock exhibitions. Because the clinical signs of MCF may be similar to some trans-boundary diseases, such as foot and mouth disease, rinderpest, and vesicular stomatitis, cases should be reported and investigated by regulatory health officials and accredited laboratories.

Permissiveness of Swine to Infection with H5N1 Highly Pathogenic Avian Influenza Viruses

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Narrative: Three different isolates of H5N1 highly pathogenic avian influenza (HPAI) virus (A/Egret/Egypt/1162-NAMR3/2006, A/Iraq/207-NAMRU3/2006, A/HongKong/156/1997) were inoculated into 3- to 4-week-old conventional influenza-naive pigs. Viruses were propagated in embryonated chicken eggs and inoculum for each virus prepared to a standard $1x10^7$ EID_{50}/ml. One ml of inoculum was administered intranasally to alert pigs via a manual pump that delivered a fine mist directly into both nostrils. A fourth set of pigs was inoculated in a similar manner with a swine influenza virus (A/Sw/MN/001170/2006), and a fifth group of pigs was inoculated intratracheally with A/Egret/Egypt/1162-NAMR3/2006. Contact pigs were placed into the same pens with inoculated pigs at 24 hrs postinoculation (PI). In trials with two of the intransally administered viruses, chickens were housed in cages suspended over the decks housing the pigs. Clinical appearance of the pigs was monitored and rectal temperatures and nasal swabs taken daily from infected and contact pigs. Inoculated pigs were euthanized at 3 and 5 days PI. Contact pigs were euthanized at 4 days postcontact. With two of the viruses, several inoculated and contact pigs were held for 2 weeks PI before euthanasia. At necropsy, blood and bronchoalveolar lavage fluid (BALF) was collected, and samples of nasal turbinate, trachea, lung, tonsil, tracheobronchial lymph node, spleen, liver, ileum and brain were selected for virus isolation studies and fixed in 10% buffered formalin for histopathologic examination.

Only very mild infection of a few inoculated pigs was achieved with each of the H5N1 viruses and virus was shed in nasal secretions only sporadically. Lung lesions were minimal. None of the contact pigs became infected as determined by virus isolation studies on nasal swabs, BALF and tissue samples. Contact chickens also were not infected. As expected, all pigs inoculated with the swine influenza virus in a similar manner became infected and shed virus that was transmitted to all contact pigs, though very few lung lesions were observed. In contrast, intratracheal inoculation of pigs with HPAI resulted in elevated rectal temperatures and multifocal lobular consolidation in cranioventral portions of lung. **In summary, although not refractory to infection with HPAI administered at high doses directly into the lung, swine appear to be minimally permissive to infection with moderate doses of these viruses when inoculated via a natural route of exposure.**

Development of a Forensic Pathology Course for Veterinary Students

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Narrative: Submissions of veterinary forensic and legal cases to the Athens Veterinary Diagnostic Laboratory has increased significantly over the past 5 years. National interest in veterinary forensics has resulted in the formation of the International Veterinary Forensic Sciences Association with student participation/interest from most Colleges of Veterinary Medicine. To nurture and encourage this interest in forensic pathology, VPAT 5220 Forensic Pathology was created at the University of Georgia College of Veterinary Medicine. The first class of this student elective course was taught in the Spring of 2009.

VPAT 5220 had 28 Sophomore students enrolled. Instructors and invited speakers included an internationally known expert on veterinary forensic sciences, an expert on wildlife forensics, the Chief of Police for the University of Georgia, and a veterinary pathologist with training in forensic investigation. The class was a one hour credit course which included lectures and laboratories. Students were taught how to perform a necropsy in one laboratory and were required to perform a legal/forensic necropsy during a second laboratory. Disposable digital cameras, special forms (chain of custody, body condition assessment, external wounds/lesions, necropsy tissue collection, etc) and forensic scales, in addition to the routine necropsy supplies were provided for the students. Each animal had several lesions that the students were required to find and document properly. One laboratory involved observing a taped mock trial and the evaluation of the trial with instructions for preparing for a trial. The ASPCA Animal Crime Scene Investigation van visited the Athens campus for one day. Students toured the van, were taught how to set up a crime scene, shown equipment used to evaluate the scene and animals, analyzed blood stains, and examined specimens from forensic cases (some of which have made national headlines). The students were evaluated on attendance, class participation, necropsy performance, and one final exam.

The students were very eager to learn and participated in all portions of the course. The faculty involved in the lectures also wanted to participate in this new course and brought a varied background of experience to the course. Student evaluations at the completion of the course were all positive with many suggestions to increase the number of lecture hours on certain topics. The students complemented the "hands-on" type of necropsy laboratory and found it beneficial for their training and understanding of these cases in general. Several of the students have already participated on a volunteer basis on other cases submitted to the Athens Diagnostic Laboratory as time has allowed.

The course was thought to be successful and met its goal of informing and encouraging veterinary students to pursue an interest in veterinary pathology and forensic pathology in particular. Students interested in veterinary forensic pathology have started a subgroup within the College's student Veterinary Pathology Club (Patheads). The student representative for the International Veterinary Forensic Sciences Association is from Georgia. The authors are willing to share information with and encourage others who would be interested in offering a course or short course in their state about veterinary forensics.

Foot-and-Mouth Disease in Feral Swine Susceptibility and Transmission

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Narrative: Foot-and-mouth disease, a highly contagious acute vesicular disease of cloven-hoofed animals, represents a dire threat to American agriculture. The FMD threat to the livestock industry may be intensified due to the grave consequences associated with introduction of FMDV into the feral swine population. The United States feral swine population is currently estimated at 4 million inhabiting at least 32 States and represents a continually increasing and difficult to manage or control disease reservoir.

Experimental studies of FMD in feral swine are limited, and data for clinical manifestations and disease transmissibility are lacking. We experimentally infected and compared the susceptibility of feral swine with that of domestic swine, gaining knowledge on virus transmission between feral and domestic swine.

Three groups of animals consisting of two sets of 2 feral swine, and one set of 2 domestic swine were inoculated in three different rooms, with 100 porcine heel bulb infectious dose 50 (PHBID50) of A24-Cruzeiro FMDV. Forty-eight hours post inoculation, four naïve feral swine were introduced and allowed to mingle with each of two inoculated feral or 2 inoculated domestic swine; and 4 naïve domestic swine were introduced and mingled with the two inoculated feral swine. Animals were monitored daily for clinical signs and fever. Serum, whole blood, oro-pharyngeal swabs, nasal swabs and air samples were collected at pre-determined time intervals through 35 dpi.

This experiment showed that feral swine are highly susceptible to A-24 Cruzeiro FMD virus by intradermal inoculation and by contact with infected domestic and feral swine. **Typical clinical signs in feral swine included transient fever, lameness, and vesicular lesions in the coronary bands, heel bulbs, tip of the tongue and snout. Feral swine transmitted the disease to in-contact domestic swine in less than 24 hrs. Feral swine showed clinical signs of FMD 24-48 hrs after contact with infected domestic swine.** Information gained from this study will be utilized in epidemiologic models of FMD, economic models of potential impacts to agriculture, and in understanding the role which feral swine may play in sustaining an FMD outbreak in domestic and wild animals.

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Evaluation of Commercial Monoclonal Antibodies for Use in Immunohistochemical Staining for Porcine and Bovine Group A Rotaviruses

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Narrative: Rotavirus is one of major enteric pathogens causing acute diarrhea in young animals. Polymerase chain reaction assay, electron microscopy, antigen-capturing ELISA, immunofluorescent staining of frozen tissue section and/or immunohistochemistry (IHC) have been employed to detect the virus in clinical specimens collected from diarrheic animals. Among those laboratory tests, IHC is frequently used as it provides diagnosticians simultaneous assessment of lesions and the target agent. The need for a new rotavirus antibody for IHC staining recently arose in our laboratory because the current monoclonal antibody (NE 9-10) supplied by the University of Nebraska was no longer available. The following study was conducted to evaluate commercially available anti-rotavirus antibodies for use in IHC for group A rotaviruses.

Four antibodies, designated 2B4 (Abcam®), 3C10 (Meridian Life Science®, Inc.), B260M (Meridian) and RV 11-2 (Rural Technologies, Inc.), were purchased from commercial vendors, evaluated and optimized for an IHC technique for group A rotavirus in swine and bovine intestinal tissues. Selection criteria were: a) monoclonal antibody; b) IgG isotype; c) specificity for VP6 inner capsid protein; d) claimed cross reactivity with rotaviruses of various species; e) potential application to tissue immunostaining; and f) US-based supplier. All antibodies were initially evaluated on a set of positive (n=20) and negative (n=10) samples of swine and bovine origin as determined by the NE 9-10 antibody using different antigen retrieval methods and visualization systems. The antibodies that provided the expected results were then serially diluted (1:100-1:32000) and applied to IHC staining to determine the optimal dilution for the best signal with the least noise. A side-by-side comparison (Chi-square test and kappa coefficience) with the NE 9-10 antibody was then conducted using 65 swine and 35 bovine samples randomly selected from submissions to the Iowa State University Veterinary Diagnostic Laboratory.

The RV 11-2, 3C10 and B260M antibodies were eliminated as they did not show positive staining on some of the positive swine and/or bovine samples. The 2B4 antibody correctly identified the previously determined positive and negative samples, and appeared to recognize the same epitope that the NE 9-10 antibody detected. With respect to antigen retrieval, the PT Link system (Dako) produced better staining intensity than did the method of 3% hydrogen peroxide block and trypsin digestion or heat-mediated antigen retrieval system based on microwave oven. The 2B4 antibody still resulted in discernable virus-specific signal at 1:16,000 dilution as compared to heat-mediated antigen retrieval and trypsin-digestion methods. The EnVision™ FLEX HRP detection system with PT link system (Dako) produced the best staining with the least amount of background staining. **Under these optimized conditions, the 2B4 antibody showed the same performance (i.e., 100% agreement) in detecting positive samples as the NE 9-10 antibody when duplicate slides were stained with each antibody for side-by-side comparison.**

Perianal Squamous Cell Carcinoma Including a New Variant of Giant Cell Carcinoma in Boer Goats.

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Narrative: During the period encompassing July of 2002 through July of 2008 in the regions of West Texas and Southern Colorado discrete to coalescing perianal tumors were observed in a total of 13 Boer or Boer-mixed, mostly white female, goats. Neoplasms measuring a few millimeters to five centimeters across were mostly concentrated at the mucocutaneous junctions of anus and vagina and were also scattered on the hairless ventrum of the tail. With the exception of one goat that developed papillary rectal carcinoma all tumors were restricted to the skin with no mucosal involvement. Histologically, the tumors varied from being well differentiated squamous cell carcinomas with typical "pearl" formation to anaplastic carcinoma (in one animal) comprising many multinucleate giant cells, some of which measured 200-300 µm in diameter. Immunohistochemical staining for pancytokeratin revealed marked reactivity of neoplastic cells including many of those multinucleated giant cells. In only one case, a distant spread of a well differentiated squamous cell carcinoma was established in the inguinal lymph node. To the extent our knowledge, **this is the first case report that describes multinucleate giant cell variant of squamous cell carcinoma in goats**, which has not been previously reported in literature.

Cattle as Potential Amplifying Hosts of Vesicular Stomatitis New Jersey Virus

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Introduction: The vertebrate amplifying host for vesicular stomatitis New Jersey virus (VSNJV) during outbreaks has not been identified. Livestock have been considered a deadend host since detectable viremia is absent in VSNJV-infected animals. Previous studies that utilized domestic swine as hosts and black fly vectors have demonstrated that black flies could become infected with VSNJV when they cofeed with VSNJV-infected black flies. Transmission of VSNJV to black flies feeding on or near virus-rich lesions was also demonstrated. In the current study, we evaluated the role of cattle as amplifying hosts for VSNJV. Cattle were chosen since they are commonly affected during VSNJV epidemics that periodically occur in the western United States.

Materials and Methods: Cattle were infected with a 2006 equine isolate of VSNJV via the bite of infected *S. vittatum* on the neck (2), lip (3) or coronary band (2). Non-infected *S. vittatum* were allowed to feed in conjunction with infected flies on all but one of the lip animals, and were processed for virus isolation. At 24 and 48 hours post infection, non-infected flies were allowed to feed on the initial inoculation locations in the presence and absence of vesicular lesions and were evaluated for virus acquisition. In a separate study we used a 1995 Colorado Bovine VSNJV strain. Fly feeding was done on the neck (3) and coronary band (2) only. The effect of spatial separation of infected and non-infected black flies was examined on the neck animals.

Results and Discussion: Co-feeding transmission rates ranged from 0-11%, and varied by location on the animal. Animals infected on the lip showed higher rates of infected and non-infected fly feeding and had viral transmission rates of 7 and 11%. Animals infected on the neck or coronary band had lower fly attack rates and failed to demonstrate cofeeding transmission. At 24 hours post infection (PI), non-infected flies feeding on neck animals acquired virus at rates of 7 and 13%, but failed to acquire virus at 48 hours PI. Non-infected flies did not become infected when feeding on any of the lip animals at 24 hours PI, but did acquire virus from one of the lip animals at 48 hours PI. Transmission of VSNJV to naïve flies feeding on coronary band vesicular lesions was not detected. In the second trial, cofeeding transmission was observed on all animals with infection rates ranging from 12.5-67% for non-infected flies feeding in the same chamber as infected flies. Non-infected flies that were physically separated from infected flies up to a distance of 7cm were able to acquire virus during feeding. The rate of transmission decreased as the distance between infected and non-infected flies increased. In this trial, acquisition of VSNJV by non-infected flies feeding at the inoculation location at 24 and 48 hours PI was not observed. **These results demonstrate that cattle can play a role in the epidemiology of VSNJV and do serve as a source of virus for biting insects.**

Progressive Accumulation of PrP^{CWD} and Spongiform Encephalopathy in the Obex of Rocky Mountain Elk (*Cervus elaphus nelsoni*) with Chronic Wasting Disease and Use as a Means of Scoring the Stage of Disease and Predicting Accumulation of Prion in Peripheral Tissues

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Narrative: Chronic wasting disease (CWD), a transmissible spongiform encephalopathy, has been reported in captive and free-ranging cervids. An abnormal isoform of a prion protein (PrP^{CWD}) has been associated with CWD in cervids and this prion protein can be detected with immunolabeling. It has been suggested in mule deer that as the disease progresses the degree of accumulation of PrP^{CWD} and severity of spongiform degeneration increases. This communication describes a method to delineate the progressive accumulation of PrP^{CWD} and increase severity of spongiform degeneration in a single section of obex. Ten nuclear areas and white matter tracts including the subependymal area of the fourth ventricle and in the thin subpial astrocytic layer of a section of obex were examined and scored in 77 free-ranging and ranch-raised elk that were positive for naturally occurring CWD. This obex score was divided into ten categories and then each category was compared to the detection of PrP^{CWD} in peripheral tissues (approximately 130) and brain (approximately 100 neuroanatomical locations) in 25 elk with natural occurring CWD. **Lymphoid tissues in early cases were at first sporadically affected, but became more uniformly affected as the disease progressed. Spinal cord was commonly affected starting in the dorsal and intermediate horns, but dorsal root ganglia were not. Myenteric plexuses and mucosa of the entire digestive tract was affected early and remained positive throughout the disease. The prion spread quickly through out the brain and nearly all areas had a degree of detectably prion midway in the disease. Adrenal medulla was affected in what was considered mid disease and then later the adrenal cortex was positive. Prion was found in the retina, nerves of the ethmoid turbinates, circumvallate papillae, heart, placenta and skeletal muscle in later stages of disease.** PrP^{CWD} was not detected in the lungs, trachea, kidney, skin and sebaceous glands. Numerous sarcocyst were examined in skeletal muscle and heart and PrP^{CWD} was not detected in them. The obex score was then compared to the obex score in 17 experimental elk representing all three genotypes with known incubation times and this score was compared to the obex scores to the elk with natural occurring CWD. **The genetics of the elk play a major role in this process or speed of spread throughout the body and brain**. Elk with a 132MM genotype had the shortest incubation period, 132ML were intermediate and 132LL had the longest incubation times with comparable obex scores to natural occurring CWD cases. **The utility of this method to estimate the distribution of prion throughout the body and brain and to estimate the duration of disease has been established.** Perhaps this data also will give some insight into the pathogenesis of CWD.

Virology Scientific Session

Sunday, October 11, 2009
California

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***** Graduate student presentation

Evaluation of Influenza Diagnostic Assays for Alcohol-Inactivated Influenza A Viruses

M. Gramer

University of Minnesota Veterinary Diagnostic Laboratory

Narrative: Importing biological material from pigs in some areas of Central and South America requires inactivation of the samples to render them non-infectious. Small feasibility studies were conducted to assess the ability of current diagnostic assays to detect influenza A viruses inactivated with alcohol.

For the alcohol inactivation studies, H1 swine influenza A viruses were obtained from the University of Minnesota Veterinary Diagnostic Laboratory (U of MN VDL) archives. Samples for the assays were prepared from the viruses as follows:
1) 1 ml of undiluted virus isolate.
2) 1 ml of virus isolated thoroughly mixed with 1 ml of 70% alcohol.
3) 1 ml of virus isolate thoroughly mixed with 0.5 ml of 70% alcohol.

Dacron-tipped swabs were suspended in each aliquot then submitted and handled for influenza A virus testing per U of MN VDL standard operating protocols. A sample of virus fluid was also submitted. Two replicates were conducted on different days with different H1 swine influenza A viruses each day.

Influenza A virus was detected in all samples in the alcohol inactivation studies by USDA-NAHLN influenza A virus Matrix RRT-PCR on both days. Influenza A virus HA and NA subtyping PCR tests and influenza HA gene sequencing attempts were successful on 2 of the 4 alcohol treated samples. Sequencing and subtyping results matched the original virus isolate on both days. Virus isolation attempts on MDCK cells were negative on all alcohol treated samples. A**ddition of 70% alcohol to influenza virus isolates successfully inactivates the virus but still allows for detection and some characterization.** Further validation on field samples is necessary.

UV_{254} Inactivation of Selected Viral Pathogens

T. D. Cutler,[1] S. J. Hoff,[3] C. Wong,[1,5] K. J. Warren,[4] F. Zhou,[1] Q. Qin,[2] C. Miller[2], J. F. Ridpath,[6] K. J. Yoon,[1] J. Zimmerman[1]

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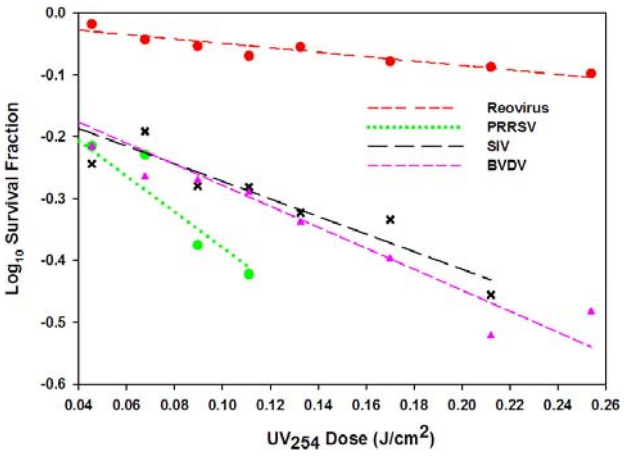
Introduction: UV_{254} inactivation has been a proven method of disinfection since the 1930s (Wells and Brown, 1936). Since then, UV_{254} inactivation of airborne pathogens has been engineered into areas where people congregate, e.g., by placing UV_{254} light tube grids into existing ventilation ductwork (Walker and Ko, 2007). Although data is available on the UV_{254} inactivation of several human viral pathogens, little data is available on the UV_{254} inactivation of viral pathogens of animals.

The goal of this research was to determine the UV_{254} dose required for inactivation of three common viral pathogens of animals (swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV) and bovine viral diarrhea virus (BVDV)) on surfaces and in the air (aerosols). Year One focused on UV_{254} inactivation of viruses under "static" conditions (virus in liquid medium) using off-the-shelf UV hardware from commercial manufacturers (American Ultraviolet Co., Lebanon IN). Year Two (in progress) will focus on inactivation of airborne pathogens.

Materials and Methods: SIV, PRRSV, BVDV, and Reovirus in media were exposed to specific doses of UV_{254} where dose is defined as (*intensity* x *exposure time*) and intensity is expressed as joules per cm^2. Reovirus, highly resistant to UV_{254} inactivation, was included for the purpose of process control and comparison. The effect of UV_{254} exposure was quantified by comparing virus titers in exposed vs unexposed controls. Three trials were conducted at exposure doses: 0.3000, 0.2500, 0.2000, 0.1500, 0.1250, 0.1000, 0.075, 0.050, and 0.0250 J/cm^2. Viruses (1.8 ml; 2 mm depth) were placed in separate wells of a modified Nunclon® MultiDish™ 8-well plate (Nalge Nunc International, Rochester, NY). The plate was modified by removal of two middle wells to accommodate the placement of a UV_{254} sensor (Technika, Phoenix, AZ). One well on each plate served as an unexposed control. Following UV_{254} exposure, the viral suspensions were placed in 2 ml cryovials and frozen at -80˚C until assayed for viral infectivity. Microtitration infectivity assays ($TCID_{50}$) were conducted on samples in random order. The rate of inactivation was determined by plotting the survival fraction of each viral against the exposure dose. The Figure summarizes the inactivation of each pathogen by dose using a linear regression model.



Discussion/Conclusion: Overall, the data indicate that PRRSV, BVDV, and/or SIV are highly susceptible to UV_{254} inactivation.

