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American Association of Veterinary Laboratory Diagnosticians

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American Association of Veterinary Laboratory Diagnosticians

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

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

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Acknowledgments

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

The Program Committee, listed below, deserves a special acknowledgement for their hard work, organization, review and editing of the abstracts, and moderation of sessions. Jay Kammerzell and Vanessa Garrison were instrumental in computerizing and organizing the review process and assisiting the AAVLD Secretary-Treasurer's Office in producing the proceedings book. Pat Blanchard, Jackie Cassarly, and Linda Ragland (USAHA) coordinated the meeting room arrangements, exhibitor booth arrangements, sponsor agreements, breaks, and all of the many other details that go into making a meeting a success.

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AAVLD Plenary Session

Saturday, Oct 25, 2008

Guilford-B

Co-Chairs: David Steffen
Gary Anderson

"One Health"

Bacteriology Scientific Session

Saturday, October 25, 2008

Guilford D

Moderators: Lindsay Oaks
Debra Royal

Influenza Scientific Session

Saturday, October 25, 2008

Guilford C

Moderator: Kristi Pabilonia
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Pathology Scientific Session

Saturday, October 25, 2008

Auditorium II

Moderator: Scott Fitzgerald
Karla Mesterhazy

***** Graduate student presentation

Tritrichomonas Scientific Session

Saturday, October 25, 2008

Guilford-E

Moderator: Scott McVey
James Kennedy

* Graduate student presentation

Virology Scientific Session

Saturday, October 25, 2008

Auditorium III

Moderator: Tim Baszler
Dick Hesse

* Graduate student presentation

Bacteriology Scientific Session

Sunday, October 26, 2008

Guilford-E

Moderators: Durda Slavic
Deepanker Tewari

* Graduate student presentation

Pathology Scientific Session

Sunday, October 26, 2008

Auditorium-II

Moderators: John Adaska
Shannon Swist

* Graduate student presentation

Toxicology and Disease Surveillance Scientific Session

Sunday, October 26, 2008

Auditorium-III

Moderators: Patricia Talcott
Catherine Barr

* Graduate student presentation

Virology Scientific Session

Sunday, October 26, 2008

Guilford-D

Moderator: Beate Crossley
Roman Pogranichniy

***** Graduate student presentation

USAHA /AAVLD Plenary Session

Monday, October 27, 2008
Guilford-ABC

Co-Chairs: David Steffen
Don Hoenig
Moderator: Alfonso Torres

Foot-and-Mouth Disease: If "When" Happened

07:30 AM **Welcome and announcements**
Dr. Alfonso Torres

07:35 AM **Status of the national FMD response plan: Coordination within the animal health community** – Jose R. Diez, Bob Hooks 142

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10:00 AM **FMD eradication efforts in South America, current status of the disease. Vaccination strategies in countries that are vaccinating** – David Ashford 146

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12 Noon **Summation**

12:15 PM **Adjournment**

AAVLD Poster Session

3 p.m. Friday, October 24, 2008 through
3 p.m. Sunday, October, 26, 2008
Guilford-ABC

**Graduate Student Presentation*

AAVLD Plenary Session

Saturday, Oct 25, 2008

Guilford-B

Co-Chairs: David Steffen
Gary Anderson

"One Health"

The AVMA One Health Initiative – Overview and Opportunities

W. Ron DeHaven, DVM, MBA
Chief Executive Officer

American Veterinary Medical Association

The 2006 *E. coli* outbreak in spinach is the perfect example of "One Health" in action. The pathogen originated in cattle, spread to feral hogs that then carried it to the spinach fields in the Salinas Valley. A strained irrigation system resulted in contamination of surface and ground water, further spreading the pathogen. Ultimately, over 200 people in 26 states were diagnosed from this outbreak of *E. coli* 0157:H7. Veterinarians, public health officials, physicians, and environmental officials all had a part in the diagnosis, epidemiology, containment, and control of this outbreak.

Well beyond such disease outbreaks, it is apparent that all veterinarians are "one-health practitioners." Whether it is the small animal practitioner vaccinating a dog for rabies or the government veterinarian ensuring the safety of our meat, every veterinarian performs public health activities. Historically we have equated health with the "absence of disease." But if we take a more comprehensive view, we realize that "health" is much more. One definition presented at a recent conference hosted by the CDC suggests that health, "is a dynamic state of complete physical, mental, spiritual, and social well-being and not merely the absence of disease or infirmity." This broader definition of health only serves to emphasize the public health role of veterinarians. For example, as we promote the health of our pets, we also contribute to the mental health of the public.

The concept of "One Health" is clearly evident in the Veterinarian's Oath that includes a statement about our dual responsibilities for the protection of animal health and promotion of public health. While all of us are "one health practitioners," the current emphasis on One Health within the AVMA is taking this role to a new level. There are many factors driving this initiative, from the alarming emergence and reemergence of zoonotic disease to the increasing demands on livestock production to meet the growing global demand for animal protein.

AAVLD members have been contributing to "One Health" as much as any group in the veterinary profession. Your critical role in conducting diagnostics on pets, poultry, livestock, and fish all contribute to the health of animals and people. While your contributions to One Health have been great, there are many opportunities to do more. Sharing information and collaborating with city, county and state public health officials and agencies could greatly enhance our ability to promptly detect the introduction of disease or other conditions affecting local populations. After such detection, the synergy realized from working together will minimize the ultimate impact on both animal and human populations. Similar benefits can be realized on a larger scale through initiatives such as collaboration between the various lab networks. This could include everything from information sharing for trend analysis to providing surge capacity across the networks.

Investigation into an outbreak of a novel inflammatory neuropathy among swine abattoir workers

Stacy Holzbauer[1,2] and Sagar Goyal[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Minnesota Department of Health, St. Paul, MN, [3]University of Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN.

Introduction. Inflammatory neuropathies have been associated with chronic diseases, prior vaccination, and infectious agents. We were notified of 10 cases of a unique inflammatory neuropathy now termed Progressive Inflammatory Neuropathy (PIN) among workers at swine abattoir, Plant A. The case-patients all worked in the primary processing area (warm room) and appeared to be clustered in the area that processes severed heads (head table). An investigation was initiated to assess exposures and prevent further illness.

Methods. Case finding was enhanced by abattoir occupational health record review, medical record searches, and statewide clinician health alert. A case-control study was conducted among consenting Plant A workers including an extensive interview, and collection of blood and throat specimens. Confirmed cases were defined as workers with symptoms of a progressive peripheral neuropathy and evidenced by electrodiagnostic testing of an inflammatory neuropathy. Probable cases were defined as workers having symptoms of peripheral neuropathy and either neuroimaging consistent with radiculitis, myelitis, or encephalitis, or an elevated cerebrospinal fluid protein. Two control groups consisting of persons not reporting neurologic illness were used: (1) a random selection of well warm room workers and (2) all well workers at the head table. An environmental assessment of the abattoir was performed. Blood and throat specimens were tested for multiple infectious agents including culture, serology, and PCR. We surveyed the 26 largest U.S. swine abattoirs to identify cases and common processing techniques.

Results. 17 Minnesota cases were identified (median age: 32 yrs; range: 21-55). Illness onsets occurred from May, 2004 - November 2007. Case-patients were more likely than warm-room controls to ever work at the head-table (odds ratio [OR], 7.7; 95% confidence interval [CI], 1.8 to 21.6) and to remove brains or remove muscle from back of heads (back heads) (OR, 17.6; 95% CI, 3.0 to 103.2). Among head-table workers, case-patients remained more likely to ever remove brains or back heads (OR, 9.0; 95% CI, 2.1 to 39.1). Workers removed brains using compressed air that liquefied brain and generated aerosolized droplets, exposing themselves and nearby workers. This technique was used in a NE and an IN abattoir; eight additional PIN cases were identified in these two abattoirs.

Conclusions. PIN is associated with processing swine heads and specifically with removing brains using compressed air. Three abattoirs using this technique have stopped brain removal; subsequently no new cases of PIN have been reported. Most likely this illness represents an autoimmune disease related to exposure to swine brain of a susceptible host.

Outbreak of a novel inflammatory neuropathy among swine abattoir workers: laboratory investigations

Sagar M. Goyal[1], Stacy Holzbauer[2,3]

[1]Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN. [2]Centers for Disease Control and Prevention, Atlanta, GA, [3]Minnesota Department of Health, St. Paul, MN.

As part of the Progressive Inflammatory Neuropathy (PIN) investigation, 120 current and former workers at swine abattoir, Plant A, consented to throat swab collection and 111 to serum sample collection. Materials from the throat swabs were pooled into 24 pools (five swab samples to one pool), which were inoculated in 10 different cell lines namely, BT (bovine turbinate), PK15 (porcine kidney), BHK (baby hamster kidney), PPK (primary porcine kidney), MDCK (Madine-Darby canine kidney), CRFK (crandell-Reese feline kidney), PAM (porcine alveolar macrophages), Marc-145 (monkey kidney), and Vero (monkey kidney). The cells were contained in 24-well plates and the amount of inoculum was 200µL/sample/well. After 7 days of incubation, a second passage was made in homologous cells. After 10 days, the supernatant fluids were harvested and by hemagglutination and were also examined by transmission electron microscopy. In addition, the cells were stained by indirect immunofluorescence using porcine polyvalent antiserum containing antibodies to several viruses including encephalomyocarditis, hemagglutinating encephalitis, transmissible gastroenteritis, porcine adeno-, porcine rota-, porcine reo-, H1N1 swine influenza, porcine tescho-, porcine entero-, pseudorabies, and porcine parvovirus. The sample pools were also inoculated in 10-day-old specific-pathogen-free embryonated chicken eggs by the allantoic route. After 5 days of incubation, the allantoic fluids were harvested and tested by hemagglutination.

None of the throat swab samples were positive for any virus by hemaggulutination, immunofluorescence, or electron microscopy. Only one sample showed cytopathic effects in CRFK cells and was subsequently identified as human herpes simplex virus. This was considered to be an incidental finding. No virus was isolated by inoculation of embryonated chicken eggs.

The111 serum samples from Plant A workers were tested for antibodies to several animal viruses. None of the samples showed serologic evidence for viral antibodies. No infectious etiologic agent was identified despite multiple attempts to identify an agent through various molecular techniques. Lack of evidence of an infectious etiologic agent combined with the epidemiologic data supports exposure to swine brain as a potential cause of PIN.

Antimicrobial resistance surveillance; the forest or the trees?

S.W.J. Reid

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Antimicrobial resistance is not a new phenomenon. In truth, it has existed and evolved wherever and whenever selection pressure has been applied through the use of antimicrobials, regardless of the motivation for their use or the target host animal species. Once in the ecosystem and established, at best, we can attempt to assess prevalence and distribution of resistant isolates in parallel to making interventions. However, if we are to minimize the impact of a phenomenon that is, in reality, emerging, re-emerging and evolving then early detection, surveillance and "freedom from" status are important epidemiological considerations. The socio-political and popularly held tenet that the use of antimicrobials in a veterinary context leads to resistance in pathogens isolated from humans is an unhelpful backdrop that must be challenged on the basis of quantitatively robust surveillance. The challenge for the scientific community is to ensure that our shared definitions, the surveillance methods, the tests, and the models are fit for purpose, a reality that is an aspiration when quantity and quality data are often lacking. If classical approaches are not practical then we must turn to other paradigms and ensure that we treat resistance as a whole ecosystem issue where pathogen evolution, host factors and variability and uncertainty are integral parts of the risk assessments we use to inform our interventions.

The role of antimicrobial susceptibility testing in the treatment of livestock disease

Peter Constable

Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN

Important considerations for the treatment of clinical bacterial diseases in ruminants are: 1) administering an antibiotic as directed on the label whenever possible; 2) using an antimicrobial with an appropriate spectrum of activity; 3) selecting an antimicrobial that attains and maintains an effective therapeutic concentration at the site of infection; 4) treating for an appropriate duration; 5) avoiding adverse local or systemic effects and violative residues. Antimicrobials are selected based on availability of labeled drugs, clinical signs in the animal, bacterial culture results for previous episodes in the herd, experience of treatment outcome in the herd, treatment cost and practicality, and withdrawal times for slaughter and milk. For many years there has been interest in optimizing treatment protocols in order to better target antimicrobial administration, with substantial reliance on susceptibility testing of bacterial isolates from animals with clinical disease.

Antimicrobial susceptibility of bacterial pathogens has most commonly been determined in livestock using the agar diffusion method, which was designed to reflect the antimicrobial concentration in serum and interstitial fluid of human patients after receiving oral or intravenous administration. Many problems exist with the currently used susceptibility breakpoints for bacteria isolated from ruminants. Accurate antimicrobial susceptibility test breakpoints should ideally be derived using MIC values for 300 to 600 isolates from representative clinical cases from a large geographic area, published pharmacokinetic/ pharmacodynamic data, and clinical and bacteriologic cure rates. The results of field studies that measure the rate of clinical cure, using clinically relevant end points such as mortality, weight gain, treatment duration, and relapse rate should be reported as a bare minimum in order to validate theoretical breakpoints. The rate of bacteriologic cure within specified time intervals, using biologically relevant endpoints such as failure to isolate the same pathogen from the affected quarter in cows with mastitis, the feces in calf diarrhea, or a transtracheal wash in pneumonia, also provides useful data. Clinical and bacteriologic cure rates may provide a clear breakpoint, or in other situations, this data can be used in conjunction with pharmacokinetic/pharmacodynamic data to suggest the most appropriate breakpoint.

Unfortunately, the ideal approach to determine accurate susceptibility breakpoints in ruminants is hampered by 3 main difficulties: 1) limited availability of contemporaneous MIC values from heterogeneous geographic locations, 2) incomplete phamacokinetic/pharmacodynamic data, and 3) complete absence of published field studies validating the theoretical susceptibility breakpoints. The effect of disease on the pharmacokinetics of antimicrobial agents has usually been ignored, but differences in the plasma concentration-time profile of 2 antimicrobials have been documented between healthy and pneumonic calves, and experimental induction of endotoxemia and pyrexia in calves changes the plasma concentration-time profile for an orally or intramuscularly administered antimicrobial. Finally, antimicrobial agents may produce beneficial effects separate to their activity against bacteria; erythromycin is a potent prokinetic agent that increases abomasal emptying rate and milk production in the immediate postoperative period after surgical correction of left displaced abomasum or abomasal volvulus in cattle, and tilmicosin decreases pulmonary inflammation in bovine pneumonia.

Mastitis

The 3 sites of infection in cows with mastitis are milk and udder parenchymal tissue in the affected quarter, and the intravascular compartment in severely affected cattle with gram-negative mastitis. We do not currently have adequate databases of in vitro MIC values for clinical mastitis pathogens, although adequate databases are available for subclinical mastitis isolates. The validity of susceptibility breakpoints derived from humans to the treatment of bovine mastitis has not been established and is extremely

questionable because bovine milk pH, electrolyte, fat, protein, and leukocyte concentrations, growth factor composition, and pharmacokinetic profiles are markedly different than those for human plasma, and because human bacterial pathogens and treatment regimens are often different from bovine mastitis pathogens and treatment regimens.

Calf diarrhea

The 2 sites of infection in calves with diarrhea are small intestine and the intravascular compartment. The results of fecal antimicrobial susceptibility testing have traditionally been used to guide treatment decisions; however, susceptibility testing in calf diarrhea probably has clinical relevance only when applied to fecal isolates of pathogenic Salmonella spp., and blood culture isolates from calves with bacteremia. Validation of susceptibility testing as being predictive of treatment outcome for calves with diarrhea is currently lacking.

Susceptibility testing in calf diarrhea has focused on using fecal isolates, although this approach is fundamentally flawed. There do not appear to be any data demonstrating that fecal bacterial flora is representative of small intestinal bacterial flora, which is the site of infection in the intestinal tract of calves with enterotoxigenic E. coli. Moreover, the predominant strain of E. coli in the feces of a scouring calf usually changes during the diarrhea episode, and fecal E. coli strains should not be considered to be representative of small intestinal E. coli strains. A clear bias present in most antimicrobial susceptibility studies conducted on fecal E. coli isolates is that data is usually obtained from dead calves, which are very likely to be treatment failures. Calves that die from diarrhea are likely to have received multiple antimicrobial treatments, and preferential growth of antimicrobial resistant E. coli strains starts within 3 hours of antimicrobial administration. Similar to susceptibility testing of mastitis pathogens, the agar diffusion break points for susceptibility testing of fecal isolates are not based on achievable antimicrobial concentrations in the small intestine and blood of calves, but on achievable antimicrobial concentrations in the plasma of humans.

Pneumonia

The site of infection in pneumonia is the lower respiratory tract, whereas the site of infection for metaphylaxis, where the goal is to minimize or prevent proliferation of Mannheimia hemolytica, is the upper and lower respiratory tract. Antimicrobial susceptibility testing has frequently been recommended to guide the treatment of respiratory disease in cattle. The utility of periodic susceptibility testing to guide treatment decisions on feedlots has not been verified and is questionable, given that strains of Mannheimia hemolytica in a single outbreak of bovine respiratory disease vary between and within an animal. A major difficulty with susceptibility testing is obtaining a representative culture of bacteria from the lower respiratory tract of cattle with pneumonia. The gold standard method is culturing affected anteroventral lung parenchyma at necropsy; however, cattle dying of pneumonia have usually been treated with antimicrobial agents, which increases the percentage of resistant isolates. Necropsy sampling is therefore strongly biased towards treatment failures. Practical methods for obtaining a representative culture of the lower respiratory tract bacteria in untreated cattle are therefore needed.

Antemortem culture of the bovine respiratory tract has used guarded nasopharyngeal swabs, guarded tracheal swabs, bronchoalveolar lavage (BAL), and transtracheal washes. Currently, endoscopic assisted BAL and transtracheal wash provide gold standard methods for obtaining a lower respiratory tract culture in live cattle. Unfortunately, both techniques are rarely performed because they are time consuming and require specific training and appropriate restraint of the animal or expensive and fragile equipment. Nasopharyngeal swabs are commonly used to collect samples from cattle in the field because the technique is rapid and inexpensive; however, nasal swabs should not be used to identify the presence of lower respiratory pathogens in individual cattle.

We do not currently have adequate databases of in vitro MIC values for bacterial isolates from cattle with pneumonia, because almost all isolates were obtained from nasal swabs of live animals or lung parenchyma of dead animals that represented treatment failures. All the pivotal studies for FDA approval

of tilmicosin, spectinomycin, and enrofloxacin used pretreatment nasal swabs and necropsy lung swabs (treatment failures) to characterize the susceptibility profile, whereas the pivotal studies for ceftiofur and florfenicol utilized pretreatment nasal swabs, transtracheal washes, and lung swabs (treatment failures) obtained at necropsy (www.fda.gov/cvm/efoi). The use of nasal swabs for susceptibility testing therefore casts doubt on the accuracy of recommended breakpoints for antimicrobials used to treat bacterial pneumonia in cattle.

Concluding comments

A popular exercise for many years has been the publication of massive tables documenting antimicrobial resistance patterns of a large number of bacterial isolates based on the results of the agar diffusion method. The author believes that such data currently has minimal clinical relevance in guiding the treatment of bacterial diseases in individual ruminants. However, because the results of susceptibility testing are repeatable, the results of population susceptibility testing do provide extremely useful information on the development or loss of antimicrobial resistance characteristics for bacterial pathogens in a population over time. An excellent example of this is the increase in ceftiofur resistance amongst Salmonella isolates from livestock over the past decade www.ars.usda.gov/Main/docs.htm?docid=17320.

Until the theoretical MIC breakpoints have been validated, this author suggests that the following statement, or something similar, be used whenever the results of susceptibility testing are reported in ruminants: "theoretical and unvalidated break points for interpretation of susceptibility testing results". In the meantime, there is an urgent need in production medicine to validate existing theoretical break points for MIC values.

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Navigating susceptibility testing results from a diagnostician perspective: appropriate and inappropriate interpretations

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CLISI breakpoints are a mix of veterinary-approved breakpoints based on veterinary clinical data, "generic" breakpoints based on pharmacokinetic/pharmacodynamic modeling, and breakpoints extrapolated from human medicine with little or no confirmatory data on the veterinary side. The breakpoints are also applied to monophasic and biphasic population MIC distributions. Blanket acceptance or rejection of all breakpoint-based testing is nonsensical. This presentation will classify susceptibility-testing outputs ranging from applications with reasonable correlation between susceptibility testing results and population outcome (e.g., respiratory disease in cattle) and applications for which no correlations have been established (e.g., enteric disease). Reasonable interpretation requires understanding the background of the breakpoint, the MIC distribution of the target population, and the true difference the antimicrobial will make in treatment outcome.

Bacteriology Scientific Session

Saturday, October 25, 2008

Guilford D

Moderators: Lindsay Oaks
Debra Royal

Development of a loop mediated isothermal amplification (LAMP) test to detect *Mycoplasma hyopneumoniae*

Albert Rovira, Maria Pieters, Eduardo Trevisol, Simone Oliveira

Veterinary Diagnostic Laboratory (Rovira, Trevisol, Oliveira), College of Veterinary Medicine (Pieters), University of Minnesota, St. Paul, Minnesota, USA

Because isolation of *Mycoplasma hyopneumoniae* from clinical samples is difficult and time consuming, many veterinary diagnostic laboratories rely on PCR for the detection of this bacterium. However, PCR testing requires the use of special equipment (a thermal cycler), which is not available in all laboratories. The objective of this study was to develop a diagnostic test for *M. hyopneumoniae* based on loop mediated isothermal amplification (LAMP) technology. LAMP is a relatively new DNA amplification technique that does not require a thermal cycler.

To design the primers, the nucleotide sequences of the 16S ribosomal RNA genes of *M. hyopneumoniae, Mycoplasma flocculare, Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis* were obtained from the Genebank and aligned with Clustal X software. A stretch of 200 nucleotides that showed the maximum differences between *M. hyopneumoniae* and the other mycoplasmas was selected as target sequence. Primers were designed with LAMP-specific software (Primer Explorer V4). The LAMP reaction was performed in a 25 µl reaction mixture containing: 1.6 µM of each inner primer, 0.2 µM of each outer primer, 1.4 mM of each dNTP, 0.8 M of betaine, 2.5 µl of 10x ThermoBuffer, 8 mM $MgSO_4$, 8 Units of Bst DNA Polymerase and two microliters of the extracted target DNA. The reaction was performed in a heat block at a constant temperature of 63 C for 60 minutes. After the reaction, visual inspection was used to determine positivity. Samples showing turbidity were considered positive.

To evaluate the analytical sensitivity, the LAMP test was run on 10-fold dilutions of a pure culture of *M. hyopneumoniae* strain 232 containing 10^3 color changing units per ml (CCU/ml). The limit of detection of the new LAMP test was 1 CCU/ml. To evaluate the analytical specificity, the LAMP test was run using DNA extracted form on a panel of pure cultures of bacteria including species commonly isolated in the respiratory tract of pigs, as well as other mycoplasmas. The panel included: *Actinobacillus indolicus, Actinobacillus minor, Actinobacillus pleuropneumoniae, Actinobacillus porcinus, Actinobacillus suis, Arcanobacter pyogenes, Bordetella bronchiseptica, Haemophilus parasuis, Mycoplasma alkalescens, Mycoplasma arginini, Mycoplasma bovis, Mycoplasma bovirhinis, M. flocculare, M. hyorhinis, M. hyosynoviae, Pasteurella multocida, Staphylococcus aureus* and *Streptococcus suis.* All these samples were negative.

The performance of the LAMP test was evaluated with 145 nasal and bronchial swabs from 20 experimentally infected piglets and 5 control piglets. The same samples were tested with previously published nested PCR (nPCR) and real time PCR (rtPCT) tests. No positive results were obtained by LAMP, nPCR or rtPCR from the 20 nasal swabs obtained before experimental inoculation, or from 5 bronchial swabs from control piglets. Out of the 120 samples obtained from inoculated piglets between 7 and 25 days post inoculation, 78 tested positive by rtPCR, 71 by LAMP and 53 by nPCR.

The new LAMP test showed good analytical sensitivity and specificity and its performance was comparable to that of PCR.

Development of a real-time polymerase chain reaction assay for diagnosis of *Mycoplasma haemolamae.*

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Introduction. *Mycoplasma haemolamae* (formally *Eperythrozoon haemolamae*) is a hemotrophic, wall-less bacterial species associated eperythrozoonosis or EPE of llamas and alpacas. It is estimated that as many as 25% of camelids in the US are infected. Most animals infected with this organism effectively control the infection and show no clinical signs, but animals that are immunocompromised or stressed may manifest acute disease, usually mild to severe anemia. Affected animals may be unable to stand and be extremely weak; in chronic infections animals may experience weight loss and lethargy. Diagnosis of *M. haemolamae* is made by either visual identification of the organism on blood cells or using a nested-set polymerase chain reaction (PCR). Both methods are time consuming and open to interpretation. Here we a combined SYBR and Taqman detection systems in a real-time PCR assay for detection of *M. haemolamae* in camelid blood.