Bovine Viral Diarrhea Virus (BVDV) Experimental Live Virus (BVDV1b and BVDV2b) Vaccines in Cattle: Immunogenicity Studies with Antibody Response to Multiple BVDV Subgenotypes

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Narrative: Bovine viral diarrhea virus (BVDV) is a diverse group of viruses causing infection and diseases in cattle worldwide. There are two major types of BVDV, BVDV1 and BVDV2. There are currently reported 12 different BVDV1 subgenotypes (BVDV1a-BVDV1l) and two BVDV2 subgenotypes (BVDV2a and BVDV2b). In the US, the three subgenotypes are BVDV1a, BVDV1b, and BVDV2a, although there is a report of a cytopathic (CP) BVDV2b. The principal BVDV subgenotype in the United States is BVDV1b based on accessions to diagnostic laboratories, typing of persistently infected (PI) cattle entering the feedlot, and a regional study of BVDV in young dairy calves. However, most vaccines in the United States contain BVDV1a and BVDV2a with a few only containing BVDV1a or BVDV1 (subgenotype not identified) strains. There are no BVDV1b vaccines currently licensed in the United States. There are antigenic differences among the BVDV1a, BVDV1b, and BVDV2a strains. The question remains: would vaccine efficacy be improved by the inclusion of the subgenotype for the predominant strain in a population.

The objectives of this study included: (1) development of BVDV1b and BVDV2b live viral vaccines for use in cattle; (2) detection of an active immune response in susceptible calves as measured by viral neutralizing antibodies in the serums to multiple strains; (3) detection of viremia post vaccination and potential for viral shedding in vaccinates; (4) ability of viral infection in vaccinates to cause skin test positive reactions post vaccination; and (4) comparison to a commercial vaccine containing MLV BVDV1a and BVDV2a strains.

There were five groups of calves: (1) nonvaccinate controls ;(2) BVDV1b CP experimental vaccine given SQ; (3) BVDV1b experimental vaccine given IM; (4) BVDV2b CP experimental vaccine given SQ; and (5) commercial MLV vaccine containing BVDV1a and BVDV2a given IM. The calves were bled weekly from day 0 to day 42. The blood leukocytes were assayed for infectious virus on the weekly samples and serums assayed for BVDV antibodies to BVDV1a (Singer), BVDV1b (TGAC), BVDV2a (125C), and the BVDV2b CP vaccine strain. Skin samples were tested for BVDV antigen using the IHC and ACE. And the day 0, 7, 14, and day 42 serums were tested by a gel-based reverse transcriptase PCR assay. The skin samples were negative at all collections to the IHC and ACE. There were no viremias detected by viral isolation, although a limited number of PCR positives were noted in the experimental vaccinates. There was no evidence of viral shedding as indicated by failure to seroconvert by the nonvaccinated control group animals that were housed together.

The experimental BVDV1b CP vaccine given either by SQ or IM induced greater antibody levels to BVDV1b at days 21,28 and 42 compared to the commercial BVDV vaccine containing BVDV1a and BVDV2a (P value-<0.05). And the experimental BVDV1b vaccine given IM induced higher BVDV1a antibody levels than the commercial vaccine at day 42. The experimental BVDV2b vaccine induced higher BVDV2a and BVDV2b antibody levels at days 14,21,28 and 42 than the commercial BVDV vaccine containing BVDV1a and BVDV2a (P=<0.05). **These results indicate the uniqueness of the experimental BVDV1b vaccine to induce higher levels of antibody to BVDV1b, the most common BVDV strain in many surveys**, than a commercial MLV vaccine containing BVDV1a and BVDV2a. Likewise the BVDV2b vaccine demonstrated ability to induce antibodies to the heterologous BVDV2a.

Evaluation of Hair as an Alternative Sample for the Detection of Bovine Viral Diarrhea Virus by Antigen Capture ELISA

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Narrative: Antigen-capture ELISA technology has been in use in some countries for almost 20 years for the diagnosis of bovine viral diarrhoea virus (BVDV) infections by the direct detection of viral antigens in peripheral blood leukocytes, blood clots and a range of tissues such as spleen, lung and various lymphoid organs. More recently, the assay has been modified to detect antigen in serum and skin tissues. These developments have lead to the pestivirus antigen capture ELISA (PACE) becoming the most commonly used assay to detect cattle persistently infected (PI) with BVDV. The high analytical sensitivity of the PACE to test skin samples combined with the availability of the assay as a commercial kit has also enhanced the adoption of this technology for the detection of PI animals and is widely used in control and eradication programs that are being developed in many countries However, in some situations there is resistance to the collection of skin biopsy ("ear notch") samples from live animals. This study involves the evaluation of a PACE test for testing of hair samples from cattle.

To determine the feasibility of using hair samples to detect BVDV antigens, new (follow-up) samples (blood, hair and skin) were initially collected from about 250 beef and dairy cattle that had previously been identified as being infected with BVDV using PACE on blood or skin samples. Each of the new samples was tested for BVDV antigens by PACE and the serum samples were tested for BVDV antibodies by VNT or AGID. Hair samples were stored under different conditions and tested at intervals to assess the stability of BVDV antigens. Additional hair samples were collected from some PI animals and were cut into small sections to determine the distribution of antigen in the hair. Later, both blood and hair samples were collected from a population of animals of unknown status to assess the sensitivity and specificity of the PACE when using hair as a sample.

The results of tests conducted on hair samples showed very high diagnostic sensitivity and specificity when compared to PACE testing on blood samples from animals that do not have maternally derived antibodies. However, similar to skin, the results for hair samples are not affected by maternally derived antibodies. When the specificity of the PACE using hair samples is considered in the context of its ability to detect PI rather than acutely infected animals, the results show that testing of hair gives higher specificity. However, these should be considered as preliminary results because hair samples have not been tested from animals infected with BVDV type 2 strains where there may be a greater likelihood of higher levels of antigen. Locally available assay kits should also be evaluated to confirm their suitability.

Based on results available to date, the testing of hair samples appears to be suitable for this purpose. The method of sample collection is faster and easier that the collection of either blood or skin samples and does not require specialised skills or sample packaging. We believe that this method can be easily applied to testing of large numbers of animals. With appropriate presentation, it is also possible to pool samples from a number of animals without any adverse impact on assay sensitivity. Further, the collection of hair samples is not invasive or disfiguring to the animal and may be appropriate for problematic species such as alpaca. **Hair samples are frequently collected for genetics testing so there is scope to rationalise sample collection for both genetics testing such as parentage verification as well as confirmation that an animal is free of BVDV infection**.

Evaluation of a Real-Time Polymerase Chain Reaction Assay for the Detection of Bluetongue Virus in Bovine Semen

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Narrative: Although there has been a long term requirement to screen the semen of cattle that have potentially been infected with bluetongue virus (BLU), until recently, there has been little evidence of the presence of BLU in the semen of naturally infected bulls. Studies conducted in the USA and Australia consistently showed that BLU was not present in the semen of bulls infected with "wild-type" strains of virus. However, if a mature animal was infected with a cell culture adapted strain of BLU, infectious virus could be detected in the semen for a short period soon after the onset of infection. Recently, following the natural infection of cattle in Europe with BLU serotype 8, virus has been readily detected in semen and has also crossed the placenta, causing foetal infections. Although these strains of BLU-8 are naturally circulating, they possess the characteristics attributed to cell-culture adapted or vaccine strains of virus.

During one of the large studies conducted in Australia, mature bulls were experimentally infected with a laboratory adapted strain of BLU serotypes 1. A total of 8 bulls were inoculated and on each occasion that a semen sample was collected, blood samples were also collected to monitor the onset and duration of viraemia. From day 7 after experimental infection, samples were collected twice weekly for 4 weeks then once a week for a further 4 weeks. Methods used to detect infectious virus in blood and semen included the inoculation of embryonated chicken eggs (ECE) followed by passage in insect and mammalian cell cultures, direct passage in both insect and mammalian cell cultures, and inoculation of sheep. For both blood and semen, a large volume of sample was examined to maximise virus detection. Serological methods (AGID, cELISA and VNT) were also employed to monitor infection. The semen samples from these bulls were stored both in liquid nitrogen and also at -80^{o}C. Recently, these samples were tested using a semi-automated method for RNA extraction and both a nested reverse transcriptase polymerase chain reaction (nRT-PCR) assay and a BLU pan-reactive real time reverse transcriptase polymerase chain reaction (qRT-PCR) assay.

BLU virus was detected intermittently in semen from a number of the mature bulls that had been experimentally infected with this laboratory-adapted strain of BLU-1. These detections occurred during or immediately after the period of detectable viraemia. The duration of viraemia varied from 17 to 31 days. Each of the virus isolation methods had comparable sensitivity if samples were passaged sufficiently. BTV was most readily detected by inoculation of ECE or sheep. When the semen samples were examined by nRT-PCR, similar results were obtained to the virus isolation methods even though a 10 fold lesser sample volume was assayed. Superior results were obtained from the semi-automated magnetic bead based nucleic acid extraction system compared to a manual column extraction method. When the semen extracts were tested in the qRT-PCR, a higher level of sensitivity was achieved than with any of the other virus detection methods. In some bulls, where infectious virus had been detected intermittently, viral RNA was detected consistently. **It was concluded that a combination of a magnetic bead based nucleic extraction method and an appropriate pan-reactive qRT-PCR will readily detect BLU in the semen of bulls and will permit a large number of samples to be tested in a short time.**

Bench Validation of a High Throughput Real-Time RT PCR Assay to Detect Very Virulent Infectious Bursal Disease Virus

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Narrative: In the first quarter of 2009 the California Animal Health and Food Safety (CAHFS) laboratory detected the very virulent form of Infectious Bursal Disease virus (vvIBDV) in a commercial layer flock. The finding which was confirmed by sequence analysis of the VP1 and VP2 gene was unexpected as vvIBDV had not been previously documented in the United States. Despite aggressive efforts to prevent spread, the virus was detected in subsequent flocks over the following weeks. Faced with the potential for rapid implementation of high throughput diagnostic and surveillance testing should the virus continue to spread, CAHFS initiated development and validation for vvIBD realtime PCR detection using cloacal swabs as the primary specimen of interest. The existing laboratory technology including serology, immuno-histochemistry, virus isolation, and/or PCR testing on bursal tissues from necropsy cases was not considered an optimum laboratory tool for surveillance and rapid response. Two previously developed single-plex realtime PCR assays (Jackwood and Sommer, 2005, Avian Diseases 49:246-251) were modified to allow high-throughput testing of cloacal swabs for the presence of vvIBDV and differentiation from endemic IBD viruses. Bench validation of the two independent assays demonstrated high analytic sensitivity and specificity. Optimal viral RNA was recovered using high-throughput magnetic bead technology, and the real time PCR assays provided excellent linearity over 7 logs of virus (r-square = 0.998 for the endemic IBDV and an r-square = 0.956 for vvIBDV). Inter-assay repeatability was within 0.3 Ct for all concentrations of virus and for both vvIBDV and endemic IBDV assays. Cross-reaction with other avian pathogens, as well as between the two IBDV assays being evaluated was not detected, indicating excellent analytic specificity (>0.99%). Clinical samples used to initially predict field performance of the realtime PCR assays included more than 200 cloacal swab and environmental samples which were obtained during a series of vvIBDV and endemic IBDV challenge studies. The studies included both high and low dose vvIBDV challenge of SPF and commercial birds as well as challenge of unvaccinated and IBDV vaccinated birds. **The real time PCR assay validation data suggests that cloacal swabs are a cost effective and reliable tool for detection of IBDv shedding and can be used to differentiate vvIBDV from endemic/vaccine IBDV strains in live birds. The IBDV real-time PCR approach of testing cloacal swabs allows vvIBDVv surveillance to be combined with Newcastle Disease virus and Avian Influenza virus surveillance in a cost effective manner by using the same specimen type, cloacal swabs, for all three surveillance programs.**

Molecular Characterization of a Novel Coronavirus, Inducing Feline Infectious Peritonitis (FIP)-Like Lesions in Ferrets

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Narrative: A systemic disease, characterized by FIP-like clinical signs and lesions, has recently been observed in ferrets across the U.S. and Europe. Clinical findings include anorexia, weight loss, diarrhea and the presence of large palpable intra-abdominal masses. Similar to FIP in cats, the lesions consist of widespread nodular foci on serosal surfaces and within the parenchyma of the abdominal and thoracic organs. A coronavirus etiology, suspected based upon immunohistochemical (IHC) staining with feline coronavirus antibody, was previously confirmed by our sequencing of a portion of the spike gene. The virus was tentatively designated ferret systemic coronavirus (FSCV). The objective of this study was to more definitively characterize FSCV at the molecular level and to elucidate its genetic relationship to ferret enteric coronavirus (FECV), a recently reported novel group 1 coronavirus associated with epizootic catarrhal enteritis in ferrets. We present here the determination and comparative analysis of the approximately 8.6 kb long 3' genomic end sequences of these two ferret coronaviruses.

Tissue samples were obtained from a ferret that had exhibited clinical signs and lesions indicative of FSCV infection and was positive by IHC for coronavirus antigen. Total RNA was extracted from tissues of this ferret and also from a fecal sample from a ferret diagnosed with FECV infection. The systemic and enteric ferret coronavirus strains analyzed were designated as FSCV MSU-1 and FECV MSU-2, respectively. To obtain the distal third genomic sequences of these two viruses, consensus coronavirus RT-PCR assays, targeting partial sequences of the spike (S), membrane protein (M), and nucleocapsid (N) genes, were initially performed. This was followed by an S-M gap-bridging PCR and a 3' RACE. The entire upstream S gene sequence plus a short 3' end portion of the polymerase (pol 1b), was derived by amplification of overlapping sequences using a combination of CODEHOP-designed degenerate forward primers and gene-specific reverse primers from already obtained sequence data.

The approximately 8.6 kb sequence obtained for FSCV MSU-1 and FECV MSU-2, comprised the 3' terminus of the pol1b gene, the full coding sequences of structural protein genes S, E, M and N, non-structural protein genes (ORF 3, 3x-like, and 7b), and partial sequence of the 3' untranslated region (UTR). The genomic organization observed for each virus was consistent with that of a typical coronavirus. Pairwise alignment of the sequences showed an overall similarity of 87.1%. For individual ORFs, the pol 1b**,** M, N, 3x and 7b genes exhibited the highest nucleotide sequence similarities (96.1-97.5%) between the two viruses. The ORF 3 region contained a single group 1 coronavirus 3c-like gene for both enteric and systemic viruses. The translation product of this gene was truncated in FSCV MSU-1. Examination of the ORF 3 region of additional FSCV and FECV strains showed that truncation of the ORF 3 protein does not appear to be a consistent feature of the systemic strains. Significant divergence between FSCV MSU-1 and FECV MSU-2 was observed in the S and the E genes, with nucleotide sequence similarities of only 79.5% and 88%, respectively. Amino acid substitutions between FSCV-MSU-1 and FECV MSU-2 appeared randomly distributed within the S protein sequence. Analysis of partial S sequences of additional systemic and enteric ferret coronaviruses showed that 21 amino acid substitutions between FSCV and FECV strains were conserved within a given virus pathotype. **Partial genomic characterization of ferret systemic and enteric strains available to us indicated that FSCV differed more significantly from FECV than is the case for FIP virus and feline enteric coronavirus (FeCoV). Phylogenetic analyses showed that both FSCV and FECV are group 1 coronaviruses that segregate into their own distinct cluster within the group and, based upon the strains analyzed thus far, appear to be two genetically distinct viruses.**

Identification of Molecular Markers for the Virulence of Porcine Reproductive and Respiratory Syndrome Virus

W. Kim, D. Sun, Y. Cho, S. Liu, V. Cooper, K. Yoon

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Narrative: Two modified-live vaccines, Ingelvac® PRRS MLV (MLV) and Ingelvac PRRS® ATP (ATP) have been widely used to control porcine reproductive and respiratory syndrome virus (PRRSV). The extensive use of the vaccines has become of great concern because the vaccine viruses are quickly reverted to virulent viruses during their replication in animals as reported in numerous studies. Since the vaccines (MLV and ATP) and their parental wild-type viruses (VR2332 and JA142, respectively) share high homologies (>99%), accurate differentiation between the vaccines and the parental viruses has been very difficult and sometimes impossible. In previous studies in our laboratory, 4 unique sequences were exclusively identified in either MLV or ATP as potential differential genetic markers for vaccine virus by analyzing the association between different viral phenotypes and sequence substitutions. The current study was conducted to determine if those unique sequences can be used as genetic markers for the virulence of PRRSV.

Four genetically modified mutant PRRS viruses were generated from the VR2332 strain by incorporating the unique sequences found in ORFs 1a, 1b, 2, 5 and/or 6 of MLV into the genome of VR2332-backboned infectious clone: SP26, SP256, SP1b256 and SP1ab256. Forty-five PRRSV-free pigs were purchased and randomly assigned to 7 rooms. Then, the pigs in each room were challenged with one of 4 mutant PRRS viruses, cVR2332 rescued from the original infectious clone, MLV or sham inoculum. The pigs were monitored daily for clinical responses (i.e. body weight and temperature) and bled twice a week until 28 days post challenge (dpc) to determine the levels of viremia and antibody response. Necropsy was performed for gross and microscopic lung scores at 10 and 28 dpc.

The pigs challenged with the mutant viruses produced significantly less severe clinical symptom and lower level of viremia as compared to pigs challenged with cVR2332 ($p<0.05$). Five MLV-specific amino acids were additively involved in the attenuation of PRRSV virulence. In particular, the pigs challenged with SP1ab256, which has all of the five MLV-specific sequences, showed viremia, lung score, and weigh growth similar to those challenged with MLV, indicating that **the five amino acids located in both nonstructural (ORFs 1a and 1b) and structural (ORFs 2, 5, and 6) genes are responsible for the virulence of PRRSV in pigs and may be used as genetic markers for viral virulence.**

Metagenomic Approaches to Molecular Identification of Pathogens

R. W. Barrette, J. M. Rowland, S. A. Metwally, M. T. McIntosh

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Narrative: The field of metagenomics has been rapidly expanding recently with regards to diagnostic platforms for identification of unknown pathogens. The marriage of molecular techniques with bioinformatics has allowed for the development of a variety of advanced platforms for genomic identification and characterization of agents such as Lujo virus in South Africa using unbiased pyrosequencing and *Reston ebolavirus* in the Republic of the Philippines by pan-viral microarray analysis. Some of the unique qualities of these methods are in their potential for discovery, identification and characterization. Here we describe some of the methods used for characterization of viruses using a metagenomics strategy involving microarrays.

We have used microarrays to characterize multiple extraneous agents using various analytical methods. These methods include simple probe population bias, feature-based metagenomic sequence reassembly, and shotgun cloning of positive-selected nucleic acid from microarrays probed with randomly amplified sample nucleic acid. Each of these methods has specific advantages in the process of identification and characterization of unknown agents.

In one example, randomly amplified Foot-and-Mouth disease virus was hybridized to a microarray for serotype characterization. Positive microarray features were sorted by mean fluorescence intensity and feature sequence information was extracted. A unique program written in Python was developed to consolidate sequence data from positive microarray features based on a positional scoring algorithm, and assembled on a related template genome. This method of 'feature based metagenomic sequence reassembly' produced a genomic 'fingerprint' which was used to characterize the virus by serotype and similarity to other subtypes of FMDV. **Employing this technique allowed us to reassemble a portion of the P1 region ~4000 bp. When compared to the actual genomic sequence, the microarray derived sequence was found to be 93% homologous to the actual target strain, based on a correct identification of 3,717 nucleotides out of a 3,983bp region.** In conclusion, this method and other metagenomic based approaches to characterize unknown agents are effective tools in the molecular identification of pathogens. Additionally, PCR based technologies used for these systems result in their high sensitivity, while the increasingly sophisticated methods for analysis continue to expand their utility. As these systems become more effective, they will continue to find their niche within mainstream diagnostics.

Pan-Viral Microarray Detection of Reston Ebolavirus in Swine From the Philippines

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Narrative: Seemingly random, sporadic fatal outbreaks of disease in humans and non-human primates by Marbug and Ebola species of *Filoviridae* have evoked interest in delineation of host tropisms and potential reservoirs. **Using a metagenomics approach we identified the *Reston ebolavirus* (*REBOV*) in pigs from two farms in the Philippines experiencing unusually severe outbreaks of porcine reproductive and respiratory disease syndrome (PRRS).** Initial diagnostic tests identified *PRRS virus* (*PRRSV*) and in some instances *Porcine circovirus type-2;* however, subsequent investigation of a lymph node cultured in Vero cells and a tonsil from a different animal cultured in SK6 cells revealed unanticipated cytopathic effects in culture. Investigation of these cultures by random amplification of cDNA followed by pan-viral microarray analysis indicated the presence of a virus similar to *REBOV* in the Vero cell culture and *Porcine teschovirus-1* (*PTV-1*, formerly known as *Porcine enterovirus-1*) in the SK6 culture. The teschovirus was confirmed to be *PTV-1* by diagnostic RT-PCR, while *REBOV* was verified by sequence analysis of a 3.7 kb portion of the viral RNA polymerase revealed by 28 positive REBOV sequence features from the microarray. **Full genome sequencing performed on three *REBOV* from two farms revealed the viruses to be more divergent from each other than from the original virus isolated in 1989.** It was also apparent that two different virus genotypes were simultaneously circulating in swine on one of the farms. **The sequence divergence between the identified viruses and all other known REBOVs indicated polyphyletic origins of the infections in swine.** Though *REBOV* is the only filovirus not known to be associated with disease in humans, its emergence in domestic swine is of concern. REBOV contribution to disease in pigs is unclear; however, moderate histopathological changes were observed in lung tissues that were double positive for REBOV and PRRSV and mild focal changes seen in lymph node that was positive for REBOV alone. **These results indicate swine as a newly identified host for this species of Ebola virus with possible implications for swine agriculture, international trade, and public health.** Furthermore, these results illustrate the important role played by unbiased metagenomic diagnostic methods for identification of unknown agents during disease investigations.