Material and methods. Primers were designed to amplify a 164 bp sequence from the *M. haemolamae* 16S ribosome gene. An internal Taqman probe, labeled with HEX and BHQ1 was also designed. Blood samples were allowed to stand overnight at 4^0C**.** Plasma or serum samples were collected and DNA extracted using the Puregene Blood Column Kit (Gentra), DNeasy Column Kit (Qiagen) or the MgMAX Viral Isolation Kit (Ambion) in combination with the King Fisher Instrument (Thermo). Blood samples from 120 camelids was tested using both the nested-set PCR and real-time. The nested-set was performed as previously described. For real-time, PCR, 1x SYBR Pre Mix Ex Taq (Takara), 0.2 µM each primer, 0.2 µM probe, 0.4µl ROX, and 5 µL template DNA were combined to a final reaction volume of 20 µL. The PCR was performed by incubating at 95ºC for10 sec, then 95ºC 30 sec, 59ºC 30 sec for 40 cycles followed by a melt cycle of 95ºC 60 sec; 55 ºC 30 sec, 95ºC 60 sec in a thermocycler (Stratagene MX3005). The results were analyzed for both SYBR fluorescence and HEX fluorescence. A sample that had a Ct_{HEX} <40 was considered positive. A sample with a Ct_{SYBR} <40 and a T_m between 80.6 ºC to 81.4 ºC was considered positive.

Results and discussion. The PCR test described in this study is suitable for clinical diagnosis for *M. haemolamae* in camelid blood. Results were analyzed using SYBR fluorescence and HEX fluorescence and the combination.When the Taqman real-time PCR used alone was compared with nested-set PCR it gave a sensitivity of 94% and a specificity of 88%; however, when SYBR and Taqman probe read-out was combined, the sensitivity was 100% and specificity was 99%. Differences among DNA extraction methods were not significant. Using the real-time PCR, results were available a full day before those of the nested-set, on average.

Conclusion. The PCR assay described in this study is suitable for clinical diagnosis for the detection of *M. haemolamae* in llama and alpaca plasma. Combining the two read-out systems makes analysis of the results simpler and improved the sensitivity and specificity to a level equivalent to the nested-set PCR.

Development and evaluation of a real-time PCR for the detection of *Actinobacillus suis* in porcine lung samples

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Actinobacillus suis, an important swine pathogen, is associated with high mortality in both high- health status and conventional herds. **In the present study, a real-time polymerase chain reaction (PCR) assay targeting the 23S ribosomal RNA gene was developed and evaluated for the detection of *A. suis* in porcine lung samples.** The performance characteristics of the assay were evaluated by testing a panel of 65 bacterial isolates comprising *A. suis* and other bacterial species, 85 lung samples from clinically sick pigs submitted to the Veterinary Diagnostic Laboratory at Iowa State University, and 75 lung samples from experimentally infected pigs. **This newly developed real-time PCR assay demonstrated 100% agreement with the culture results. Additionally, it provided 100% sensitivity, 100% specificity, 100% positive predictive value, and 100% negative predictive value compared to the conventional culture method.** The high sensitivity, rapidity and reproducibility of this real-time PCR make it a feasible technique for the timely detection of *A. suis* directly from clinical samples. Future studies will be directed to ascertaining whether this *A. suis* real-time PCR assay can be used on clinical specimens other than lung tissue.

Accurate diagnostic tests that efficiently identify Johne's disease positive sheep and goats

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The bacteria that causes Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is shed very late in the clinical disease of infected sheep and goats. This is a cell-mediated disease; therefore antibody is produced late in the infected animal. Animals infected with MAP develop chronic wasting and death after this bacterium targets the mesenteric lymph nodes and intestines of animals. Adults transmit the MAP bacteria to their fetus *in utero* or young via colostrum, milk and feces.

This diagnostic testing was initiated to accurately and efficiently identify Johne's disease positive small ruminates from positive flocks and herds for eventual elimination of these positive animals and offspring. Four antemortem and two postmortem tests for detecting MAP-positive animals are being compared:

- the bovine serology ELISA (IDEXX Herdchek) using 0.250 S/P cutoff on sheep and goat serum, plasma and milk samples;
- the bovine serology ELISA (IDEXX Pourquier) using 0.300 S/P cutoff on the above sheep and goat samples;
- culture of feces, milk and colostrum samples;
- the johnin intradermal skin test for status of cell-mediated immune response to MAP infection.
- culture and histopathology of tissues.

An increased sediment inoculum and time in culture is being used on liquid culture media: BACTEC™ MGIT™ para TB liquid medium with the fluorometric manual read method; and all acid-fast positive tubes are subcultured to Herrold's egg yolk agar (HEYA) with or without mycobactin J (to determine if the isolate is MAP or the MAP bovine strain).

Our samples are coming from three cooperator producers, each with one MAP culture positive index animal. The producers include a 2000 ewe range flock, a 40 ewe farm flock, and one 20 doe farm herd. To date 137 serum samples and 44 milk samples have been assayed with the two bovine serology ELISA tests. IDEXX Herdchek ELISA identified 51 MAP antibody positive serum samples and IDEXX Pourquier identified 33, with 29 samples positive with both tests. Herdchek identified 14 Johne's antibody positive milk samples, and Pourquier 11 positive milk samples, with agreement on seven samples. The Herdchek test identifies positive animal samples (15) earlier in infection; the Pourquier test identifies positive animals (3) later in the course of the disease. Both tests identify positive serum samples from lambs or kids (colostral antibodies) up to three months of age from culture positive dams. To date 36 of 180 culture samples are positive for MAP. All of the positive samples originated from antibody positive animals. The average time in culture until identified as acid-fast is 2.6 months (range: two weeks to 10 months). Seventy-five of 180 culture samples were set up from tissues, fecals and milk pellets from thin, antibody positive animals. Only one of 26 milk pellets is culture positive. Fecals from two lambs (3 and 4 months of age) are culture positive. Both asymptomatic lambs are from culture positive ewes. A johnin positive test indicates an animal's exposure to MAP. This test is more easily used on a small number of animals that can be confined for several days. This test can be used to identify lambs and kids (6/6) that are exposed from their dams, or environmental exposure. This test becomes negative as the animal becomes clinical (15/15). Eleven of fifteen animals' tissues were acid-fast positive on histopathology. The four paucibacillary animals were thin with serum S/P's greater than 1.00. At certain times, milk samples are more easily collected and tested if serum samples are not available. **Antibody ELISA tests are the most efficient and accurate tests for both the serum and milk samples from adults and, serum from young offspring from known culture positive herds and flocks.**

Evaluation of Two Direct-PCR Commercial Kits for Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Bovine Feces

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Two commercial direct-PCR kits for extraction and detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from bovine feces were evaluated with 300 archived samples. The infection status of these archived samples was previously established with a liquid culture system using a semi-quantitative days-to-detection method.

Fecal samples were evenly distributed between four shedding categories: negative, low, moderate, and high. Kit A identified 192 of 225 culture positive fecal samples (relative diagnostic sensitivity 85.3%) and correctly identified all 75 culture negative samples (relative diagnostic specificity 100%). For Kit A, the predictive value (PV) of a positive test was 1.00 and the PV for a negative test was 0.69. Kit B correctly identified 195 of 225 culture positive fecal samples (relative diagnostic sensitivity 86.7%) and 74 of 75 culture negative samples (relative diagnostic specificity 98.7%). For Kit B, the PV of a positive test was 0.99 and the PV of a negative test was 0.71. Within each MAP shedding category, relative diagnostic sensitivity was greatest for heavy and moderate shedders for both direct-PCR kits (Kit A = 98.7%; Kit B = 97.3%) and had dropped noticeably for low shedders (Kit A = 59.2%; Kit B = 65.8%). One heavy shedder was missed by both direct-PCR kits; this sample had a mean days-to-detection of 19.3 days as determined by liquid culture. One medium (Kit A) and 3 medium (Kit B) shedders were missed by direct-PCR. Further characterization of these samples is needed to determine why they were missed. A strong relationship (Kit A, r = 0.85; Kit B, r = 0.84) existed between direct-PCR Ct values and days-to-detection. The average cycles to threshold (Ct) values for Kit A were: 27.03 heavy shedders (SD = 3.08; range = 21.14-35.75), 33.49 moderate shedders (SD = 2.77; range = 27.14-38.88), and 36.54 low shedders (SD = 2.72; range = 25.53-42.47). Kit B average Ct values were: 24.49 heavy shedders (SD = 4.51; range = 16.33-38.55), 31.11 moderate shedders (SD = 3.26; range = 24.06-38.32), and 36.15 low shedders (SD = 2.82; range = 24.11-44.93).

The two direct-PCR commercial kits exhibited low sensitivity for identifying low shedders, suggesting that they might not be suitable for determining Johne's negative herd status or other stringent requirements including international trade. However, with its respective high degree of sensitivity (>0.97) and specificity (>0.98) for identifying heavy and moderate shedders, direct-PCR will be a useful tool for effective management and control of Johne's disease by identifying high-risk animals so they can be removed from herds in the most rapid turnaround time among current MAP tests. Direct-PCR results can be provided in a manner of 1-2 days instead of 6-8 weeks (liquid culture) or 3-4 months (solid agar). Further studies are needed to address how to correlate quantified results by direct-PCR with shedding categories as determined by our current semi-quantification liquid culture method.

Diagnosis of *M. paratuberculosis* infection via extra-cellular antigen capture and confirmation

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Introduction. Infection by *M. paratuberculosis (Mptb)* is a continuing problem for many domestic agriculture species. US dairy herd Johne's disease prevalence now approaches 80% and the organism's potential association with Crohn's disease in humans is also of concern. Diagnosis of the infection via culture of feces or tissue can be lengthy, laborious and costly due to the slow-growing nature of the causative organism and the need to genetically identify isolates. Commercial liquid culture methods (Trek, MGIT) rely on expensive and space-intensive machines to detect the proliferation of the organism in the sample. An *Mptb* antigen detection assay that does not rely on these machines and that triages the results to minimize the need for PCR identification of all isolates was developed (JTC MAC ELISA). This assay captures and confirms antigens as secreted *Mptb* proteins using two different preabsorbed polyclonal antibodies for high specificity. The performance of this antigen detection assay was compared to the Becton Dickinson MGIT 960 culture system.

Materials and Methods. *Samples.* More than 500 fecal samples from six dairy operations (including one believed uninfected) were collected. The infection test status of a majority of cattle had been previously determined. Fresh fecal samples were processed using the BD MGIT 960 fecal processing method per manufacturer's instructions. On Day 3 of processing, 0.1ml of the concentrated processed sample was inoculated to each of two MGIT Para TB medium tubes. One of these "sister-samples" proceeded through the standard MGIT 960 culture assay, the other through the JTC MAC ELISA.
MGIT pathway. Samples in this pathway followed the standard MGIT 960 Instrument workflow except that samples flagged as positive by the MGIT 960 instrument were removed by day 40 vs. 42. All MGIT 960 flagged positive samples were further tested by AFB staining and multiplex PCR.
MAC ELISA pathway. Samples were incubated in racks at 37°C for 49 days. They were tested at days 28 and 49 with the four layer sandwich ELISA (bound to a microtiter plate is polyclonal antibody against *Mptb* extracellular antigens; the second layer is a 300µl inoculum from MGIT ParaTB media; the third layer is the detector antibody against the *Mptb* antigens and the last layer is sheep anti-rabbit IgG conjugated to HRP). All MAC ELISA positive samples were further tested by AFB staining and multiplex PCR. All negative MAC ELISA samples after 49 days of incubation had AFB staining performed. Ten percent of sister samples with negative results on both MGIT and ACE pathways were randomly selected and evaluated by AFB staining, multiplex JTC PCR and blood agar plate to confirm test negative status.

Results. Thirty eight percent of MGIT pathway samples signaled positive; 66% of these were found to be *Mptb* by multiplex JTC PCR. The MAC ELISA found 31% samples to be positive; 96% of these were confirmed as true positives by multiplex JTC PCR. Sixty-five percent of the confirmed positive samples were detected by MAC ELISA at the first test (28 days). In the closed herd believed to be uninfected, both methods identified the same animal as infected (i.e. JTC PCR confirmed the positive result for each sister-sample). Both methods signaled another sample as positive that subsequently was identified as *M. avium* ss. *avium* by JTC multiplex PCR. All quality control PCRs of samples signal-negative for both methods were negative.

Conclusions and Discussion. The MAC ELISA's false positive rate was significantly lower than what was seen with samples monitored by the MGIT 960 signaling instrument. **Use of capture antibodies for detection of extracellular secreted *Mptb* antigens serves as an efficient, inexpensive basis to find and identify this pathogen in liquid cultures**.

Evaluation of the difference in signal time of cattle feces in the MGIT 960 as an indicator of the final result for detection of *Mycobacterium avium* subsp. *paratuberculosis*

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Introduction. For the detection of *Mycobacterium avium* subsp. *paratuberculosis* (*M. ptb*) from cattle feces in the MGIT (Mycobacteria Growth Indicator Tube) 960, the instrument signals when the level of oxygen in the prepared tube reaches an established threshold over a 49 day incubation period. Upon signaling, the tube is removed from the instrument, vortexed and scanned back into the instrument to await a second signal before confirmatory testing is conducted. The median difference of the duration in days between the first and second signal of each sample was compared to the final result to determine if the difference in signal times may aid in indicating the final result.

Materials and Methods. The days to detection for both the first and second signal times of 437 cattle fecal samples, QC samples and process controls removed from the MGIT between August 2006 and March 2008 were tracked and related to their final reported result.

Results. At the Colorado Department of Agriculture there are three main reporting categories for samples that signal twice in the MGIT;

- **Positive.** Specimens that are acid-fast positive and either grew in subculture on HEY with mycobactin J (HEYJ) or are confirmatory PCR positive. The median difference between the first and second signal time of 189 samples reported out as positive was 4 days (range was 0 to 28 days, 74% ranged between 3 and 5 days).
- **Acid fast, negative *M. ptb* (AFNMPTB).** Specimens that are acid-fast positive and confirmatory PCR negative or grew in subculture in less than 2 weeks. The median difference between the first and second signal time of 18 samples reported out as AFNMPTB was 2.5 days (range was 1 to 6 days, 61.1% ranged between 1 and 3 days).
- **Non acid fast signal positive (NASP)**. Specimens that were acid-fast negative and either did not grow in subculture on HEYJ or were confirmatory PCR negative. The median difference between the first and second signal time of 143 samples reported out as NASP was 2 days (range was 0 to 33 days, 60.8% ranged between 1 and 3 days).

Additionally, two types of controls are utilized when testing feces**;**

- **QC samples (known suspension of ATCC 19698).** The median difference between the first and second signal time of 50 QC samples was 3 days (range was 1 to 5 days, 86% ranged between 3 and 5 days).
- **Process controls (*M. ptb* containing feces).** The median difference between the first and second signal time of 37 process controls samples was 4 days (range was 0 to 6 days, 78.3% ranged between 3 and 5 days).

Discussion/Conclusion. Due to the lack of normal distribution of the data, based on the Anderson-Darling test, statistical significance of the data could not be assessed. However, when evaluating the median difference between the first and second signal of the three reporting categories there is a notable difference between the *M. ptb* positive category and the *M. ptb* non-positive categories, NASP and AFNMPTB. Although not solely an indicator of the result, evaluating the difference in days between the first and second signal along with the confirmatory test results may be informative when reporting a result.

Detection and isolation of Sheep strain *Mycobacterium avium* subsp. *paratuberculosis* from goats using the MGIT 960

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Introduction. The MGIT 960 has been shown to support the growth of the cattle strain of *Mycobacterium avium* subsp. *paratuberculosis* (*M.ptb*) but little is known of the system's ability to support growth of the sheep (S) strain of *M. ptb*. Recently, the S strain has been isolated from two different pygora goats using the MGIT 960.

Materials and Methods. In December 2006, feces from a two year old male pygora goat was submitted to the Colorado Department of Agriculture for Johne's liquid culture using the MGIT 960. After being stored at -80 degrees Celsius for 5 days, the sample was decontaminated using the instrument manufacturer's recommended fecal sample processing protocol including the addition of 200 ug/ml of naladixic acid to the Para TB medium.

Results. At 14 days post-inoculation, the sample signaled positive for the first time in the MGIT 960, and after the second signal in the MGIT 960, the sample was subcultured onto a Herrold's egg yolk medium (HEY) with mycobactin J slant and acid-fast stained. The acid-fast stain was positive, but no growth was observed on the slant for 12 weeks post-inoculation. The sample was subcultured from the MGIT tube a second time with no growth observed at 8 weeks. The sample was PCR positive using the Tetracore® culture confirmation protocol and VetAlert™ Johne's Real-Time PCR. Due to the inability of the sample to grow on HEY, the sample was suspected to be S strain. A portion of the original MGIT media was submitted to the National Veterinary Services Laboratories in Ames, Iowa and was confirmed as S strain by growth on Modified Middlebrook 7H10 media and *M. ptb* by PCR. Since this initial positive sample in the MGIT, several more fecal samples have been submitted from the same herd of pygora goats and a second sample (a one year old male) signaled positive in the MGIT at 17 days post-inoculation. This sample was reported as positive for the S strain of Johne's disease based on the inability to grow on HEY with mycobactin J, growth on Modified Middlebrook 7H10 media and positive for *M. ptb* using Tetracore's direct fecal PCR and confirmatory PCR.

Discussion/Conclusion. Until now, detecting the S strain of *M.ptb* in the MGIT 960 was thought to be difficult, especially within the manufacturer's recommended 49 day protocol for bovine fecal samples. Observations in our laboratory shows that it is possible to detect the S strain in goats in a reasonable time period utilizing the MGIT 960.

Influenza Scientific Session

Saturday, October 25, 2008

Guilford C

Moderator: Kristi Pabilonia
Kyoung-Jin Yoon

Results of two sampling methods for the detection of avian influenza in wild birds in the highly pathogenic avian influenza early detection data system directed by the USDA in Utah

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Many of the viruses causing human influenza pandemics have shared genetic markers with Avian Influenza viruses. As of June 2008, 383 people worldwide have been diagnosed as infected by Highly Pathogenic Avian Influenza (HPAI) H5N1 of which 241 infections have been fatal. In addition, an enumerable number of birds of several domestic species have been culled in an effort to control the spread of this disease or have died as a result of the infection. In 2006, the USDA-APHIS began a surveillance program, the HPAI Early Detection Data System (HEDDS), to detect Avian Influenza (AI) in wild birds in the United States. The Utah Veterinary Diagnostic Laboratory (UVDL) was one of several participating laboratories. Surveillance concentrated on the detection of AI H5 and H7 subtypes; the two AI types that are commonly associated with HPAI. In 2006, cloacal swabs were collected by Utah Department of Natural Resources and USDA Wildlife Services from hunter-harvested birds, surveys of healthy birds and mortality events and from avian cases submitted to the UVDL. Field samples from birds of the same species, type, collection date and site were pooled (range 1 to 5, mean 4.5 samples per pool) and the RNA was extracted per the National Veterinary Service Laboratory (NVSL) testing protocol at the UVDL. RNA from the pooled samples was screened for the presence of AI matrix gene and matrix positive pools were tested for the presence of H5 and H7 subtypes by real time PCR. In 2007, the USDA/NVSL sampling protocol for the program was modified; oropharyngeal, tracheal, and cloacal swabs were collected. Most importantly, samples were processed individually. **In 2006, 92 of 449 (20.5%) pools representing samples from 2020 birds tested positive for AI matrix. In 2007, 196 of 1050 (18.7%) birds tested positive. When the sample results from 2007 were mathematically analyzed as pools using the 2006 pooling criteria, the number of positive samples in a pool averaged 1.03 per pool or 23% (4.5 birds per pool with a 95% CI of 0.64 to 1.4 positive samples). Therefore in 2006, using the upper level of the confidence limit for positive samples per pool, no more than 129 (1.4 x 92) of 2020 individual birds (6.4 %) were likely to have been detected as positive for AI.** Even though the prevalence of AI in 2006 is an estimate, the increase from 6.4 % in 2006 to 18.7 % in 2007 of AI in the wild bird species in Utah is unusual since previous studies demonstrated that total AI prevalence rates in a region tend to remain consistent from year to year although the prevalence of individual subtypes does vary. **In 2006, 6/449 pools (1.3%) were H5 positive and in 2007, 19/1050 (1.9%) of the individual bird samples were H5 positive. When the results for 2007 are mathematically analyzed as pools, then 19/219 (8.6 %) were positive for H5.** Data may reflect a true increase in the prevalence of AI (including H5 subtype) among birds in Utah from 2006 to 2007. Alternatively, data more likely demonstrates that individual bird testing is more sensitive than pooled sample testing for the detection of AI and subtypes.

Influenza in dogs: transmission from horses during the Australian equine influenza outbreak

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Respiratory disease due to a Type A Influenza virus has been described in dogs in the USA in recent years. The virus is closely related to strains of H3N8 equine influenza virus and has a presumptive but unidentified equine origin. This virus has become adapted to dogs and is transmitted from dog to dog. Investigations in the UK have also described a retrospective association of pneumonia in dogs with influenza virus infection and influenza infections have been identified serologically in dogs that were likely to have close contact with horses during the 2003 outbreak in the UK. Unlike the situation in the USA, there is to date no evidence of continuing circulation of an influenza virus of equine origin in the canine population in the UK. In late 2007, Australia experienced the first occurrence of influenza virus infection in horses. During this large outbreak, respiratory disease was observed in dogs that were in close proximity to infected horses. Because of the situations described in the UK and USA, investigations were undertaken to exclude influenza virus infection.

A number of infected dogs were identified. The first dog observed had been inappetant and depressed, had a slight nasal discharge and a persistent cough for several days. This dog was held near a large stable complex where there were infected horses. Over the following 2 weeks, dogs present in stables where there were infected horses, or whose owners were handling infected horses, were examined and a history collected. Nasal swabs and clotted blood were collected from each of the dogs, including any others with which they had contact. Convalescent sera were collected 3-4 weeks later in some cases. Samples were also collected from dogs at 2 other locations in the Sydney region.

From a total of 40 dogs, there were 10 with clinical signs consistent with influenza, including anorexia, depression and, in a number of cases, a harsh cough that has persisted for several weeks. All of the affected dogs recovered. **Of the 40 dogs sampled, 23 were seropositive** in both an influenza Type A blocking ELISA and a haemagglutination inhibition assay using the current Influenza A/Sydney/2007 virus as the test antigen. A single dog had a high titre in the HI test but was negative by ELISA while there were another 3 dogs that reacted in the ELISA but were negative in the HI test. Seroconversions were observed for 4 dogs. HI titres ranged from 16 to 256. Each of the seropositive dogs were in close proximity to EI infected horses but did not always have direct contact. **There was no evidence of lateral transmission of the virus to other dogs that did not have contact with horses. Nasal swabs collected from one dog gave a positive result in a real time reverse transcriptase PCR (qRT-PCR) assay. When re-sampled on subsequent days, this dog gave a positive result on 2 other occasions**. It remained clinically normal and was seropositive on day 16 after the first positive swab. Attempts to isolate virus from these swabs were unsuccessful. **Nucleic acid sequencing was undertaken on the haemagglutinin, matrix and neuraminidase genes of virus from two of the dog samples and from a nasal swab collected from an infected horse in the same stable. There was complete homology between the sequences from each of the 3 sets of samples.**

This study is believed to be the first to show a direct linkage between active influenza virus infection in dogs and horses. The virus infecting the dogs did not have any of the changes in the nucleotide sequence that have been identified in viruses from dogs in the U.S. These changes may be critical to the adaptation of equine influenza viruses to dogs as there is no evidence of continuing circulation of virus in dogs in Australia.

Influenza A virus whole genome RNA amplification

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The high mutation frequency of the influenza A virus genome underlies its widespread infection and pathogenicity. Rapid and accurate whole genome sequence information is required for effective surveillance of emerging variants. Currently, field viruses need to be propagated in cultured cells or embryonated chicken to generate enough sequencing template. We have therefore developed a workflow to expedite isolation of type A influenza virus genomic RNA and a whole genome amplification process that will provide genome sequence information in hours instead of days.

The workflow consists of the MagMAX™ Viral RNA Isolation Kit and Path-Amp Flu A RT-PCR (reverse transcription PCR) reagents. As an example validating the workflow: Influenza A virus RNA was isolated and purified directly from wild duck cloacal swab samples with the MagMAX-Express 96 Magnetic Particle Processor, a magnetic bead-based high throughput system that isolates RNA from up to 96 samples in approximately 15 min. The wild duck cloacal samples were also used for virus isolation in embryonated chicken. The MagMAX purified viral RNA was used for type A influenza virus detection using the matrix gene TaqMan® PCR assay (Spackman et al, J *Clin Microbiol* 2002 **40(9):**3256–3260) and the AgPath-ID™ One Step RT-PCR Kit. Subsequently, selected RT-PCR positive samples were used to amplify all eight full-length segments of the viral genome in one tube using Path-Amp Flu A RT-PCR reagents; this step was completed in approximately 5 hours. The PCR products obtained were purified with MagMAX-Express 96 Magnetic Particle Processor in approximately 10 min, thus **the amplified influenza genome was ready for sequencing in approximately 6 hours.**

Our results show successful whole genome amplification with purified RNA from 6 of 12 cloacal samples and all 12 influenza positive allantoic fluid samples, all of which were RT-PCR positive. Interestingly, 3 cloacal samples which were RT-PCR positive and virus isolation negative were successfully amplified with Path-Amp Flu A reagents. In addition, analysis of a large number of allantoic samples showed that the gene segments of **all N subtypes** (N1–9) and **all H subtypes available** (H1–7 and H10–11) were **successfully amplified**. **Furthermore, the RT-PCR amplification was specific and universal for type A Influenza virus. Successful amplification was obtained from a broad range of hosts including swine, equine, human, and duck. This workflow provides a fast and reliable solution for Influenza A virus surveillance and management that includes the ability to subtype RT-PCR positive, virus isolation negative samples.**

Validation of a real-time RT-PCR assay for the detection of H7 avian influenza virus

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Between April 2006 and March 2007, more than 164,000 cloacal (CL) and fecal swabs were collected from apparently healthy, dead and hunter-killed wild aquatic birds in all 50 states and tested for presence of avian influenza (AI) virus by real-time RT-PCR (rRT-PCR) at National Animal Health Laboratory Network (NAHLN) laboratories, the National Wildlife Research Center, Ft. Collins, CO and the National Wildlife Health Center, Madison, WI. Specimens were screened for AI virus by the matrix (M) rRT-PCR assay with subsequent testing of M-positive specimens by the H5 and H7 rRT-PCR assays. Matrix positive H5/H7 negative specimens were subsequently tested for isolation of AI virus in chicken embryos to determine the prevalence and subtype of non H5/H7 AI circulating in North American wild birds. Conventional serologic subtyping and/or sequence analysis of the hemagglutinin gene of AI viruses identified H7 AI viruses not detected by the USDA 2002 H7 rRT-PCR assay. Consequently, an alternative H7 assay (2008 H7 assay) was developed by the Southeast Poultry Research Laboratory for the detection of North and South American H7 lineage viruses. Analytical and diagnostic sensitivity (DXSn) and specificity (DXSp) validation data and assay performance characteristics were determined for the 2008 H7 assay.

Diagnostic sensitivity (DXSn) and specificity (DXSp) of the 2008 H7 rRT-PCR assay compared to virus isolation was determined to be 97.5 % and 82.4 %, respectively, based on testing a total of 1,578 diagnostic poultry specimens. The 2008 H7 assay was shown to detect 10^1 EID_{50} per reaction or 10^3 - 10^4 copies of transcribed RNA. In addition the assay was evaluated with AI reference viruses (H1-H15), poultry respiratory pathogens, and 239 wild bird viral isolates. Of the 239 wild bird viruses tested 82 were identified as H7 subtype AI virus by the 2008 H7 rRT-PCR assay and serologic or molecular subtyping procedures. The remaining 157 non-H7 viruses tested negative by the 2008 H7 rRT-PCR assay. Performance characteristics of the 2008 H7 assay using different real-time platforms and chemistries showed the Ambion MagMAX™ RT-PCR chemistry was more sensitive on Applied Biosystems (AB) and Roche 96-well instruments than the Qiagen One-Step® RT-PCR chemistry and the Qiagen RT-PCR chemistry produced better results with the Cepheid Smart Cycler instrument than AB or Roche 96-well instruments. The 2008 H7 rRT-PCR assay has recently been accepted as the official USDA Real-Time RT-PCR assay for the detection of H7 in clinical specimens.

Viral variation resulting in false negative results in H5 and H7 real-time RT-PCR tests

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Real-time RT-PCR (RRT-PCR) for the diagnosis of avian influenza virus and the determination of the hemagglutinin subtype by RRT-PCR, particularly the H5 or H7 subtype, is a common laboratory procedure. Monitoring of the performance of molecular diagnostic tests need to be routinely performed because of the potential for sequence changes in the target resulting in reduced sensitivity or false negative results, particularly for the highly variable targets like the influenza hemagglutinin gene. Two recent examples of false negative test results were evaluated and alternative test procedures were developed to address the issue. The first example included several H5N1 highly pathogenic avian influenza viruses from Hong Kong in 2007 that were observed to not be detected with the NAHLN H5 test. On sequence analysis of these isolates, 3 nucleotide changes were observed at the probe site. A series of experiments were performed using de novo synthesized DNA plasmids to demonstrate that these three changes were the cause of the test failure. It was shown that at the normal annealing temperature of 57 C, the H5 probe did not detect plasmids with 2 or 3 nucleotide mismatches, although the samples were detected if the annealing temperatures were lowered to 54C or 52C respectively. To look for ways to improve test performance three alternative probes were evaluated, including replacing inosine at the three mismatched positions, replacing inosine at one position and a mixed base at the second position, or moving the probe 3 nucleotides downstream and also replacing the two mismatches with inosine. All three new probes were able to detect all H5 viruses tested at the 57 C annealing temperature, but the probe with the two changes was recommended. A second case of false negative results was observed with North America H7 wild bird viruses that was not observed with the NAHLN H7 test. The false negatives were due to sequence differences in the primer and probe, and therefore a new RRT-PCR test was developed using the much larger sequence database. The Pan-American test was able to identify all the viruses tested from North and South America and was used to replace the existing H7 test.

Use of genomic interspecies microarray hybridization to detect differentially expressed genes associated with H5N1 avian influenza virus infections in ducks

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The Asian H5N1 highly pathogenic avian influenza (HPAI) viruses have changed from producing mild respiratory infections in ducks, to some strains producing severe disease and mortality. The objective of this study was to examine the differences in host response to infection with H5N1 HPAI viruses with different pathogenicity in ducks by determining gene expression in tissues of infected ducks using a chicken genome microarray. The use of cDNA microarrays offers a highly effective system to study transcriptional responses during host-pathogen interaction. By using genomic interspecies microarray hybridization we can detect a large number of genes, provided that the microarray for a fully sequenced genome of a close relative is available. A 44K, 60-mer oligonucleotide, whole chicken genome microarray was used to compare gene expression in spleens from Pekin ducks infected with two different HPAI viruses, A/Ck/HK/220/97 and A/Egret/HK/757.2/02. An important number of differentially expressed genes associated with infection were detected and demonstrated the complexity of the patterns of gene expression in ducks in response to HPAI. Semi-quantitative RT-PCR was used to confirm the regulated expression of several of the differentially expressed genes. **The results obtained suggest that different mechanisms are potentially induced by avian influenza viruses to modulate the host response to infection.** The differentially expressed genes identified in this study are candidates for further hypothesis-driven investigation of the mechanisms involved in resistance to AI viruses in ducks.

Federal and State transport plan for movement of eggs and egg products from non-infected commercial table egg premises in a high pathogenicity avian influenza control area - the FAST eggs plan

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Introduction. Creation of a **"**Rapid Decision-Making Tool**"** to allow movement of eggs and egg products from non-infected premises within an avian influenza Control Area is the objective of a cooperative agreement between APHIS and researchers at Iowa State University. Components of the Rapid Decision-Making Tool include the following: a) a Rapid Approval Program Biosecurity Checklist for Egg Production Premises and Auditors; b) Surveillance Program using RRT-PCR; c) GPS coordinates for Egg Premises and Infected Premises in a Control Area; and d) an Analysis Algorithm that includes all three of the previous components.

Rapid Approval Program Biosecurity Checklist for Egg Production Premises and Auditors. An all-inclusive Model Biosecurity Program Checklist for Egg Premises was developed from biosecurity plans currently being used by 4 large egg production companies in the Midwest. This comprehensive list includes 124 separate biosecurity measures that are available to large commercial egg producers. To determine which items are important to prevent the introduction of avian influenza onto an egg premises, we asked a panel of 10 poultry veterinarians with expertise in egg production and avian influenza to score each of the 124 items on the original list. Panelists scored each biosecurity item on a scale of 1 (least important) to 4 (most important). Biosecurity measures with an average score of 3.2 or higher (49 individual items) would include all important measures needed for an avian influenza biosecurity program.

Surveillance Program using RRT-PCR and Flock Performance Indicators. The absence of infection will be documented by requiring each house on the farm to be tested each day and found to be negative by the real time reverse transcriptase – polymerase chain reaction (RRT-PCR) or other suitable procedure as determined by the Incident Commander. Oropharyngeal swabs from a minimum or five chickens from the daily mortality and/or from euthanized sick birds from each house (flock) will be tested each day. Veterinary diagnostic laboratory personnel will perform RRT-PCR testing on these samples immediately upon receipt and electronically send test results to the Incident Commander by the end of each day.

Participating facilities will be required to submit daily information on egg production, mortality, and feed consumption for each chicken house. This data will be submitted directly to the web-based server and will be available each day to the Incident Commander.

GPS Coordinates for Egg Premises and Infected Premises in the Control Area. GPS coordinates of participating commercial premises and nearby backyard flocks will be collected in a format compatible with that used by Center for Epidemiology and Animal Health at Fort Collins, Colorado.

Analysis Algorithm. An algorithm that combines the exposure audit and surveillance data with location analysis will be used to estimate the premises' risk of exposure to HPAI. On-going surveillance data and location analysis will be entered daily. All data will be processed using pre-designed analysis protocols. A qualitative (high, medium, low) or quantitative assessment of this flock's exposure risk can be estimated. State or Federal animal health officials can then review the analysis and determine whether to allow movement of shell eggs and liquid egg products or modify a premises' status.

Exposure of American black vultures (*Coragyps atratus*) to select viruses in Mississippi

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The black vulture, *Coragyps atratus*, is an important scavenger in the Southeast United States. As a scavenger, they have contact with many species, potentially being exposed to numerous diseases. Little research has been conducted on exposure to diseases in black vultures. As part of a damage removal program, 498 birds (19% males, 81% females) were collected from a roost in Columbus, MS in 2007. Birds were sampled and necropsied to determine exposure to pathogens. Forty five randomly selected serum samples from each sex were submitted to the Mississippi Poultry Diagnostic Lab for testing of Newcastle disease (NDV), infectious bronchitis virus (IBV), Reovirus, infectious bursal disease (IBD), chick anemia virus (CAV), *Mycoplasma gallisepticum*, and *M. synoviae*. All 498 samples were submitted to the National Wildlife Research Center in Fort Collins for testing of Avian Influenza, and West Nile Virus. Forty (20 each males and females) were also tested for Laryngotracheitis. Exposure rates ranged from 0-16%, with all positive samples being from males, except one. **A total of 10 birds seroconverted to these diseases, with 3 birds having exposure to 4 diseases (IBV, IBD, Reo, and NDV), 2 exposed to 2 (IBD and Reo) and 5 others showing titers to 1 pathogen. Multiple exposure individuals mirrored vaccination practices in poultry production. Low numbers of male birds and their higher exposure rates point to a potential sexual selection pressure due to current poultry practices.** The impact of this reduced male population needs to be examined.

Pathology Scientific Session

Saturday, October 25, 2008

Auditorium II

Moderator: Scott Fitzgerald
Karla Mesterhazy

***** Graduate student presentation

Right-sided heart failure in young Holstein cattle: an emerging problem on the Colorado Front Range

Donal O'Toole[1], Gregory M. Goodell[2], Scott Smith[2], Chris Koeller[2], Chris Malherbe[2], Patricia Schultheiss[3], Katherine L. Gailbreath[4], Gary J. Haldorson[4]

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Cor pulmonale occurs in dairy breeds of cattle, but English language reports of the disease are rare. Recognized causes in the Holstein breed are heart failure secondary to chronic pneumonia, and brisket disease. A syndrome of rapidly progressive right sided heart failure was recognized in dairies along the Front Range of Colorado (altitude: >1,600-m). In some operations it was the most important single cause of annual mortality in heifers aged <1-year. Cattle are aged 6 – 12 months and present with abrupt onset dyspnea, tachycardia, jugular venous distention and pulsation, and lethargy. Clinical progression is rapid (2 days – 2 weeks). Affected heifer calves die. Tissues from 9 affected cattle were examined by diagnostic pathologists at the University of Wyoming (3), Colorado State University (3), and Washington State University.

This presentation focuses on lesions in affected heifer calves. Grossly there is marked dilation of right atrium and ventricle, with hypertrophy of right ventricular myocardium. The normal ratio of right ventricular free wall to total ventricular free wall in cattle is 0.251 ± 0.011; in fatal brisket disease of beef cattle the ratio is typically >0.40. The RVFW/TVFW ratios in two affected Holsteins with typical clinical signs and lesions were 0.397 and 0.487. Other gross lesions in affected Holsteins are marked dilation of the pulmonary trunk, hepatomegaly, ascites, weight loss with or without saponification and serous atrophy of fat, and anteroventral bacterial pneumonia. Histologically, the principal findings in heart are hypertrophy of cardiocytes in right ventricular free wall and atrium with endomyseal and septal fibroplasia. Myonecrosis and post-necrotic scarring are present in some calves, but such lesions are small and widely scattered. Arterial changes in lungs include medial hypertrophy, intimal hyperplasia, thrombosis and adventitial hyperplasia. Changes in liver are consistent with right-sided heart failure. They consist of atrophy and loss of periacinar and mid acinar hepatocytes, with congestion, early fibroplasia, and lipidosis in surviving mid-acinar hepatocytes.

The cause of the syndrome is unknown. A preliminary analysis of pedigrees did not reveal a common genetic pattern among affected cattle. A logical explanation for the syndrome is that it is brisket disease. Some lines of Holsteins are reported to be particularly sensitive to pulmonary hypertension and its complications, but most of this scant published literature is based on cases in Peru. Features atypical of brisket disease compared to the condition in beef breeds are rapid progression of clinical signs, marked dilation of the pulmonary trunk and right atrium, and (in some cattle) severe histological changes in pulmonary arteries and arterioles in lungs.

Reproductive tract examination at slaughter of repeat breeding beef cows and heifers in south central Florida

John Roberts[3], Max Irsik[1], Hilary Swain[4], Gene Lollis[4], Diane Kitchen[5], Heather Stockdale[6], Oscar Morales[5], Claus Buergelt[1], Donald Schlafer[7], Bradley Austin[2], Joel Yelich[2], Christy Waits[4], Travis Heskett[1]

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Introduction. Reproductive efficiency of beef cattle grazed in south central Florida is below the national average by producer's estimates. While death of young calves and abortion is a significant contributor to decreased reproductive efficiency baseline information on the reproductive health of non-productive cows and heifers from commercial herds, which has not collected systematically in the past, was gained from this study.

Materials and Methods. Reproductive tracts were recovered at slaughter from 112 repeat breeder non-pregnant cows and heifers and 38 pregnant cows from two ranches in south central Florida. Gross examination of the entire reproductive tract and histopathology from the external os of the cervix, uterine body, uterine horns, oviduct and ovary were performed. Swabs from the uterine body were cultured with routine aerobic technique and special technique for *Campylobacter* spp., *Mycoplasma* spp., and *Ureaplasma* spp.. *Tritrichomonas foetus* was isolated by inoculation of InPouches TM and positive results were confirmed with PCR.

Results. Congenital or developmental defects that were identified in 12/112 tracts included: cervical disease-2, infantile tract-5, persistent hymen-1, freemartism-1, uterine aplasia-1, uterine didelphis-1 and para-uterine cyst-1. Neoplasias (granulosa cell tumor-2, leiomyoma-1) were diagnosed in 3/112. Bacterial metritis-6 and mummified fetus-2 were identified in 8/112. *Tritrichomonas foetus* metritis was identified by culture or PCR in 6/112. Uterine lymphangectasia was diagnosed in 1/112. Ovarian cysts were identified in 3/112. Diseases of the oviduct or fimbria were identified in 6/112. Ovarian degeneration or dysplasia was identified in 40/112. No significant gross or microscopic pathology was observed in 31 of 112 non-pregnant cows or heifers. Aerobic culture of swabs inoculated from the uterine body of 111 non-pregnant and 21 pregnant cattle yielded various combinations of 26 bacteria species. *Campylobacter* spp., *Ureaplasma* spp. or *Mycoplasma* spp. were not identified. Single bacterial isolates were obtained from 21/111 non-pregnant uteri and 2/21 pregnant uteri. Multiple bacterial isolate were obtained from 50/111 non pregnant uteri and 8/21 pregnant uteri. *E. coli* was isolated from 42/111 non-pregnant and 9/21 pregnant uteri. Various *Streptococcus* spp. were isolated from 32/111 non-pregnant and 5/21 non-pregnant uteri. Various *Staphylococcus* spp. were isolated from 32/111 non-pregnant and 4/21 pregnant uteri. No bacterial or trichomonad growth was achieved from 40/111 non-pregnant and 11/21 pregnant uteri.

Conclusion. Ovarian degeneration or dysplasia was the most common lesions in non-pregnant cows. Cervicitis, metritis and salpingitis were usually minimal or not present in non-pregnant cows. *Tritrichomonas foetus* infection was present in low percentage of cows but was always associated with significant metritis. The uteri of pregnant and non-pregnant cows often contained a commensal bacterial population that did not result in endometrial inflammation. Future studies into conception failure in beef cattle should focus on ovarian pathology.

Osteopetrosis in Red Angus Cattle

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Osteopetrosis is a rare, heterogeneous group of diseases due to defective osteolcast function resulting in inadequate resorption of bone and modeling of primary trabeculae. Its morphological hallmark is endochondral new bone in medullary cavities. Most forms of the disease are genetic in animals and people, but an osteopetrosis-like disease occurs sporadically in cattle as a result of congenital infection with bovine viral diarrhea virus (BVDV). Inherited osteopetrosis was relatively common in American Angus cattle in the 1970s, but has largely disappeared. We report the emergence of congenital osteopetrosis in Red Angus cattle with laboratory confirmation of 10 cases in three states (KS; MO; WY) since 2006.

Two widely-used related sires were identified as presumptive carriers and of the ten affected Red Angus calves; five were the result of embryo-transfer from one superovulated presumed carrier dam following sire-daughter breeding. Calves were small and either premature or stillborn. The following gross changes were present: brachygnathia inferior, impacted molars, short long bones and/or vertebrae, medullary cavities filled with bone; thick calvarium; malformed and focally compressed cerebral hemispheres; posterior herniation of cerebellum. The primary histologic lesions were medullary cavities filled with primary spongiosa, and reduced numbers of osteoclasts. The 10 affected calves were negative for bovine viral diarrhea virus by viral isolation, immunohistochemical staining and/or fetal serology.

The trait responsible for osteopetrosis in Red Angus cattle has not been identified. DNA from affected calves is being used for gene discovery and assay development. **The purpose of the presentation is to remind diagnosticians of the characteristic appearance of osteopetrosis in Angus cattle.** Until the putative osteopetrosis gene is characterized, we propose the following diagnostic criteria for the disease in Red Angus cattle: 1. Defective osteoclast function manifested as marrow cavities filled with endochondral bone. 2. Prematurity or stillbirth. 3. Brachygnathia inferior. 4. Genetic relationship to defined carriers (http://redangus.org/genetic-defects/carriers/). 5. Negative for BVDV.

An outbreak of *Mycoplasma bovis* infection in a herd of American bison (Bison bison)

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A disease outbreak of high morbidity and high mortality in bison was investigated. The bison had clinical signs of lameness, swollen joints, respiratory distress and lethargy. Fifty three out of 193 animals died (27.5 %). The cows between 5 to 10 years of age were the most affected group (40 out of 88: 45.5 %). Several animals were necropsied. There were abscesses in the lung and liver, fibrinosuppurative pleuritis and polyarthritis, and disseminated microabscesses in various organs. No significant bacteria were isolated by routine aerobic cultures from lung and liver abscesses from two representative cases. However, PCR assays using lung and synovial fluid were positive for *Mycoplasma bovis*. Histologically, the lesions were characterized by areas of necrosis with variable mineralization rimmed by granulomatous inflammation and bands of fibrous connective tissue. No new animals were introduced into the herd in the last four years. The bison isolate was compared to two field isolates of *M. bovis* from cattle and a laboratory control strain of *M. bovis* by two restriction fragment length polymorphism typing techniques, and found to be identical. Further, the 16s ribosomal DNA was amplified from all the three bacteria and the amplified product was cloned into pCR8/GW/TOPO TA plasmid vector. **The 16s ribosomal DNA sequences of the bison isolate, *M. bovis* from cattle and the laboratory control were compared and found to be more than 99% homologous**. We think that a cattle herd adjacent to the bison herd was the most likely source of infection. **We conclude that in bison, *M. bovis* can cause disseminated infection with a high morbidity and mortality, and the bison isolates are similar to bovine *M. bovis* isolates.**

Pathologic characteristics of alpaca acute respiratory distress syndrome

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Between June and November 2007, a large number of alpacas throughout the US experienced a syndrome of acute respiratory distress that largely remains without a specific viral etiology. The clinical disease varied from subclinical, mild infection to severe distress with fatalities. Total of eight alpacas submitted to Colorado State University, Veterinary Diagnostic Laboratory during that period fit the clinical criteria. Out of these eight alpacas, six females in late gestation succumbed to the infection with similar histologic lesions that varied in both of their severity and duration. Gross lesions comprised acute pulmonary congestion and edema with pleural effusions and acute to chronic interstitial pattern. Histologic lesions consist of any combination of the following lesions in any given animal: fibrin deposition/hyaline membrane; bronchoalveolar junction necrosis/hyperplasia; pneumocyte type 2 hyperplasia/giant (syncytial) cells; alveolar leukocytosis predominantly macrophages/neutrophils; vasculitis with perivascular hemorrhage; equivocal eosinophilic cytoplasmic inclusion bodies; and finally atelectasis with multifocal fibrosis. Testing for blue tongue (BT), epizootic hemorrhagic disease (EHD), and corona virus was negative. No significant bacteria were isolated from the affected lungs.

Isolation of *Helcococcus ovis* from sheep with pleuritis and bronchopneumonia

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Helcococcus ovis is a newly established species in the genus *Helcococcus*. The clinical significance of this organism in sheep has not been reported. Here we report isolation of *H. ovis* from a 6-month-old mix-breed ewe lamb that died of respiratory disease. Pathologic examination revealed severe, focally extensive, chronic necrotizing pleuritis with intralesional coccobacilli and mild, multifocal, subacute mucopurulent bronchopneumonia, indicating a bacterial etiology. *H. ovis* was isolated in heavy growth from the lung tissue. **This is the first report of isolation of *H. ovis* associated with infection in sheep with pleuritis and bronchopneumonia**.

Unusual encephalopathy in weaned lambs: a consequence of water deprivation?

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Following the detection of an unusual encephalopathy in weaned lambs in several flocks, the records of the histology archive at VLA Lasswade from 1994-2007 were searched for ovine submissions meeting the case definition of (1) histological lesions of middle laminar cerebrocortical neuronal necrosis and white matter vacuolation and (2) absence of autofluorescence of cut surfaces of brain when exposed to ultraviolet light. The case records of these cases were analysed, where available, for age of onset, morbidity / fatality rates, nature and duration of clinical signs, response to treatment and possible predisposing or causative factors. Fourteen submissions meeting the case definition were recorded from 11 flocks. All cases occurred in crossbred weaned lambs. Detailed information was available for 6 flocks.