Simultaneous Detection of Multiple Pathogens Using High-Throughput Nanoliter Real-Time PCR

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Narrative: Multiple pathogen detection systems can be of great help to accurate and rapid differential diagnosis, simultaneous surveillance on various infectious agents, and studying interactions among different pathogens involved in multifactorial diseases. Although various multiplex technologies have been developed and applied for these purposes, their applications are somewhat limited due to the complexity of test development and maintenance: optimization of multiple assays in a multiplex system is very challenging; and addition of a new assay to the optimized system requires significant time and effort for additional validation. Therefore, a new detection system in which multiple individual assays can be optimized and run independently, yet simultaneously and economically, needs to be explored.

High-throughput nanoliter real-time PCR (OpenArray™, BioTrove) uses conventional SYBR Green or TaqMan real-time PCR formats. However, the system can run up to 56 real-time assays simultaneously and economically as independent reactions in parallel since the total reaction volume for each assay is only 33 nanoliters, which is nearly 1000 times less than that of conventional real-time PCR. In the current study, the nanoliter real-time PCR system was evaluated for multiple pathogen detection in comparison with individual TaqMan real-time PCR assays which had been routinely used in the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) for various bacterial, viral and protozoan agents of various species. Primers and probes (labeled with FAM or VIC) for 28 real-time assays were prepared in a plate for the nanoliter PCR and the system was validated and optimized using 55 clinical samples archived from previous submissions to ISU-VDL and known positive controls for the target agents. All procedures were performed as per manufacturer's recommended protocol.

All of the 28 assays prepared in the nanoliter PCR simultaneously detected each target agent without generating false-positive results. Because the analytic sensitivity of the nanoliter PCR was 10-100 times lower than that of conventional TaqMan PCR assays, a universal extraction system was developed to prepare concentrated RNA or DNA of viral, bacterial, and protozoan pathogens from various sample matrices such as serum, oral fluids, tissues, and feces. Combined with the universal extraction system, the nanoliter PCR showed sensitivity and specificity equivalent to the corresponding individual TaqMan PCR assays. **In conclusion, high-throughput nanoliter real-time PCR can simultaneously detect multiple infectious agents and be a valuable tool for veterinary diagnostics and ecological research.**

Use of High Throughput Instrumentation and Kits for Real-Time RT-PCR Testing of Serum Samples for Porcine Reproductive and Respiratory Syndrome Virus

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Introduction: The ability to process and evaluate increasingly larger numbers of samples is critical to the timely ability to report the presence of targeted agents in numerous biosurveillance programs evaluating the status of animal production facilities. In the United States, the National Animal Health Laboratory Network (NAHLN) has been a leader in developing high throughput real-time PCR capacity and capabilities for participating NAHLN-certified diagnostic laboratories. Unfortunately, the application of these high throughput systems has not been demonstrated with many of our endemic disease agents that would be considered routine molecular diagnostic agents and targets. We will discuss the incorporation of an integrated, 96-well high throughput protocol for setting up and completing RNA recovery and subsequent real-time RT-PCR detection, and its application in detecting Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in a PRRSV challenge study involving three groups of 200 piglets and 9 separate bleeding dates per group. An average of 1,727 samples were assayed per group of piglets, for a total of 5,182 serum samples evaluated over a six-month period of time.

Material and Methods: As part of an experimental PRRSV challenge study, serum samples were collected from three groups of 200 piglets on 9 separate bleeding dates (one prior to infection [dai 0] and eight post-infection on dai 4, 7, 11, 14, 20, 28, 35, and 42). In each group, 190 piglets were infected at 21 days of age with NA PRRSV (strain 97-7895; a highly pathogenic strain from USDA/NADC) on dai 0. Ten piglets per group were uninfected reference controls and kept in separate pens within the same facility. A Biomek® NXp Workstation (Beckman Coulter) with multichannel liquid handling capabilities using procedures developed for a 96-well robotics format was used in clean room liquid handling manipulations for setting up RNA recovery procedures using Ambion's MagMAX™-96 Viral RNA Isolation Kit (AMB1836-5) and dispensing RT-PCR master mix solutions from the Applied Biosystems AgPath-ID™ NA & EU Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) multiplex reagents (AM1028) in a one-step multiplex qRT-PCR kit. The RNA recovery process was completed on serum samples using a 96-well magnetic particle processing system (Kingfisher-96) according to the MagMAX™-96 Viral RNA Isolation kit instructions. The PRRSV TaqMan qRT-PCR reactions were completed with a QST 7500 Real-Time PCR System (Applied Biosystems) in a 96-well format according to the manufacturer's instructions.

Results: All PRRSV-challenged piglets demonstrated positive PRRSV Ct values (<37) by dai 4 [average Ct for group 1was 19.5; group 2 was 20.3; group 3 was 17.9]. In group 1, all pigs remained positive (Ct<37) until dai 19 when 6 pigs became negative (Ct≥40), while group 2 had 1 pig negative on dai 21 and group 3 had 2 pigs negative on dai 21. Group 1 demonstrated 35 pigs were negative (Ct≥40) by dai 28 [group 2-32; group 3-1], 10 pigs were suspect (Ct>37<40) [group 2-16; group 3-1], and 137 pigs were positive (Ct<37) [group 2-123; group 3-172]. By dai 42, 164 pigs were negative (Ct≥40) in group 1 [group 2-123; group 3-45], 7 pigs were suspect (Ct>37<40) in group 1 [group 2-13; group 3-19], and 3 pigs were positive (Ct<37) in group 1 [group 2-33; group 3-108].

Discussion/Conclusions: The integration of high throughput sample processing technologies with automated liquid handling workstations and then connecting these processes with high throughput real-time qRT-PCRs was demonstrated in this PRRSV challenge study. The ability to process thousands of serum samples for viral RNA and then complete qRT-PCRs without obvious cross-contamination was accomplished and demonstrated the applicability of the high throughput molecular diagnostic procedure as a workable surveillance method for detecting PRRSV in swine serum samples.

Detection of Porcine Circovirus (PCV) 2 and Anti-PCV2 Antibodies in Porcine Oral Fluid Samples

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Introduction: Previous studies demonstrated the use of pen-based oral fluids for the surveillance of porcine respiratory and reproductive syndrome virus (PRRSV), porcine circovirus (PCV) 2, and other pathogens.[1,2,3] The objectives of this research were to describe the excretion patterns of PCV2 and anti-PCV2 antibody in oral fluid samples from experimentally inoculated pigs.

Materials and Methods: The onset, level, and duration of qPCR-detectable PCV2 and anti-PCV2 antibody in serum and oral fluid was evaluated over time. Eleven week-old animals were housed separately in four rooms, i.e., treatment groups, each holding 6 pigs. Group 1 served as negative control animals. Group 2 was inoculated on DPI 0 with PCV2a (strain ISU- 40895) both intramuscularly (2 mls) and intranasally (2 mls). Group 3was inoculated on DPI 0 with PCV2a (strain ISU- 40895) both intramuscularly (2 mls) and intranasally (2 mls) and every 4 weeks thereafter throughout the end of the study. Group 4 received IM+IN exposures of two PCV2a strains (ISU- 40895 and ISU-4838) and one PCV2b strain (PVG4072) on DPI 0 and every 4 weeks throughout the study. Serum from each animal and one oral fluid sample per group was collected every other day through DPI 14 and weekly through DPI 98. Serum and oral fluid samples were assayed for the presence of PCV2 by qPCR and anti-PCV2 antibody by ELISA. In addition, antibody isotypes (IgG, IgA, and IgM) were quantified in oral fluid samples..

Results: There were no significant differences among inoculated groups in the level of anti-PCV2 antibody in serum. Anti-PCV2 antibody was detected in oral fluids and persisted though out the 98 day study. In separate assays, anti-PCV2 IgG, IgA, and IgM were present in oral fluid by DPI 14 and persisted through the end of the study. The data presented here suggests that oral fluid could be used to monitor pig populations for PCV2 circulation and anti-PCV2 antibody.

Discussion: All groups of inoculated pigs were viremic by DPI 8 (first sample tested), with persistent, but declining, levels of virus throughout the 98 day observation period. PCV2 was detected in oral fluids in all inoculated groups by DPI 2 (first sampling) with peak levels between DPI 10 and 21. Levels declined thereafter, but persisted through the end of the 98 day study. Analysis of PCV2 qPCR results found no statistically significant differences among inoculated groups either in oral fluid or serum specimens

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Increased Prevalence of Neuropathogenic Strains of Equine Herpesvirus-1 in Equine Abortions

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Narrative: A panel of 426 archived equine herpesvirus (EHV)-1 isolates collected from aborted equine fetal tissues, submitted to the Livestock Disease Diagnostic Center between 1951 and 2006, was analyzed using real-time Taq-Man® allelic discrimination PCR. Based on previous findings, isolates possessing adenine at nucleotide position 2254 (A_{2254}) in ORF30 were classified as having a non-neuropathogenic genotype and those with guanine at 2254 (G_{2254}) were designated as the neuropathogenic genotype. The resultant data demonstrated that viruses with the neuropathogenic genotype existed in the 1950s and isolates with this genotype increased from 3.3% in the 1960s to 14.4% in the 1990s. **The proportion of EHV-1 isolates from 2000 to 2006 with G at position 2254 was 19.4%. Although diagnostic laboratory submissions constitute passive disease surveillance, the findings suggest that viruses with the neuropathogenic genotype are continuing to increase in prevalence within the latent reservoir of the virus. This could lead to greater risks for costly outbreaks of equine herpesvirus myeloencephalopathy** Two of the 426 isolates failed to react with either probe in the allelic discrimination assay. These isolates were found to possess an adenine to cytosine substitution at position 2258 ($A_{2258}\rightarrow C_{2258}$) in ORF30, in addition to $A_{2254}\rightarrow G_{2254}$. **This non-synonymous $A_{2258}\rightarrow C_{2258}$ change is predicted to replace tyrosine with serine at amino acid position 753 in the viral DNA polymerase, clearly suggesting that additional research is required before the genetic basis of the neuropathogenic phenotype in EHV-1 is fully understood.**

AAVLD Poster Session

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Seasonal and Geographical Patterns of and Risk Factors Associated with Canine Viral Squamous Papillomas

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Narrative: Virally induced squamous papillomas (VSP) are common, typically benign growths of the skin and mucous membranes of young dogs and less commonly older or immunosuppressed dogs. Up to four different papilloma viruses including canine oral papilloma virus (COPV) are suspected to be responsible for these lesions which typically regress with only rare reports of possible progression to malignant lesions. **Other risk factors or patterns of seasonality or geographic clustering have yet to be reported. Data on canine viral squamous papillomas diagnosed at the Colorado State University Veterinary Diagnostic Laboratory from May 2008 through April 2009 were retrieved retrospectively** from the database consisting of 200 lesions from 192 dogs. Several factors including age, breed, sex, month of submission and state of submitting veterinarian were analyzed to identify possible disease patterns which might warrant further investigation. The mean age was 3.72 years with a range of 2.5 months to 13 years. Sex was available in 188 cases where males accounted for 103 (54.8%) of cases and females 85 (45.2%) of cases (neutered or intact). Of the 77 breeds represented, Labrador Retriever (10.05%; 19/189) and Golden Retriever (8.99%; 17/189) were the most common. Of the lesions, 24.7% (45/182) were located on the face (including lip, muzzle and nose) and 22.5% (41/182) were located on the paw (including digit, pad and interdigital) followed by the oral cavity (12%; 22/182), limbs (11%; 20/182), ears (8.8%; 16/182) and body (8.8%; 16/182). The highest numbers of sample submissions were during the months of May (11.5%; 23/200) and September (11.5%; 23/200). Grouping submissions by quarter, most were from quarter 2 (Mar-May) (26.5%; 53/200) and quarter 3 (June-Aug) (26.5%; 53/200). Most sample submissions were from Colorado (51%; 100/197) with the remaining 49% representative of 25 states in the US and 2 from Alberta, Canada. **This data appears to support the lack of a breed or sex predilection for papilloma virus. Possible peaks of cases were identified in late spring and early fall; however, data appears more evenly distributed when evaluated by quarter.** Further exploration of seasonal incidence of virally induced squamous papillomas ideally from a single state, for multiple years evaluated against a control set of submissions from the same state of other mucosal and cuteanous lesions is required to determine if statistically significant trends exist.

Time to Seroconversion to Vesicular Stomatitis Virus in Sentinel Cows in Southern Mexico

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Narrative: Vesicular stomatitis (VS) is a disease of livestock and some wildlife species caused by vesicular stomatitis virus (VSV). VS epidemics are frequent in certain regions of the United States and such epidemics inflict severe economic losses to affected regions of the country. A prospective cohort study design was used to assess VSV-seroconversion in four free-ranging dairy cattle herds of southern Mexico. Two of the herds were located in highlands (≥500 m) and the other herds two were located in lowlands (<500 m). Ninety-two sentinel cows were tested, on average, every 10 weeks using serum neutralization (SN) test. The proportion of sentinel cows that seroconverted was higher in herds located in highlands (39/43), compared to herds located in lowlands (7/49). Seroconversion was most frequent during the rainy season and beginning of the dry season. Hazard for VSV-seroconversion was higher for sentinel cows in highlands compared to cows in lowlands, whether cut-off values of 1:80 (HR= 3.56, CI95% HR=2.43-5.58, P<0.01) or 1:160 (HR= 3.63, CI95% HR= 2.37-6.21, P<0.01) were used. **These results support the hypothesis that environmental conditions in Mexican highlands are more favorable for occurrence of the disease than in lowlands. These findings will help to understand the dynamics of the disease in the endemic setting most closely located to the United States, and ultimately, to design control and prevention strategies that are effective in preventing the occurrence of future virus incursions into the United States.**

Near Real-Time Surveillance of Avian Influenza in Italy

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Narrative: Avian influenza (AI) is one of the most important global threats to public and animal health. AI viruses are classified as low pathogenic (LPAI) and highly pathogenic (HPAI), based on severity of the disease in birds. Although LPAI strains do not impose a serious concern to human health, some subtypes can result in high mortality for some avian species and, under favorable conditions, they may evolve to highly pathogenic strains for birds and humans. Thus, AI surveillance must include active collection and analysis of data associated with both LPAI and HPAI-induced disease.

Early detection and control of AI epidemics has been impaired by the absence of surveillance systems capable of incorporating, visualizing, sharing, and analyzing multiple streams of epidemiological and phylogenetic data in near-real time. Here we present a system for global surveillance of AI referred to as BioPortal that has recently become operational in Italy. Densely populated poultry areas of Italy have experienced frequent AI epidemics, indicating conditions favorable for mutation of LPAI strains into HPAI forms, as reported in 1997 and 1999.

The BioPortal is updated with epidemiological information and sequencing data from new AI cases in near-real time. Historical databases for LPAI and HPAI outbreaks and tools to conduct phylogenetic and time-space clustering analyses are publicly available through the BioPortal, which also contains global AI data from public sources. Time-space and phylogenetic relations of the H5 and H7 LPAI cases reported in 2007 and 2008 in Italy suggests that outbreaks were caused by multiple introductions of AI viruses.

The BioPortal is the first example of a publicly available system for analysis of molecular and epidemiological data applied to near-real time surveillance of animal diseases, and creates the foundations for a system for surveillance of AI and other major animal diseases at a global scale.

The Cador *T. equigenitalis* PCR Kit is a Fast and Sensitive Method for *Taylorella equigenitalis* Routine Diagnostics

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Introduction: *Taylorella equigenitalis* is the infectious agent of contagious equine metritis in horses. The symptoms range from temporary infertility to severe inflammation of the uterine lining. It is spread through direct transmission during mating. *T. equigenitalis* testing is important to identify infected animals before breeding. The *cador* T. equigenitalis PCR Kit is a ready-to-use system for the detection of *T. equigenitalis* DNA using polymerase chain reaction (PCR). The assay was co-developed by QIAGEN and the Veterinary Laboratory Agency (VLA). Analytical and clinical validation was conducted by the VLA.

A large prospective clinical study on more than 2000 specimens conducted by Beaufort Cottage Laboratories demonstrated complete concordance with routine microbiological culture and suitability of the *cador* T. equigenitalis PCR Kit to be used in standard laboratory conditions, without any false-positive results.

Materials and Methods: The *cador* T. equigenitalis PCR Kit contains components for the specific amplification of a conserved region of the *T. equigenitalis* genome. The amplicons are detected by measuring the FAM™ fluorescence on QIAGEN's Rotor-Gene Q-series, Applied Biosystems Sequence Detection Systems or Stratagene's Mx3000-instrument series. For analytical and clinical validation sample material was supplied by the VLA. Genital swabs collected according to the Horserace Betting Levy Board recommendations were tested by PCR following standard culture. Crude lysates prepared directly from clinical swabs were used for analysis with the VLA "in house" PCR assay. For subsequent analysis with the *cador* T.equigenitalis PCR Kit samples were prepared using the QIAamp DNA Mini Kit on the QIAcube.

The prospective clinical trial at Beaufort Cottage Laboratories was conducted on 2072 routine pre-breeding genital swabs collected from mares and stallions during 2009, together with stored positive material. Swabs were cultured for *T. equigenitalis* using standard microbiological techniques. Bacterial lysates were isolated from the swabs and examined using the *cador* T.equigenitalis PCR Kit.

Results: Analytical sensitivity of the *cador* T. equigenitalis PCR Kit is below 0.6 copies/ µl. Detection is highly specific for *T. equigenitalis*. Neither *T. asinigenitalis* nor other potentially cross-reacting pathogens are detected. The prospective trial conducted at Beaufort Cottage Laboratories demonstrated that there was 100 % concordance between positives and negatives obtained by the two methods. Real time PCR also detected *T. equigenitalis* DNA from swabs that were negative using standard microbiological culture after 6 months secure storage at +4°C but from which *T. equigenitalis* had been isolated following collection. The relative sensitivities of real time PCR and bacterial culture were similar using serial dilutions, with a threshold of 10-3 equivalent to 3 colony-forming units.

Discussion/Conclusion: The *cador* T. equigenitalis PCR Kit is a reliable, sensitive and time-saving alternative to the currently used bacterial culture. The performance of the *cador* T.equigentalis assay has been validated by the VLA and was successfully used in standard laboratory conditions in a large prospective trial by Beaufort Cottage Laboratories.

A High-Throughput Workflow for the Extraction of Aquaculture Pathogen DNA/RNA from Aquatic Tissues

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Narrative: An increasing number of new and emerging aquatic diseases affecting the shrimp or fish industry such as White spot syndrome, Yellowhead disease, Infectious Salmon anemia and Infectious hematopoietic necrosis have posed challenges for aquatic management and health. Predictably, the development of a sensitive, reliable and high-throughput DNA/RNA extraction and amplification workflow will assist aquatic health management to develop methods to reduce or prevent spread of such diseases. Current methods include lightly agitating tissue to release pathogens or soaking tissue in lysis buffer to lyse cells and release nucleic acid. These methods are time consuming and do not effectively release pathogenic nucleic acid from tissue samples. In this study, we describe a high-throughput, sensitive and reliable bead-based extraction protocol to purify DNA/RNA from aquatic tissues such as fish and shrimp. This method homogenizes ~0.5g tissue with stainless steel beads and a Biospec™ bead beater along with MagMAX™ lysis solution to help break open pathogenic and endogenous cells and release nucleic acid. This solution is then processed utilizing MagMAX™ chemistries and MagMAX™ Express instrumentation to extract and purify nucleic acid for downstream real time PCR analysis. **This workflow is capable of processing 96 samples or more within 3 hours of starting the procedure.**

Diagnostic Sample Types for Real-Time PCR Detection of Equine Pathogens

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Narrative: It has become common practice to test numerous sample types for pathogens using real-time PCR; unfortunately, negative PCR results in certain samples are not reliable for the exclusion of tested pathogens, which might have a negative impact on the wellbeing of an animal. The suitability of sample types for detection of suspected pathogens must be taken into consideration before collection and testing.

A retrospective analysis of pathogen detection and sample types correlation was evaluated over a four year period. A total of 10,178 equine samples were submitted to the Lucy Whittier Molecular and Diagnostic Core Facility, University of California at Davis during 2005-2008, of which 653 were PCR positive for 31 different pathogens. This review focuses on samples submitted for the testing of *Anaplasma phagocytophilum* (etiological agent of granulocytic anaplasmosis), *Neorickettsia risticii* (agent of Potomac Horse Fever), *Clostridium difficile*, *Streptococcus equi* subsp. *equi* (etiological agent of strangles), Equine Herpesvirus-1 (EHV-1), Equine Herpesvirus-4 (EHV-4), and Equine Influenza Virus (EIV). None of the submitted blood samples were PCR positive for *S. equi*, EHV-4, and EIV; whereas, nasal secretions were PCR positive at 8.8%, 4.7%, and 3.6% of tested samples for *S. equi,* EHV-4, and EIV, respectively. Whole blood and feces are good samples types for the detection of *N. risticii*, 9.1% and 7.1% respectively were PCR positive. Whole blood is the best sample type and most often submitted for *A. phagocytophilum* (15.7% were PCR positive). EHV-1 was positive at 2.0% in whole blood and 5.2% in nasal secretions.

A formative decision must be made in determining the optimum sample type(s) for PCR detection of equine pathogens. Sample type collection must be based on the type of infection (acute, chronic, latent), location of infection, clinical symptoms, and differential diagnosis.

Comparison of Four Methods to Quantify Equine Herpesvirus-1 Load by Real-Time PCR in Nasal Secretions of Infected Horses

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Narrative: The objective of this study was to compare the performance of four methods to quantify equine herpevirus-1 (EHV-1) by real-time PCR in nasal secretions from experimentally and naturally infected horses.

Nasal secretions were collected on the challenge day and daily thereafter for 13 days from 4 experimentally infected horses. Additional, nasal swabs were collected from 30 horses with clinical signs consistent with natural EHV-1 infection. Absolute quantitation of EHV-1 target molecules was performed using standard curves for EHV-1 (95%, r^2=0.994) and equine glyceraldehyde-3-phosphate dehydrogenase (98%, r^2=0.999) and DNA yield. The four different methods for expression are: EHV-1 glycoprotein B (gB) gene copies per million nucleated nasal cells, EHV-1 gB gene copies per entire swab, EHV-1 gB gene copies per 1 µl of purified DNA, and EHV-1 gB gene copies per 1 ng of template DNA.