Flock	Total number of lambs	Number affected (%)	Number died (%)	Number humanely killed	Management
A	150	14 (9.3%)	0	1	Pasture
B	200	10 (5%)	5 (2.5%)	1	Housed
C	446	51 (10.9%)	0	1	Housed
D	105	5 (4.8%)	0	2	Pasture
E	16	1 (6.3%)	0	1	Housed
F	110	10 (9.1%)	0	2	Housed

Blindness was the main clinical sign in flocks A, B, C, E and F. In flock D, affected lambs were dull, depressed, head pressing and teeth grinding and one showed signs of blindness. Lambs in flocks C and F were treated with moxidectin at time of housing. Parenteral administration of B vitamins including thiamine, shortly after the onset of clinical signs in flock C and at unknown intervals in flocks D and F, had no clinical effect. Lambs in flocks E and F had liver Vitamin B12 levels below the reference range but no histological evidence of ovine white liver disease. Similar clinical signs and histological findings were reported in lambs by Scarratt *et al* (1985) and by Jeffrey and Holliman (1990) within 48 hours of housing, in the former case following lack of water for 24 hours. For one flock, the submitted history stated that water was not available for the initial period of housing in a shed recently treated with creosote. Definite evidence of lack of water supply was not obtained for the other flocks described here. Clinical signs were noted in flocks B, C, E and F within 2-4 days of housing and the farmer stated that lack of familiarity with the ball-valve drinkers in flock B may have resulted in inadequate water intake. Drinking water for flock A was supplied via cattle troughs which may have impeded access to water, and the water supply for flock D was very likely to have frozen in the days prior to observation of clinical signs. **The observations suggest that water deprivation is the most likely aetiology, possibly associated with lack of familiarity with the means of water supply at housing.** Osweiler and others (1995) suggested assessing aqueous humour sodium ion concentration may aid the diagnosis of water deprivation / salt intoxication in cattle. As water deprivation was not suspected from the initial histories, sodium ion levels were not determined in these lambs. Analysis of sodium ion concentration may be a useful diagnostic aid in lambs developing blindness after housing.

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Aerosol inoculation of opossum (*Didelphis virginiana*) and experimental lateral transmission of *Mycobacterium bovis*

Karla Mesterhazy[1], Scott Fitzgerald[1], Steve Bolin[1], John Kaneene[2], John Kruger[3], James Sikarskie[3], Konstantin Lyashchenko[4]

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Introduction. The *Mycobacterium tuberculosis* complex consists of five species, included within the complex is *Mycobacterium bovis (M. bovis)*. This complex is able to produce tuberculosis in a wide range of species including humans, thus making research, surveillance, and control of *M. bovis* important in the eradication of tuberculosis. An endemic focus of *M. bovis* in northern Michigan is contributing to a regional persistence in wild and domestic animal populations. This study investigates the role of the opossum (*Didelphis virginiana*) as a wildlife reservoir. Opossums are a known host of tuberculosis in the state of Michigan and previous studies have shown them to be susceptible to *M. bovis* by aerosol inoculation. This project aims to answer whether or not wild opossum are contributing to disease spread by assessing intra-species lateral transmission after aerosol inoculation.

Materials and Methods. One wild caught, pregnant female opossum bearing eleven pups and one additional male pup outside of this litter was obtained. Four pups received aerosol inoculation of *M. bovis* (inoculated), four served as non-inoculated in-contact pups (exposed), and four pups plus the mother were housed separately (controls). Animals were monitored daily and offered a commercially available dry cat food and water ad lib with weekly supplements of apples or moist cat food. Weight measurements were taking every two weeks until the animals were sacrificed. *M. bovis* was administered at a concentration of 1×10^6 cfu via nebulization to approximately ten week old, sedated, laterally recumbent, ear notched pups for ten minutes (five minutes per side). Inoculated pups were housed separately for one week prior to the forty-five days of co-habitation in a BL-3 Horsfall isolator. One non-inoculated (exposed) pup was housed with one inoculated pup making four replicate groups. At day eighty-four post inoculation or post exposure, pups and controls were sacrificed. A complete post-mortem examination was performed. Serum was collected and sent for antibody testing via the rapid test (RT). All major organs were weighed, sampled for *M. bovis* culture, and trimmed for histopathology. Slides were stained with H&E and Ziehl-Neelsen's acid-fast stain followed by light microscopy examination.

Results. On gross and histological examination all inoculated opossums had marked, multifocal, granulomatous pneumonia. The average bi-weekly weight gain between each group was not remarkably different, inoculated (425g), exposed (385g) and controls (502g). The percent total body weight to the lung, liver and kidney were almost twice as much in the inoculated group when compared to the other two groups. Identification of *M. bovis* in the lung was successful in all the inoculated opossums and was additionally identified in the liver, kidney, and spleen (pooled sample) in half of the inoculated group. Antibody was detected only in the inoculated group to the RT antigen cocktail (ESAT-6, CFP10, Acr1, MPB83).

Discussion/Conclusions. In conclusion, the inoculated group was infected with *M. bovis* and confirmed by gross, histological, culture and antibody tests. However, *M. bovis* was undetectable in the control and exposed groups. **We conclude that there is no significant lateral transmission after aerosol inoculation of *M. bovis* between wild opossum; therefore there is little risk for natural spread of the disease between individuals of this species.**

Tritrichomonas Scientific Session

Saturday, October 25, 2008

Guilford-E

Moderator: Scott McVey
James Kennedy

* Graduate student presentation

Evaluation of a real-time PCR test for *T. Foetus* on 1300+ samples utlizing a crude DNA extract of preputial smegma and trichomonas culture media

Lee Effinger and Julie Weikel

Oregon Department of Agriculture, Animal Health Division, 635 Capitol Street NE
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Between September 2007 and June 2008 the Oregon Department of Agriculture tested crude DNA extracts from1309 samples of either direct smegma (N=302) or smegma/preputial wash incubated in trichomonas culture media (In-Pouch™ or Diamond's media) (N=1007) utilizing a real time PCR assay.

The PCR assay is a *T. foetus* specific 5' Taq nuclease assay using a 3' minor groove binder-DNA probe targeting conserved regions of the internal transcribed spacer (ITS-1) region as outlined by McMillen and Lew (Vet. Parasitology 141 (2006) 204-215).

DNA was extracted from both types of samples (1 ml) using a crude extraction technique involving centrifugation and heat lysis.

Samples from 40 different herds were tested. 13 of these herds had the same animals sampled on multiple dates (N=234 bulls).

PCR results on trichomonas culture media post incubation agreed with the culture reading result of the same media on 96.2% of the samples **(**N=969/1007**).** 13 samples read as positive by culture were negative by PCR. 9 of these 13 were confirmed to be Tetratrichomonas by traditional gel electrophoresis and RFLP. 3 others were either not able to be identified by traditional methods or additional testing was not performed. This discrepancy between the culture reading and the real time PCR indicates that this PCR is capable of distinguishing between true *T. foetus* infection and colonization with at least one common commensal trichomonad. One sample which was culture read positive was negative using the crude DNA lysate. This same sample, upon extraction and cleanup using the Qiagen DNA easy extraction kit, was positive with this real time PCR. 25 samples were positve by PCR but were interpreted as negative by culture. Of these 25, six were confirmed as T. foetus by traditional gel electrophoresis and/or RFLP. When the confirmed commensal trichomonads and the confirmed *T. foetus* samples which were culture read negative are factored in, **the real time crude extract DNA PCR was 97.7 % effective in identifying *T. foetus* infected bulls compared to culture and/or isolate identification.**

235 smegma samples were collected and approximately 1 ml used to inoculate trichomonas culture media. The remaining smegma was taken to the laboratory and tested within 48 hours of collection by PCR. The trich culture media was incubated and read at various trich testing sites within the state. After completion of culture and determination of status by the reading site, the culture media was sent to the ODA lab for PCR testing. **PCR results on the direct smegma done within 48 hours of collection agreed with the PCR result of the same sample incubated in culture media in 98.3% of the samples. Preliminary data utilizing *T. foetus* specific real time PCR indicates its usefulness in rapidly detecting *T. foetus* infected bulls utilizing crude DNA lysates from direct smegma.**

Additional experiments to address DNA extraction methodology and the utilization of an Internal Positive Control are anticipated to provide an even more reliable and sensitive assay for rapid detection of *T. foetus* infected bulls.

Serial sampling and comparative testing of bulls for *Tritrichomonas foetus* in two infected Nebraska beef herds by culture, real time PCR and gel PCR

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Introduction. An apparent epizootic of *Tritrichomonas foetus* (TF) venereal protozoal infections in US cattle herds is driving increased demand for and utilization of TF diagnostic assays in both veterinary clinics and veterinary diagnostic laboratories. Three consecutive TF negative cultures on preputial scrapings collected at least one week apart in sexually rested bulls is the currently recommended diagnostic standard to define TF-negative bulls. PCR using gel-based or real time amplicon detection has recently become available as a TF diagnostic option. Under the data-supported assumption of greater diagnostic sensitivity and lower target detection limit of a single PCR versus a single culture, several states currently may accept a single PCR negative test result or three consecutive negative cultures to define bull TF-free status for regulatory animal health purposes. To our knowledge, no data is available that compares serial three-sample culture versus three-sample PCR TF diagnostic findings.

Materials and Methods. Preputial scrapings were collected three times at approximately weekly intervals in Spring 2008 from 58 non-virgin herd bulls in two large Nebraska cow-calf operations experiencing severe 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 days at 37^0C using the In-Pouch^{TM} TF system with daily 100X microscopic inspection. Aliquots of each 4 day old culture were then subjected to gel-based (g) and real-time (rt) PCR. Both the gPCR and the rtPCR targeted the same TF-specific 5.8S ribosomal RNA and internal transcribed spacer (5.8S-ITS) region of the genome. For each TF assay, a bull was classified as TF-positive if one, two, or three samples were test-positive. Samples were coded to blind analysis until study completion.

Results Forty three of 58 bulls were culture, gPCR and rtPCR negative on all three serial samples. Fifteen bulls were TF-positive one or more times by one or more of the three TF assays. On the initial bull sampling, eight, eight and nine bulls were TF-positive by culture, gPCR and rtPCR, respectively. Based on all three samplings, 11, 13 and 12 bulls were classified as TF-infected by culture, gPCR and rtPCR, respectively. Only seven, eight and five bulls were consistently positive on all three samplings by culture, gPCR and rtPCR. Two bulls each were uniquely positive by gPCR and rtPCR, with test positivity occurring once in each bull (first sampling for one bull, second sampling for three bulls).

Discussion. There was good overall agreement in how the three TF assays classified bulls based on three serial tests. However, use of either a single gPCR or rtPCR in place of three serial cultures would have resulted in three culture-positive TF infected bulls being incorrectly classified as TF-free (i.e., false negative bull status). These findings suggest that TF control at the herd or state level may be compromised if results of a single PCR assay are used to define individual bull TF status, especially if bulls originate from infected herds.

Conclusion. More than one sample (eg three serial samples) should be collected and tested from high risk bulls regardless of whether culture, PCR or culture and PCR are used for TF diagnosis.

Evaluation of a real-time PCR assay of pooled specimens for the detection of *Tritrichomonas foetus* infection

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Tritrichomonas foetus is a sexually transmitted pathogen of naturally bred cattle that is known to cause infertility and abortions. This protozoan parasite has a predilection for the preputial folds and crypts of the bulls and is transmitted to the cows during coitus. Although culturing and microscopic visualization is the routine test for detection of *T. foetus*, PCR based detection has a greater sensitivity, specificity, and reduced turn-around-time than culture. However, PCR testing of individual specimens is relatively more expensive than culture, and therefore is a constraint for testing of herds for surveillance, control and eradication of *T. foetus*.

In this study, our goal was to evaluate the sensitivity of real-time PCR based detection of *T. foetus* in pooled specimens compared to individual specimen real-time PCR results using the same set of specimens. In all, 1255 submitted specimens (preputial washes received in InPouch media) were included in this study. The specimens were incubated at 37°C for 48 hours prior to screening for the presence of *T. foetus* DNA by a 40-cycle real-time PCR assay according to the method of McMillen *et al* (Veterinary Parasitology, 2006). The specimens were tested individually and in parallel by randomly pooling upto five specimens per group, thereby producing 251 pools. A cut-off Ct value of 38.5 and below was used as the criterion for considering a specimen to be positive upon real-time PCR. **Of the 251 pools, 43 (17.13%) were positive and 201 pools (80.08%) were negative for the presence of *T. foetus* DNA**. All of the individual specimens which made the 201 negative pools were tested by PCR and found to be negative. When individual specimens belonging to the 43 positive pools were tested; one, two, three, four, or five positive specimen(s) were present in the various pools. Of these 43 positive pools, 30 pools had one positive, 3 pools had two positive, 4 pools had three positive, 3 pools had four positive and 3 pools had five positive specimens**. Comparison of the PCR results on the pooled specimens (5 specimens per pool) with the individual specimen PCR results revealed a sensitivity of 100% and specificity of 97.21%.** In resource-limited settings and large scale epidemiological studies of *T. foetus* infections, pooling of specimens could be cost-effective compared to individual specimen testing by PCR, depending upon the prevalence of the disease in the population.

Comparative evaluation of two real-time PCR assays for the detection of *Tritrichomonas foetus* infection in cattle

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Trichomoniasis is a sexually transmitted reproductive tract disease of cattle caused by the flagellate protozoan parasite, *Tritrichomonas foetus*. Bulls harbor the protozoa in the preputial folds and crypts and transmit the organism to cows during breeding. *T. foetus* infection leads to reduced fertility, abortion, and decreased calving rates, causing significant losses to the cattle industry. Culturing and microscopic visualization of the live organisms in the preputial washes/scrapings is considered to be the gold standard test for the diagnosis of bovine trichomoniasis. Conventional PCR and real-time PCR assays have improved the sensitivity and rapidity of *T. foetus* detection.

In this study, our goal was to evaluate two different real-time PCR assays and perform inter-laboratory comparisons by testing at TVMDL and NMDA-VDS laboratories. The TVMDL internal transcribed spacer-1 (ITS-1) target detection real-time assay was based on a recent publication by McMillen *et al* (Veterinary Parasitology, 2006), whereas the NMDA-VDS 18S rRNA target detection real-time assay was developed in-house. Both assays have established sensitivities below one organism (due to multiple gene copies present in a single cell). Therefore, we were interested in determining if there would be differences in results when the two methods were utilized in testing identical specimen extracts. Both laboratories tested 188 clinical specimens that were submitted to the laboratories during routine testing. **Of these samples, 62(32.97%) were determined positive by TVMDL and 59(31.38%) were positive by NMDA-VDS.** The 5.31% variation between the two labs can be attributed to differences in nucleic acid extraction procedures, the real-time PCR procedures, or to post-run analysis and interpretation. **In summary, two real-time PCR assays utilizing different primers and probes were run in parallel in two separate labs. Results demonstrated a high correlation of sensitivity and specificity between the two PCR assays. Therefore, based on this information both real-time PCR assays can serve as a useful, accurate and rapid screening tool for the detection of bovine *T. foetus* infections.**

Comparison of direct microscopic examination of In-Pouch™ bags and real time PCR for detection of *Tritrichomonas foetus*

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Requests for *Tritrichomonas foetus* testing have increased substantially at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) over the past few years. This increase in testing is due to several things, including requirements for negative test results before an animal can be imported into certain states or countries, and the diagnosis of *T. foetus*-associated decrease in reproductive efficiency (repeat breeding, increased calving interval) in at least one beef herd in Oklahoma.

Traditionally, culture of *T. foetus* has required that samples be inoculated directly into appropriate culture medium (such as In-Pouch™ bags, Biomed Diagnostics, White City OR) and be transported to the laboratory as quickly as possible and such that the samples are not subjected to temperature extremes. Once in the laboratory, pouches are incubated at 37C, and each pouch is examined under low power microscopy several times (generally days 2, 5, and 7) until the pouch has been incubated for a total of 7 days. Depending on the importation requirements, a total of 3 separate tests may be required on each animal, resulting in at least 3 weeks' delay until the animal can be moved. Collection of 3 samples from some animals can be challenging for the practicing veterinarian, and visual examination of the pouches is labor intensive and time consuming for the laboratory. Additionally, diagnostic sensitivity of this method may be low, and is dependent on many factors from collection of an appropriate sample, appropriate sample handling/transport to the laboratory, and diligence of the person performing microscopy to detect organisms. A more sensitive, less subjective test such as a PCR-based test may help increase diagnostic sensitivity and decrease time to detection of infected animals.

A protocol for real time PCR was obtained from Texas Veterinary Medical Diagnostic Laboratory-Amarillo. Samples submitted to OADDL for *T. foetus* culture were examined microscopically as usual; in addition, aliquots of the In-Pouch™ fluid were removed each time the pouches were examined (unless an adequate volume of fluid was not present, in which case an aliquot was removed at day 7 only). The aliquot was centrifuged, supernatant removed and the sample stored frozen until processed for real time PCR. All day 7 aliquots were analyzed by real time PCR; any positive samples' previous aliquots were also analyzed (positive by either microscopy or real time PCR).

To date, 116 samples have been tested by both culture and real time PCR. Six samples were positive by real time PCR (day 7 and day 2 aliquots); three of these samples were positive by direct microscopic examination. One hundred ten samples have been negative by both direct exam and real time PCR. **These results indicate that real time PCR may be more sensitive for detection of *T. foetus* than microscopic exam, both in detecting organisms as soon as day 2 and detecting positive animals that are considered to be negative by microscopy.**

Comparison of two commercial DNA extraction kits and crude heat lysates for the detection of *Tritrichomonas foetus* by conventional and real-time PCR

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Rapid, sensitive, cost effective, high throughput assays for the detection of bovine venereal trichomoniasis are essential for federal control/eradication programs. Many currently employed molecular based detection methods require the use of costly, labor intensive DNA extraction methodology. The purpose of this study was to investigate limits of detection of *Tritrichomonas foetus* following high throughput magnetic bead extraction methodology (Ambion) and standard spin column extraction (Qiagen) of *T. foetus* in Diamond's medium. Additionally, limits of detection of a real time PCR assay for T foetus from crude heat lysates of preputial washes as compared to standard nucleic acid extracts was also investigated. Samples tested following DNA extraction with commercial methodology consisted of serially diluted *T. foetus* spiked with freshly collected pooled, *T. foetus* negative preputial washes and pooled preputial washes previously cultured for four days in Diamond's medium. Controls included log serial dilutions of *T. foetus* in Diamonds media. Extracted DNA from treatment groups were screened utilizing conventional PCR for amplification of the conserved regions of the 5.8S rRNA gene and ITSRs for *T foetus* and *T. species* detection. Amplicons were detected by capillary electrophoresis on the Agilent bioanalyzer. **The detection limits of laboratory cultured *T. foetus* determined by conventional PCR assays were similar with the two extraction methodologies (limits of detection is 100-10 cells/ml). The addition of fresh pooled preputial washes or four day cultured preputial washes resulted in one to two logs reduction in sensitivity of detection by conventional PCR (limits of detection is 1000-100 cells/ml).**

For determination of real time PCR detection of *T. foetus*, crude extracts consisted of serially diluted *T. foetus* suspended in uncultured, pooled *T. foetus* negative preputial washes. Controls included serially diluted *T. foetus* in PCR grade water. Extracted DNA from experiments described above and crude lysates were screened in triplicate with a quantitative real time Taqman PCR assay for the detection of *T. foetus*. Additionally, a comparison of the sensitivity limits of conventional PCR and real time PCR detection assays were performed with DNA extracts. **Detection of *T. foetus* with the a quantitative real time Taqman PCR assay improved detection by 1-2 logs over conventional PCR and the addition of fresh pooled preputial washes or PCR detection of crude lysates yielded the best sensitivity (10-1 cells/ml). Results validate the use of magnetic bead extraction methodology for high throughput robotic detection of *T. foetus* with either conventional or real time PCR. Initial equivalency data on detection limits of real time PCR following crude and commercial extraction methodology is also provided.**

Development and validation of a high resolution RFLP map for the identification of non-*Tritrichomonas foetus Protozoa* from Bovine preputial samples

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Recent advances in rapid molecular diagnostic techniques such as real time PCR for the detection of *Tritrichomonas foetus* have improved sensitivity and turn around time over conventional culture methods. However, the presence of non-*Tritrichomonas foetus* protozoa following conventional culture can result in false positives that lead to discrepant results when molecular techniques are utilized as confirmatory tests. Moreover, discrepant results complicate evaluation of sensitivity and specificity of novel molecular assays and may lead to confusion and reduced confidence of new detection methodologies with veterinarians, regulatory officials, and animal producers and subsequent delay in the implementation of more sensitive molecular based assays for diagnosis of venereal Trichomoniasis. PCR amplicon base pair size, RFLP analysis, and DNA sequencing currently are utilized for resolution of discrepant results. The confidence and cost of accurate identification of non-*T. foetus* protozoa utilizing these methods varies and is additive. To resolve discrepant results quickly, affordably and with high confidence, **we incorporated three methodologies to develop and validate a high resolution RFLP (HR-RFLP) map for commonly encountered non-*T. foetus* protozoa in diagnostic samples from three states (Idaho, Oregon and Utah).** This map is derived from conventional PCR amplification with primers that amplify the conserved region of the 5.8S rRNA gene and ITSRs of trichomonadid protozoa (TFR1 and TFR2) resulting in an amplicon which is subsequently digested with restriction enzyme HpyCH4IV. Instead of conventional agarose gel electrophoresis, we employed high resolution capillary electrophoresis utilizing the Agilent Bioanalizer for rapid (30 minutes), accurate DNA fragment size determination to within 2-5 base pairs utilizing only 1 ul of digested amplicon. Direct DNA sequencing of the PCR amplicon was employed to definitive identification of genus and species and validate the HR-RFLP map. The map contains fragment data from The PCR control Tritrichomonas foetus, *Pentatrichomonas hominis* and various *Tetratrichomonas species*. Utilizing this method, the *T. foetus* PCR amplicon of approximately 375 bp yields two fragment of approximately 230 and 151 bp, *P. hominis* PCR fragment of approximately 338 bp yields 3 fragments of approximately 160, 132 and 42 bp. Data from various *Tetratrichomonas species* yielded generally two fragments of varying bp sizes which will be presented based on genetic relatedness and PCR amplicon size. **Implementation of this HR-RFLP map will aid in 1) the resolution of discrepant diagnostic samples and confirmation of suspect diagnostic samples following PCR, and 2) the explanation of discrepant samples when determining the sensitivity and specificity of newly developed molecular techniques aimed at near immediate detection to *T. foetus* from bovine preputial samples.**

A decrease in the prevalence of bovine trichomoniasis in New Mexico correlates with the implementation of mandatory molecular based testing

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Trichomoniasis is a sexually-transmitted disease of cattle, and is caused by the protozoan parasite *Tritrichomonas foetus.* Cows recover from the infection but can become reinfected by breeding to chronic carrier bulls. Infection with *T. foetus* can decrease fertility and subsequent calving rates. These factors, combined with the culling of carrier bulls, result in significant production loss. Trichomoniasis is recognized with increasing frequency as a major problem in beef cattle in most western states. Screening for infected individuals has become a requirement in many states and for importation into Mexico. In 2006 the implementation of mandatory testing for bovine trichomoniasis in NM generated a need for a sensitive, rapid, and high throughput method of testing. The initial testing method developed at NMDA/VDS was a standard PCR that used published primer sequences specific to *T. foetus*, but the method was modified for higher throughput by transferring samples to a 96-well format. **Standard PCR is a major improvement over microscopic examination in sensitivity, specificity, and efficiency. However, the development of a real-time PCR assay for the detection of *T. foetus* in cattle provides a superior test to standard PCR by eliminating the need for agarose gels and providing a shorter amplification cycle with increased sensitivity.**

The results reported here are part of a validation study performed to enable the New Mexico Department of Agriculture Veterinary Diagnostic Services (NMDA/VDS) to bring on line a novel real-time PCR assay with an internal positive control (IPC). The IPC included in the new method ensures the differentiation between a true negative test and a failed PCR reaction, further reducing the rate of false negative results due to inhibition. The new real-time PCR assay has increased sensitivity over standard PCR and microscopic examination. Real-time PCR can detect 0.125 organisms per reaction compared with 1.25 organisms per reaction detected by standard PCR and microscopic examination can miss positive samples that can be detected by PCR (of 100 preputial samples tested, 7 positive samples were detected by microscopic examination compared with 20 positive samples detected by real-time PCR) and increased specificity over microscopic examination (it is difficult to speciate Trichomonads by microscopic examination alone).The validation study compares results for the same preputial samples tested by enrichment culture followed by microscopic examination, the standard PCR, and the new real-time PCR assay developed at NMDA-VDS.

In addition, we report the results of molecular testing of New Mexico cattle for *T. foetus* beginning in March of 2006 when mandatory testing was implemented through June of 2008. Initial testing showed a 6.3% prevalence of infection (n= 4,545). As of June 2008, the prevalence of *T. foetus* infection is 2.8% (n=4,197). Given the demonstrated sensitivity advantage of RT-PCR over standard PCR we conclude that the decrease in the prevalence of *T. foetus* infection was NOT due to a change from standard PCR to RT-PCR as the validated testing method, but **rather that implementation of mandatory molecular diagnostics and subsequent improved management methods (e.g., culling of infected bulls) has led to better control of trichomoniasis in cattle in New Mexico. The decreased prevalence of bovine trichomoniasis in NM correlates with the implementation of mandatory molecular based testing.**

Virology Scientific Session

Saturday, October 25, 2008

Auditorium III

Moderator: Tim Baszler
Dick Hesse

* Graduate student presentation

Multiplex real-time PCR test to aid the diagnosis of calf diarrhea

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Calf diarrhea causes a significant economical loss to the bovine industry. Since multiple infectious agents can be involved in calf diarrhea and the detection of each of those causative agents by traditional methods is laborious and expensive, a multiplex real-time PCR was developed to identify the 5 most important bovine enteric pathogens [i.e., bovine coronavirus (BCV), group A bovine rotavirus, *Salmonella spp*, *Escherichia coli (E. coli)* $K99^+$, and *Cryptosporidium parvum*]. Then, the multiplex PCR was validated and optimized in comparison with other traditional diagnostic tests (individual PCR for BCV; antigen-capturing ELISA for rotavirus group A; bacterial culture and latex agglutination test for *E. coli* K99; bacterial culture after pre-enrichment and serotyping for *Salmonella spp*; microscopic exam with special staining for *Cryptosporidium*) using 243 fecal or intestinal samples archived from previous submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) or experimental animal inoculation studies. In addition, the test was used on 201 fecal samples submitted to ISU-VDL between March and May 2008 (i.e., spring calving season) to assess the significance of the 5 agents in calf diarrhea.

The newly developed bovine diarrhea multiplex real-time PCR simultaneously detected all 5 target pathogens directly from fecal or intestinal samples and was more rapid and sensitive than the traditional tests. The test showed 90-97% agreement with other conventional diagnostic tests. The estimated detection limit of the multiplex PCR was 0.05 $TCID_{50}$/ml for BCV and rotavirus, 5 and 0.5 CFU/ml for *E. coli* $K99^+$ and *Salmonella* respectively, and 50 oocysts/ml for *Cryptosporidium*. Among the 201 fecal samples tested during the 2008 spring calving season, 137 samples (68%) were positive for at least one of the 5 agents: BCV was detected in 66 samples (33%); bovine rotavirus group A was detected in 62 samples (30%); *Salmonella spp*. was detected in 12 samples (6%); *E. coli* $K99^+$ was detected in 22 samples (11%); *Cryptosporidium* was detected in 63 samples (31%). In addition, more than 2 agents were simultaneously detected in 53 samples (39%) out of the 137 positive samples.

In conclusion, the multiplex real-time PCR can be a tool for a timely and accurate diagnosis of calf diarrhea associated with BCV, bovine rotavirus group A, *E. coli* ($K99^+$), *Salmonella* and/or *Cryptosporidium*.

Pan-viral diagnostic microarrays for the identification of unknown agents.

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Introduction. Foreign animal and emerging infectious diseases represent threats to public and animal health and present a significant challenge to the diagnosis of disease. A classical diagnostic approach is to assess epidemiological findings to limit the differential list of suspect diseases and then run specific diagnostic tests for those pathogens. Such an approach relies heavily on previously identified disease manifestations and antigenic or genetic properties of known pathogens. In emerging diseases, pathogens have diverged significantly from known agents such that they may manifest different clinical pictures and/or may be difficult to isolate or detect by standard methods. While such instances are rare, they are quite confounding, and there exists a need for broader methods of detection in order to identify divergent or emerging pathogens. To aid in such complex animal disease investigations, we have designed pan-viral DNA microarrays capable of detecting emerging viruses and foreign animal disease viruses.

Materials and Methods. A multi-tiered bioinformatics search of the more than 540,000 viral nucleotide sequences present in GenBank was used to design a comprehensive pan-viral microarray consisting of approximately 12,000 different virus family, genus or species specific oligonucleotide features. The pan-viral microarrays (FADDL PanVira4) were then commercially synthesized by Combimatrix Corp. Two cases of unknown etiology were analyzed using the Combimatrix 12K arrays. Samples were randomly amplified by reverse transcriptase polymerase chain reaction (RT-PCR), and indirectly labeled with Cy3 and Cy5 dyes. Microarrays were hybridized based on a modified protocol, as described by D. Wang et. al., 2002. Hybridized arrays were scanned using a GenePix 4200AL scanner and GenePix Pro software (v.6.1).

Results. Microarrays with Cy5 labeled samples from "infected" animals and Cy3 labeled controls were found to have divergent hybridization patterns. Here we describe a case which was found by microarray to be co-infected with two unique viruses. The data were found to be comprised of primarily two probe populations from two distinct families. The first population was found to be members of the *Flaviviridae* family. Within this grouping, the majority were found to be probes specific to bovine viral diarrhea virus (BVDV). The second grouping was a smaller set of probes from the *Paramyxoviridae* family, and was found to primarily consist of probes specific to bovine parainfluenzavirus-3 (PI3). Viral nucleic acid was recovered from the microarray, and PCR utilizing primers based on the sequence of positive features resulted in a product of the expected size (~800bp). Sequence analysis confirmed that the sample contained PI3.

Discussion/Conclusion. Microarrays and genomic techniques for the identification of unknown pathogens have been gaining interest. Although the current systems do not replace traditional diagnostic techniques, they have proven to be a powerful and valuable complement to standard diagnostic. The viruses identified in the microarray analysis were consistent with clinical signs of animals co-infected with BVDV and PI3

Diagnostic investigation of acute respiratory syndrome in alpacas

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In the spring of 2007 alpaca producers began noting cases of an acute respiratory disease affecting alpacas nationally. The clinical presentations ranged from mild upper respiratory disease with influenza-like presentation to severe respiratory disease resulting in death. Though referred to as a viral disease, an etiologic agent was not identified. The syndrome is variously referred to as Acute Respiratory Syndrome (ARS), Acute Respiratory Distress, or "the snots" within the alpaca industry. In the fall of 2007, a cluster of cases were reported, anecdotally linked to alpacas returning to home farms from one or more regional shows. The disease at that time included respiratory signs affecting females in contact with the alpacas returning from shows, increased severity with high mortality among pregnant females, with some associated stillbirths or premature deliveries. In the spring of 2008, reports of abortion and weak births in females that had reported cases of ARS in the prior year began surfacing. Full-diagnostic work-ups were performed on cases submitted to the California Animal Health and Food Safety Laboratory during the fall outbreak. Necropsy findings, generally reported marked diffuse acute to subacute bronchointerstitial to interstitial pneumonia with hyaline membrane formation, marked terminal airway and alveolar epithelial hyperplasia, interstitial lymphocytic infiltrates. Microbial and Mycoplasma cultures were negative. A combination of immunohistochemistry, PCR, and serology were used to rule-out BVD viruses, BRSV, Bovine Herpesvirus, Bovine Coronavirus, Bluetongue virus, Influenza virus, Equine herpesvirus 1 and 4, West Nile virus, Paramyxovirus, and Chlamydia. Though micro-array analysis was attempted, insufficient quantity and/or quality of RNA and DNA were available from the tissue samples available, and no results were obtained. A Coronavirus was recovered from lung tissue using CRFK cell cultures. The virus was not recovered using any of the equine, bovine, human, primate, rabbit, and camelid cell lines attempted. The virus was identified by transmission Electron Microscopy, and confirmed as a Coronavirus by sequence analysis of the RNA dependent RNA polymerase. **The Coronavirus recovered is genetically distinct from the Coronavirus previously reported to cause diarrhea in New World camelids.** In the absence of fulfilling Koch's postulates, and to test for an association between antibody response and disease, serum was obtained from alpacas in ARS affected-herds (n= 37) and alpacas (n = 144) in herds with no history of ARS. **Serum virus neutralization antibody titers, using the isolated alpaca Coronavirus as virus, demonstrated that animals in herds with a reported history of ARS are approximately 50-fold more likely to be positive for antibody to the Coronavirus (OR = 49.3, 95% CI: 18.127,134.097, p<0.001).** Though this work is preliminary, and the recovered Coronavirus has not been definitively linked to ARS or possible-associated reproductive risks, the virus recovered is reported here to generate interest in additional diagnostic investigation and epidemiologic follow-up, ultimately to assist in the understanding and future diagnosis of ARS in alpacas.

Rapid detection of *Classical swine fever virus* in blood by real-time RT-PCR: Evaluation, selection and optimization of commercial RNA extraction kits

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Classical swine fever (CSF) is a highly contagious disease of pigs and wild boar. The etiological agent of CSF is a pestivirus belonging to the family flaviviridae. CSF is considered a foreign animal disease and a high consequence pathogen in the US. Due to the highly infectious nature of CSFV and its naive status in the US swine herd, an outbreak of CSF could severely threaten animal health and cause large economic losses to agriculture and trade. Routine surveillance and early detection of the virus prior to the onset of clinical signs of the disease are crucial to control the spread of the disease. Real time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) is a highly sensitive molecular diagnostic test which has the potential to detect CSFV in pre-clinical animals. However the accuracy of rRT-PCR is compromised by many factors including genetic variability of the virus, sample quality, reagent integrity, and the presence of naturally occurring PCR inhibitors present in certain sample types.

In this study we used rRT-PCR to detect CSFV in whole blood (WB) from infected pigs. The RNA was extracted from WB as well as different fractions of WB including red blood cells (RBC), leukocytes (WBC) and serum, and analyzed by rRT-PCR to determine the relative distribution of the virus between each fraction. Eight different commercial RNA kits including six single tube-based kits and two magnetic bead-based kits were tested. The rRT-PCRs were carried out using the CSF assay developed at FADDL. The efficiencies between different extraction methods were compared based on the sensitivity of detection of Armored RNA (encapsidated synthetic RNA containing a target sequence specific for CSFV) or intact CSFV serially diluted in WB as starting materials.

CSFV was found to be readily detectible in all fractions of WB including RBC, WBC and serum. The presence of inhibitors in WB however was detected by rRT-PCR. None of the commercial RNA kits was able to completely remove PCR inhibitors from WB. To further improve the capacity of the commercial RNA kits to remove PCR inhibitors from WB we tested several modifications to the original protocols. The RNA extracted from WB using the modified protocols exhibited higher sensitivity of detection of the virus by rRT-PCR with cycle threshold (C_t) values 0.5 – 3 units lower as compared to the RNA extracted by standard methods. CSFV was detectable in WB on day 2 post inoculation (PI) in all animals (100%) infected with highly and moderately virulent virus Haiti and Brescia strains, respectively, and sporadically (one out of three animals from two separate studies) on day 1 PI in pigs infected with Haiti.

Results of this study indicate WB as the most appropriate sample for the detection of CSFV by rRT-PCR, although inhibitors for PCR are present. Use of smaller sample volume (30 □l) and additional washing steps including a high salt-EDTA wash were shown to improve the performance of the commercial RNA kits. The RNA kits that performed well with these modifications include the Viral RNA Mini Kit (Qiagen) and the MagMAX-96 Total RNA Isolation kit (Ambion). Based on the Ct values corresponding to the viral RNA extracted from different fractions of infected blood higher concentration of the virus was found in the WBC fraction followed by RBC and serum. The modified RNA extraction protocols described in this study may be useful for routine surveillance and early detection CSFV in swine.

Porcine high fever disease in Vietnam 2007; PRRS and other disease agents

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Introduction. Swine disease outbreaks causing high mortality, respiratory distress, high fever, constipation and diarrhea first occurred in the northern Province of Vietnam (bordering China) in March 2007. The disease spread rapidly to the central and southern Provinces by July. In the central Province alone, a total of 32,014 pigs were affected and culled and more than 8,000 pigs died from the disease. Tissues collected from 7 affected pigs in 5 districts from the northern and central Provinces were sent for analysis to FADDL, PIADC in collaboration with the FAO.

Materials and Methods. The diagnostic approach was to first rule out African swine fever (ASF) and classical swine fever (CSF), and to further investigate the presence of other viral or bacterial agents. Foot-and-mouth disease (FMD) and swine vesicular disease (SVD) were tested to ensure safe handling of specimens at NVSL in Ames, IA. For virus isolation (VI), specimens were inoculated on monolayers of swine primary peripheral blood macrophages (PBM), SK-6 (swine kidney cell line), LK (secondary lamb kidney), MARC-145, IBRS-2 and Vero cell line. A number of realtime and conventional PCRs were run on the original samples and positive viral cultures. Sequence analysis of PRRS virus (PRRSV) included the ORF6, ORF5 and nsp2 regions, and was conducted on original tissue and a pool of positive PBM cultures. Animal inoculation studies were conducted in the BSL-3Ag facility at PIADC to determine virulence of the isolated PRRSV and to identify other possible agents present in the original tissues. Pigs (4-6 weeks of age) were inoculated by different routes with either homogenate of original tissues, PRRSV positive PBM ($10^{4.5}$ $TCID_{50}$) or sham inoculated. Naïve pigs were introduced to each inoculated group 2 dpi (days post inoculation). Pigs were monitored for 28 days and samples were collected.

Results. Tissues were negative for CSF, ASF, FMD and SVD by VI and PCR. PRRSV was isolated from 7 of 7 pigs and identified by electron microscopy and RT-PCR. PCV-2 was detected by PCR from 4 pigs from the central Province. *Escherichia coli* and *Streptococcus equi* subspecies *zooepidemicus* were cultured from all samples and *Streptococcus suis* from one sample. Sequence analysis of PRRSV isolates showed close identity to the recently reported Chinese strains with the identical deletions in nsp2 region. Amino acid sequence in the nsp2 region revealed 95.7%-99.4% identity to Chinese strain HUN4, 68-69% identity to VR-2332 and 58-59% identity to strain MN184. Pigs inoculated with PRRSV and contact pigs developed persistent fever between 2-17 dpi and 2 of 5 pigs were coughing and had mild neurological signs and swollen joints. There were mild to moderate bronchopneumonia, enlarged lymph nodes, fibrinous pericarditis and polyarthritis. PRRSV was isolated starting at 2 dpi and PRRS antibody was first detected at 8 dpi. Homogenate-inoculated pigs developed high fever and septicemia, and acutely died within 72 hours. *Streptococcus equi* subspecies *zooepidemicus* was cultured and PRRSV was re-isolated. In addition, a subsequent submission from the central Province was positive for CSFV.

Conclusion. PRRSV and *Streptococcus equi subspecies zooepidemicus* were isolated and PCV-2 was detected from pigs presumed to be infected with porcine high fever disease in Vietnam. Animal inoculation studies showed that PRRSV caused high fever, respiratory distress and was transmissible to contact pigs. PRRSV isolates were sequenced and found to be nearly identical to a recent Type 2 Chinese isolate, having a similar deletion in the nsp2 region. It is proposed that the cause of the swine deaths in numerous parts of Vietnam is a combination of opportunistic pathogens where PRRSV is a major factor.

Western blot analysis for detection of recombinant NSs protein: An approach to determine DIVA status in ruminants for Rift Valley Fever

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Introduction. Rift Valley Fever virus (RVFV), a member of the Bunyaviridae family, is a mosquito-borne pathogen. The corresponding disease, usually affecting livestock, has been reported throughout sub-Saharan Africa and the Arabian Peninsula. Epidemics are characterized by widespread abortion-storms and high mortality in newborn animals resulting in significant economic losses to producers. Humans are also susceptible to RVFV infection primarily through mosquito vector transmission, which can be fatal.

The RVFV non-structural gene from the small genome segment (NSs) has been found to function as a virulence factor. The NSs protein product is responsible for down regulating host mRNA production resulting in reduced antiviral response of the host. Attenuated vaccines containing large deletions in the NSs gene have been shown to confer protective immunity in both cattle and sheep.
A current challenge in RVFV research is the development of an assay which will distinguish between infected from vaccinated animals (DIVA). Our hypothesis is that Western blot analysis against recombinant NSs protein will yield positive results when using sera from sheep previously infected with RVFV as the primary antibody source and may lead to the development of a DIVA companion diagnostic test for RVFV vaccines containing deletions in the NSs gene.

Materials and Methods. The recombinant NSs gene was expressed from either pCDNA3.1 (Invitrogen) or pQE9 (Qiagen) using COS-1 cells or *E. coli* M15 cells, respectively. Approximately 0.5-1 □g of purified NSs protein extract was loaded onto a 13% acrylamide gel for SDS-PAGE separation. Following electrophoresis, proteins were transferred to a nylon membrane (Amersham Biosciences) then blocked using 3% BSA in Tris-buffered saline containing 0.05% Tween-20 (TBST) for 3 hr. The source of primary antibody was sheep sera (1:7000 dilution; sheep sera #14 28 DAI) from a sheep infected with virulent RVFV ZH501 and was incubated with the membrane overnight at 4^{o} C. Following primary antibody incubation, the membrane was washed with TBST then incubated with HRP-conjugated rabbit-anti-sheep secondary antibody (1:12,000 dilution; ZYMED). Bands were visualized using chemiluminescent detection (Pierce Biotechnologies) according to manufacturer's instructions.

Results. The sera obtained from RVFV infected sheep demonstrated positive detection of recombinant NSs protein when using Western blot analysis and chemiluminescent detection. Using non-infected sheep sera (RVFV negative), detection of this protein was not observed.

Discussion/Conclusion. It is possible to use whole animal sera as a source of primary antibody against recombinant NSs protein, and therefore determine DIVA status of the animal(s) in question. Our Western blot results also suggest that NSs protein detection can be used to evaluate immunogenicity of NSs in RVFV infected animals, even though this protein is directly responsible for immune suppression in the host.

Small ruminant lentivirus enhances PrPSc accumulation in cultured sheep microglial cells

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Sheep scrapie (Sc) is the prototypical transmissible spongiform encephalopathy (prion disease), which has a fundamental pathogenesis involving conversion of normal cellular prion protein (PrPC) to disease-associated prion protein (PrPSc). Sheep microglial cell cultures, derived from a *prnp* 136VV/171QQ near-term fetal brain, were developed to study sheep scrapie in the natural host and to investigate potential cofactors in the prion conversion process. Two culture systems, a primary cell culture and a cell line transformed with the large T antigen of SV40, were developed and both were identified as microglial in origin as indicated by expression of several microglial phenotype markers. **Following exposure to PrPSc, sheep microglial cells demonstrated relatively low (transformed cell line) to high (primary cell line) levels of PrPSc accumulation over time.** The accumulated PrPSc demonstrated protease resistance, an inferred beta-sheet conformation (as determined by commercial ELISA), specific inhibition by anti-PrP antibodies, and was transmissible in a dose-dependent manner. **Primary microglia coinfected with a small ruminant lentivirus (caprine arthritis encephalitis virus-Cork strain) and PrPSc demonstrated an approximate twofold relative increase in PrPSc accumulation as compared to primary microglia infected with PrPSc alone.** The results demonstrate the *in vitro* utility of PrPSc-permissive sheep microglial cells in investigating the biology of natural prion diseases, and show that small ruminant lentiviruses enhance prion conversion in cultured sheep microglia.

Systems for the rapid detection of DNA and RNA viruses using high throughput real time PCR

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The polymerase chain reaction (PCR) has been applied to the diagnosis of animal diseases for more than 20 years. In the last 5 years, assays have been refined and streamlined to support both large-scale testing as well as reliable quantification of pathogen loads. Real time PCR assays are now widely used for the detection of DNA viruses (qPCR) or RNA viruses by the inclusion of a reverse transcriptase step (qRT-PCR). qPCR/RT-PCR overcomes most of the limitations of agarose-gel-based PCR, especially the potential for cross contamination, high labour demand and limited capacity. Purification of viral DNA and RNA remained a rate limiting step until recently. Systems to semi-automate nucleic acid extraction and support a high throughput are now available. Over the last 12 months, we have had the opportunity to couple qRT-PCR and a rapid nucleic acid extraction system to support several different applications. A start-to-finish system has been progressively refined to minimise specimen handling, maximise throughput and reduce test times without compromising sample integrity or biosecurity. While designed to achieve high throughout and rapid turn-around, these methods are equally applicable to the efficient and economical testing of small numbers of samples. Examples from each end of this spectrum, of testing for both DNA and RNA viruses, follow.

Firstly, in 2007, Australia experienced its first occurrence of influenza virus infection in horses. There were more than 50,000 infected horses on 8500 farms. The extensive testing program to support diagnostic, surveillance and "proof of freedom" activities relied heavily on the use of an Influenza A (qRT-PCR). To achieve the necessary performance levels, **key considerations were sample type, extraction method and chemistry, PCR master mix, preparation of reaction 'cocktails', thermocycler specifications and an integrated workflow from specimen receival to set-up and completion of testing** and ultimately disposal of samples. Testing of urgent specimens could be completed in **less than 3 hours of receipt and a high throughput capability allowed more than 70,000 samples to be tested, with 30,000 tested in a 4 week period**. An important consideration was that these performance levels could be achieved with the use of a modest number of staff and equipment for test procedures and in 2 shifts per day. With 'round the clock' employment of 3 technicians per shift, **3500 samples could be tested per day**, outstripping the capacity of field personnel to collect samples.

Secondly, the EMAI Virology Laboratory is engaged in the testing of imported prawns for freedom from several viruses. In addition to the range of considerations outlined for the qRT-PCR for EI, the prawn testing presented additional challenges. The primary sample received at the laboratory is shelled frozen prawn. These samples are tested for both DNA and RNA viruses. To avoid the numerous complications of homogenising large numbers of tissue samples, a simple sample collection procedure was devised. This modified sampling procedure provided material that could be immediately introduced to the standard workflow and equipment. The standard extraction equipment and chemistry were used to provide purified nucleic acid to test for both DNA and RNA viruses in a multiplexed qPCR Modifications to the assay conditions and use of appropriate reaction mixtures now allow the **concurrent detection of 2 DNA viruses and 1 RNA virus in the same reaction from the same extract**.

Finally, the systems devised for the high-throughput capability were not expected to suit testing of small numbers of different samples for a variety of different viruses. However, with minor modifications to workflow and planning, these systems were as effective and efficient for small scale applications. For example, **viral DNA or RNA could be extracted at the same time and in the same plate, from a diversity of materials including transport medium, serum, blood, tissue extracts, semen and faeces. With standardised reaction chemistry and conditions, a number of 'single target' assays for DNA and RNA viruses could be run concurrently, maximising the use of laboratory resources.**

Bacteriology Scientific Session

Sunday, October 26, 2008

Guilford-E

Moderators: Durda Slavic
Deepanker Tewari

* Graduate student presentation

Evaluation of TaqMan® PCR workflow for rapid and sensitive detection of *Trichomonas foetus* in culture and smegma samples

Darcy A. Myers, Rohan Shah, Quoc Hoang, WeiWei Xu, Angela M. Burrell, Weiwen Ge, Mangkey Bounpheng

Applied Biosystems, Austin, Texas

Bovine trichomoniasis is a venereal disease caused by the protozoan, *Tritrichomonas foetus*. The disease results in infertility, abortions, and reduced weaning weight which leads to substantial economic losses worldwide. Mandatory regulatory testing programs in some states in the USA are now required prior to the breeding season or before cattle movement. Current standard testing programs require culture of genital secretions (preputial smegma) in media such as Diamond's or In Pouch TF, followed by microscopic identification at 24 h intervals for up to 6 days. However, the sensitivity (detection rate of ~70–96%) and specificity of these culture methods are suboptimal. We have, therefore, evaluated the use of a TaqMan PCR workflow to detect *T.foetus*.

The TaqMan PCR workflow consists of MagMAX™ Nucleic Acid Isolation Kits, Path-ID™ PCR reagents, and a *T. foetus* specific TaqMan Assay (McMillen and Lew, *Vet Parasitology* (2006) **141**: 204–215). DNA isolated from smegma samples cultured in Diamond's medium (180 samples) was used as template for *T.foetus* detection. DNA isolation for all samples was completed in ~1 h using the magnetic bead-based high-throughput instrument, MagMAX-Express 96. Subsequent TaqMan PCR was completed in < 2 h, with total time from samples to results of ~ 3 h.

Eight samples were culture positive, and of these, 7 were PCR positive, for a *T. foetus* TaqMan PCR sensitivity of 87.5%. Two PCR positive smegma samples examined by direct PCR (without culture) were positive, suggesting that it may be possible to isolate DNA directly from smegma. **Eleven of 173 culture negative samples were TaqMan PCR positive (in our laboratory and in an independent laboratory), and a third corroborative test may be required to determine if these are true or false positives.**

These results suggest that the TaqMan PCR workflow is a rapid and sensitive method for *T. foetus* detection. Further evaluation will include more smegma and corresponding culture samples to validate the use of smegma for direct TaqMan PCR. This will eliminate the need to culture smegma samples and reduce cost and time associated with *T. foetus* detection.