The study results showed that all four calculation methods yielded comparable results between experimentally and naturally infected horses and that the different methods were significantly correlated with each other. For the 4 different calculation methods, the mean daily viral loads ranged from 1,098 to 4.7 x 10^8 EHV-1 gB gene copies per million nucleated cells, from 1,150 to 6.5 x 10^8 EHV-1 gB gene copies per swab, from 5.9 to 3.1 x 10^6 EHV-1 gB gene copies per 1 µl of extracted DNA and from 0.093 to 4.2 x 10^4 EHV-1 gB gene copies per 1 ng of template DNA. Results of the four different calculation methods showed a highly significant positive correlation between each other (r=0.85-1, p<0.001). A Spearman rank correlation test was used to assess the relationship between the four different viral load quantitation methods. A value of $P < 0.05$ was considered significant.

Reporting of quantitative results for EHV-1 viral load in nasal swabs collected from infected horses constitutes an important advance in both the research and diagnostic fields, allowing one to determine the infectious risk of affected horses, disease stage or response to antiviral therapy. However, protocols that normalize the PCR results against a pre-selected volume of DNA or nasal secretions are likely to be more prone to variations than protocols that calculate the load for the entire swab, incorporate a house-keeping gene or use a constant amount of extracted DNA.

Applications of Universal Internal Positive Control for Pathogen Nucleic Acid Purification and Research

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Introduction: Quantitative, real-time, reverse transcriptase PCR (RT-PCR) is a powerful tool for research involving amplification of pathogen nucleic acid from diverse biological and environmental samples. RT-PCR is simple, fast, robust, accurate, sensitive, and adaptable for high-throughput applications. The successful use of RT-PCR in research depends on two critical processes, nucleic acid purification and nucleic acid amplification. Confidence in RT-PCR results requires the use of good laboratory techniques and internal positive controls to ensure that the critical processes are functional. False positive and false negative results may compromise the confidence of results. The use of reliable reagents and internal positive controls ensures that negative results are correctly identified; a true negative result is due to lack of target nucleic acid as opposed to a false negative result due to the inability to amplify target nucleic acid due to the presence of PCR inhibitors. We have developed a TaqMan® chemistry-based PCR workflow consisting of reliable reagents and universal exogenous innocuous internal positive controls to minimize false negative results for pathogen research.

Materials and Methods: The TaqMan® chemistry-based PCR workflow consists of our MagMAX™ Nucleic Acid Isolation Kit, Path-ID™ PCR reagents, exogenous non-competitive Xeno™ RNA internal positive control, and a bluetongue (BTV)-specific TaqMan® primers and probes.[1] Xeno™ RNA nucleotide sequences possess no significant homology to the current annotated public sequences data, thus can serve as a universal internal positive controls for many TaqMan® assays. Dilutions of BTV vaccine were spiked into bovine (unique samples = 8; n = 3) and ovine (unique samples = 8; n = 3) whole blood to mimic varying titer BTV-positive samples. Xeno™ RNA was spiked into the MagMAX™ lysis solution and used for RNA purification of BTV-spiked blood samples. Purified RNA was used for BTV/Xeno™ RNA TaqMan® PCR using Path-ID™ PCR reagents. Addition of hematic was also added to PCR to mimic inhibition.

Results: Bluetongue amplification was present in all BTV vaccine spiked samples; negative samples did not result in any amplification. The Xeno™ RNA was reliably and reproducibly amplified in all samples; Xeno™ RNA Ct standard deviation <1 Ct. The consistently amplified Xeno™ RNA indicated the lack of PCR inhibitors in all 16 unique blood samples. The hematin inhibition mimic results indicated that a Xeno™ RNA Ct shift of >3 Ct was indicative of PCR inhibition. A shift of >3 Ct reduces the ability to amplify BTV nucleic acid by one log (10-fold reduction).

Discussion/Conclusions: The results of this evaluation suggest that our TaqMan® chemistry-based PCR workflow provides highly reliable results. The Xeno™ RNA exogenous control provides confidence in the nucleic acid purification and identification methods. The Xeno™ RNA is noncompetitive with target assays and can be used with many assays, providing a universal internal control application.

Reference:

[1] Shaw A.E. et al. (2007) Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. *J Virol Methods* Nov; 145(2):115-26.

Adaptation of a Real-Time RT-PCR Based Assay for the Detection of Bluetongue Virus (BTV)

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Introduction: Bluetongue (BT) is a non-contagious disease in cattle, sheep and other ruminants. The infectious agent is the Bluetongue virus (BTV, a dsRNA virus of the family *Reoviridae*) which is transmitted by certain species of *Culicoides* midge. BTV is classified into 24 serotypes and found in both tropical and temperate zones. It can cause severe symptoms such as high fever, hyperaemia, salivation, cyanotic (blue) tongue and loss of condition. Infection of cattle has also been shown to lead to a loss of milk yield and abortion. Since 1998 several outbreaks of BTV 8 and other serotypes have been seen in Europe. Currently, control measures are limited to vaccination, movement restrictions and the control of midges.

In 2008 QIAGEN acquired a license to commercialize a veterinary molecular Real-time RT-PCR based test for the detection of Bluetongue virus developed by the Institute for Animal Health (IAH). This assay will be available from QIAGEN as a complete solution, including optimized reagents (including controls), protocols for Real-time RT-PCR, and recommendations for nucleic acid preparation. The Real-Time RT-PCR assay will be validated by the IAH in conjunction with manual and automated technologies for RNA extraction from a large number of samples.

Material and Methods: The *cador* BTV Real-time RT-PCR assay is an adaptation of the existing Shaw et al (2007) pan-BTV real-time RT-PCR assay, and was initially developed on the Rotor-Gene Q PCR cycler using different serotypes (e.g. serotypes 1, 2, 4, 8, 9,16) found in Europe and other regions. It is a pan BTV assay for the detection of all known BTV serotypes and topotypes. Reliable detection of BTV was demonstrated for different starting sample materials, including whole blood, using manual (e.g. QIAamp RNA Blood Mini Kit, QIAamp Viral RNA Mini Kit) as well as automated solutions (e.g. BioRobot Universal).

Results: Sensitive detection was shown for all 24 BTV serotypes using the *cador* BTV Real-time RT-PCR assay in combination with different extraction methods. All samples which were tested positive with the regular BTV assay offered by IAH were also found being positive using the *cador* BTV Real-time RT-PCR assay.

The analytical sensitivity for the cador BTV RT-PCR Kit is below 1 copy/µl and has an analytical specificity for a large number of serotypes and strains.

Discussion/Conclusion: The *cador* BTV RT-PCR Kit allows highly sensitive and specific detection of Bluetongue viral RNA from a diverse selection of serotypes and topotypes. It is validated by IAH and QIAGEN on a large number of samples. The *cador* BTV RT-PCR Kit will be available as a complete solution offered by QIAGEN.

Reference:

[1] Shaw, A.E. et al. (2007) Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segement 1. Journal of Virological Methods 145:115-126.
[2] Mackay, I.M. (2004) Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 10: 190.

Real-Time PCR Detection of Veterinary Pathogens - Solutions That Can Adapt to Changing Needs

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Introduction: Veterinary pathogen detection by nucleic acid (NA)-based analysis is widely replacing immunological and culture based methods due to higher sensitivity and speed. A key factor for successful amplification and detection of pathogenic NAs is an effective and reproducible sample preparation methodology providing reliable purification and maximum recovery of DNA and RNA. A way to achieve standardization and reproducibility of nucleic acid isolation is the employment of automated procedures. Real-time PCR techniques further improved work efficiency in veterinary molecular diagnostics. For various pathogenic targets, premixed and validated real-time PCR assays are commercially available, further decreasing hands-on time and error risk. Alternatively, self-designed or literature-derived real-time PCR systems can be used for pathogen identification. In both cases, methods need to be based on high-performing and reliable PCR reagents. However, once established such methodology may need to be adapted to changing requirements. For example the type and number of samples used to detect a certain pathogen may change over time, as well as the requirements related to the type and nature of pathogen itself, like in an outbreak situation. Sample types described below were taken as a case for testing of the adaptability of different sample preparation and PCR-based detection methods.

Materials and Methods: Viral NA and bacterial DNA were isolated from veterinary samples including whole blood, serum and bacterial culture using automated, high-throughput sample preparation with the One-For-All Vet Kit on the BioSprint 96 instrument, and additionally using the QIAGEN instruments QIAsymphony SP and QIAcube in combination with the appropriate kits. The purified NAs were evaluated by real-time PCR targeting Bovine Viral Diarrhea Virus (BVDV) and *Taylorella equigenitalis*, using premixed commercial PCR reagents (QIAGEN *cador* reagents), or for Porcine Circovirus (PCV-2), using the PCR primers and probe described by Brunborg *et al.*, J. of Virological Methods 122 (2004), p.171 in combination with a generic real-time PCR master mix chemistry following the supplied protocols.

Results: Viral RNA was purified from individual blood samples derived from BVDV infected cattle and was detected using the *cador* BVDV RT-PCR Reagent. Reliable and linear detection of BVDV RNA was achieved after pooling of one BVDV positive and 10 or 50 negative blood samples. *Taylorella equigenitalis* DNA was isolated from bacterial culture and detected with the *cador* T. equigenitalis PCR Reagent over a 4-log range with high linearity ($R^2 > 0.996$) and precision. The PCV-2 DNA was isolated from porcine serum with high repeatability and precision. Straightforward establishment of the PCV-2 assay was accomplished using the generic real-time master mix. Comparable results were obtained for different sample preparation procedures using the BioSprint 96, QIAsymphony SP and QIAcube platforms.

Discussion/Conclusion: 1) Three automation platforms addressing different throughput show comparable results – demonstrating adaptability to changing requirements; 2) *cador* PCR reagents provide easy-to-use and validated premixed reagents for reliable veterinary pathogen detection and guarantee immediate results; 3) For upcoming and self designed pathogen tests, generic pre-optimized master mixes offer a high-performing chemistry, allowing establishment of new tests with minimal optimization.

Development of Duplex Real-Time RT-PCR for Rapid Detection and Differentiation of the Classical North American Genotype Porcine Reproductive and Respiratory Syndrome Viruses (PRRSV) and the Highly Virulent PRRSV from China

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Narrative: Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important swine diseases in the world. Since the first PRRS virus (PRRSV) was isolated from pigs in China in 1996, it was found that all PRRSV isolates in China belong to the Northern America (NA) genotype. Recently, a highly pathogenic (acute, atypical) PRRSV emerged in Southern China in the spring of 2006, inducing a >50% morbidity rate and >20% mortality rate. Since infections of PRRSVs with the NA genotype and the highly virulent PRRSV cause similar clinical symptoms, it is difficult to differentiate between the highly pathogenic and the NA strains. A critical need for the PRRSV surveillance and control program in China is rapid detection and differentiation of the classical NA genotype PRRSVs and the highly pathogenic PRRSV. Therefore, a SYBR green duplex real-time RT-PCR was developed and evaluated for its capability to detect and differentiate the classical NA and the highly pathogenic PRRSV strains. The primers were designed based on the 87nt deletion in the NSP2 gene of the highly virulent PRRSV. This newly developed duplex real-time PCR was able to differentiate between the highly pathogenic PRRSV and all known NA genotypes of PRRSVs. The analytical sensitivity of this assay was approximately 10^1 $TCID_{50}$ per reaction. The assay was highly specific for PRRSVs because no positive results were obtained when other common swine pathogens were tested. **These results demonstrated that the newly developed duplex real-time RT-PCR can be used for rapid detection and differentiation of the classical NA genotype and the highly pathogenic PRRSV.**

Evaluation of a High-Throughput Protocol for the Purification of *Tritrichomonas foetus* DNA from Bovine InPouch TF™ Cultures

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Narrative: Significant economic losses associated with bovine trichomonaisis, caused by the protozoan *Tritrichomonas foetus,* have compelled many states in the USA to implement mandatory regulatory testing programs. Current standard testing programs require culture of genital secretions (preputial smegma) from breeding bulls in media such as In Pouch TF. Because the number of culture samples diagnostic labs are required to process is increasing, many have started using homebrew PCR assays to screen cultures instead of the traditional microscopic identification. This creates a need for a fast, less labor-intensive protocol for DNA extraction and isolation from the cultured smegma that both removes PCR inhibitors and is suitable for automation. We optimized and evaluated a protocol for the high throughput purification of DNA from bovine InPouch TF cultures using the magnetic bead-based MagMAX™-96 Viral RNA Isolation Kit with the MagMAX™ Express (MME) and the MagMAX™ Express-96 (MME-96) instruments, Applied Biosystems' automation platforms.

To evaluate our protocol, we used the MagMAX™ -96 Viral RNA Isolation Kit to purify DNA from (N=48) bovine InPouch TF samples with our optimized InPouch TF culture protocols using the manual protocol (without automation in a 96-well plate), MagMAX™ Express (automated magnetic bead-handling for 24 samples), and MME-96 (automated magnetic bead handling for 96 samples) instrument methods. Qiagen's filter-based QiaAmp DNA Mini Kit was also used for DNA purification for performance comparison evaluation. Purified DNA (8 µl) from samples were then used for TaqMan® PCR specific for *T.foetus* DNA (McMillen et. al. 2006), bovine endogenous control, and an exogenous internal control (Ambion's Xeno™DNA). Varying volumes of purified DNA (10, 5, and 1 µl) were used for TaqMan® PCR to evaluate the purity of DNA and presence of PCR inhibitors.

Results of this evaluation demonstrate that amplification of all DNA targets (*T.foetus*, bovine endogenous control, and Xeno™DNA) from the three MagMAX™-96 Viral RNA Isolation Kit methods and the QiaAMP DNA Mini Kit manual protocol vary less than one cycle (1 Ct) for all samples tested (N=48). In addition, no PCR inhibition was observed. In our hands, the MagMAX™ Kit DNA purification methods produce equivalent PCR results to the QiaAMP DNA Mini Kit manual protocol but are a minimum of one and one half hours faster. The MagMAX™ Express instruments enable robust and rapid DNA purification in approximately 45 minutes. **In conclusion, our optimized InPouch TF culture DNA purification protocol on the MagMAX™ Express instruments provides a robust, simple, and fast, high-throughput workflow solution for bovine trichomonaisis DNA extraction and isolation.**

Effects of High Temperatures on the Survival of *Tritrichomonas foetus* in InPouch TF™ Media

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Narrative: The objective of this study was to evaluate the effects of high temperatures on the survival of *Tritrichomonas foetus* (*TF*) in culture media. Two sets of thirty-six commercially available sampling kits (InPouch TF™) were inoculated with two different strains of *TF* (36 pouches for each strain) and subjected to temperatures of 37° C, 46.1° C, and 54.4° C for 1, 3, 6, and 24 hours. Six un-inoculated pouches exposed to 37° C for the entire treatment period served as negative controls. Pouches were removed from the incubators and held at 22.2° C until all temperature treatments were complete. Pouches were submitted to Texas Veterinary Medical Diagnostic Laboratory (TVMDL) for culture and polymerase chain reaction (PCR) testing. **Culture results of pouches exposed to 37° C for all treatment times were positive while those subjected to 46.1° C for 1, 3, 6, and 24 hours were positive, positive, negative, and negative, respectively. Pouches subjected to 54.4° C for 1, 3, 6, and 24 hours were positive, negative, negative, and negative, respectively. All 72 inoculated samples were positive by PCR. All un-inoculated pouches were culture and PCR negative. These findings suggest high temperatures can affect the ability to culture *TF* positive samples.** While high temperatures may affect the growth rate of the organism, they do not appear to negatively influence the ability of PCR to identify the organism. **Therefore, samples submitted to diagnostic laboratories for *TF* testing during warm seasons should be protected from high temperatures or submitted for PCR testing.**

A Novel One-Step RT-PCR Mix that Provides Greatly Improved Sensitivity and Much Shorter Time to Results in Real-Time PCR for the Detection of RNA Viruses and in Gene Expression Analysis in Bacteria or Animals

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Narrative: Real-time PCR is a powerful tool for the diagnosis of veterinary disease. The advantages of this technology include speed of sample preparation and testing, high target specificity, and potential quantification of pathogen DNA or RNA. With RNA based detection methods, a cDNA copy of the RNA target must first be generated for the PCR amplification. Reverse transcription is frequently performed as a separate reaction step, with a portion of the generated cDNA being transferred to another tube for PCR. This two tube process can be time consuming and greatly increases the potential for sample contamination,

The preferred method for many applications is to use a one-step RT-PCR method, combining reverse transcription and PCR in a single tube. Here we report the development of a novel one-step RT-PCR mix which has been optimized for use in rapid cycling real-time one-step RT-PCR. This novel formulation allows single tube detection of a single RNA transcript in under one hour, with the reverse transcription incubation step often requiring as little as 3 to 5 minutes. This one-step RT-PCR mix was tested against numerous commercially available "gold standard" enzymes and one-step RT-PCR kits for the detection of Avian Influenza Matrix RNA and the Avian influenza H5 serotype**. It was demonstrated that this novel mix could improve detection sensitivity by up to 6 PCR cycles compared to "gold standard" kits, with significantly earlier detection across the entire dynamic range of the assays.**

Similarly remarkable results were observed in numerous gene expression analysis studies and in studies examining the detection of mRNA transcripts from bacterial pathogens. **Combining the use of this unique one-step RT-PCR mix with lyophilized beaded reagents can provide an unparalleled level of sensitivity, specificity, repeatability and reproducibility in the detection of RNA viruses.**

Isolation of *Rothia*-Like Organism From a Goat Abscess

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Narrative: A swab sample from a 4-month old goat abscess consistent with *Corynebacterium pseudotuberculosis* infection was submitted to the Ohio Animal Disease Diagnostic Laboratory for bacterial culture. After 24 hours of aerobic incubation, heavy growth of a Gram-positive tetracoccal bacterium was isolated on blood agar. The colony was dry, flat, non-hemolytic, and entire with a size ranging from pinpoint to 1-2 mm in diameter. DNA sequence analysis on the 16S rRNA gene indicated that the bacterium is closely related to bacteria in the *Rothia* genus. **Phylogenetic analysis demonstrated that the organism is a new species that has not been reported before.**

Development of PARACHEK® 2 for High-Throughput Detection of Johne's Disease in Milk and Serum Samples

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Narrative: Paratuberculosis, also known as Johne's Disease, is caused by the presence of *Myobacterium avium spp paratuberculosis* in the small intestine of ruminants. It occurs worldwide and affects animal health of beef and dairy herds. The PARACHEK® is the original Johne's absorbed ELISA and is a reliable and useful tool for effective detection, control and management of paratuberculosis. The ELISA is able to detect antibodies against *M. paratuberculosis* in serum and milk prior to the onset of clinical signs. In order to simplify the use and increase throughput, we have developed the PARACHEK® 2 which is more user-friendly and enables automation. It contains a one component substrate and incubation times were adapted for user-friendliness. To evaluate the performance of PARACHEK® 2 and compare it to PARACHEK®, we have analysed 583 negative and 93 positive cattle and 164 negative and 36 positive sheep serum samples, respectively. In addition a set of 351 negative and 16 positive milk samples from cattle were tested. All samples were collected from animals with known fecal culture status for *M. paratuberculosis*. The fecal culture was done using the TREK ESP para-JEM Culture System II. The agreement is expressed by the Cohen's Kappa coefficient and interpreted using the Landis and Koch table. **The agreement between PARACHEK® 2 and PARACHEK® is almost perfect with kappa values of 0.83 (ovine serum), 0.92 (bovine serum) and 0.93 (bovine milk).** The PARACHEK® 2 also has two options for detection, a kinetic protocol for a high plate to plate reproducibility or an end-point protocol enabling high throughput and automation. The PARACHEK® 2 was automated on a Beckman Coulter Biomek® FXP Laboratory Automation Workstation equipped with a 96-well plate washer and a plate reader which allows for a throughput of up to 16 plates in one working day (8.5 hours) starting from serum or milk samples. The results of the fully automated system were compared to manual processing of the samples. **The agreement is almost perfect with a kappa value of 0.96. These results demonstrate that the PARACHEK® 2 can be easily run on an automated system with the same excellent performance as with manual processing of the samples using the original PARACHEK®. This enables laboratories to save time and free staff for other work.**

BD BACTEC™ MGIT™ 960 Para TB System Performance with Growth Medium Antimicrobials for Highly Contaminated Samples

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Introduction: When selecting growth medium antimicrobials to suppress contaminant overgrowth from very highly contaminated bovine feces cultured for Johne's diagnostics, the inhibitory effects on recovery of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) must be considered. High concentrations of nalidixic acid are especially useful against *Bacillus* endospores, and ceftriaxone is effective against the Gram-negatives that have unintentionally appeared in proficiency test samples in recent years. This study tested the effects of three antimicrobial cocktails formulated to manage highly contaminated samples on the performance of an automated liquid culture system with respect to MAP detection times, recovery rates, and contamination rates.

Materials and Methods: Ten processed bovine fecal samples were inoculated at three dilution levels each into the BD BACTEC™ MGIT™ 960 Para TB System according to manufacturer's instructions, five replicates per condition, to test three antimicrobial cocktails in the growth medium. Three were high shedder and seven were low or very low shedder samples, and nine of the 10 samples were highly contaminated by bacteria (≥ 1E7 cfu/gm). In addition to the standard 18-19 µg/ml vancomycin and 6-7 µg/ml amphotericin B, the cocktails imparted to the growth medium were (1) 200 µg/ml nalidixic acid (NAL-200), (2) 75 µg/ml nalidixic acid and 6 µg/ml ceftriaxone (N75-C6), or (3) 50 µg/ml nalidixic acid and 8 µg/ml ceftriaxone (N50-C8). Ongoing cultures were incubated in the instrument beyond the 49-day protocol in order to identify delayed growth. After 62 days, terminal negative cultures were examined microscopically for acid-fast bacilli. MINITAB ver. 15.1 statistical software was used to analyze MAP times-to-detection (TTDs) by ANOVA-type general linear model and recovery and contamination rates by binary logistic regression.