Application of pooled polymerase chain reaction testing in the face of an outbreak of *Tritrichomonas foetus* in Southern Colorado

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In the spring of 2007 a battery of bulls from a Southern Colorado ranch was identified as infected with *Tritrichomonas foetus (T. foetus).* The herd was from an area where the incidence of T. foetus was quite low with only 3 positive cases identified in the past 6 years. The initial diagnosis was made based on a positive culture of a preputial scraping (pps) with PCR confirmation. Following this initial diagnosis a surveillance of at risk herds in geographical proximity was initiated. In order to provide the highest degree of sensitivity of detection, minimizing the number of collections, and maintaining cost awareness to the owners a pooled PCR strategy was adopted. During the year following the initial diagnosis 2628 bulls were tested using a pooled PCR testing strategy. A total of 657 pools were constructed with an average pool size of 4 bulls, of these 657 pools 64 pools were found positive. From the 64 positive pools 65 bulls were identified as infected with *T. foetus*. Individual bulls from positive pools were identified by PCR on frozen residuals from the original laboratory submission. Positive bulls were removed from the herd and sold as "slaughter only". Herds where positive bulls were identified were required to remove for disposal all positive bulls and to submit follow-up samples one week after the identification of the initial positive bull/bulls, no additional positives have been identified on follow-up testing after initial pooled screening and removal of individually identified positive bulls. The use of PCR on pooled preputial scrapings is a cost effective yet highly sensitive strategy that may be applied to screen herds for infection and to monitor herd status after the initiation of a trichomoniasis control and eradication program.

Cattle, Deer and Bovine Tuberculosis: Current Research in Michigan

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Bovine tuberculosis (bTB) is a contagious disease caused by the bacterium *Mycobacterium bovis* capable of infecting humans, wildlife and livestock. While once common in US cattle, the disease has historically been rare in wildlife. However, in 1994 bTB was found to be endemic in free ranging white tailed deer (*Odocoileus virginianus*) populations in northern Michigan and evidence suggests transmission to cattle. A key component to transmission of bTB between deer and livestock appears to be shared resources, such as livestock feed. To evaluate the extent to which deer and livestock share resources, we fitted 27 deer with GPS collars programmed to record locations every 2 hours for one year. Capture locations were 4 beef cattle farms in and around the bTB infected zone in Michigan's Northeastern Lower Peninsula. In addition, data has been collected on farming practices employed by study sites and nearby farms that may be frequented by deer. To date 11 collars have been recovered, with a total of over 36,000 data points. Analysis is underway to describe co-use of pasture by deer and cattle, and proximity of deer habitat use to stored cattle feed. **Preliminary results indicate up to 30% of recorded deer locations are in areas of cattle use, cultivated crops and hay fields. In addition, approximately 2.25% of locations were within farm yards, near buildings or fenced feed.** Further analysis is pending to determine overlapping seasonal and daily use of habitat by cattle and deer. We hope to use the final results to recommend mitigating measures for livestock owners to reduce the risk of exposing livestock to bTB.

Experimental inoculation of coyotes with *Mycobacterium bovis*: susceptibility and shedding

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Introduction. Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is endemic in white-tailed deer (*Odocoileus virginianus*) in the northeast corner of Michigan's Lower Peninsula. Several other wildlife species have also been found positive for bTB in that area. Of these other wildlife species, coyotes (*Canis latrans*) are infected with a prevalence around 30%. If coyotes shed the bacteria coupled with the high prevalence, the potential of serving as a transmission host to other animals is also high. The objective of this study was to investigate the susceptibility to bTB and shedding of *M. bovis* in coyotes.

Materials and Methods. Four captive raised coyotes were orally inoculated on Day 0 with 1.0 X 10^5 CFU/ml of *M. bovis* from isolates cultured from Michigan white-tailed deer. Oral and nasal swabs were taken fortnightly and feces was collected weekly for testing by culture. Fecal samples were also tested by exposing two guinea pigs per coyote. On Day 140, the coyotes were euthanized and tissues evaluated for *M. bovis* infection by histology and culture. The guinea pigs were necropsied on Day 152 and tissue samples also evaluated by histology and culture.

Results. Ten sets of swabs and 19 fecal samples were collected from each coyote over the 18 weeks. Swabs and feces were negative on culture. Tissues samples of coyotes and guinea pigs were also negative on culture. For histology, the retropharyngeal lymph node from one coyote had a lesion and a single acid fast bacillus. All other tissues from coyotes and guinea pigs were negative.

Discussion/Conclusion. A single dose of 1.0 X 10^5 CFU/ml of *M. bovis* did not result in infection in these coyotes. Due to the lack of infection, we still do not know the risk coyotes pose for shedding *M. bovis*. The dosage used in this study may not be comparable to what may actually occur in the field where a coyote may feed multiple times from an infected deer or other animal with unknown number of bacteria. While work is being conducted on field samples from coyotes in Michigan, the results of this study indicate that dosages of higher concentration or multiple dosages may be necessary to study the pathogenesis of bTB in coyotes. Conducting this additional research will help in assessing the role of coyotes in the spread of bTB.

Comparison of antemortem diagnostic serologic assays for the detection of M*ycobacterium bovis* in domestic cats

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Introduction. *Mycobacterium bovis* is an acid fast bacillus (AFB), intracellular bacterium which persists within chronic granulomatous lesions. An endemic focus resides within the wildlife population of northern Michigan contributing to a regional persistence within both wild and domestic animals. Investigation of additional reservoir species identified the domestic cat as a contact reservoir for susceptible animals. Current feline antemortem diagnostic tests for identification of *M. bovis* infection are largely inconsistent. This project investigates the antemortem tests available to avoid indiscriminate culling of cats on infected premises. The objective of this study was to experimentally stimulate specific pathogen free cats with Sensitinogen (a heat-killed *M. bovis* product) and produce a detectable serologic response in order to compare and contrast the following antemortem serologic tests, rapid test (RT), multiple antigen print immunoassay (MAPIA), and purified protein derivative (PPD) single skin test.

Materials and Methods. Eight, six month old, specific pathogen free, purpose breed, domestic short hair cats were purchased. Six cats were injected subcutaneously twice with Sensitinogen on day 0 and Day 30. Serum was collected every 30 days for 150 days total. On day 134, a PPD tuberculin skin test was given in the right pinnea and read at 48 and 72 hours. Two representative cats were euthanized and complete necropsies were performed. The skin at the injection sites, lymph nodes, and tonsil were harvested, processed for histopathology, stained with H&E, Ziehl- Neelsen's acid-fast stain, and examined by light microscopy.

Results. The PPD single skin test gave a measurable difference between stimulated and control groups, with an average of 2 times change in skin thickness. RT was able to weakly identify four reactors within the stimulated group after the injection of PPD, all controls were negative implying high specificity. MAPIA appears more sensitive then the RT, as a strong response was noted among all the stimulated cats to MBCF and 16/83 antigens. The average band intensity of the stimulated cats prior to PPD was close to 30 times more intense when compared to controls and 51 additional feline serum samples.

Discussion/Conclusions. In conclusion, 0.1mL bovine PPD ID injection did give a measurable difference (approximately two times) between vaccinated and control groups, the RT was able to identify four out of six stimulated cats after the injection of PPD, but all unstimulated cats were negative indicating high specificity. MAPIA appears more sensitive than the RT and a strong response was noted with the MBCF and 16/83 antigens. **We conclude that MAPIA is a more sensitive diagnostic test in detecting stimulated cats over the RT, and that the PPD single skin test was proven to have a measurable difference between stimulated and nonstimulated cats.**

Use of infrared thermography as an alternative method to evaluate the comparative cervical test (CCT) in cattle sensitized to *Mycobacterium bovis* or *M. avium*

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Introduction. The comparative cervical test (CCT) is a critical component of the Cooperative State–Federal Tuberculosis Eradication Program. Conducting the test requires rounding up animals for the purified protein derivative (PPD) injections and then rounding them up for a second time in 72 hrs for reading the test results by palpation and skin thickness measurements. A positive response consists of swelling and induration at the injection site. The swelling may be associated with inflammation and thus, skin temperature changes. Infrared thermography (IRT) remotely measures skin surface temperatures and may be an alternative technique to reading the CCT. The objective of this study was to determine if infrared thermography could be used to correctly classify which cattle were sensitized for *M. bovis* or *M. avium* through their responses to the avian and bovine PPD.

Materials and Methods. Fifteen domestic cattle were evaluated by infrared theromgraphy. Nine were sensitized to *M. bovis*, four were sensitized to *M. avium,* and two were not sensitized. The CCT was conducted on all of the animals. Thermal images were taken at the time of the injection of the avian and bovine PPD and at 24 ±3 hr intervals until 72 hrs after the PPD injections using a Forward Looking Infrared camera. For images at 24 hrs and 72 hrs, the animals were loose in their pen. Using the images from 72 hrs, the methods used to classify an animal as a reactor or non-reactor to the avian PPD or bovine PPD included having both avian and bovine PPD injection sites on one image, using area max to cover both sites with the area max measurement mode, and listing the site that had the maximum temperature between sites and was over 37°C as the reactor site. If the temperature was under 37°C, the animal was classified as non-reactor to the avian or bovine PPD. Results from the reading by palpation and skin thickness measurements were also collected and compared to the IRT results.

Results. Using the parameters described above, 86% of cattle were correctly classified to the category of sensitization using IRT. The results from the skin thickness measurements had 80% correctly classified. The bovine sensitized cattle and control cattle could also be correctly classified at 24 hrs using IRT if the temperature cut off was raised to 37.5°C. One avian sensitized animal was not correctly classified at 24 hrs.

Discussion/Conclusion. IRT was able to correctly classify 86% of the animals and all of the bovine sensitized cattle were correctly classified. These results indicate that infrared themograhy may be a non-invasive and objective way to read the CCT. It may also provide results for the CCT within 24 hrs instead of the typical 72 hrs. Further research is still needed to understand how other factors, such as weather and breed, may affect skin temperature and influence the parameters used to identify between reactors and non-reactors.

Detection of Brucellosis from swine meat juice samples

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Introduction. Meat juice, a liquid released from a meat sample after it is frozen and allowed to thaw at room temperature, contains antibodies that may reflect the individual disease status of the animal from which it was derived. Detection of antibodies from swine meat juice is currently used to determine exposure status to trichinae, pseudorabies, toxoplasma, PRRS, salmonella and, experimentally, to mange. The objective of this project was to compare meat juice samples to serum samples using several brucellosis serology tests in order to investigate the possibility of utilizing meat juice samples for brucellosis surveillance in swine.

Materials and Methods. Diaphragm and serum samples from 35 swine were collected from depopulated herds in Iowa and Georgia that were declared positive for *Brucella suis* biovar 1. These samples remained frozen until analyzed. Bacterial culture for *Brucella suis* was performed on a variety of tissues to confirm the disease status of the individual animals from which the diaphragm and serum samples were collected. The frozen diaphragm samples were allowed to thaw at room temperature, and the juice that accumulated in the plastic bags in which they were frozen was harvested. A portion of this meat juice was filtered using a .22µM filter to remove any potential *Brucella* organisms so that further testing could be conducted outside of a biosafety level 3 laboratory. The serum, filtered meat juice, and unfiltered meat juice were tested and compared using the fluorescence polarization assay (FPA), particle concentration fluorescence immunoassay (PCFIA), and complement fixation (CF) serology tests.

Results. A sample was called positive if it was positive on at least one of the three serology tests and negative if it was negative on all three tests. When these criteria were utilized, the meat juice and serum results matched 80.0% of the time with sensitivity and specificity both at 80.0%. The positive predictive value (PPV) and negative predictive value (NPV) were 96.0% and 40.0% respectively. Using the 21 Iowa samples and comparing only the CF and FPA tests, the results matched 81.0% of the time with sensitivity and specificity at 82.4% and 75.0% respectively. The PPV and NPV of the Iowa samples were 93.3% and 50.0% respectively.

Discussion/Conclusion. Serum is the ideal ante mortem sample to test for brucellosis. However, if serum could not feasibly be collected, meat juice could be utilized as an alternative post mortem sample to assess the brucellosis status of swine. A reduction in the detection of brucellosis was noticed in the meat juice samples when comparing it to serum, but this could be attributed to a dilution effect because of the increased amount of extra cellular fluid in this sample type. Positive swine with low serum titers may not be detected and could be classified falsely as negative because of this potential dilution effect.

PCFIA was run only on filtered meat juice and not the unfiltered since the reader for this test was not located in a biosafety level class 3 laboratory. The PCFIA test often gave results of negative or suspect on samples that were positive on the CF or FPA. Utilizing only the results of the CF and FPA tests did not appear to reduce the effectiveness in the detection of brucellosis in the meat juice when the PCFIA results were not utilized.

Meat juice could be utilized on a limited basis for brucellosis surveillance in commercial, transitional or feral swine, when serum samples are not able to be collected.

Evaluation of a "no wash ELISA" assay for high throughput serological diagnosis of brucellosis in ruminants

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The control and eradication of brucellosis is highly desirable but heavily resource intensive as high throughput serological testing is required. The aim of this study was to meet the needs of high throughput screening laboratories involved in this process through the development of a new assay. An existing cELISA used for the serodiagnosis of brucellosis in ruminants was converted to an AlphaLISA homogenous proximity based assay. **This assay requires no separation steps and can be performed in low volume microtitre format.** The *Brucella* AlphaLISA was validated on a panel of bovine, ovine and caprine sera from infected and uninfected animals. The diagnostic sensitivities (>96%) and specificities (>98%) obtained compared well to those from cELISA, iELISA and FPA performed on the same samples. **The AlphaLISA met the testing criteria set for ELISAs as defined by the OIEELISA standards and had an analytical sensitivity similar to that of the parent cELISA**. The method was also used on a small panel of serum samples from cattle that were experimentally infected with *Y.enterocolitica* O:9. Some false positive reactions were obtained as was also the case with results from FPA, iELISA, cELISA, CFT and SAT. Despite this, the methodological advantages of the AlphaLISA mean that this assay is well suited to high throughput serodiagnosis. **This report is the first description of the use of AlphaLISA to detect pathogen specific antibodies.** Furthermore, the relative ease with which the cELISA was converted to this platform indicates that this technology is ready to meet the high throughput testing requirements for the diagnosis of many other diseases.

Bovine tuberculosis: analyzing the parameters of the interferon gamma assay and improved diagnosis with new antigens

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Bovine tuberculosis (TB), a zoonotic disease with a major economic impact, continues to be a significant problem with a global perspective. The BOVIGAM® interferon gamma (IFN-γ) assay constitutes a laboratory-based tuberculosis test and is widely used complementary to the tuberculin skin test. The assay consists of a first step culturing whole blood with antigens and stimulating leucocytes to produce IFN-γ which is quantified by ELISA in a second step. The first step measures the cell-mediated immune response (CMI) and critically depends on the sample quality, stimulation reagents and culture conditions.

The CMI is known to be impacted negatively by stress. We have stimulated fresh blood from animals with or without stress with mitogens resulting in significantly lower IFN-γ production in stressed animals and therefore potentially leading to false negative results. **These results furthermore emphasize the utility of a positive control for stimulation.**

Tuberculosis-specific stimulation is currently done with tuberculins. We have analyzed tuberculins from different sources to define an optimized and standardized tuberculin concentration for the use with the BOVIGAM® interferon gamma (IFN-γ) assay. **The results show that sensitivity and specificity of PPDs from each supplier can be optimized by titrating PPD B vs. PPD A activity in the BOVIGAM® interferon gamma (IFN-γ) assay for positive and negative animals, respectively. The use of alternative antigens, e.g. a cocktail of recombinant antigens for stimulation resulted in improved diagnostic sensitivity and specificity.**

These recent developments in measuring the CMI therefore represent excellent tools for control and eradication of bovine tuberculosis.

Herd level prevalence of *Mycoplasma* mastitis and findings in infected dairy herds in Utah – results of a statewide survey

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Mycoplasma is an important cause of dairy cattle disease, including mastitis. A survey was undertaken to determine herd level prevalence and offer an outreach and disease reduction program to *Mycoplasma*-positive herds in Utah.

All 285 dairy farms in Utah were asked for written permission to collect bulk tank milk samples (5 from each tank) at 3-4 day intervals for mycoplasmal culture. Most farms ship to 3 major milk buyers; producer-dealers and independents and could provide self samples. Samples were collected frozen with ID's coded for confidentiality and transported frozen to a laboratory in Greeley, CO for mycoplasmal culture. Positive farms were contacted for follow up visits, completion of a questionnaire and farm-specific recommendations.

78% of farms in Utah (222 farms) provided 5 samples from 292 tanks (n=1460). 16/222 farms **(7.2%) were positive for *Mycoplasma***. The proportion of all bulk tank samples (some farms had multiple tanks) that were positive from infected herds ranged from 5-100%.

Some *Mycoplasma*-positive farm owners could not answer all questions; response numbers varied. Most positive farms (8/15) milked > 750 cows; 4 milked > 2000. 9/11 herds (82%) had BTSCC 140-240,000/ml. 7/11 herds (64%) had actual milk production between 21,000 and 26,000 lb (9534 – 11,804 kg) per 305 d. Findings (proportion of 12 farms with owner able to answer) included: **Clinical mastitis "not curing" and in 2 or more quarters 92%, moving from quarter to quarter 75%**, cows droopy ears 67%, cows head tilt 58%, calves either sign 92%, **common towel to > 1 cow milked 58%**, cloth towels machine washed 100%, dryer 92%. Closed herd 17%, buy bulls only 17%, no fenceline animal contact 67%, mastitis treatment: ceftiofur 67%, cephapirin 50%, pirlimycin 42%, flunixin 33%. Open ended question for comments resulted in responses that **milk had "bubbles, grit or sand" in it, 25%**. Purchased animal biosecurity (10 open herds): vaccinate 90%, segregate 50%, milk culture 30%, "hope" 10%.

Producers were telephoned before follow up visits and recommendations were discussed. By the time of the farm visit, 8/12 (67%) had cultured cows for *Mycoplasma*, 6 (75%) of those were culling all cows found positive. Participation in the statewide Utah survey was excellent, and the 7% herd prevalence of *Mycoplasma* was higher than most of the US (most regions have 2-5% prevalence). Long-term impact will be further measured when another round of farm visits is made during 2008.

Preliminary investigation of a humoral and cell-mediated immunity ratio for diagnosis of paratuberculosis in beef cattle

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Infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis* (*Mptb*), the etiologic agent of paratuberculosis, causes reduced production worldwide. Diagnostic tests for paratuberculosis in cattle include assays that directly detect viable organisms (bacterial culture of feces) and components of the *Mptb* genome (polymerase chain reaction; PCR) or indirectly detect the organism through measurement of specific immune responses.

One thousand three hundred and twenty-four adult beef cattle from 5 populations were tested for paratuberculosis using 2 antibody enzyme-linked immunosorbent assays (ELISA) and radiometric bacterial culture of feces. Samples from cattle in 2 of the 5 herds (n = 226) were tested for interferon-gamma (IFN-γ) using an available ELISA. A ratio of humoral to cell-mediated immunity was generated using results from 1 antibody test and the IFN-γ ELISA. Latent class analysis was used to estimate accuracy of the 4 paratuberculosis assays (2 antibody ELISA, 1 IFN-γ ELISA and fecal bacterial culture) within a Bayesian framework. Determination of test accuracy and paratuberculosis prevalence in the latent class analysis allowed for estimation of predictive value positive (PVP) functions. The estimated PVP functions were used to iteratively assign paratuberculosis status to the cattle with immunity ratio results. Accuracy of the immunity ratio was determined for 28 cutoffs based on the probabilistically assigned paratuberculosis status.

Area under the receiver-operating characteristic (ROC) curve was estimated as 0.778 (95% PI, 0.657 – 0.889). The Youden (sensitivity + specificity – 1) peaked at immunity ratios of 0.5 (J = 0.48) and 1.0 (J = 0.46). Sensitivity and specificity at an immunity ratio cutoff of 0.5 were 0.65 (95% PI, 0.44 – 0.85) and 0.83 (95% PI, 0.78 – 0.88), respectively. Sensitivity and specificity at the 1.0 cutoff were 0.55 (95% PI, 0.33 – 0.77) and 0.91 (95% PI, 0.87 – 0.95), respectively. **An immunity ratio can be used to diagnosis paratuberculosis in beef cattle and it might have higher estimated sensitivity than currently available screening tests.**

Six-gene-multiplex PCR for *Escherichia coli* O157:H7 identification

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Enterohemorrhagic *Escherichia coli* causes serious human foodborne illnesses. In North America, serotype O157:H7 is responsible for an average of 17 outbreaks per year involving >75,000 human *E. coli* infections. The primary reservoir of *E. coli* O157 is cattle and other ruminants. In cattle, the organism colonizes the hindgut and is shed in the feces, which serves as a major source of contamination of food products. Isolation of *E. coli* O157 from fecal or food samples is by enrichment, immunomagnetic separation, and plating on selective medium. Identification often includes PCR detections on different combinations of five virulence genes: *eaeA, stx1, stx2, fliC* and *hlyA*. Traditionally, two separate PCR procedures, a multiplex for *eaeA, stx1, hlyA* genes, and another for *fliC, stx2* gene are used because of the inability to separate *stx1* (614 bp) and *fliC* (625 bp). We had developed a multiplex PCR to detect all five genes in one reaction, yet the *rfbE* gene was not included. The O-antigen of *E. coli* is encoded by the *rfb* gene cluster consisting of 12 genes, and the fifth gene, *rfbE* is specific for serotype 0157.

We report here a newly developed multiplex PCR procedure for identification of *E. coli* O157:H7. Six major virulence genes, *fliC*, *stx1*, *stx2*, *eaeA*, *rfbE*, and *hlyA* were included. The new procedure detects all six virulence genes in the same reaction and generates six distinct bands with product sizes of 949, 655, 477, 375, 296, and 199 bp, respectively. The procedure was validated with a total of 217 Shiga toxin producing *E. coli* (STEC) isolates, including three ATCC strains, 84 cattle isolates, 57 human isolates , and 73 non-O157 Shigatoxigenic *E. coli* strains. Sensitivity tests with different dilutions of an *E. coli* O157:H7 pure culture indicated that the procedure can detect about 10 bacterial cells per reaction. Our result indicated that the procedure can be used for identification of *E. coli* O157:H7 strains from both animal and human samples, with high sensitivity and specificity.

Evaluation of diagnostic methods for detecting *Leptospira* in cattle kidney samples obtained from an abattoir

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This study compared the results obtained from four currently available diagnostic methods for the detection of *Leptospira* infection in cattle. Fifty bovine kidney samples collected from an abattoir were tested by dark field microscopy (DFM), fluorescent antibody staining technique (FA), polymerase chain reaction (PCR), and culture to detect *Leptospira* infection. Buffered–formalin-fixed representative specimens were also examined by routine histopathology for microscopic lesions. Kidney homogenates spiked with *Leptospira interrogans* serovar hardjo at different dilutions were also similarly tested to compare the effectiveness of testing methods. The spiked samples were positive for *Leptospira* by all the testing methods used. For abattoir samples, the proportions of samples with a positive result by each of the other methods were: PCR (0/50; 0%), DFM (30/50; 60.0%), and FA (31/50; 62.0%). Cultures of all 50 kidney samples became contaminated, precluding the isolation of *Leptospira*. On histopathology 33/50 (66%) samples had some degree of interstitial nephritis. Eight (8/33; 24%) had moderate to severe interstitial nephritis and 25/33 (76%) had a mild degree of interstitial nephritis. While all methods were efficient in detecting the organism in spiked samples, the tests had variable efficacy in detecting organisms from harvested bovine kidneys. **Therefore, further refinements or development of more optimal tests are required to detect infection in tissues of naturally infected animals.**

Isolation of *Arcanobacterium hippocoleae* from an aborted equine fetus

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Arcanobacterium hippocoleae is a newly established bacterial species in the genus of *Arcanobacterium*. The bacterium was previously isolated from the vaginal discharge of a horse in the United Kingdom, but the clinical significance was not known. Here, we report isolation and identification of *A. hippocoleae* from the lung of an aborted equine fetus. The initial histopathological examination revealed a bronchopneumonia indicative of a bacterial etiology. Heavy and pure growth of *A. hippocoleae* was obtained from the fetal lung. **This is the first time that the organism was isolated in the United States of America. Importantly, it is the first time that the bacterium was clearly associated with a clinical disease in a horse.**

Pathology Scientific Session

Sunday, October 26, 2008

Auditorium-II

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Shannon Swist

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10:30 AM **Cases of cerebral nematodiasis in canaries and emus due to *Baylisascaris procyonis* larval migration (neural larva migrans) diagnosed in the state of California, year 2007 * -** Alexandre Paulino Loretti, Sriveny Dangoudoubiyam, James Koobs, Jackie J. Gai, Kevin R. Kazacos 103

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***** Graduate student presentation

Neuropathology of naturally occurring *Trypanosoma evansi* infection of horses

Aline Rodrigues[1], Rafael Fighera[2], Tatiana Souza[2], Claudio Barros[2]

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Introduction. *Trypanosoma evansi* is a flagellate protozoan parasite which causes disease in a variety of mammalian species. An outbreak of equine trypanosomiasis caused by *T. evansi* resulted in the death of at least 100 horses in Southern Brazil, where the disease was previously unreported. Twenty-three affected horses were clinically evaluated, and nine horses (39%) presented with severe, fatal, neurologic disease. Here we describe the central nervous system (CNS) pathology in nine horses naturally infected by *T. evansi*.