Results: Mean MAP TTDs differed significantly by antimicrobial cocktail formulation, with the shortest detection times in NAL-200 and the longest in N50-C8 ($P < 0.001$). Of the 450 total culture tubes, 286 (63.6 %) were MAP-positive within 49 days, 4.4% were lost to contamination, and 32.0% were negative for growth after 49 days. The high number of negatives reflects the use of diluted inocula from low and very low shedder fecal samples. Of these negatives, 10.4% (15) went positive from MAP growth post-protocol (49-62 days). From 150 cultures tested per antimicrobial cocktail, results with NAL-200, N75-C6, and N50-C8 were, respectively, positives within 49 days: 97, 97, and 92; of these, positives in the final week of protocol (42-49 days): 1, 14, and 26; lost to contamination: 4, 9, and 7; negative for growth after 49 days: 49, 44, and 51; and of these, post-protocol detections (49-62 days): 1, 7, and 7 cultures. Numbers of MAP-positives within 49 days, cultures lost to contamination, cultures negative for growth at 49 days, or post-protocol detections did not differ significantly by antimicrobial cocktail formulation ($P < 0.05$). Numbers of MAP-positive detections in the final week of incubation (42-49 days) differed significantly by cocktail formulation, with the fewest from NAL-200 and the most from N50-C8 ($P < 0.05$). None of the cultures that were still negative for growth after 62 days were AFB-positive by smear microscopy (no false negatives).

Conclusions: MAP recovery rates and contamination rates were comparable with the three antimicrobial cocktails. Mean MAP detection times differed by cocktail, with NAL-200 yielding the shortest TTDs and as a result the fewest detections during the final week of incubation (42-49 days), and N50-C8 yielding the longest TTDs. Between antimicrobial cocktails that contained ceftriaxone, the N75-C6 formulation was as effective as N50-C8 but had less impact on detection times.

Investigation of Wild Freshwater Fish Die-Off in New Jersey

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Narrative: A die-off was reported in a freshwater lake in New Jersey involving various species of fish including common carp (*Cyprinus carpio*), yellow perch (*Perca flavescens*), and others during April and May 2009. Fish were submitted to Animal Health Diagnostic Laboratory to investigate the cause of death. Necropsy revealed following lesions: cutaneous, branchial, swim bladder, and fin hemorrhaging; cutaneous ulcerations; and multifocal areas of hepatic necrosis. The intestine was hemorrhagic and filled with yellow fluid. Microscopically, necroinflammatory lesions involving the skin and muscle were observed. These lesions were suggestive of hemorrhagic septicemia of viral or bacterial origin. Kidney, spleen, and liver were subjected to virus isolation. Kidney was swabbed and cultured to isolate bacteria. Cytopathic virus was not isolated. Hemolytic bacterial colonies were recovered on plates. Isolated bacteria were subjected to morphological and biochemical tests. **Recovered bacteria were identified as *Aeromonas hydrophila*.** The micro-organism isolated is responsible for motile aeromonad hemorrhagic septicemia of fish. Fish do not eat during winter and their immune system is weak. This weak immunity coupled with other factors like rising water temperature during spring and spawning stress makes fish highly susceptible to *Aeromonas* infection. The severity of infection suggests that fish were under stress. **Although *A. hydrophila* is not a severe threat to fish, it is fatal in stressed fish.**

The Effects of Common Semen Extenders Inoculated with Various Bacterial Strains on Semen Quality in Horses

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Narrative: Equine semen that is packaged and shipped is almost always extended with a skim milk or egg yolk-based semen extender. These semen extenders typically contain antibiotics to prevent bacterial growth prior to insemination. Because equine semen is shipped with great frequency, and the potential for bacterial growth in extenders has not been well studied, it poses an important question about the quality and bacterial status of the semen being shipped.

The purpose of this presentation is twofold. Initially, to report the growth of various bacteria including *Streptococcus equi* subsp. *zooepidemicus, Pseudomonas aeruginosa, Taylorella equigenitalis,* and *Klebsiella pneumoniae* in extended semen using INRA 96® (+/- Timentin), E-Z Mixin®, and VMDZ. INRA 96® is a milk-based extender containing Penicillin, Gentamycin, and Amphotericin B. E-Z Mixin® is also milk-based with no added antibiotics. VMDZ is an egg-yolk based extender containing Amikacin and Potassium Penicillin G. The bacteria (at a 0.5 MacFarland standard) were added to the extenders at the time the semen was added, and CFUs of bacterial growth were then evaluated. Culture results indicated that each extender decreased bacterial CFUs over the 72 hours. VMDZ was by far the most effective extender against all types of bacterial populations. In addition, *Taylorella equigenitalis* had a greater than 50% chance of growth at 24 hours in INRA 96® extender alone. For the second part of this study, using CASA (computer-assisted sperm analysis) and SCCA (sperm chromatin condensation assay), the quality of the inoculated semen in each of these extenders was analyzed.

It is the object of this presentation to inform veterinarians about the potential for disease spread in semen extenders, and also how semen quality may be affected by extenders over time.

Evaluation of the *in vitro* Susceptibility of *Leptospira* Strains to Cefovecin Sodium (Convenia®)

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Narrative: The *in vitro* activity of cefovecin sodium (Convenia®) was evaluated against selected *Leptospira* isolates that were in the bacterial stock culture collection at Veterinary Medicine Research and Development, Pfizer Animal Health. Four *Leptospira* isolates including serovars: canicola, grippotyphosa, pomona, and copenhageni were tested for susceptibility to cefovecin sodium by a broth microdilution method in 96-welled plates. Two of the *Leptospira* tested including serovar canicola, strain Hond Utrecht IV; and serovar pomona, strain NVSL are of canine origin. Results indicated that the minimum inhibitory concentration (MIC) values for cefovecin sodium were 1.5 µg/ml for serovar canicola, 0.5 µg/ml for serovar pomona, 0.5 µg/ml for serovar grippotyphosa, and 0.13 µg/ml for copenhageni. **These preliminary results are promising and indicate the feasibility of additional indications for cefovecin sodium (Convenia®) for the treatment of Leptospirosis in companion animals.**

Real-Time qPCR Pathogen Detection Using a Novel Nanofluidic System

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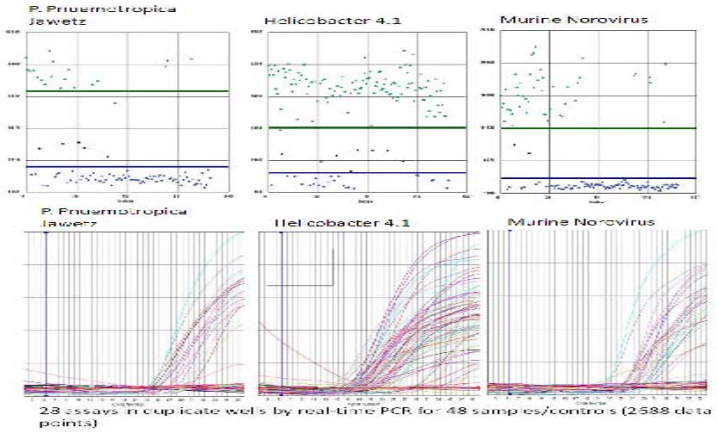
Narrative: With an increasing frequency of both the number of infectious diseases and the diversity of pathogenic agents in the veterinary environment, there is a fast growing need for rapid and sensitive assays. Utilization of qPCR for pathogen detection represents a powerful tool in veterinary and medical microbiology, particularly in areas where traditional methods alone have proven insufficient.

Using the OpenArray system, we were able to generate thousands of qPCR data points in one day. This format eliminates the need to multiplex or pool PCR assays prior to PCR. The OpenArray platform accommodates the use of end-point and real-time PCR technology.

Previously, we have demonstrated utility of the OpenArray for the detection of rodent infectious agents. The set of assays validated in the OpenArray contained primer and probes representing 20 common viruses and bacteria. Evaluation of the results has shown that the analytical sensitivity for the OpenArray and 96-well format was similar. Furthermore, the amplification in the OpenArray appeared to be resistant to potential inhibition associated with nucleic acid extraction from feces and also high concentrations of nucleic acid in the sample.

A recently developed OpenArray DLP Real-Time PCR application allows a quantitative approach to health monitoring for post-quarantine or routine health monitoring of sentinel mice. Rodent samples, which had previously been determined to be positive for at least one agent by the 96-well format in combination with standard practices, were positive for the same and sometimes additional agents by the OpenArray. **Direct testing of quarantined rodents using the OpenArray Real-Time qPCR system provides a more sensitive and efficient process of pathogen detection and significantly reduces the quarantine period.**



Comparative Histopathologic Characteristics of Highly Pathogenic Avian Influenza (HPAI) in Chickens and Domestic Ducks in 2008 Korea

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Introduction: The first documented outbreak of HPAI occurred in Korea during fall/winter of 2003–2004; since then, two more outbreaks have occurred. The latest outbreak of H5N1 HPAI, occurred in April and May 2008, and killed more than 50% of infected ducks, whereas previously the HPAI mortality rate in domestic ducks was low. The histopathologic findings of the latest outbreak were also more severe in ducks, whereas findings in chickens were similar to those observed previously.

Material and Methods: Chickens and domestic ducks suspected of HPAI infection were collected from all areas of Korea during April and May 2008. All cases diagnosed as HPAI were reviewed. Chickens included layer, broiler, and Korean native breeds. We compared characteristic lesions occurring in chickens and domestic ducks naturally infected with H5N1 HPAI virus in April and May 2008.

Results: Infected chickens generally exhibited pale-green, watery diarrhea, depression, neurological signs, and cyanosis of wattles and combs. Infected ducks generally exhibited neurological signs, watery diarrhea, and fever. Gross petechial or ecchymotic hemorrhage affected the heart, proventriculus, liver, muscle, fat, and pancreas in chickens, and muscle and brain in ducks. Necrotic foci were present in the pancreas of both species and in the heart of domestic ducks. Histopathologically, chickens exhibited multifocal malacia of brain, multifocal lymphohistiocytic myocarditis, multifocal necrotic pancreatitis, and hemorrhage of several organs and tissues; ducks exhibited lymphohistiocytic meningoencephalitis with multifocal hemorrhage, multifocal necrotic pancreatitis, and severe necrotic myocarditis with mineralization. In addition, the tracheal epithelium, myocardiocytes, renal tubular epithelium, blood vessel endothelial cells, Purkinje and ependymal cells, and neurons of both species immunoreacted with anti-influenza A nucleoprotein antibody.

Conclusions: The characteristics histopathologic findings of 2008 HPAI were multifocal necrotic encephalitis and necrotic pancreatitis accompanied with lymphohistiocytic myocarditis, and hemorrhage in various organs and tissues in chickens, and severe necrotic myocarditis and necrotic pancreatitis with mineralization accompanied with lymphohistiocytic meningoencephalitis in ducks. The high mortality of domestic ducks was intimately associated with heart failure resulting from increased H5N1 HPAI viral cardiotropism.

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Necrotizing Sialometaplasia of Parotid Gland in a Dog: A Case Report

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Introduction: Necrotizing sialometaplasia (NSM) is a self-limiting, benign, inflammatory disease of the salivary glands with clinical and histologic features that often closely resemble malignancy. The differential diagnosis must be done specially with squamous cell carcinoma or mucoepidermoid carcinoma. It is generally agreed that ischemia is the main etiological factor, but the pathophysiology of the ischemia is unclear. In this study, we report the first case of NSM of parotid gland in a dog.

Materials and Methods: Unilateral swelling of parotid salivary gland in a seven-year-old cocker spaniel was biopsed and submitted to Animal Disease Diagnostic Center, NVRQS. The dog also had otitis externa. The biopsed tissue was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 □) were cut and stained with Hematoxylin and Eosin for histological examination.

Results: Grossly, the mass was moderately firm and large, about 12 x 6 cm in size. The main histological features were as follows: extensive necrosis accompanying with hemorrhage and fibrosis, marked squamous metaplasia with keratin pearls in the glandular and ductal epithelium, preservation of salivary lobular morphology, and variable inflammation. There were no evidences of neoplasia. And there was fibrinoid necrosis of arteries within the necrotic area of the gland and some of them contained thrombi. The lesion recurred four months after local surgical excision and otitis also persisted. Etiologic factors remain obscure but extension of chronic otitis externa could be a contributing factor.

Conclusions: Based on the characteristic histopathological examination, the present case was diagnosed as NSM. This is the first report of NSM at the parotid gland of dog as far as we know.

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Does Asinine Herpesvirus-5 Cause Renal Disease in Miniature Donkeys?

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Narrative: Asinine herpesvirus-5 (AHV-5) has not been documented to cause renal or systemic disease in donkeys (*Equus asinus)*. A two-year-old, 89 kg, female Miniature donkey developed clinical signs of lethargy, anorexia, cyanosis, and lack of gastrointestinal motility prior to euthanasia for deteriorating status. Necropsy examination revealed non-collapsing lungs with multiple pinpoint to 0.2 cm nodules distributed throughout the pulmonary parenchyma and pleura. Both kidneys were enlarged with irregularly distributed patches of soft, raised, variably sized (0.2 cm -1.0 cm) foci that extended as radial streaks into the medulla. Histologically, the lung interstitium had multifocal to coalescing aggregates of pyogranulomatous inflammation with numerous large multinucleated giant cells. A number of pulmonary vessels contained fibrin thrombi and plump, disorganized endothelial cells. The renal cortex had aggregates of pyogranulomatous inflammatory cells with large multinucleated giant cells and vasculitis. Syncytia identified in inflammatory aggregates labeled positively by immunohistochemistry for lysozyme and negatively for broad spectrum cytokeratin indicating the cells were likely macrophagic in origin. The distribution of the pyogranulomatous nephritis was suggestive of embolic spread, likely from the lungs. Differential diagnoses for gross and microscopic lesions in the lung included equine herpesvirus 1 and 4, influenza A, equine arteritis virus, equine adenovirus, and equine rhinovirus. Bacterial and fungal infections were also considered. No viruses were isolated from submitted specimens. There was no growth of bacteria on aerobic culture of kidney, and a streptococcal species was cultured from the lung. Fungi were not observed with special stains. Lung and kidney sections were processed and DNA was extracted from tissue homogenate and a small region of polymerase gene of herpes virus was amplified by polymerase chain reaction (PCR). Sequencing of the amplicons established a ~99% homology with AHV-5a in the lung and kidney sections. Asinine herpesvirus 5 is a gamma herpesvirus that causes characteristic lesions of interstitial pneumonia with syncytia in donkey lungs. Given the similarity of gross and histologic lesions to that of AHV-5a, the severity of lesions, and the detection of AHV-5a by PCR, we concluded that AHV-5a was involved in the renal lesions in this miniature donkey. **This case offers evidence for an important role of AHV-5 respiratory disease leading to severe systemic infection in donkeys**.

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Enteroviral Polioencephalomyelitis in an Indiana Swine Herd

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Narrative: Porcine *Enterovirus* group 1 (recently reclassified into the *Teschovirus* genus) is a single stranded, nonenveloped RNA virus within the Picornaviridae family. *Teschovirus* infection is considered endemic in North American swine production facilities. Despite this widespread distribution, only sporadically is it implicated in clinical disease, most notably a nonsuppurative polioencephalomyelitis. An Indiana pork producer reported losses ranging from 5-7% of a nursery group of 400 crossbred pigs. The pigs were 5-6 weeks old and had signs of progressive neurological disease characterized by ataxia, paraparesis, and opisthotonus. Six pigs were submitted alive to Purdue University Animal Disease Diagnostic Laboratory in lateral recumbency with extensor rigidity of the thoracic limbs, but alert and responsive to stimulus. At necropsy, lesions were not observed in the central nervous system. Spinal cord and brain were removed aseptically and submitted to virology. Significant microscopic findings were mostly restricted to the gray matter of the thalamus, brain stem, and spinal cord. These lesions consisted of gliosis, glial nodules, perineuronal microglial satellitosis, neuronophagia, and prominent lymphoplasmacytic perivascular cuffs that extended focally into the surrounding neuropil and craniospinal ganglia. Multifocally, neuronal cell bodies were necrotic. ***Teschovirus* was isolated from pooled samples of spinal cord and brain. Virus isolation from the central nervous system, in addition to the observed microscopic lesions, confirmed the diagnosis of enteroviral polioencephalomyelitis. It is crucial to recognize the characteristic lesions of porcine *Teschovirus* infection and differentiate them from those of other porcine viral encephalomyelitides, such as pseudorabies or hog cholera.** Although these other viral infections result in lymphocytic perivascular cuffing and gliosis, lesions tend to be widely disseminated in the central nervous system, whereas Teschan viral lesions are typically restricted to the gray matter, especially in the ventral spinal columns.

Embryonal Rhabdomyosarcoma in a Dog

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Narrative: A 2-year-old intact male Australian Shepherd dog developed a rapidly growing mass on the left side of its neck and face with involvement of the left orbit. The ipsilateral mandibular lymph node was enlarged. Hematologic and serum biochemistry results and thoracic radiographs were within normal limits. A punch biopsy specimen of the cervical mass resulted in a tentative diagnosis of pleomorphic sarcoma. Due to the extension of this growth, the owner elected euthanasia. At necropsy, there was left exophthalmia with firm swelling of the left side of the neck and face. Soft tissues in the affected area were expanded by a dark red, malodorous mass with a central cavity that contained viscous, brown fluid. A connection between the retrobulbar and neck masses was not apparent. Regional lymph nodes were enlarged, firm and pale yellow.

Histologically, the architecture of the left cervical lymph nodes was completely effaced by a neoplastic proliferation of variably sized round to polygonal cells with distinct cell limits, eosinophilic to basophilic cytoplasm, central or eccentric vesicular nucleus or nucleus with reticular chromatin and variably sized nucleolus. Nuclear and cellular pleomorphism was marked, with frequent karyomegalic and multinucleate giant cells. In addition, there was a proliferation of haphazardly arranged atypical spindle to stellate cells with abundant cytoplasm and a large oval to round and vesicular nucleus with nucleoli. Some cells contained multiple nuclei in a row (strap cells). The stroma was variable and collagenous. Tumor cells were also present in the soft tissue surrounding affected lymph nodes as well as in numerous thin-walled vessels. A similar neoplastic growth was observed in the contralateral mandibular lymph node and the left retrobulbar mass, but not in other tissues examined. Phosphotungstic acid hematoxylin revealed equivocal striations in a few spindle cells. **Immunohistochemistry labeled neoplastic cells with antibodies to vimentin, desmin, sarcomeric actin, muscle actin, and myoglobin and did not react with antibody to MyoD1 and smooth muscle actin**.

Few, well-characterized rhabdomyosarcomas have been reported in domestic animal species. The tumor has three subtypes: embryonal, alveolar, and pleomorphic. **This tumor was classified as the embryonal subtype.** Although neoplastic cells were pleomorphic, the presence of numerous round cells fits better with embryonal subtype than with pleomorphic rhabdomyosarcoma. Embryonal rhabdomyosarcoma is the most commonly diagnosed subtype in dogs and may occur in animals as young as 1.5 years of age. This tumor proved to be malignant based on the invasive behavior and the presence of nodal metastases.

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Pleuritis and Pneumonia Incited by *Paecilomyces lilacinus* in an Aquarium-Held Loggerhead Sea Turtle (*Caretta caretta*)

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Narrative: *Paecilomyces* spp. are saprobic, ubiquitous, non-pigmented environmental fungi that occur worldwide. Infections caused by these species, known as paecilomycoses, are rare and usually limited to immunosuppressed humans or animals. This disease commonly presents as chronic granulomatous lesions in various organs, most typically in the lungs. In rare cases it is diagnosed in humans and animals without underlying immunosuppressive disease.

A 12 year-old female loggerhead sea turtle (*Caretta caretta*) with a history of abnormal buoyancy and decreased appetite of several weeks duration was found dead and was necropsied. The sea turtle was maintained at a commercial aquarium in a multi-taxa exhibit tank (7.75 m diameter x 1.2 m deep) holding 72,000 liters of synthetic saltwater at 25 to 26.1ºC and 20 ppt salinity. Gross postmortem findings included firm, hemorrhagic lungs with multiple, firm, yellow granulomas throughout the lung parenchyma. The urinary bladder was severely distended and contained a concretion measuring 17.8 x 7.6 x 7.6 cm. The bladder wall was mildly thickened with areas of hemorrhage on the mucosal surface. Multiple formalin-fixed tissues were submitted to the Connecticut Veterinary Medical Diagnostic Laboratory (Storrs, CT) and were routinely processed, embedded in paraffin, sectioned at 4 um, mounted on glass slides and stained with hematoxylin and eosin. Selected sections were stained with hematoxylin-phloxine-saffronin, Grocott's methenamine silver, Von Kossa, periodic acid-Schiff, Brown and Brenn Gram or Prussian blue stains. Swabs of tissue samples of lung were streaked onto Sabouraud dextrose agar and inhibitory mold agar with gentamicin (0.05 g/L), and cultures were incubated at 30ºC. Molds recovered by culture were sent to the Fungus Testing Laboratory (San Antonio, TX) for species identification. Histopathologic evaluation of formalin-fixed tissue samples revealed severe, multifocal and focally extensive, heterophilic and granulomatous pleuritis and pneumonia with intralesional non-pigmented, septate hyphae having parallel walls as well as demonstrating diagnostic fruiting structures. The microscopic morphology of the fungus recovered in culture was identical to that seen in tissues, confirming *Paecilomyces lilacinus* as the fungus seen in pulmonary lesions. Microscopic features included finely-roughened conidiophores giving rise to dense clusters of phialidic conidiogenous cells supported on metulae and chains of oval to subglobose conidia borne from these conidiogenous cells. There was concurrent ulcerative bacterial cystitis and renal lesions. Another environmental fungus, *Beauvaria bassiana,* was also isolated from the lung but not compatible histologically, and not considered significant. **This case demonstrates the infrequently encountered phenomenon of fruiting or conidial production in tissue, and reiterates the importance of having a histological correlate to fungal culture when environmental fungi are isolated.**

Histopathologic and Ultrastructural Findings of Chronic Experimental Cardiotoxicity Induced by Monofluoroacetate Producing-Plant *Palicourea marcgravii*.

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Introduction: *Palicourea marcgravii* is a poisonous plant that under natural conditions causes peracute heart failure and sudden death in cattle in Brazil where the plant is widely distributed and causes important economic losses. Monofluoracetic acid (MFA), the toxic principle, inhibits aconitase in the citric acid cycle causing cell death. The same principle is present in other plants from South Africa and Australia, where toxicoses are reported in ruminants. No significant heart lesions had been reported in either natural or experimental poisoning by *P. marcgravii*. This study reports for the first time vacuolar changes in cardiomyocytes due to the ingestion of *P. marcgravii*. Additionally, we describe for the first time smooth endoplasmic reticulum (SER) swelling due to the consumption of a MFA-producing plant.

Materials and Methods: Daily, for 28 days, two clinically healthy 5-8-month-old, female, brown Swiss goats received 0.2 g/Kg of fresh *P. marcgravii* leaves admixed with their regular diet. A negative control animal received the same diet excluding *P. marcgravii* leaves. At the end of the experiment, animals were euthanized with barbiturate overdose. Samples of heart, liver, kidneys, lungs and CNS were collected in 10% buffered formalin for routine paraffin embedding and HE staining. Subsequently, formalin fixed myocardium was routinely processed for transmission electron microscopy.

Results: Clinical signs of lethargy and heart failure started around day 10 in both experimental animals. Animal # 1 was markedly lethargic at day 15 and died the following day. Resolution of clinical signs in animal # 2 started at day 20 and it was euthanized at day 28 along with the negative control. Grossly, the heart from animal # 1 contained pale areas in the interventricular septum and left ventricular free wall. There was pericardial effusion, pulmonary edema, hydroperitoneum and a nutmeg liver. Necropsy findings from animal # 2 and negative control were unremarkable. Light microscopy of heart from animal # 1 revealed diffuse and extensive cytoplasmic vacuolation of cardiomyocytes and Purkinje cells and multifocal individual cardiomyocyte necrosis and loss. There was centrilobular congestion and necrosis of hepatocytes in liver. Sections of the heart from animal # 2 revealed multifocal and marked vacuolation of Purkinje cells and cardiomyocytes, with occasional individual cell necrosis of cardiomyocytes. The remaining tissues from animal # 2 as well as all tissues from the negative control were unremarkable. Ultrastructurally, cardiomyocytes from animal # 1 contained distorted and markedly dilated SER.

Discussion: One-third of the *P. marcgravii* dose needed to induce peracute heart failure was used in this study aiming to assess the effect of chronic *P marcgravii* ingestion. Small foci of myocardial necrosis, with lymphocytic infiltration and fibroplasia, have been described in sheep due to the ingestion of fluoroacetate and other plants, such as *Dichapetalum cymosum*, in South Africa, and *Acacia georgina*, in Australia. However, widespread vacuolar changes in cardiomyocytes and Purkinje cells as seen in this study have not been described. Although electron microscopic changes do not appear to have been reported for MFA poisoning, one would expect ultrastructural findings primarily in the mitochondria since MFA interferes with the citric acid cycle. Surprisingly, SER changes were observed with chronic ingestion of *P. marcgravii*, presumably the result of chronic MAF poisoning.

Conclusion: Chronic *P. marcgravii* toxicosis causes severe vacuolar degeneration of cardiomyocytes associated with dilation of SER in goats.

Mast Cells in Canine Enteritis and Some Neoplasms

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Narrative: In humans, mast cells play an important role in gastrointestinal pathology, especially in the intestinal response to bacterial infections, in antigen presentation to T cells and in tumor progression and angiogenesis. Report on the role of mast cells in inflammation and neoplasm progression in domestic animals is rare to absent. To evaluate the involvement of mast cells in canine intestinal inflammation, cases diagnosed as inflammatory bowel disease (IBD, n=9) and enteritis (n=10) were evaluated and compared with intestine from dogs with no microscopic evidence of enteritis. Mast cell count (MCC) were also compared in canine cutaneous hemangioma (HM, n=12), hemangiosarcoma (HS, n=12), mammary adenoma (AD, n=9) and adenocarcinoma (AC, n=12). The specimens were processed for routine histology and stained with H&E, and Toluidine blue stains. MCC in lamina propria, submucosae and tunica muscularis in sections of intestine and in HM, HS, AD and AC were made. A monoclonal antibody against endothelial markers, Factor VIII and VEGF were used to visualize and determine micro-vessel density (MVD). **MCC in both IBD and enteritis was significantly higher ($p<0.05$) than in the controls. Total MCC and MCC on invasive edges also were significantly higher ($p<0.01$) in canine AC than AD**. Although not significant, MCC was also higher in HS (8.6±3.3) than HM (5.5±2.8) and in IBD (24.8±9.5) than in enteritis (17.6±8.7). **There was a positive correlation between MCC and MVD in canine adenocarcinoma. The preliminary results indicate that mast cells may play an important role in canine enteritis and neoplasm progression.** Detailed similar studies in dogs and in other species of animals are under investigation.

Effects of Prolonged Formalin-Fixation on the Immunohistochemical Detection of Infectious Agents in Domestic Animals

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Narrative: Immunohistochemistry is commonly used to detect and characterize infectious agents in diagnostic pathology.[1] The principal advantage of immunohistochemistry over other antigen-detection techniques is the ability to identify the agent within the context of histologic lesions. However, epitope-masking due to formalin-fixation, especially prolonged fixation, has been considered a limiting factor in the use of diagnostic immunohistochemistry.[2] Only a few studies have evaluated the immunohistochemical detection of single infectious agents[3,4] or cellular antigens[5] following prolonged formalin-fixation. Therefore, the goal of this study was to evaluate the effects of prolonged formalin-fixation on the immunohistochemical detection of 21 infectious agents including 14 viruses, 4 bacteria, 2 protozoa, and one fungus. Approximately 5-mm-thick tissue slices were fixed in 10% neutral-buffered formalin, then processed and paraffin-embedded at day 1 or 2, and at approximately weekly intervals. Porcine circovirus was evaluated following up to 4 weeks of fixation. Porcine rotavirus and transmissible gastroenteritis virus were evaluated following up to 5 weeks of fixation. All other antigens were evaluated following up to 7 weeks or more of fixation. Three pathologists graded immunoreactivity according to a 4-tier grading system as negative, weak, moderate, or strong. Canine parvoviral immunoreactivity was markedly decreased following 2, 7, and 10 weeks of fixation in myocardium, small intestine, and spleen, respectively. Bovine respiratory syncytial virus immunoreactivity was markedly decreased following 7 weeks of fixation. *Bartonella henselae* had an abrupt loss of immunoreactivity following 9 weeks of fixation. Despite variation among time points, immunoreactivity remained moderate to strong throughout the study period for the other 18 antigens. **These results suggest that prolonged formalin-fixation generally does not limit immunohistochemical detection of infectious agents**. However, the effects of prolonged fixation depend on the targeted antigen and the selected antibody, and therefore, should be evaluated for each antigen-antibody combination. The results of this study further validate the utility and reliability of immunohistochemistry in diagnostic pathology.

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Warty Dyskeratoma in a Dog

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Narrative: Warty dyskeratoma, first described in human beings in 1957, commonly manifests as an umbilicated mass with a keratinized plug and is typically limited to the head, neck or face. Involvement of the hard palate has been described. Although a rare lesion, it most frequently occurs in patients 50-70 years of age. It is considered to be of follicular epithelial origin. In human patients, there is evidence of an acquired genetic mutation in ATP2A2, resulting in loss of SERCA2 staining immunohistochemically and loss of, or reduction in keratinocyte adhesion.

Warty dyskeratoma in dogs is a very rare, benign tumor originally described in 1987. It is histologically identical to the human lesion. Too few cases have been described to provide useful information regarding breed or sex predilection.

An older (estimated to be 7-11 years of age) neutered male Shih Tzu was presented for evaluation of a cutaneous mass on the right lateral cervical region. The patient was under treatment for chronic atopic dermatitis and physical examination revealed severe lichenification and hyperpigmentation consistent with chronic atopic dermatitis. A single 3.5 x 2.5 cm freely movable coalescing plaque of nodules was present in the right lateral cervical region. The lesion had expanded in size over two weeks. The lesion was removed and fixed in formalin. Tissues were embedded in paraffin, sectioned at 5 um and stained with H&E. Extending from a central focus of marked epidermal and follicular epithelial proliferation into the underlying hypodermis was a flask-shaped cystic mass with several other epithelium-lined cysts within a background of dense fibrous connective tissue. Cysts were lined by squamous epithelium with peripheral short pegs interposed by pale connective tissue, and were filled with abundant acantholytic and apoptotic keratinocytes and small amounts of keratin. **Histologic findings were compatible with warty dyskeratoma.**

Fatal Respiratory Disease Associated with Chemical Injury in Pre-Weaned Lambs

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Narrative: Rapidly fatal respiratory disease and sudden deaths in 3 - 8 week old lambs were investigated in 2 flocks. In flock 1, 10 of 210 lambs developed dyspnea and tachypnea and died within 1-2 hours of completion of tailing, castrating and drenching. In flock two, 6 of 300 lambs were found dead the day following collection for worming drenching, castration and tailing. Different drenches were used in the 2 flocks however both products included copper, selenium and cobalt. Three lambs from flock 1 and 6 lambs from flock 2 were submitted for necropsy examination. In 1 / 3 lambs from flock 1 and 5 / 6 lambs from flock 2, profuse straw-colored pleural fluid and variable consolidation of lungs were found at necropsy. Pulmonary edema and epicardial hemorrhages were detected in another lamb from flock 1. There was no significant bacterial isolate from these lambs. Histological examination of the lungs of these 7 lambs revealed multifocal extensive coagulative necrosis of airway epithelium, resulting in formation of detached rafts of necrotic ciliated epithelial cells, often sparing the underlying bronchial basal epithelial layer. Additional features were patchy parabronchiolar alveolar hemorrhagic necrosis and marked protein-rich pulmonary edema. One other lamb from each flock had severe fibrinous pleuritis and bronchopneumonia from which *Mannheimia haemolytica* was isolated in profuse pure growth.

The airway epithelial lesions were consistent with a surface-acting chemical injury as a consequence of inhalation of a necrotising compound. Copper and selenium concentrations were analyzed in lung and liver from the 7 lambs with airway necrosis, the 2 lambs with *Mannheimia* pneumonia and 3 other lambs of similar age from different flocks with (n=2) or without (n=1) pneumonia but no history of drenching. The hepatic copper and selenium concentrations were well within the reference ranges in all lambs. The pulmonary copper concentrations were considerably elevated in the 7 lambs with airway necrosis compared with normal and pneumonic lung from lambs of similar age, whereas the selenium levels were not as markedly elevated (results tabulated below).

Lamb group	Lung copper (μmol/kg DM) **Mean** (range)	Lung selenium (μmol/kg DM) **Mean** (range)
Airway necrosis (n = 7)	**667** (274 – 1543)	**31.8** (15.6 – 45.6)
Unaffected (n = 5)	**65** (28 – 98)	**16.2** (9.3 – 31.6)

An association between high local concentrations of copper and tissue necrosis is well recognized in a wide range of tissues (Howell and Gooneratne 1987, Suttle 1981, Wisniewski et al. 1965). **The findings suggest that the severe pulmonary lesions in these lambs are likely to have resulted from inadvertent inhalation of the drenches, and hence exposure of airway epithelium to high local concentrations of copper, and possibly selenium, resulting in airway epithelial necrosis.**

Gastrointestinal Leiomyosarcoma in an Egyptian Fruit Bat (*Rousettus aegyptiacus)*

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Narrative: Tissue from a reportedly ten-year-old Egyptian fruit bat (*Rousettus aegyptiacus)* was submitted for histopathologic evaluation to the Animal Disease Diagnostic Laboratory. The bat was found recumbent in its exhibit at the Potawatomi Zoo. Physical examination revealed dehydration and suspected hepatomegaly or a cranial abdominal mass. Despite supportive therapy, the animal died. Post-mortem examination by the submitting veterinarian noted a pale tan mass associated with the right renal cortex. Sections of the lung, liver, spleen, kidney, heart, pancreas, urinary bladder, and multiple sections of the gastrointestinal tract were placed in 10% neutral buffered formalin solution, and submitted to the Animal Disease Diagnostic Laboratory.

In microscopic sections of the small intestine, the tunica muscularis and serosa were segmentally effaced by haphazardly arranged, interlacing fascicles composed of pleomorphic spindle cells. The neoplastic cells were arranged in small, interwoven and streaming bundles, and focally infiltrated into the adjacent pancrease. The neoplastic cells also segmentally effaced the gastric tunica muscularis and serosa, focally expanded and infiltrated the renal capsule, and focally effaced a section of skeletal muscle of undetermined origin.

A tentative diagnosis of spindle cell sarcoma was made. Differential diagnoses included malignant schwannoma, leiomyosarcoma, and gastrointestinal stromal tumor (GIST). An immunohistochemical panel was performed and over 95% of the neoplastic spindle cells had strong, diffuse cytoplasmic labeling for alpha-smooth muscle actin and vimentin, weak to moderate cytoplasmic, and less frequently nuclear, labeling for S-100, weak to moderate cytoplasmic labeling for laminin, and no labeling for desmin or c-kit. **The morphology of the neoplastic cells and presence of strong and diffuse cytoplasmic labeling for vimentin and alpha-smooth muscle actin support a diagnosis of leiomyosarcoma.**

Few neoplasms have been reported in fruit bats and include pulmonary sarcomatoid carcinoma, a papillomavirus-associated basosquamous carcinoma, and a microchip-associated leiomyosarcoma. To the author's knowledge, this case documents the first primary gastrointestinal leiomyosarcoma in an Egyptian fruit bat, and also validates multiple additional immunohistochemical markers available for use in the diagnosis of chiropteran neoplasms.

1-OH-Pyrene and 3-OH-Phenanthrene as an Exposure Biomarker for Polyaromatic Hydrocarbons in Dairy Cattle

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Narrative: Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous environmental contaminants which are present as mixtures in air and food. Toxic doses of some PAHs such as phenanthrene (PH) and pyrene (PY) are low compared to benzo(a)pyrene (BaP), but both PH and PY are found in relatively high concentrations in air, animal feed and food. Most of PAHs are metabolized to hydroxylated compounds by the hepatic microsomal NADPH-dependent cytochrome P450 monooxidase system and excreted into urine and feces. We determined the amounts of PH and PY in muscle and their respective hydroxylated metabolites, 3-OH-PH and 1-OH-PY, in urine of dairy cattle (n=24). We also evaluated the relationship between the parent compounds and their metabolites.

The concentration of PH in muscle ranged from 0.0007 to 0.0048 μg/g (0.0018 ± 0.0017) and that of PY ranged from 0.0004 to 0.0041μg/g (0.0012 ± 0.0012). The amounts of 3-OH-PH ranged from 0.0001 to 0.0059 μg/mL (0.0029 ± 0.00037) and 1-OH-PY ranged from 0.0005 to 0.0036 μg/mL (0.0019 ± 0.00023). The correlation (R^2) between the content of PY in muscle and 1-OH-PY in urine was 0.657 and that between the content of PH in muscle and 3-OH-PH in urine was 0.579. The correlation between the content of PY and PH in muscle was 0.8857, while that between 1-OH-PY and 3-OH-PH in urine was 0.834. **This study suggests that 1-OH-pyrene and 3-OH-phenanthrene could be used as biomarkers for PAHs exposure in dairy cattle.**

Chronic Yew Toxicity in a Holstein Heifer

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Narrative: Twenty-six 5-month-old Holstein calves were accidentally exposed to discarded branches of yew bushes (*Taxus* sp.). Several calves were found dead approximately 24 hours after exposure; however, a few calves died several days after exposure. One calf died 18 days after the initial exposure to *Taxus* spp. and was examined on the farm via necropsy. Gross lesions included ascites and dilated and flaccid myocardial ventricles. Sections of formalin-fixed heart were submitted to the Animal Disease Diagnostic Laboratory for histopathologic examination; fresh rumen contents were also submitted for toxicologic testing. Histologically, broad swaths of myocardium were replaced by fibrous connective tissue, suggesting previous myocardial necrosis. Minimal mononuclear inflammation was also scattered throughout areas of fibrosis. Rumen contents were submitted for qualitative analysis. Taxines, the toxic components of *Taxus* spp., were identified in the rumen contents. The heifer's diet included 4 pounds per day of a grain mixture balanced by a professional nutritionist and was devoid of ionophores. The heifer grazed an orchard grass and clover pasture, and known cardiotoxic plants in Indiana, including *Eupatorium* spp., were not available on the pasture where the animal was kept. Based on the clinical history, the gross and histologic lesions, and the identification of taxines in the rumen contents, chronic yew toxicity was considered the cause of death in this calf.

Ingestion of taxines is known to cause acute and subacute toxicity in humans and animals; however, a chronic clinical course and severe histologic lesions have not been previously associated with yew toxicity. **Although only one calf was necropsied, this case suggests that yew toxicity can result in a prolonged clinical course and can cause histologic myocardial lesions.**

Hepatitis and Mortality in Neonatal Calves Co-Infected with Bovine Herpesvirus-1 and Bovine Viral Diarrhea Viruses

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Narrative: Tissues were submitted from six 4 to 20 day old Jersey calves with a clinical history of depression, anorexia and respiratory signs for 3 to 4 days which was unresponsive to treatment with Nuflor™. These calves had received a modified live viral vaccine containing infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD)1, BVD2, bovine respiratory syncytial virus (BRSV) and parainfluenza (PI)3 viruses by intranasal route at 3 to 5 days of age. Prior to instituting intranasal vaccination, the mortality rate of calves on this dairy was 4%; following administration of the vaccine, the mortality rate for neonatal calves increased to 20%. Surviving calves were tested for persistent BVDV infection by antigen-capture, enzyme-linked immunosorbent assay (AC-ELISA) using ear notch samples and all samples were negative.

All six calves submitted had histopathologic diagnoses of bronchopneumonia or bronchointerstitial pneumonia. Evidence of bovine herpesvirus-1 (BHV1/IBR) infection as determined by positive IBR FA staining and PCR amplification of BHV1 DNA was positive in all six lung samples. In addition, large amphophilic/eosinophilic intranuclear inclusions suggestive of BHV1 were identified in respiratory epithelial cells in one case. Two calves had significant hepatocellular lesions with intranuclear inclusions in hepatocytes. Five calves had evidence of concurrent BVDV infection as evidenced by positive FA staining of lung. One calf had BVDV IHC staining of pericytes in the myocardium. One calf had concurrent PI3 and coronavirus FA staining in lung. One calf had intralesional fungal hyphae in lung, and *Aspergillus* sp. was cultured from lung of another calf. Moderate lymphoid hypoplasia was observed in the spleens of two calves. These lesions are consistent with viral disease with or without secondary bacterial involvement.

Five calves had histopathologic evidence of nephritis. *E. coli* was cultured from the kidney in one case. The calves had not been treated with potentially nephrotoxic drugs such as gentamicin or tetracycline. The pathogenesis of the renal lesion may be a combination of viral and/or bacterial infection with dehydration.

The necrotizing hepatic lesions, bronchointerstitial pneumonia and intranuclear inclusions found in these calves were previously described for 11 and 15 day old dairy calves infected with both BHV1 and BVDV (Palfi et al., 1989). **We hypothesize that the BVD1 and BVD2 viruses suppressed the ability of these neonatal calves to limit the BHV1 virus infection resulting in spread to lung and liver causing pneumonia and hepatocellular necrosis, secondary bacterial, fungal, PI3 and/or coronavirus infection.**

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Isolation and Genetic Analysis of Bovine Viral Diarrhea Virus from Infected Indiana Cattle

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Narrative: Bovine Viral Diarrhea Virus (BVDV) is known to cause disease and have an economic impact on ruminant populations across the world. Not excluded from these affected areas is Indiana, a state that is heavily involved in both the beef and dairy industries. BVD virus was isolated and genetically analyzed from 44 cases of Indiana cattle submitted to the Purdue ADDL. BVD viral RNA was detected in the 5'-untranslated region (5'-UTR) and N^{pro} region using reverse transcriptase polymerase chain reaction (PCR) in all cases followed by sequencing analysis of the PCR product. Genetic analysis revealed a total of three subgenotypes to be present from the representative counties in Indiana. Cythopathic (cp) and noncytopathic (ncp) BVD virus biotypes were also established. Results indicate that of the cases that exist throughout Indiana 8.89% are cp BVDV 1A, 6.67% are ncp BVDV 1A, 4.44% are cp BVDV 1B, 44.44% are ncp BVDV 1B, 11.1% are cp BVDV 2A, and 24.44% are ncp BVD 2A viruses. The cases were then classified into one of six clinical categories according to the signs and lesions reported: acute symptomatic, hemorrhagic, bovine respiratory distress, abortion, persistently infected (PI), and mucosal disease (MD). **An interesting finding is that only ncp BVDV type 1B viruses were isolated from BVDV-positive cases with reproductive clinical signs. Bovine hemorrhagic disease associated with BVDV type 2 virus was found in 9.1% of cases, and 13.6% of the positive cases were found to have MD. A majority of the BVDV positive cases were located in the southern part of Indiana.** Awareness and prevention of BVDV requires a thorough understanding of the disease prevalence, spread and pathogenesis in the cattle population.