Material and Methods. The epidemiologic and clinical data were obtained during on-site visits to the farms where the outbreaks occurred. Nine horses with neurological signs were necropsied. The brain and spinal cord were fixed in 10% buffered formalin and processed for routine histopathologic examination and immunohistochemistry.

Results. Nine horses developed neurologic signs, which included marked ataxia, blindness, circling, hyperexcitability, obtundity, proprioceptive deficits, falling down, head tilt, head pressing, and paddling movements with the four limbs. Clinical courses ranged from 2-20 days. In eight of the nine horses, neurologic signs were preceeded by wasting and anemia. Seven out of the nine horses had asymmetric flattening of gyri and locally extensive areas of yellow discoloration and leukomalacia in the cerebral white matter. **Histologically, a necrotizing panencephalitis with a white matter predilection was observed, characterized by marked edema, demyelination, and lymphoplasmacytic perivascular infiltrates of up to 20 rows of cells.** The plasma cells occasionally contained eosinophilic globules in their cytoplasm (Mott cells). Mild to moderate meningomyelitis or meningitis were observed in the spinal cord of 5/7 horses. ***T. evansi* was demonstrated immunohistochemically in the perivascular spaces and neuropil of formalin-fixed paraffin-embedded brain tissue of 8/9 affected horses.**

Discussion. The encephalic lesions described in this report are similar to those found in one previous equine report of *Trypanosoma evansi* infection and similar to the lesions described in horses infected with *Trypanosoma brucei brucei* and humans infected with *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. In addition to horses, *T. evansi* appears to cause encephalitis in other animal species, including cattle and hog deer (*Cervus porcinus)*. In the present report, the organisms were present within blood vessels, in the perivascular spaces, and free in the brain parenchyma, indicating that they are capable of crossing the blood-brain barrier. It is believed that a determining factor for the development of fatal encephalitis in these horses was the introduction of the agent to a naive population and the use of subcurative doses of diminazene aceturate and other trypanocidal drugs. Although uncommon, trypanosomiasis should be considered in the differential diagnosis for encephalitis in horses in regions where the disease is enzootic.

Gross and microscopic study of laryngopharyngeal lesions in Thoroughbred horses in Southern California

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In Thoroughbred racehorses, abnormalities of the upper respiratory tract are a recognized cause of suboptimal racing performance. Knowledge of these lesions has largely been derived from endoscopic examination of the laryngopharynx of horses, which routinely does not include the sub-epiglottic area. Therefore, information about prevalence of sub-epiglottic lesions is very limited. Anecdotal evidence of an increasing number of cases of sub-epiglottic ulceration in Thoroughbred racehorses at Southern California racetracks prompted us to conduct a longitudinal prevalence study focusing on the morphologic abnormalities in the sub-epiglottic region and particularly on sub-epiglottic ulcers and soft palate free border ulceration (kissing lesions).

The whole larynx, pharynx and soft palate were collected from 91 horses euthanized due to catastrophic leg injuries, and subjected to gross examination. Histology was performed on 56 of these horses and tissues examined included the soft palate free border, the epiglottis (including a portion of the gloss-epiglottic fold) and selected sections of representative lesions throughout the laryngopharynx.

Thirteen out of the ninety-one horses (14.3 %) had at least one laryngopharyngeal lesion. A few horses had more than one lesion and the total number of lesions found was 16. The most prevalent lesions were mucosal ulceration and scarring (n=9), 7 of which were located in the sub-epiglottic area. Other lesions found with a much lower prevalence included arytenoid chondropathy, epiglottic entrapment, and absence of the left arytenoid cartilage.

The prevalence of laryngopharyngeal morphologic abnormalities (14.3 %) noted by gross and microscopic pathology examination in the present study was higher than those reported by other authors by means of endoscopic examination (0.7-3.8 %). Furthermore, in the sub-epiglottic area alone, the prevalence of lesions was 7.7 % (7 out of 91 horses). This high prevalence suggests that an important number of sub-epiglottic lesions are likely to be missed by routine endoscopic examination on the standing horse. The pathogenesis of the sub-epiglottic ulcers and soft palate kissing lesions is unclear, but trauma and bacterial agents are suspected to play a role in originating and/or perpetuating the conditions. The clinical significance of such lesions is unknown and further studies are required in this regard.

Feline intestinal sclerosing mast cell tumor: 50 cases (1997-2008)

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Mast cell tumor (MCT) is a common round cell tumor in both the cat and dog and the second most common cutaneous tumor in the cat. Visceral forms of MCT are more common in the cat as compared to the dog with splenic and intestinal forms most commonly reported. While these tumors typically display similar microscopic features regardless of anatomical location, a sub-group of intestinal mast cell tumors in the cat have been recognized in our diagnostic laboratory characterized by a striking amount of associated stromal connective tissue. Fifty cases of feline intestinal mast cell tumors with this unique histological feature were reviewed. Case evaluation included age, breed, gender, anatomical and microanatomical location of the lesion, presence or absence of mucosal ulceration, cell pattern and morphology, mitoses, eosinophilic infiltrate, and metastasis when applicable. Ages ranged from 2-18 years old with a median age of 8. Eleven different breeds were represented with Domestic Short Hairs over-represented at 28/50 (56%). There was no gender predilection. When the specific anatomical location was known, lesions most often involved the small intestine at 35/46 (76%) and less often the large intestine at 10/46 (22%) with a single case involving the stomach (2%). Lesions most commonly demonstrated transmural involvement (46/50; 92%) and mucosal ulceration was present in 29/50 cases (58%). Cell morphology varied from round to polygonal to spindled with a predominance of polygonal to spindled variants. Intracytoplasmic granules, typical of mast cells, were identified in all cases yet were variably present and often poorly discernible on routine H&E. Histochemical staining with Toluidine Blue on 10/50 cases revealed metachromatic granules consistent with mast cell granules. Neoplastic cells were occasionally arranged in sheets but more often formed, at least in part, a trabecular pattern (47/50; 94%) admixed with and separated by moderate to abundant amounts of dense stromal collagen consistent with sclerosis. This stromal component comprised at least 30% of the tumor mass in 35/50 cases (70%). The number of mitotic figures (MF) across cases was uniformly low with an average of 0.6 MFs /5 high power fields. Evaluation of associated infiltration by eosinophils revealed moderate to marked numbers in 44/50 cases (88%). Of the 50 cases, 30 had regional lymph node submitted for evaluation. Of these, 20/30 (67%) had lymph node involvement consistent with metastatic disease. Liver was submitted in 9 of the 50 cases of which 6 (66%) demonstrated metastatic disease. **To the authors' knowledge, this is the first case series to characterize a sclerosing variant of intestinal mast cell tumor in the cat.**

Intussusception in association with *Streptococcus equi subspecies equi* (Strangles) in two horses

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Mississippi State University, College of Veterinary Medicine

Well-defined complications associated with *Streptococcus equi equi* (strangles) include guttural pouch empyema, metastatic abscesses, pneumonia, pleuritis and purpura hemorrhagica. With respect to purpura hemorrhagica, petechiae and subcutaneous edema involving all four limbs are best recognized, clinically, often accompanied by pyrexia, anorexia, lethargy, reluctance to move and, occasionally, colic. Infarction and necrosis of the intestinal wall with hemorrhage has been described at necropsy (Pusterla, 2003). Horses presented for emergency colic may not be recognized as exhibiting potential sequelae of *Streptococcus equi equi*, particularly if no history of strangles on the farm is available and limb edema is absent or minimal.

A three-year-old Quarter Horse mare had a 48 hour history of colic. The mare had a serosanguineous abdominal tap and a heart rate of 90. At surgery, a jejuno-ileocecal intussusception was found which was reduced, but the horse was subsequently euthanized. Gross and histopathologic findings included severe fibrinonecrotic ileitis and serosal hemorrhage. Vessels in the intestines, predominantly in the long intussuscepted segment, had severe fibrinoid vasculitis. Severe suppurative lymphadenitis in the submandibular and retropharyngeal lymph nodes cultured *Streptococcus equi equi*.

A three-year-old Quarter Horse filly presented with a heart rate of 90, temperature of 97.8^{o}F, respiratory rate of 40 and no GI sounds in any of the four quadrants. The mucous membranes had a toxic line and the horse was 10% dehydrated. A nasogastric tube produced 25 L of spontaneous reflux. Segmental dilatation of the small intestine was palpated on rectal examination. Ultrasonography revealed a target lesion indicative of an intussusception. Abdominal tap produced serosanguineous fluid with a total protein of 8. The horse was euthanized. Gross and histopathologic findings included acute to subacute lymphadenitis with abscessation in the retropharyngeal lymph nodes. The guttural pouch had moderate to severe empyema and eustachitis. Both lymphadenitis and guttural pouch empyema were due to *Streptococcus equi equi*. The horse had six discrete intussusceptions in the jejunum and ileum. The lamina propria and submucosa at the sites of intussusception and at additional intervening sites had acute multifocal severe leukocytoclastic vasculitis. Vasculitis extended from submucosa to serosa and easily explained the perturbed intestinal motility and subsequent intussusceptions. Additionally, a large segment of transmural hemorrhage and edema with mucosal necrosis and ulceration was in the distal ileum and was associated with striking leukocytoclastic vasculitis.

Specific antibodies for *Streptococcus equi equi* M protein (SeM) were detected at a very high level of 1:25,600 (Equine Biodiagnostics, Kentucky) in an antemortem serum sample. According to the laboratory, such levels are often found in horses with a metastatic abscess or purpura hemorrhagica (immune-mediated vasculitis) following exposure to *Streptococcus equi equi* or strangles vaccine.

Purpura hemorrhagica has been most often associated with leukocytoclastic vasculitis involving the skin. So-called infarctive purpura hemorrhagica was described in five horses in which leukocytoclastic vasculitis occurred in numerous tissues including small intestine (Kaese, 2005). Deposition of IgM or IgA immune complexes associated with streptococcal M protein in vascular walls is the pathogenesis of the vascular compromise and infarction in multiple tissues. **No previous report of intussusception was found in other published cases of *Streptococcus equi equi* or purpura hemorrhagica.** Infarctive purpura hemorrhagica in horses has been compared to an immune complex disease (Henoch-Schonlein purpura) in humans in which intussusception is listed as a rare occurrence.

Peripheral primitive neuroectodermal tumor (pPNET/Ewing's sarcoma) in the lumbar vertebra and liver of a dromedary camel (*Camelus dromedarius*) with progressive hindlimb paralysis

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Introduction. Peripheral primitive neuroectodermal tumors (pPNETs) and Ewings sarcoma (ES) are a closely related family of aggressive tumors in persons designated the ES family of tumor or EFT which as a group are composed of primitive small round cells of putative neuroectodermal phenotype generally occurring outside the nervous system. The EFT group in man includes three major tumor types: the intraosseous pPNET (ES), the extraosseous pPNET, and the thoracopulmonary Askin's tumor. pPNETs have been reported very rarely in few mammalian species other than man, including three dogs and a Colobus monkey. The authors here describe the first reported case in a camel of an intraosseous pPNET, similar to a human ES, involving the lumbar vertebra and causing progressive hind limb paralysis, with subsequent metastasis to the liver.

Materials and Methods. Necropsy examination of a 9 year-old dromedary camel with posterior paralysis was performed within 6 hours of euthanasia, and representative tissue samples were immersed in 10% neutral buffered formalin, routinely processed for paraffin wax embedding, sectioned at 4-5µm, stained with H&E, and examined by light microscopy. Samples of formalin-fixed bone (vertebra) were decalcified 48-72 hr in a 12% (by weight) hydrochloric acid solution and then were processed and stained as described. Immunohistochemistry (IHC) on selected sections (vertebra, liver) was performed on an automated immunostainer using a biotinylated secondary antibody and a horseradish peroxidase-conjugated streptavidin-biotin conjugated chromagen according to the manufacturer's protocol with cross-reacting antibodies against neuron-specific enolase (NSE), synaptophysin, vimentin, cytokeratin, glial fibrillary acidic protein (GFAP), CD3, and CD79a.

Results. Significant gross findings were limited to the 3rd lumbar vertebra, spinal cord at L3-L5, and liver. Lesions were not observed in the brain, pelvis, ribs, or long bones. The body of L3 was invaded by a firm 1.25 cm x 3.0 cm x 2.5 cm dense white, necrotic and hemorrhagic mass that infiltrated the boney roof of L3 extending dorsally and longitudinally within the vertebral canal along the spinal axis. The mass locally compressed but did not appear to invade the spinal cord which was locally softened and discolored tan and gray. In the liver, multiple, thickly encapsulated, variably cystic and multilocular masses ranging in size from 1-2 mm up to 4cm in diameter were scattered randomly throughout the parenchyma. Microscopically, hepatic and vertebral masses consisted of uniform sheets of primitive round to polygonal cells with mitotic figures variably arranged in a fibrillar background as perivascular pseudorosettes and few neuroblastic Homer-Wright rosettes. Immunohistochemically, tumor cells were uniformly positive for vimentin and were variably positive for NSE and GFAP. There was severe white matter (axonal) degeneration in the spinal cord at L3 – L5.

Discussion/Conclusion. Histomorphologic and IHC findings in this case are consistent with a pPNET variably exhibiting neuroblastic, glial, and ependymomatous differentiation likely reflecting the tumor's primitive multipotential neuroepithelial nature. To the authors' knowledge, this is the first reported case in the camel of a pPNET, presumably intraosseous in origin (L3) with hepatic metastasis, morphologically similar to ES in persons.

The pathology of equine serum hepatitis (Theiler's Disease)

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We interrogated the diagnostic files of the California Animal Health & Food Safety (CAHFS) laboratory system for cases of equine liver disease with a diagnosis of the so-called serum hepatitis (Theiler's disease). This was an opportunity to review the pathology of a large number of cases of a condition whose cause is unknown and the diagnosis of which is thus never certain. Thirty two cases of serum hepatitis were diagnosed at the San Bernardino, Davis and Tulare branches of CAHFS by different pathologists between 1991 and 2008. Four cases were excluded on review of sections: two of these were cases of chronic hepatic venous congestion; one was a case of cholangiohepatitis, and the liver changes in the fourth were minor and inconsistent with liver failure. A wide range of clinical histories emerged from this review, including administration of products of equine origin (n= 6), but also several cases in which there was no history of iatrogenesis (n=22). Clinical signs included depression (n=28), anorexia (n=11), colic (n=5) and nervous signs (n=13). Gross pathology included small and flabby livers, hemoglobinuric nephrosis and/or presence of haemoglobin in urine (n=15), icterus (n=13) and photosensitivity (n=2). The microscopic pathology included acute, global destruction of hepatocytes with hemorrhage and only mild cholangiolar activation (n=10), and more chronic cases with florid hepatocellular and cholangiolar cell reactivity as well as prominent stromal infiltrates of lymphocytes and plasma cells (n= 18); in these cases canalicular cholestasis was prominent. The presence of Alzheimer type 2 astrocytes was prominent in most cases with clinical neurological signs (n=10). The review revealed nothing about the nature of the causal agent. **Beginning with Theiler's original series, there have been clusters of cases associated with administration of serum, plasma, gonadotrophins and products of equine origin, but transmission trials using blood from fatal cases has not reproduced the disease. The fact that no administration of products of equine origin had occurred in the cases reported herein suggests that this is not a pre-requisite for this condition to occur.**

Naturally occurring Influenza infection in a ferret (*Mustela putorius furo*) colony

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In a routine diagnostic case submitted to the Iowa State University Veterinary Diagnostic Laboratory, pooled tissue samples from two ferrets were submitted for examination. The ferrets represented a population of approximately 1,000 of which 8% of the animals were exhibiting respiratory signs including sneezing, coughing, and crusting of the eyes and nose. Mortality at the time of first submission was 0.6%.

Histopathological examination of lung sections revealed bronchointerstitial pneumonia with bronchitis. Pulmonary congestion was also evident. **An immunohistochemistry (IHC) stain and PCR for influenza A virus was positive on lung tissue and further sequence analysis of the HA gene indicated high homology with a reassortant H1N1 swine influenza (SIV) group represented by A/swine/MN/02**. No bacteria were isolated from this submission and virus isolation attempts for influenza virus were negative. A fluorescent antibody (FA) test for Canine Distemper was also negative.

A field investigation undertaken 2.5 weeks following the initial submission further characterized the influenza virus present within the colony. Additional history taken on the farm revealed the presence of multiple avian species (including ducks, geese, peacocks), additional small mammal species raised for purchase (raccoons, skunks and fox), a cow/calf herd, horses and llamas. Additionally, the farm was located approximately 0.25 miles from the nearest swine operation. At the time of visit, clinical signs of severe dyspnea, sneezing, and crusting of the eyes and nose were still apparent within the colony. Four ferrets displaying the acute clinical signs listed above were euthanized. Nasal swabs, blood and tissues were collected to further characterize the influenza strain affecting the colony. Blood samples from 4 ferrets not showing acute clinical signs were also obtained for serological analysis.

Histopathological results from the second submission were similar (bronchointerstitial pneumonia with bronchitis) to those originally described during the first submission. Also similar to the first submission, an IHC for influenza was positive, no bacteria were isolated and the FA for Canine Distemper was negative. PCR for influenza was positive for an H1N1 (or H1) strain in all lung tissues and bronchoalveolar lavage (BAL) samples and in 3/4 nasal swabs. Virus isolation was also positive for Influenza A on 3/4 samples which was confirmed to be H1N1. Serum samples were submitted for a hemagglutination inhibition (HI) assay in which a variant H1N1 reference strain (SIV 99) was utilized. HI titers ranged from 1:10 – 1:160 indicating exposure to influenza virus.

Although ferrets have been used extensively to research the virulence and transmissibility of human and swine influenza virus stains[1,2], **little to no published information exists on naturally occurring outbreaks of influenza in ferrets. Attention to the potential role of wildlife in carrying and/or transmitting an influenza virus to domestic animals is needed**

Nor98-like Scrapie in the United States of America

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This paper describes the first six sheep diagnosed with Nor98-like disease in the United States and serves to acknowledge the increased efforts of diagnosticians and the U.S. Department of Agriculture program to control and eradicate scrapie disease. Classical scrapie, a fatal neurodegenerative disease affecting the central nervous system of sheep and goats, is among a number of diseases classified as transmissible spongiform encephalopathies (TSEs). Recently, a distinct strain of scrapie was diagnosed in sheep in Norway[1] and has been identified in numerous countries of the European Union (EU). The disease has been identified among other names as Nor98 or Nor98-like scrapie. Distinctions between classical scrapie and Nor98-like scrapie are made on signalment, clinical signs, histopathology and immunodiagnostic results. In the past, the classical scrapie disease was confirmed by examination of the brain tissue for a triad of histopathological signs – vacuolation, loss of neurons and gliosis – and, more recently, by immunohistochemical (IHC) or biochemical detection of abnormal prion protein (PrPSc) in the brain, or lymphoid tissues. In the case of Nor98-like scrapie there is generally little or no vacuolation in the brain and, to date, no lymphoid accumulation of PrPSc has been detected. Classical scrapie typically has the most intense PrPSc immunostaining at the obex (motor nucleus of the vagus), while this area is spared in Nor98-like scrapie. Alternatively, Nor98-like scrapie consistently has PrPSc immunostaining in the spinal nucleus of the trigeminal nerve and variable, but often an intense immunostaining for PrPSc in the cerebellum. Thus the diagnosis of Nor98 and Nor98-like disease can be based on immunohistochemistry identifying abnormal prion protein in regions of the brain not typically associated with classical scrapie. Additionally there is a distinct diagnostic western blot pattern for Nor98 and Nor-98 like disease consisting of three or more protein bands with the unglycosylated band being less than 15 kd, compared to classical scrapie in which the unglycosylated band is greater than 15 kd. Nor98 and Nor-98 like disease is associated with older sheep, usually greater than four years of age, while sheep in the range of three to five years of age are more commonly affected by classical scrapie. Clinical signs are uncommon with Nor98 and Nor98-like disease but when present most often include ataxia without pruritis. Genotypes known to provide sheep with resistance to classical scrapie are not spared from Nor98 and Nor98-like disease.

1. Benestad SL, Sarradin P, Thu B, Schönheit J, Tranulis MA, Bratberg B., Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet. Rec. 2003 Aug 16;153(7):202-8.

Cases of cerebral nematodiasis in canaries and emus due to *Baylisascaris procyonis* larval migration (neural larva migrans) diagnosed in the State of California, year 2007

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Neural larva migrans or cerebrospinal nematodiasis is the migration of helminth larvae into the brain of mammals and birds. It causes extensive tissue damage and inflammation, and results in severe and usually fatal neurological disease. The ascarid *Baylisascaris procyonis*, the intestinal raccoon roundworm, is the most common cause of neural larva migrans. Other species involved include *B. columnaris* (the skunk roundworm), *B. melis* (the badger roundworm), and *B. transfuga* (the bear roundworm).

Two outbreaks of neural larva migrans in birds due to *B. procyonis* were diagnosed in California from April until July 2007. One of the outbreaks happened in a privately owned outside aviary in which canaries and doves were housed together; 12 out of 22 canaries died or were euthanized after 3-4 days of illness. Clinical signs included loss of balance and equilibrium, torticollis, star gazing, and inability to fly or walk. The other outbreak occurred in a farm where 18 emus (9 chicks and 9 adults) were kept in a 20 acre pasture; 5 out of 9 emu chicks died, were euthanized, or disappeared (missing birds were presumably dead) 1-10 days after the first clinical signs were observed. Sick chicks were seen being attacked by adults, were ataxic, walking backwards, and unable to stand without assistance; 2 canaries and 3 emu chicks were necropsied. No significant gross lesions were found. Microscopic lesions in the brain were confined to the brain stem, within the white matter tracts/fasciculi and grey matter nuclei in the pons/medulla oblongata. There were multiple foci of malacia infiltrated by macrophages, reactive astrogliosis around these necrotic foci, vacuolation of the neuropil, areas of hemorrhage, presence of swollen axons and axonal spheroids, and lymphocytic perivascular cuffing. Since the distribution and morphology of the brain lesions were consistent with those of neural larva migrans, serial histological sections of the brain were examined in search for nematode larvae. **Few larvae morphologically consistent with *Baylisascaris* sp. larvae were found in the brain of 1 canary and 1 emu chick**, in the region of the medulla**.** There was no inflammation surrounding these larvae, and the neuropil around these parasites was normal. Few larvae of *Baylisascaris* sp. were also noted within the myocardium and skeletal muscle of 1 emu chick in association with nonsuppurative myocarditis and myositis, respectively. **Larvae of *Baylisascaris* sp. were found in the brain of a canary by brain squash** but none was found in the brain of an emu chick. **These larvae tested positive for *Baylisascaris* sp. by PCR**. The submitters reported that raccoons were commonly seen in the vicinity where the canary aviary was located, and that both raccoons and active latrines were present near the area where the emus were fed.

Identification of the species of *Baylisascaris* larvae in a brain squash or on histopathology is difficult since other closely related species are morphologically similar. The PCR assay used here is able to differentiate between *B. procyonis* and *B. transfuga* but not between *B. procyonis* and *B. columnaris*, or between *B. procyonis* and *B. melis* so identification of the particular species of *Baylisascaris* involved in cases of neural larva migrans is best determined epidemiologically. **Based on the history of exposure to raccoon feces in both outbreaks, we conclude that *B. procyonis* was the species of *Baylisascaris* involved.** Environment, enclosures and feedstuff could be contaminated with dried raccoon feces containing *B. procyonis* infective eggs, with the transmission occurring by the fecal-oral route. **To the best of our knowledge, there are no published reports of neural larva migrans in canaries.**

Retrospective evaluation of the occurrence of *Brucella canis* positive dogs, 1997-2008

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Brucella canis is an intracellular pathogen adapted to long-term infection of macrophages. The reproductive failure and infertility resulting from this disease is a potential source of economic loss to the commercial kennel industry. Discovered as a disease of laboratory Beagles in the 1960's, there are recently reported cases in a variety of breeds, particularly in kennels of large multi-breed producers. Examination of records of the University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL) regarding canine abortions revealed 5 proven and an additional 2 suspected cases of *Brucella* infection among 54 abortions submitted for workup during the period January 1997 to March 2008. **Placentitis and histiocytic fetal pneumonia were the most often observed lesions when *Brucella canis* was isolated.** However, although placenta was the most useful diagnostic tissue, histologically and bacteriologically, testing of this organ was done in less than 50% of all cases, and bitch's serum was made available for testing in less than 20%. Retrieval of results of over 6300 serologic tests conducted at the VMDL during this period revealed an overall **seroprevalence of 5.8% on initial screening** by the card agglutination test. The files contained a substantial number of samples submitted from other states, and these had a similar seroprevalence to samples originating in Missouri. Over 80% of all sera were also positive when tested after 2ME treatment. This procedure is thought to eliminate false positive reactions resulting from exposure to cross-reacting lipopolysaccharides of other bacteria, but several instances were noted in which previously ME test-positive dogs became negative over time, while remaining positive on the original screening test. The percentages of positive sera varied between years, but no trends were noted. Bacterial isolations were an effective means of establishing a positive diagnosis, and over 40% of blood cultures tested during the last 3.5 years were positive. The findings indicate that *Brucella canis* is a frequent infection and cause of abortion in dogs in the mid-west; positive dogs were most frequent in submissions containing large numbers of samples.