Experimental Infection of Colostrum-Deprived Calves with Bovine Viral Diarrhea Virus Type-1 Isolated from Free-Ranging White Tailed Deer (*Odocoileus virginianus*).

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Narrative: Bovine viral diarrhea virus (BVDV) has been associated with vast losses to the cattle industry in the United States. Even though BVDV has been isolated in white-tailed deer (WTD) and previous studies suggest that persistent infection and clinical lesions are possible as a result of experimental infection,; the role of WTD as a reservoir is not fully understood. It is unknown whether or not BVDV strains from WTD can infect cattle. This study investigates if colostrum-deprived calves can be experimentally infected with a bovine viral diarrhea virus type1a isolated from free ranging white-tailed deer (WTD) in Indiana and if they can develop lesions and shed the virus.

Twelve male Holstein neonatal calves (8 inoculated and 4 controls) were kept on 6 isolation units containing 2 calves from the same group for a total of 29 days. The experimental group was inoculated intranasally with a 2 ml virus suspension of BVDV1a NCP with a titer of 10^6 $TCID_{50}$ /ml, while the control group received a virus-free sham inoculum. Daily physical examinations were performed. Real time PCR (RT-PCR) assay was done on whole blood (buffy coat) and nasal, rectal and saliva swabs collected on days 3, 7, 10, 14 and 21 post inoculation (PIN) for detection of BVDV RNA. Virus neutralization (VN) assay and ELISA were done on serum collected on days -5, 0, 3, 7, 14 and 21 PIN for detection of BVDV-specific host antibodies. On days 14 and 21 PIN, 4 calves from the experimental group and 2 calves from the control group were humanely euthanized, necropsied and tissue samples were collected for virus isolation (VI) and histopathology.

RT-PCR was positive for BVDV in the buffy coat of, and the nasal and saliva swabs collected from all 8 experimental calves. The rectal swabs were negative at all times for the 12 calves (8 experimental calves and 4 control calves). Serum virus neutralization was positive in 4/8 experimental calves, and serum ELISA test result was positive on day 21 post infection in 3/8 experimental calves. The experimental group had microscopic evidence of thymic atrophy and lymphoid depletion of Peyer's patches. The control group remained seronegative for the duration of the study and no lesions or virus was found in samples obtained at necropsy.

The results of the study established that calves can be infected intranasally with BVDV-1a strain from WTD, and that infection results in the development of histological lesions in lymphoid tissues. In addition, BVDV shedding into the environment may be possible based on presence of BVDV RNA in nasal and salivary secretions following experimental intranasal inoculation.

An Outbreak of Epizootic Hemorrhagic Disease in a Captive Deer Herd

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Narrative: Epizootic hemorrhagic disease (EHD) is a severe disease of wild and domestic deer. The causal agent is epizootic hemorrhagic disease virus (EHDV) that is related to bluetongue virus. From late September to early October in 2008, a captive herd of white-tailed deer (*Odocoileus virginianus*) experienced an outbreak of a severe infectious disease that resulted in the deaths of 35 of 51 animals of various age and gender. The clinical history, gross lesions, and histopathologic lesions were consistent with EHDV infection. Lung and spleen tissues from multiple deer were positive for EHDV and negative for bluetongue virus by RT-PCR. A virus was isolated from the tissue pools of lung and spleen. Viral neutralization assay indicated that the virus cross-reacted with EHDV II virus and RT-PCR for eight serotypes of EHDV further confirmed the causal agent is EHDV II virus. **This case indicates the persistence of EHD virus infection in white-tailed deer in Ohio after a disease outbreak occurred in wild and captive white-tailed deer throughout the state in 2007.**

Characterization and Sequence Analysis of 2009 Foot-and-Mouth Disease Outbreak in Iraq

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[1] USDA, APHIS, Veterinary Services, National Veterinary Services Laboratories, Foreign Animal Disease Diagnostic Laboratory (FADDL), Greenport, NY [2] , Transboundary Animal Disease Laboratory, State Company for Veterinary Services, Baghdad, Iraq. [3] US Army Veterinary Corps, Veterinary Command. [4] Office of Agricultural Affairs-FAS/USDA, US Embassy, Baghdad, Iraq.
[5]College of Veterinary Medicine and Biomedical Sciences, Colorado State University

Narrative: Foot-and-Mouth Disease Virus (FMDV) is a high consequence pathogen responsible for a highly transmissible disease that is endemic in two thirds of the globe. In March 2009, FADDL received 37 samples from the recently recognized outbreak in various providences of Iraq. At least nine of the 18 provinces of Iraq were involved in this outbreak, namely: Babil, Dhiqar, Erbil, Sulimania, Ninewa, Dahouk, Basra, Maysan, Mosul and Baghdad). The diagnostic procedure included virus isolation (VI), real time RT-PCR for detection of FMDV nucleic acid and serotyping using Antigen ELISA (AgELISA) test. Out of the total number of received samples, 18 were positives by both VI and real time RT-PCR. All were characterized as FMDV serotype A in AgELISA test.

Further sequence analysis of the complete genomic region coding for the capsid proteins (polyprotein P1) revealed that the same isolate was found in all provinces where FMDV was diagnosed (Babil, Thiqar, Basra and Messan). Specific clusters of the positive samples were identified by locations. BLAST analysis against over 3,400 sequences in Genbank and sequence alignment against over 700 FMDV type A sequences, revealed that the Iraq 2009 FMDV isolate VP1 protein coding region is more than 99.98% identical to the 2006-2007 A virus isolated from Landhi buffalo dairy colony (located in the suburbs of Karachi in the Sindh province of South-Pakistan). Additional sequence information, genetic distance and phylogenetic analysis of the complete P1 polyprotein showed highest similarity to FMDV A/BAR/08 isolated in Barhain during 2008 outbreak. Both strains have been classified as part of the ubiquitous A/IRAN/05 genetic lineage.

Here we present and discuss the data that suggest that the Iraq 2009 FMDV epidemics comes from a single viral strain, classified as serotype A virus, genotype IRAN 05 that belongs to the previously described Asia topotype that is endemic in Turkey, Iran and Pakistan and currently circulating in the Middle East region.

Prionics Test Kit Combinations for Effective Testing During the Recovery-Phase of Outbreaks of Foot-and-Mouth Disease and Classical Swine Fever

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Prionics Lelystad BV, Platinastraat 33, NL-8211 AR Lelystad

Narrative: Through the years Prionics has developed a set of different test kits (formerly known as the Ceditest kits) that are fit for use during the recovery-phase of outbreaks of Food-and Mouth Disease and of Classical Swine Fever. Different test kit combinations can be used within either vaccination policies or non-vaccination policies.

For Classical Swine Fever outbreaks controlled by eradication we provide the PrioCHECK CSFV Ab and Ab 2.0 for screening and confirmation. For CSF outbreaks controlled by vaccination with the CSF E2 vaccine we present the novel PrioCHECK CSFV Ab Erns test. Results on samples from infection-studies performed in Denmark, Germany, Belgium and the Netherlands show good specificity and sensitivity for the CSF virus along with a low sensitivity for the BVDV types.

For Food-and Mouth Disease outbreaks controlled by eradication we market the "all-species" PrioCHECK FMDV Ab type O kit, which can be used with the PrioCHECK FMDV-NS as confirmation test. For FMD outbreaks controlled by vaccination we present the PrioCHECK FMDV-NS and 3D test kits for screening and confirmation. The first results with the PrioCHECK FMDV 3D indicate higher analytical sensitivity levels on cattle-sera dilution ranges.

Each of the FMDV NS products can be used on sera samples from cattle, swine, sheep, goat and other cloven-hoofed animals: One test for all species regardless of the vaccination status.

Competitive Evaluation of the Next Generation IDEXX Bovine Viral Diarrhea Antigen Enzyme-Linked Immunosorbent Assay

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Introduction: Bovine viral diarrhea virus (BVDV) is a highly infectious disease of cattle that can cause significant economic losses within the livestock industry. Cattle that are persistently infected (PI) with BVDV constitute the mechanism by which BVDV spreads among cattle herds. Detection and elimination of PI cattle are necessary for control of BVD. IDEXX Laboratories produces a USDA-licensed ELISA test kit for the detection of BVDV, and has recently validated changes designed to enhance the performance of the test. The purpose of this study was to compare the performance of the improved IDEXX BVD Antigen ELISA to an improved version of another commercially available BVD Antigen ELISA (competitor A).

Materials and Methods: The improved IDEXX BVD Antigen ELISA test kit, and the improved test kit of competitor A were used for this study. Each test was performed according to the package insert using ear notch tissue or cultured BVD virus as a sample type. Each of the samples used in the study was previously confirmed as BVD positive or negative by polymerase chain reaction (PCR) testing. In total, 100 BVD negative and 107 BVD positive ear notch samples were tested, as well as tissue culture fluid samples from 43 different isolates. Samples representing BVD types 1a, 1b and 2 were included in the study. Analytical sensitivity was measured for each test using serial dilutions prepared from ear notch or culture samples.

Results: The IDEXX BVD Antigen ELISA correctly identified all of the samples, resulting in 100% sensitivity and 100% specificity in this study. Competitor A demonstrated 100% specificity, but detected only 94 of 107 BVD positive ear notch samples, for 88% sensitivity. Dilution studies showed that the IDEXX BVD Antigen ELISA detected BVD samples (type 1a, 1b or type 2) when diluted in the range of 1:64 or greater. Competitor A's ELISA did not detect virus in any samples diluted beyond 1:8. All 43 tissue culture isolates were detected by each test method.

Discussion/Conclusion: The sensitivity of any BVD diagnostic is critical to ensure that all PI animals are detected and removed from the herd. The next generation IDEXX BVD Ag ELISA demonstrated 100% sensitivity and 100% specificity in this study, while Competitor A's ELISA demonstrated 100% specificity and 88% sensitivity. The next generation IDEXX BVD ELISA showed significantly higher analytical sensitivity across all strains tested (>1:64) compared to (1:8) for the competitor ELISA. The competitor ELISA failed to detect any positive sample that tested at an S/P ratio of 2.0 or lower on the next generation IDEXX BVD Ag ELISA. This finding suggests that competitor A's test kit may have an increased risk of yielding false negative results on weak PI reactors. The evaluation demonstrates that the performance characteristics of the next generation IDEXX BVD Antigen ELISA make it a preferred choice for BVD control and eradication.

High Genetic Diversity of Bovine Viral Diarrhea Viruses Isolated Recently in the Republic of Korea

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Narrative: Bovine viral diarrhea (BVD), which is characterized by reproductive problem, enteric disease, mucosal disease (MD) and unthrifty in cattle and sheep, is uncontrollable and endemic throughout the world. BVD viruses have been highly prevalent in Korean native cattle farms, causing a huge economic loss. BVD viruses isolated before 2007 in Korea have been classified into 5 genetic subtypes (1a, 1b, 1c, 1n, 2a). Interestingly, it is observed that the viruses have been classified into various subtypes and become more diverse genetically over times. The following study was conducted to investigate genetic diversity and emergence of new subtypes in the Korean native cattle farms using the recently isolated viruses.

Materials and Methods: Feces and tissue samples (n=199) were collected from 74 nationwide Korean native cattle farms affected by diarrhea, abortion and mucosal diseases in 2007-2008. A part of 5' non-coding region (NCR) (n=23) and a whole E2 region (n=12) were amplified and subjected to sequencing. Subsequently, genetic diversity was investigated by comparing the viral sequences with sequences of numerous BVD viruses from other countries or previously isolated in Korea.

Results and Discussion: Phylogenetic analyses using the part of 5'NCR and E2 sequences revealed that most of the recent Korean isolates were classified into a variety of genetic subtypes including 1a, 1c, 1b, 1n, 2a. Surprisingly, four isolates (08GB01, 08GB08, 08GB45-1 and 08GB45-2) belonged to type 1, but, were not classified into any of fifteen genetic subtypes (1a-1o). **The results suggested that the four isolates may be classified as novel genetic subtypes. Meanwhile, two viruses (08GB01 and 08GB08) formed an independent group between subtypes (1g and 1h) of Austrian viruses, which was notable because no Korean viruses have been reported to be close to Austrian viruses before. Therefore, further epidemiological study may be needed to determine the origin of the viruses.**

Our study demonstrated that BVD viruses in Korean native cattle population have evolved continuously, which leads to expansion of genetic diversity.

Detection of Avian Influenza (AI) Viruses in Experimentally Infected Poultry Litter

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Introduction: Avian influenza viruses (AIV) are type A influenza virus from the Orthomyxoviridae family. They are enveloped viruses with segmented single-negative-stranded RNA genome. AIV can infect a wide variety of animals, from wild birds to humans. Although poultry is not a natural host of AI, infections in poultry are current. Recently it has become more apparent that human infections with AIV can occur, and that direct contact with affected poultry is necessary for an effective transmission. However, further knowledge regarding the poultry-human interface is required to determine what is the probability of AIV transmission to humans based on the survivability of AIV in the poultry house environment. In particularly information on the survivability of viruses in poultry litter is needed to predict the risk to avian influenza exposure associated with the poultry husbandry environment. In this work the survivability of poultry adapted virus A/Ck/CA/139/01(H6N2) when in contact with broiler litter was evaluated under controlled conditions. The virus was detected by virus isolation and real-time reverse transcriptase PCR (real time-RT PCR).

Material and Methods: One hundred and sixty grams of litter were inoculated with 1 ml of chicken embryo allantoic fluid containing a viral titer of $10^{7.4}$ $EID_{50,}$ and as a negative control the same amount of litter was inoculated with 1 ml of non-infected allantoic fluid. The litter treatments were incubated at 25 and 35^0 C in two environmental chambers. Ten grams of litter were collected in triplicates from each group at 0, 6, 24, 48, 72, 96 and 120hrs post-inoculation (PI) and inoculated into 30 ml of Brain Heart Infusion (BHI) with an antibiotic cocktail containing penicillin (1000u/ml), gentamycin (250µg/ml), amphotericin B (25µg /ml), kanamycin (500µg /ml) and streptomycin (1mg/ml). Litter samples (10 g) were mixed and centrifuged, the supernatant was separated and filtered through a 30µm pore size paper filter. The filtrate was further clarified by centrifugation and filtration through a 0.45µm pore size syringe filter. Filtrate samples were incubated at 4^0 C overnight to permit the overgrowth of bacteria. Samples where bacteria grew were filtrated a second time. The filtrates were used for virus isolation in embryonated chicken eggs and for RNA extraction for real time RT-PCR analysis.

Results: Virus was isolated from samples incubated at 25^0C and 35^0C from 0 to 24hrs PI. No virus isolation was obtained after 24hrs PI. No virus was isolated from litter inoculated with non-infected allantoic fluid. However, viral RNA was detected in samples incubated at 25 and 35^0C. Real-time PCR cycle threshold (C_T) values for samples incubated at 25^0C ranged from 21.95 to 29.22 for samples collected 0 to 120 hrs PI, and C_T values from samples incubated at 35^0C ranged from 22.4 to 33.82 for samples collected 0 to 120 hrs PI. Viral RNA was detected through the complete sampling period (120hrs) an indication that viral RNA can persist after virus inactivation. Therefore, real-time RT PCR can be used to prove that virus was present but not to confirm an active infection.

Conclusion: Under controlled conditions the H6N2 virus survived up to 24 hours in contact with broiler litter. This result demonstrates that the AI virus can be easily inactivated. However, in the field, viral shedding from a large population of birds may prolong the period that infectious virus circulates in the environment.

Lack of Prion Accumulation in Lymphoid Tissues of Scrapie-Affected Sheep with the AA136, QR171 Prion Protein Genotype

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Introduction: Sheep scrapie is a transmissible spongiform encephalopathy which can be transmitted horizontally through the shedding of an infectious conformer (PrP^{Sc}) of the normal cellular prion protein (PrP^{c}). Genetics profoundly influence the susceptibility of sheep to scrapie. PrP^{c} amino-acid polymorphisms A136V, R154H, Q171R, and Q171H are predictive for relative susceptibility (V136, R154, Q171) or resistance (A136, H154, R171) to classical scrapie in natural settings and in experimental oral inoculation studies. The objective of the study is to compare the clinical course and PrP^{Sc} tissue distribution in sheep with high resistance and high susceptibility genotypes after inoculation of scrapie-affected sheep brain homogenate directly into the brain.

Materials and Methods: Five sheep each of genotype VRQ/VRQ, ARQ/ARQ(H), VRQ/ARQ, or ARQ/ARR were inoculated via intra-cerebral route with a 10% brain homogenate derived from a ARQ/ARQ sheep affected with scrapie. Sheep were euthanized in the terminal phase of scrapie development. Tissues collected at necropsy were examined by light microscopy, immunohistochemistry (IHC) and Western blot.

Results: All inoculated sheep succumbed to scrapie. Clinical signs, microscopic lesions, and Western blot profiles were uniform across genotypes and consistent with manifestations of classical scrapie. Mean survival time differences were associated with the 171 polymorphic site with VRQ/VRQ and ARQ/ARQ sheep surviving 18 and 19 months, whereas VRQ/ARR and ARQ/ARR survived 56 and 60 months, respectively. Labeling of PrP^{Sc} by IHC revealed similar accumulations in central nervous system tissues across genotypes. Labeling of PrP^{Sc} in lymphoid tissue was consistently abundant in VRQ/VRQ, present in 4/5 ARQ/ARQ and VRQ/ARR, and totally absent in ARQ/ARR sheep.

Discussion: The results of the study demonstrate the susceptibility of sheep with the high resistance genotype ARQ/ARR to scrapie by the intra-cerebral inoculation route, with attendant PrP^{Sc} accumulation in CNS tissues, and concurrent lack of PrP^{Sc} in lymphoid tissue. Our data suggest that genetic resistance to scrapie is associated with failure of PrP^{Sc} to accumulate in lymphoid tissue, and not associated with permissiveness of CNS tissue to PrP^{Sc} amplification.

Application of a Real-Time Reverse Transcriptase Polymerase Chain Reaction and an Antigen Capture Enzyme-Linked Immunosorbent Assay to Detect Animals Infected with Bovine Viral Diarrhea Virus

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Department of Pathobiology & Population Medicine, Mississippi Veterinary Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University

Narrative: Bovine viral diarrhea virus (BVDV) is a major viral pathogen of cattle and the infection can result in significant economic loss. Persistently-infected (PI) animals are the main source of BVDV transmission. Early identification and removal of PI animals is an essential measure to a successful BVD control program. Different diagnostic tests, including immunohistochemistry (IHC), antigen capture enzyme linked immunosorbent assay (ACE), and reverse transcriptase polymerase chain reaction (RT-PCR) have been used to detect the virus in serum or ear notches. To establish a cost-effective, sensitive, and specific testing scheme, a real-time RT-PCR (RRT-PCR) assay was developed and used in conjunction with a commercially available ACE test kit to monitor BVDV status in herds. Upon arrival in the laboratory, samples were pooled with a size of 25 or less per pool. Pooled samples were screened by RRT-PCR and individual samples in positive pools were then tested using ACE. A total of 22,932 animals were tested, 85 PI animals were identified. Among the identified PI animals, 94% were infected with BVDV-1, 3.53% with BVDV-2, and 3.53% with both genotypes. At an average prevalence rate of 0.30% in Mississippi, application of the RRT-PCR/ACE scheme reduced the testing cost by 57% and 64.17% compared to IHC and ACE, respectively. Further investigation indicated that pooling samples at a size of 25 did not compromise the sensitivity or the specificity of RRT-PCR. **In summary, the RRT-PCR/ACE method is cost-effective and suitable for monitoring BVDV herd status in areas with a low prevalence.**

AAVLD/USAHA Joint Plenary Session

Monday, October 12, 2009
Atlas Ballroom

EMERGING VECTOR-BORNE DISEASES: WHAT IS THE RISK?

08:00 - 8:15 **Welcome and Announcements**
N. James MacLachlan, University of California-Davis

08:15 - 9:00 **Climate Change and Emerging Diseases: A Search for Patterns and Predictions in a Changing World -** E. Paul Gibbs, University of Florida

09:00 - 9:45 **Impact of Bluetongue in Europe - A Recent Example of an Emerging Disease -** Vincenzo Caporale, Istituto Zooprofilattico Sperimentale dell' Abruzzo, Italy

09:45 - 10:00 **Emergence of Bluetongue and Related Orbiviruses in the United States -** David Stallknecht, University of Georgia

10:00 - 10:30 **Break**

10:30 - 11:15 **Unique Challenges to North America Posed by Emerging Diseases -** Terry McElwain, Washington State University

11:15 - 11:45 **USDA Perspective -** John Clifford, Veterinary Services, USDA-APHIS

11:45 - Noon **Summation**

**** Please note: There are no corresponding abstracts for this session.**

AAVLD Exhibit Floor Plan

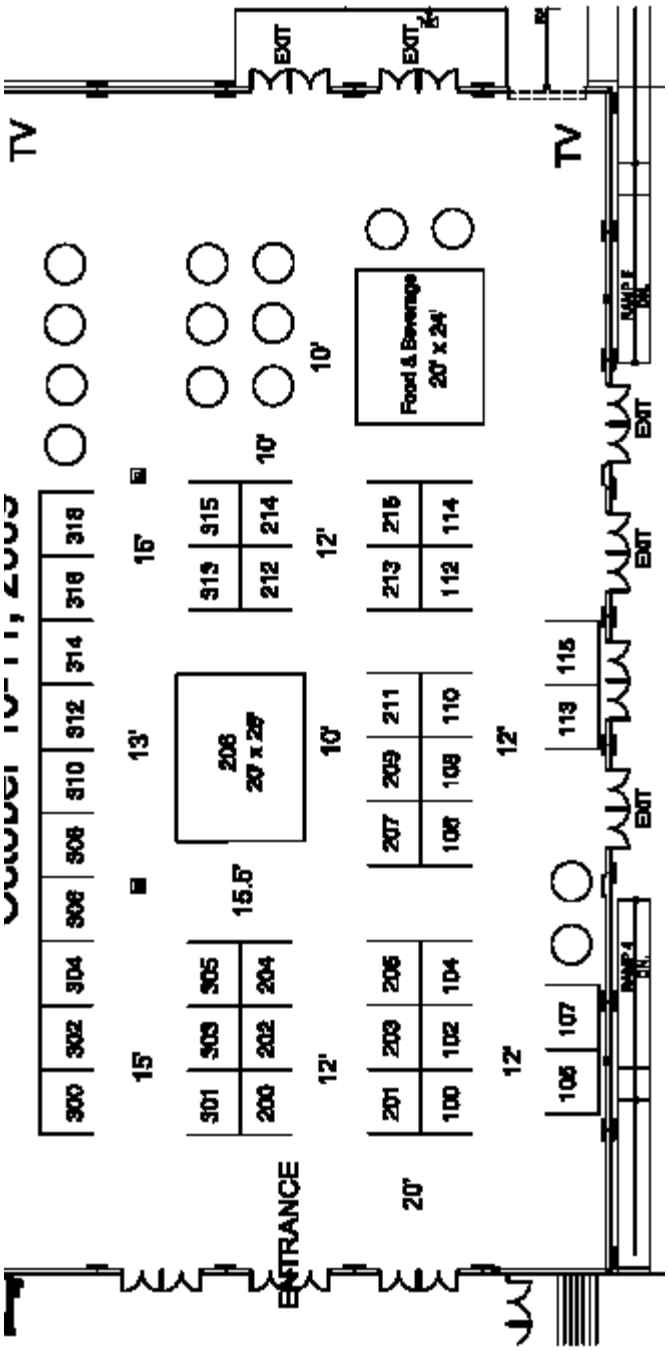
Town & Country Resort & Convention Center Grand Exhibit Hall

October 10-11, 2009

Exhibit Hours

Saturday	Sunday
11:00 am—1:00 pm	9:45 am—10:45 am
3:00 pm—6:00 pm	Noon—2:00 pm

Lunch concession in Exhibit Hall both days



Grand Hall Foyer

Booth	Exhibitor
100	Applied BioSystems - Animal Health
104	Quality Systems Integrators
105	Inverness Medical Professional Diagnostics
106	Prionics USA, Inc.
107	Computer Aid, Inc.
108	CDC/EPI-X
110	VETQAS (Veterinary Laboratories Agency)
114	Synbiotics Corporation
115	Feedback Stat
200	IDEXX Laboratories
201	TREK Diagnostic Systems
203	Ventana Medical Systems, Inc.
204	National Institute for Animal Agriculture
205	Automated Technologies Inc. (ATI)
206	Qiagen, Inc.
207	Centaur, Inc.
209	Global Vet Link
211	NCE-Crawford-Emcotek
212	Smiths Detection Diagnostics
213	Tetracore, Inc.
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215	Hydrol-Pro Technologies, Inc.
300	ITL Animal Health Care
301	Bio-Rad Laboratories
303	Merrick and Company
305	Advanced Technology Corp. VADDS
306	BioGX
308	Advanced Animal Diagnostics (Entira)
310	Fort Supply and Technologies, LLC
313	Enfer Diagnostics
314	BioSAFE Engineering, LLC
315	Biosearch Technologies, Inc.
316	FBI - Laboratory Division
318	Luminex Corporation

American Association of
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Exhibit Directory

Town and Country, San Diego, CA
October 7-14, 2009

Advanced Animal Diagnostics (Entira)

Booth **308**

1912 Hwy 54 East, Suite 205
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www.advancedanimaldiagnostics.com
Contact: Rudy Rodriguez
919-544-0089
rudy@advancedanimaldiagnostics.com

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Booths **113-115**

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BioGX

Booth **306**

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205-250-8055
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BioGX, Inc. offers tests for rapid real-time PCR based detection across numerous applications, including, but not limited to Animal Pathogen Testing, Food Safety and Quality Control, Brewing and Wine Industry Quality Control, Recreational Water Monitoring, Sewage Effluent and Drinking Water Testing, Agriculture (including GMO), and Environmental Monitoring. We provide custom development of customer-specific testing applications across numerous instrument platforms and detection chemistries, and we offer custom PCR internal amplification control kits and incorporation services. BioGX also provides conversion of existing molecular assays to a convenient bead format in easy to use dispensers utilizing Cepheid's (Sunnyvale, CA) proprietary technology. We recently released a one-step RT-PCR kit for multiplex real-time PCR that provides more rapid and sensitive performance than various "gold standard" mixes. BioGX is an authorized Industrial Distributor of Cepheid SmartCycler real-time PCR instrumentation, mastermix reagents, and accessories, and provides assay development services for the Cepheid GeneXpert platform.

Bio-Rad Laboratories

Booth **301**

2000 Alfred Nobel Drive
Hercules, CA 94547
www.bio-rad.com
Contact: Wendy Lauer
510.741.5653
Wendy_Lauer@bio-rad.com

Company Representatives: Wendy Lauer, Camille Armijo

Bio-Rad Laboratories is a world leader in creating advanced tools for biological exploration and clinical diagnostics. Its mission is to create innovative products and services that advance scientific research and development efforts, and support the commercialization and discovery of new technologies. Bio-Rad's BSE test is the most widely used test in Europe and Japan and is recognized for its high level of sensitivity and specificity. Additionally, the Bio-Rad TSE test is the fastest and easiest test method to adapt to mass screening programs. The Bio-Rad TSE test is the only test in the United States approved by the USDA for BSE, scrapie for sheep and goats and chronic wasting disease for mule deer, white tailed deer and elk. Bio-Rad also offers a rapid ELISA for rabies testing.

BioSAFE Engineering, LLC

Booth **314**

485 Southpoint Circle
Brownsburg, IN 46112
www.biosafeengineering.com
Contact: Jack Hoff
317-858-8099 x204 Corp Office
609-581-7816 Eastern Office
jhoff@biosafeengineering.com

BioSAFE Engineering LLC of Brownsburg, IN is the world leader and U.S. manufacturer of decontamination and disposal systems for infectious, bio-hazardous waste. Our product line includes WR^2 Tissue Digestors, EDS Effluent Decontamination Systems, and STI Medical Waste Treatment systems in a wide range of capacities to meet the specific application. Our patented WR^2 alkaline hydrolysis process used in our tissue digestor technology is unique throughout the industry and is recognized by the USDA, Canadian Food Inspection Agency, and European Union as the only green decontamination process to have the capability to inactivate and destroy prions. All products are designed to reduce operating costs while at the same time provide a safe, environmentally-friendly method of infectious waste decontamination and disposal.

Biosearch Technologies, Inc.

Booth **315**

81 Digital Drive
Novato, CA 94949-5750
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. (Biosearch) is a privately held company and is a leader in the design and manufacture of probes, primers and other molecular tools of the Life Science market. Other products include fluorescent dyes (CAL Fluor®, Quasar®, and Pulsar® dyes), versatile dark quenchers for FRET applications (Black Hole Quencher® or BHQ® dyes), compact, fortified probes with duplex stabilizing technology (BHQ*plus*™ Probes) , and a line of standard and specialty DNA synthesis reagents. Biosearch also offers RealTimeDesign™ software, a sophisticated web-based program for qPCR assay design available at no cost to the user.

As an industry proven B2B OEM manufacturer we are able to conform to all applicable GMP standards per 21CFR Part 820. Our products and services cater to the pharmaceutical/diagnostic IVD, biotech, public health, homeland defense and biodefense sectors. Biosearch also has extensive experience supplying bulk qualified reagents and fully assembled and ready-to-inventory assays for RUO and IVD markets.

Centaur, Inc.

Booth **207**

P. O. Box 25667
Overland Park, KS 66225-5667
www.centaurunavet.com
Contact: Mark Metrokotsas
913.390.6184
centaurunavet@aol.com

Centaur sells diagnostic products converted from human healthcare to animal health applications. Examples include our 45-minute equine infectious anemia test, a test for Tuberculosis in non-human primates, a test for canine heartworm, a test for IgG in foals, and tests for two different blood analytes.

Centaur's newest pharmaceutical products include three different strengths of iodine formulations; two formulations of isopropyl alcohol; three different topical sprays; and bulk liquids to treat metabolic disorders, to serve as laxatives, to preserve tissues and to serve as delivery vehicles for custom-compounded medicinals. Centaur also has creams to treat inflamed, abraded tissue and solutions to help identify parasites.

Computer Aid, Inc.

Booth **107**

470 Friendship Road
Harrisburg, PA
Contact: John Kucek
717.856.8294
john-kucek@compaid.com

Computer Aid, Inc. (CAI) is a $250 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.

Enfer Diagnostics

Booth **313**

8678 West Bent Tree Drive
Peoria, AZ 85383
www.enferdiagnostics.com
Contact: Cliff Cain
623.561.7799
cliff.cain@enferdiagnostics.com

Enfer Diagnostics (www.enferdiagnostics.com) is a specialized provider of advanced diagnostics to improve animal disease management worldwide. Enfer is committed to delivering only the most accurate diagnostics that industry and government disease eradication demands.
Enfer has incorporated AnDiaTec's veterinary diagnostic testing portfolio (www.andiatec.com) into its family of products. AnDiaTec specializes in unique and proprietary reagents for use in human and veterinary diagnostics and detection systems, and performs environmental analytics in their commercial lab located near Stuttgart, Germany.

Enfer and AnDiaTec's capabilities include the design and implementation of leading-edge analytical systems for the food and agricultural industries, and independent testing and research to provide microbiological, chemical, and environmental analyses to the food, pharmaceutical, medical device, and chemical industries. A commitment to quality by both companies is further supported by ISO:17025 lab accreditation.

Enfer Diagnostics and its partners participate in many government-sanctioned working groups and are participating in several international disease eradication programs.

CDC/EPI-X

Booth **108**

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.

FBI – Laboratory Division

Booth **316**

2501 Investigation Pkwy, Room 3130 HMRV
Quantico, VA 22135
www.fbi.gov
Contact: Stephen Goldsmith
202.615.1190
stephen.goldsmith@ic.fbi.gov

The Federal Bureau of Investigation (FBI) is the lead agency for the investigation of terrorism in the United States. Agroterrorism is an attack against the agricultural infrastructure of a country and is designed to intimidate, coerce, or cause panic in the population in the furtherance of political or social objectives. The FBI is dedicated to preventing, detecting and investigating potential attacks on the agricultural infrastructure. However, the response to an agroterrorism attack requires coordination between federal law enforcement agencies; federal animal, plant, and food health agencies; and other federal, state, and local agencies. Some of the BFI efforts to combating agroterrorism include: liaison with other government agencies, ensuring the FBI's ability to analyze contaminated evidence, and numerous outreach efforts to the agricultural community.

Fort Supply and Technologies, LLC

Booth **310**

1773 W 200 N
Kaysville, UT 84037
www.fort-supply.com
Contact: Malcolm Harvey
435.535.3039
malcolm@fort-supply.com
Contact: Terrill Weston
435.881.5311
terrill@fort-supply.com

Fort Supply Technologies is a leader in providing field rugged hand held computers with easy to use programs for electronically capturing and managing animal information.

Global Vet Link

Booth **209**

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Ames, IA 50010
www.globalvetlink.com
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515.296.0860
khenry@globalvetlink.com

Company Representatives: Kevin Maher

GlobalVetLink is the nation's innovator of moving a paper-based regulatory process to real-time, online technology since 1999. GlobalVetLink offers veterinarians, state animal health authorities, and diagnostic laboratories a solution to collaborate within a secure, web-based platform. Our applications include animal health certification, an online EIA system, Equine Passport Permits (in specified regions), diagnostic laboratory test submittal and resulting, animal movement data, veterinary feed directives, and more all by the click of a mouse. Fast, secure, online.....we make paperwork, paperless.

Hydrol-Pro Technologies, Inc.

Booth **215**

40420 Free Fall Avenue
Zephyrhills, FL 33542-5838
www.hydrolpro.com
Contact: Kim Etherington
813.780.6753
kim@hydrolpro.com

Company Representatives: Kevin Morris, Kim Etherington

Hydrol-Pro Technologies featuring the BioMEER system of advanced alkaline hydrolysis for carcass processing for destruction of TSE's and recovery of energy from carcass waste. Dry (low BOD) or wet output versions available. A low-cost, effective alternative to incineration. New energy efficient Bio-effluent De-con systems are also offered.

New for 2009, large mobile (100K lb.) Emergency Response Carcass Processing System.

IDEXX Laboratories

Booths **200-202**

One Idexx Drive
Westbrook, ME 04092
www.idexx.com/production
Contact: Mary Spear
207.856.8059 207.749.4117
mary-spear@idexx.com

The Production Animal Services (PAS) Division of IDEXX Laboratories, Inc. develops, manufactures and sells a wide range of ELISA-based detection systems for animal health and quality assurance applications. Laboratories around the world use IDEXX PAS HerdChek*, FlockChek*, CHEKIT* and Pourquier* products for the detection and monitoring of diseases affecting livestock and poultry, and our xChek* data management software simplifies and organizes test results. For more information, please contact us at phone: (800) 548-9997 or (207)-556.4300; email: PASweb@idexx.com; or visit our website at www.idexx.com/production

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Inverness Medical Professional Diagnostics

Booth **105**

10 Southgate Road
Scarborough, ME 04074
www.invernessmedicalpd.com
Contact: **Animal Health**
207.730.5722
animalhealth@invmed.com

Inverness Medical Innovations, Inc. is committed to being the global leader in developing and marketing diagnostic tests. Binax, a wholly owned subsidiary of Inverness Medical, received USDA-licensure to manufacture, market and distribute the BinaxNOW® Avian Influenza Virus Type A Antigen Test Kit in the United States. Test results are available in 15 minutes compared to traditional methods, which take hours or days to complete. No specialized equipment or training is necessary.

The BinaxNOW AI Antigen Test is an easy-to-use, rapid immunochromatographic test (ICT) that detects influenza A nucleoproteins in chicken, turkey and duck samples using cloacal, tracheal and oropharyngeal swabs. This antigen test is capable of detecting all hemagglutinin subtypes, including H5 and H7. Storage is 2-30°C with shelf life up to 24 months.

For more information about the BinaxNOW® Avian Influenza Virus Type A Antigen Test Kit, contact Inverness Medical Innovations, Inc. at +1.207.730.5722, Animalhealth@invmed.com or http://www.invernessmedicalpd.com/poc/animalhealth

ITL Animal Healthcare

Booth **300-302**

1925 Isaac Newton Square East, Ste 480
Reston, VA 20190
www.itlanimalhealthcare.com
Contact: Susan Tamborini
888.411.2851
itlanimalhealthcare@itlus.com

ITL Animal Healthcare is unveiling a breakthrough innovation for collecting blood samples from livestock. In doing so, ITL Animal Healthcare is introducing an unprecedented level of safety into the process of drawing blood for genetic and disease testing. The new system also simplifies the process without compromising sample integrity or requiring changes to laboratory processes.

ITL Corporation is a global company that advances best practices through the design of its blood collection devices. ITL Animal Healthcare combines this experience with expertise in livestock sampling systems to better meet the needs of veterinarians, animal handlers, producers and laboratories from around the world.

To learn more about ITL Animal Healthcare or its new biological sampling system for livestock, visit Booth #300-302.

Luminex Corporation

Booth **318**

12212 Technology Blvd
Austin, TX 78727
www.luminexcorp.com
Contact: Tom Copa, Business Unit Manager
512.381.3276
tcopa@luminexcorp.com

Luminex is a manufacturer of multiplexed bead based test kits, instruments and reagents. Our xMAP® technology allows diagnosticians to detect up to 500 proteins or nucleic acid markers in a single sample in a single well of a microtiter plate. The Luminex platform is a cost effective and open platform. Labs can run pre-developed kits or purchase raw reagents and develop homebrew tests in their labs.

Merrick and Company

Booth **303**

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 **204**

13570 Meadowgrass Dr, 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 get 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 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 **211**

5425 S. Semoran Blvd, Suite 10-B
Orlando, FL 32822
www.animal-cremation.com
Contact: Brian Gamage
800.228.0884 x1891
gamage@crawfordequipment.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 approved many 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 **106**

9810 Hupp Drive
LaVista, 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 as a spin-off from Zurich University, 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). 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 Research and Development facilities in Switzerland and the Netherlands and has regional hubs in key markets such as Argentina, Germany, Italy, the Netherlands, and the USA. Prionics is also represented by distribution partners around the world.

Qiagen, Inc.

Booth **206**

19300 Germantown Road
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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.

Quality Systems Integrators

Booth **104**

PO Box 91
Eagle, PA 19480
Website address:
Contact: Renee Cabrey
610.458.0539
reneecabrey@qsi-inc.com

Quality Systems Integrators (QSI) offers affordable software solutions to efficiently manage documents and training records. Our TMSWeb Quality Management Compliance System is completely web-based and simplifies the tedious tasks surrounding compliance to AAVLD requirements and other certifications such as ISO and electronic records & electronic signatures. TMSWeb provides access control, workflow, job certification training & document management, and is fully integrated with e-mail systems. Additional software modules are available for Testing and Quizzing, Forms, Auditing and Change Control. Reasonably priced, our TMS quality solutions can save you time and money by streamlining your processes and enhancing productivity.

Smiths Detection Diagnostics

Booth **212**

459 Park Avenue, Bushey
Watford, Hertfordshire
United Kingdom WD23 2BW
www.smithsdetection.com/diagnostics
Contact: Carmelo Volpe
+44 1923 658 338
carmelo.volpe@smithsdetection.com

Smiths Detection Diagnostics offers the world's first truly portable fully automated sample preparation and PCR system designed specifically for the needs of the veterinarian or animal health professional. The Bio-Seeq Portable Veterinary Diagnostic Laboratory comprises a portable briefcase-sized instrument and disposable sample preparation units. On site diagnostics provides rapid results, quicker decision making reduced costs. Combined these can significantly reduce the effect of an outbreak.

The system builds on Smiths Detection's global leadership in biological agent detection for security applications, coupled with its experience in the development of rugged handheld devices for field use. Equipment from Smiths Detection puts laboratory power that requires minimal training into the hands of emergency responders, security teams and government agencies around the world.

Synbiotics Corporation

Booth **114**

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www.synbiotics.com
Contact: John Trabucco
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customerservice1@synbiotics.com

Synbiotics Corporation develops, manufactures and markets veterinary diagnostics and related products for the companion animal, large animal and poultry markets worldwide.

Further Synbiotics is the worldwide leader in canine reproductive services as well as canine semen freezing and storage.

Headquartered in Kansas City, Missouri, Synbiotics manufactures and distributes its products through its operation in San Diego, CA, and a wholly owned subsidiary; Synbiotics Europe in Lyon, France. For information on Synbiotics and its products, visit the Company's website at www.synbiotics.com.

Tetracore, Inc.

Booth **213**

9901 Belward Campus Drive #300
Rockville, MD 20850
www.tetracore.com
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240.268.5417
bkijowski@tetracore.com

Company Representatives: Dr. William Nelson, Dr. Beverly Mangold, Tracy Fecteau, John Kelly, and Brian Kijowski

Providing Advanced Molecular and Immunological Detection.

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 to specific 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.

TREK Diagnostic Systems

Booths **201**

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.

Ventana Medical Systems, Inc.

Booth **203**

1910 Innovation Park Drive
Tucson, AZ 85755
www.ventanamed.com
Contact: Terry Haikara
800.227.2155 x3867 or 520.906.2952
thaikara@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.

VETQAS
(Veterinary Laboratories Agency)

Booth **110**

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New Haw, Surrey KT15 3NB UK
www.vla.gov.uk/eqa/eqa-home.htm
Contact: Peter Cousins
0044 (0) 1932 357332
p.cousins@vla.defra.gsi.gov.uk

VETQAS® is the new name for the Proficiency Testing (PT) services provided to you by the Quality Assurance Unit of the Veterinary Laboratories Agency for over twenty years.

Our new name, ***Vet***erinary **Q**uality **A**ssurance **S**ervice, reflects our commitment to continue providing you with first class proficiency testing for veterinary diagnostic laboratories. With more and more laboratories becoming accredited to ISO17025 and increased emphasis on quality assurance we felt that a recognizable name for the services we provide was overdue. It signals our continuing expansion of the services we offer to our customers. We believe our new **VETQAS**® name will make it easier for you to recognize our schemes and service provision.

Over the next couple of years we will add new schemes for farm animal diseases as well as expanding the number of schemes covering molecular biology, avian diseases and equine diseases. Many of these are being developed in response to requests from our customers worldwide.

VMRD, Inc.

Booth **214**

4641 Pullman Albion Road
Pullman, WA 99163
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.

American BioResearch (ABR, Inc.). ABR conducts 9 CFR and EMEA extraneous virus testing on cell lines, virus seeds, cell culture reagents and supplements, and other virology related services. ABR and VMRD have worked together for many years and we are dedicated to continuing to refine ABR's services. Visit www.ambio.net for more information.

Upcoming AAVLD/USAHA meetings:

2010: November 10-17, Hilton Minneapolis, Minnesota

2011: September 29–October 5 Adam's Mark - Buffalo, New York

AMERICAN ASSOCIATION OF VETERINARY LABORATORY DIAGNOSTICIANS

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The AAVLD Foundation is a non-profit foundation, which aims to raise funds for the advancement of veterinary diagnostics through scholarship programs, guest lectures, seminars, awards and research programs. Contributions to the Foundation are tax-exempt (501(c)(3))

If you would like to become a Foundation donor, please fill out the form below and send with check, money order or VISA/MC information to: AAVLD Foundation, PO Box 1770, Davis, CA 95617
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