Diabetes associated with glucagon secreting cell hyperplasia in an adult Blue and Gold Macaw (*Ara Ararauna*)

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Naturally occurring diabetes mellitus is rare in birds but has been reported in budgerigars, cockatiels, toco toucan, two macaws and a Conure. Some of these cases of diabetes were spontaneous but others were associated with glucagon secreting tumor, pancreatitis and hemochromatosis. Here we describe a naturally occurring diabetes in a Blue and Gold macaw (*Ara Ararauna*) associated with hyperplasia of glucagon secreting cells in the pancreas.

An adult female Blue and Gold macaw had a history of several months of polyuria and polydipsia. Several assays performed on the plasma glucagon of the Blue and Gold macaw ranged from 684-2179 pg/dl (normal 299-1190 pg/dl) but insulin levels were within normal ranges. Serum biochemical abnormalities included elevated blood glucose 1067 mg/dl (normal, 286-332 mg/dl), elevated triglycerides, cholesterol and glucosurea, anemia and chronic weight loss. The bird was treated with insulin with positive results but died later due to oral candidiasis, severe loss of weight and seizures.

The bird was necropsied and tissues were collected for histopathology. Significant lesions were confined to all lobes of the pancreas with some variation. There was severe hyperplasia and hypertrophy of islet cells. The islet cells contained foamy and faintly staining basophilic cytoplasm and the nuclei were occasionally vesicular and hypertrophied. Immunohistochemistry of the pancreas for insulin, glucagon and somatostatin revealed that most of the hyperplastic and hypertrophied islet cells stained positive for glucagon.

A review of the first five years post-deployment pathology and toxicology finding for search & rescue dogs deployed to terrorist attack sites on Sept. 11, 2001

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Narrative: On Sept. 11, 2001, terrorist attacks were made upon the World Trade Center, New York City, and the Pentagon, Washington, D.C. Many dozens of search & rescue (S&R) dogs were deployed to these sites within hours to days and exposed to large amounts of airborne toxicants and particulate matter. For the last 7 years we have conducted long-term surveillance for the cause of death, pathology and toxicology in both deployed S&R dogs as well as non-deployed control S&R dogs.

Only dogs which died or euthanized within 5 years of Sept. 11, 2001 were included in this study. Dogs were necropsied by local veterinarians by a standardized protocol, and their tissues forwarded to DCPAH, Michigan State University, for histopathology and toxicologic evaluation. A total of 23 dogs were included; 18 were deployed and 5 were non-deployed controls. The mean age at death of deployed dogs was 10.1 ±3.2 years; the mean age of non-deployed dogs was 11.1 ± 2.8 years. **Proliferative conditions including tumors were the most common cause of death in deployed dogs** (38.9%, 7 of 18 dogs), while degenerative conditions were the most common cause of death in non-deployed dogs (60%, 3 of 5 dogs). The cardiovascular system was the most commonly affected system in deployed dogs (38.9%, 7 of 18 dogs), while the central nervous system was most commonly affected in non-deployed dogs.

Incidental pathology, not directly related to the dogs' death, was evaluated and was most common in the pulmonary tissue. **Seventeen of 18 deployed dogs, and 4 of 5 non-deployed dogs, exhibited varying degrees of anthracosis and refractile particulate matter within their lungs** microscopically. These findings represent inhaled matter such as smoke, ash, and pulverized airborne materials, which were of major concern since S&R dogs are not fitted with any respiratory protection during their S&R activities, unlike their human counterparts. Interestingly, **no primary pulmonary tumors, inflammatory conditions, or chronic degenerative conditions** - which one might expect following inhalation of potentially toxic or carcinogenic materials - **were identified in any of the S&R dogs**.

Toxicologic testing included PCB testing of abdominal fat, inductively coupled plasma-atomic emission spectroscopy for trace minerals and heavy metals on both liver and kidney tissues, and gas chromatography and mass spectrum analysis of liver for organic toxic compounds. **No significant PCB or heavy metal concentrations were identified in any dog's tissues.** Fifteen out of 23 S&R dogs had euthanasia compounds identified from their livers, but no non-therapeutic toxic organic compounds were identified.

The long-term surveillance is funded for two additional years, taking this study out to 9 years post deployment. Additional specialized electron-microscopic and x-ray dispersive studies of the fixed lungs are underway to more fully characterize the fibrous and non-fibrous particulate matter within the lung tissues. We hope these additional studies will allow us to quantitate and differentiate the inhaled components between the two deployed sites, as well as provide insight into potential problems faced by human rescue workers who also were deployed to these sites.

Proventricular Dilatation Disease associated with *Bornavirus* in four Psittacine birds

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Proventricular Dilatation Disease (PDD) is a condition found primarily in psittacines. The disease is characterized by regurgitation of food, passing of undigested seeds in feces, neurological signs, anorexia, weakness, loss of weight and death. PDD has also been described in other species of birds such as raptors, toucans, Canada geese, canaries, finches, *etc*. The pathology of PDD includes dilation of the proventriculus (70 % of the time) and distention of the duodenum with lymphoplasmacytic inflammation in the central, peripheral and autonomic nervous systems, as well as, adrenalitis and myocarditis. Even though the disease has been known since the 1970's, the cause of this condition has not been determined.

PDD was diagnosed in four psittacines from one aviary based on characteristic gross and microscopic lesions. The four birds were: 2 adult Canindae Macaws, a one-year old Vinaceous Amazon Parrot and a four-month old Harlequin Macaw. The birds had clinical signs ranging from regurgitation, loss of weight, weakness and lethargy, to sudden death. All birds had moderate to severely distended, thin walled proventriculi and one bird had enlarged adrenal glands upon postmortem examination. Histopathology revealed lymphoplasmacytic inflammation in various organs as described above.

Using high throughput pyrosequencing and real time PCR, a virus related to Borna disease virus was identified in the brain, adrenal gland and gastrointestinal tract of the birds diagnosed with PDD. Borna disease virus was not detected in the four other birds that were unaffected by PDD. **This is the first description of an association between PDD and Borna disease virus in psittacine birds.**

BVDV – induced bovine congenital tremor associated with hypomyelination in 23 British herds

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Introduction. This paper describes the clinical, neuropathological and virological changes of calves from 23 herds in England and Wales with congenital tremor associated with severe diffuse neuraxial hypomyelination caused by persistent BVDV infection.

Materials and Methods. Investigations were carried out at VLA laboratories on 34 affected calves which were identified over a 17 year period from 1991 to 2007, from 23 British herds. Calves which were presented alive were examined clinically prior to being euthanased. Others were received dead, having died or been euthanased by a practitioner, and details of clinical signs were given by the farmer and/or the practitioner. Tests for BVD virus infection were undertaken on blood and/or tissue samples, with supplemental genotyping of the isolates from seven affected calves and two clinically-normal BVDV-infected cohort calves from one of the herds. Histopathology was performed on the brains of all calves by examination of haematoxylin and eosin stained sections. Selected sections were also stained for myelin using Luxol fast blue, and for pestivirus antigen by IHC with a monoclonal antibody (15C5).

Results. Tremor was the principal presenting sign for affected calves in all but two of the herds. In the latter a range of congenital neurological signs including incoordination or inability to stand from birth was seen. Other neurological signs reported included recumbency or inability to stand unassisted, nystagmus, apparent blindness, fitting and opisthotonus. Details of the herd types were recorded for 21 of the herds, all except one being Holstein Friesian dairy herds, one also having Brown Swiss cattle. One was a suckler herd. Histopathologically, severe diffuse deficiency of stainable myelin was a consistent feature in all affected calves. In addition there were abnormalities of white matter macroglial nuclei. In only one calf was cerebellar dysgenesis evident. Pestivirus labelling was demonstrated in each of the brains and consisted of intense punctuate and linear cytoplasmic neuronal cell body labelling which was especially frequent in the granule cell and pyramidal cell layers of the hippocampus. Extensive diffuse cytoplasmic staining for pestivirus antigen was also observed in pericytes and white matter glia. BVD virus was not consistently demonstrated by virus isolation, antigen ELISA or fluorescent antibody testing on the earlier cases, which was probably due to inhibition by colostral antibodies. For all the more recently examined calves RT-PCR testing was consistently positive for BVDV. The BVD virus isolates from four of the herds where virus classification was carried out were identified as BVDV 1a, similar to those previously typed from animals in England and Wales, whilst three virus isolates from a fifth herd, including two cohort calves, were identified as BVDV 1b.

Discussion/Conclusion. Diffuse neuraxial hypomyelination due to infection with BVDV was the cause of congenital neurological disease in all the calves examined in this study. The identification of pestivirus antigen within CNS neurons was indicative of persistent viral infection. Virus classification undertaken on seven of the affected and two unaffected cohort calves identified a range of BVDV 1a and 1b isolates which indicated that the occurrence of hypomyelination was unlikely to solely relate to the virus strains. Since hypomyelination due to foetal infection with BVDV is considered to be relatively rare in cattle the timing of the transplacental infection in these herds was most likely the critical factor.

Toxicology and Disease Surveillance Scientific Session

Sunday, October 26, 2008

Auditorium-III

Moderators: Patricia Talcott
Catherine Barr

* Graduate student presentation

The state of veterinary diagnostic toxicology:
Toxicology and analytical chemistry survey results

Toxicology Working Group of the National Animal Health Laboratory Network (NAHLN Tox)

Stephen Hooser[1], Robert Poppenga[2], Co-Chairs

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The Toxicology Working Group of the National Animal Health Network (NAHLN Tox) was established in May, 2007 in recognition of the need for a National plan to support, coordinate, and establish formal lines of communication among existing state veterinary analytical toxicology laboratories and appropriate governmental agencies. It is comprised of professionals from state veterinary diagnostic laboratories who analyze and diagnose chemical toxicoses and deficiencies in animals. A great strength of the current system is that veterinary toxicology capabilities with highly trained professionals and established analytical methods already exist within AAVLD-accredited veterinary diagnostic laboratories in many states. However, the personnel, instrumentation, state-of-readiness, and surge capacity of these state/university analytical toxicology laboratories are variable. To more fully document the readiness of existing veterinary toxicology and analytical chemistry laboratories to meet current and future threats to human and animal food safety, a survey was undertaken in 2008. The survey consisted of 30 questions with comments. A total of 41 responses were received. While those laboratories with analytical toxicology sections indicated an ability to detect commonly encountered chemicals, it is apparent that most individual laboratories are ill-prepared to respond to a major chemical contamination event due to personnel and/or equipment limitations. A minority of laboratories possessed the necessary equipment for broad-based chemical screens or investigation of non-routine chemical contamination incidents, and very few had the ability for analysis of chemicals such as blue-green algae toxins, ricin, shigatoxin, or tetrodotoxin. Only 1/3 of the respondents indicated that they currently participate in a national surveillance program with a significant focus on broad-based chemical detection (FERN). Only 15% of respondents indicated that they had received increased funding for toxicology over the last 6 years, while 27% indicated that funding had decreased. The majority of respondents indicated that they had insufficient surge capacity to effectively deal with a major incident. **Over 90% of respondents indicated that current funding was inadequate to purchase and maintain state-of-the-art instrumentation or to hire and retain sufficient numbers of knowledgeable and trained personnel necessary to rapidly respond to routine or extraordinary analytical toxicology needs.** Slightly over ½ of the respondents indicated that they were planning new equipment purchases within the next two years, although there was a relative lack of intent to improve capabilities to detect organic compounds compared to metals. A majority of respondents indicated that important components of a national network include broadly available basic capabilities with comprehensive regional capabilities, improved inter-laboratory communication capacity and coordinated method development efforts. **Clearly, the existing veterinary toxicology and analytical chemistry laboratories provide valuable services to their states for analysis of common toxicants, however, more resources are needed to protect the nation's human and animal food supplies from intentional or accidental chemical contamination.**

Pathology and mortality associated with graded levels of melamine fed to young broiler chickens

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Introduction. Recently, there has been concern about the intentional adulteration of protein ingredients used in pet foods, poultry rations and human foods with melamine. The LD_{50} of melamine is greater than 3000 mg/kg in rats suggesting that it is not a potent toxin. The combination of melamine and cyanuric acid in pet foods is suggested to result in crystal formation in the kidneys of cats and dogs leading to acute renal failure. Melamine was reported to have been incorporated into poultry rations at low levels on 30 commercial broiler farms. No clinical signs of disease or mortality were reported. The objective of this study was to evaluate the toxic effects of melamine in broiler chickens.

Materials and Methods. A study was conducted to determine the toxicity of melamine in young broiler chicks fed dietary treatments from 1 to 21 days of age. One hundred seventy-five day old male commercial broiler chicks were assigned to 7 dietary treatments with 5 replicate pens of five chicks assigned to each treatment. The diets contained 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% melamine.

Results. There was no difference ($P>0.05$) in feed intake (FI) among controls and chicks fed 0.5% melamine. Feed intake was reduced ($P<0.001$) in chicks fed diets containing ≥ 1.0% melamine. Body weight gain (BWG) decreased significantly in birds fed ≥ 1.0% melamine. Sixteen percent to 36% mortality was observed in chicks fed ≥ 2.0% melamine and 85% of the mortality occurred during the first 10 days of the experiment. Gross pathology was evident in broilers that died of melamine toxicity and this included pale enlarged kidneys and a brown discoloration of the bile. Microscopic examination of a wet mount of the abnormal bile revealed numerous spherical brown crystals. In the seven broilers that died during the first 5 days of treatment, renal histopathology revealed a severe accumulation of eosinophilic to basophilic casts in the collecting tubules and ducts with an associated moderate heterophil infiltration of the collecting tubules and ducts. The tubular casts were composed of what appeared to be single to aggregated spherical crystalline structures. After the initial mortality most birds survived to the end of the experiment, with surviving broilers fed 2 to 3% melamine showing only mild renal pathology at the termination of the study. The maximum dose of melamine was delivered in the 2.5% melamine treatment with an average daily dose of 1140 mg/kg.

Conclusion. Melamine at concentrations ≥ 2% in the feed is toxic to young broiler chickens and chicks which survive beyond 10 days of age have a greater resistance to melamine toxicity. The pathology associated with melamine toxicity is noted primarily in the kidneys with the deposition of crystalline casts in the collecting tubules and ducts. In the current study melamine alone caused renal toxicity in chickens without any other potential synergistic intoxicants such as cyanuric acid. Melamine is not a highly toxic substance in chickens and toxicity in chickens is in a comparable dose range as reported in rats.

Survey of recent food animal toxicoses from contaminated feeds

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Introduction. The FDA Center for Veterinary Medicine (CVM) announced in 2003 a comprehensive and risk-based animal feed safety program, the Animal Feed Safety System (AFSS). Part of the program was to determine background information on feed contamination incidents from sources the federal government does not routinely receive; using in part available data from AAVLD accredited laboratories.

Materials and Methods. A voluntary retrospective survey was initiated among 41 American Association of Veterinary Laboratory Diagnosticians (AAVLD) laboratories requesting information about poisoning incidents from feed contamination in the past 5 years. Requests for information included the following 8 questions: 1) Where the incident occurred, 2) Whether a contaminant was found, 3) Level of contaminant found, 4) Type of feed affected, 5) Animal species affected, 6) Number of animals affected, 7) Disposition of the feed and 8) Disposition of the animals. Twenty laboratories initially indicated they would participate and 14 were able to complete the survey. Responses were recorded by region, corresponding to the six USA regions for the AAVLD. These regions are designated as NE, SE, NC, SC, NW and SW.

Results. Fourteen states returned data on 504 instances of feed related poisonings. The top 10 diagnoses amounted to 446 (88.5%) of the total diagnoses reported. The 10 most frequent toxicants across all reporting regions in decreasing order of prevalence were copper, cantharidin, deoxynivalenol, ionophores, fumonisins, ammonia/NPN, nitrate, organophosphates, aflatoxins and ergot. The 4 mycotoxins in the top 10 accounted for 125 reports. The next 10 most reported toxicants in decreasing order were carbamate insecticides, arsenic, cocklebur, lead, zearalenone, ethylene glycol, fluoride, gossypol, oxalate and roquefortine. This group accounted for 26 additional diagnoses, so the total diagnoses from the top 20 toxicants was 472 (93.7%) of the 504 total reports. The most frequent bovine poisonings were NPN, ionophores, nitrates, copper and organophosphates. Copper was the most frequent toxicant for both sheep and goats. Nearly all (94/95) cantharidin poisonings were in horses, followed by fumonisins and ionophores. The top 5 diagnoses in swine were deoxynivalenol, fumonisins, OP insecticides, zearalenone and aflatoxin. Response totals were heaviest from the NC region (280 reports; 6 labs) followed by SC (169 reports; 1 lab) and SE (40 reports; 4 labs). The three remaining regions reported less than 10 cases from each of those remaining regions (NE 6 reports - 1 lab; NW 4 reports - 2 labs; SW no reports).

Discussion/Conclusion. Diagnoses reported were primarily from familiar sources and were consistent with risk factors for each species. History was a limiting factor in some cases, especially regarding disposition of affected animals and contaminated feed. Ability to efficiently search multiple year diagnostic data and cost of assays were limiting factors in complete evaluation of contamination incidents. Considering the limitations noted, respondents provided substantial information about prevalence and distribution of feed related poisonings as reported through diagnostic laboratories. **A relative lack of data about "disposition of feed" and "disposition of animals" reflected that this information is often not shared with the laboratory or may not be requested early in the course of a problem and later is difficult to reconstruct.** A solution to this would be targeted funds to confirm contaminations, expanded field response in the states and a continuing prospective study to accumulate feed contaminant data annually to provide continuing input on feed related poisonings and to document them in more detail. Such information could assist in defining the changing risks of feed contaminants to livestock.

Managing asymptomatic feeder cattle with confirmed lead exposure for food safety

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A 5000 head backgrounding feedlot in Alberta unknowingly ground an implement battery through the feed mixer and fed the lead contaminated feed to a portion of the cattle in the yard. The feedlot was maintained in quarantine while an initial disease investigation, measuring blood lead levels, was undertaken to determine the extent of the lead exposure in the feedlot and to formulate a plan for the disposition of the exposed and unexposed animals. An acceptable level of blood lead for an animal was set at <0.11 ppm based on regulations elsewhere in the world and discussions with the Canadian Food Inspection Agency. Animals determined not to have been exposed to the lead contaminated feed (blood lead levels <0.11 ppm) were subsequently released from quarantine. All 487 animals with an initial blood lead level above 0.11 ppm (range: 0.11-0.89 ppm) were maintained in a voluntary quarantine and enrolled in a project to investigate the depletion of body lead burdens in these animals. These animals were grouped into low (0.11- 0.30 ppm), medium (0.31 – 0.50 ppm), and high (0.51 – 0.89 ppm) exposure levels based on initial blood lead levels. Blood was collected from all animals at monthly intervals for a period of six months. Liver biopsies were collected using tru-cut biopsy needles at enrollment and at month 2, 4, and 6 post enrollment from 30 randomly chosen animals (10 from each exposure level). Four animals from each exposure level were randomly chosen for euthanasia each month. Blood, kidney, liver, skeletal muscle, diaphragm, and bone were collected for tissue lead analysis and the GI tract visually examined for evidence of lead fragments. At the end of the study period those animals with a blood lead level below 0.11 ppm were cleared for slaughter at a federally inspected abattoir. The offal from all slaughtered animals was condemned and did not enter the food chain. Based on bone lead results obtained from sacrificed animals some of those cleared for slaughter were deboned. Eighteen animals were euthanized at the end of the study due to blood lead levels remaining above 0.11 ppm (lead fragments were found in the GI tract of 17/18 of these animals).

Lead fragments were found in the GI tract of 73% of all animals sacrificed, regardless of the exposure group, during the course of the project. Skeletal and diaphragm muscle lead levels of sacrificed animals were low (<0.03 ppm) regardless of the blood lead levels at the time of sacrifice. The bone lead levels ranged from <0.03 ppm to 32.0 ppm. Liver biopsy lead levels correlated poorly to liver tissue lead levels. The blood lead depletion curves showed that the animals could be divided into two groups. One group had a blood lead half-life of approximately 70 days. The second group had a blood lead half-life of approximately 200 days. We speculate that the longer depletion curves of this group represent animals that retained significant amounts of lead fragments in their GI tract.

There is currently a lack of information concerning the management of cattle exposed to lead with respect to food safety. Results from this field study will provide much needed information on post-exposure blood and tissue lead concentrations. This information can be used to make management decisions and formulate regulations for the disposition of exposed animals in future accidental lead exposure cases.

Clinical findings and serum cardiac troponin I concentrations in horses after intragastric administration of sodium monensin

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Monensin is an ionophore antibiotic used in the dairy and beef industries to decrease the incidence of ketosis, improve feed efficiency (growth promotion), and help control ruminal acidosis, and in the poultry industry as a coccidiostatic agent. In the horse however, it is highly toxic and accidental, feed-related outbreaks of monensin toxicosis are well documented. The proposed LD_{50} of monensin for horses is reported to be 2-3 mg/kg based on a single dose study in non-fasted horses. Monensin's toxic effect is caused by abnormal movement of sodium, potassium, and calcium ions into muscle cells, with myocardial necrosis believed to be the result of myocyte osmolar imbalance and mitochondrial dysfunction. Cardiac disease and heart failure are the most common and serious consequences of monensin ingestion in horses. Specific biochemical detection of cardiac disease and monensin poisoning in horses has previously relied upon measurement of creatine kinase myocardial band (CKMB), but few veterinary laboratories assay CKMB and the specificity of this test for detecting cardiac disease is unknown in horses but poor in other species. Recently, we have reported that cardiac troponin i (cTni), measured by either Access® Immunoassay or point of care analyzer i-STAT® 1 (Heska™), is an accurate method of detecting cardiac disease in the horses. The purpose of this study was to describe the clinical signs and cTni changes over time following administration of monensin at different doses, all below the reported LD_{50}, mixed in either water or corn oil, and administered to either fasted or fed horses.

Six adult horses were administered sodium monensin, 1.0-1.5 mg/kg via gastric gavage. Anorexia and/or diarrhea occurred within 24 hours after monensin administration in all 6 horses**. Cardiac disease, determined by both elevations in heart rate and increase in serum concentrations of cardiac troponin i (cTni), occurred in 4 horses. Development of and severity of cardiac disease was likely affected by monensin dose, vehicle (water or corn oil) mixed with monensin, and/or whether the monensin was administered to fed or fasted horses. The 2 horses in this study that recieved the higher doses of monensin after witholding feed were the most severely affected. Initial increases in cTni concentrations occurred between 24 and 72 hours after monensin administration**. **The 2 horses with the highest cTni died or were euthanized within 5 days after monensin administration and had severe cardiac disease. One horse had increased cTni concentrations from day 2 to day 16, but no apparent change in ventricular contractile function was evident. The 4th diseased horse did not return to normal cTni range until day 27 post monensin administration and the ventricular function was still abnormal at euthanasia 9 months later. cTni measurements could be useful in managing farm outbreaks of accidental monensin feeding by the early identification of horses with cardiac disease.**

Zinc phosphide rodenticide toxicity in Oregon wild geese (*Branta spp*.)

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Epizootic mortality of migrating Cackling geese (*Branta hutchinsii*) during the late winter/early spring has been recognized for over a decade in the Willamette valley of Oregon. In fall of 2004 and January 2005, two epizootics involving a total of over 300 geese, were investigated. Similar smaller die-offs were documented during 2007 (3) and 2008 (1), with mortalities ranging from 5 to 67 birds. In each instance toxic levels of zinc phosphide were determined to be the cause of the epizootic.

Cackling geese were mainly affected but mortalities in Taverner's geese (*B. h. taverneri*) and Western Canada geese (*B. canadensis. moffitti*) were also documented in some outbreaks. Most birds were simply found dead. Occasionally clinical signs such as weakness, inability to raise the head or to control head movements, impaired locomotion, and convulsions were observed. Clinically affected birds generally died within a few hours of capture. At necropsy, most birds were in good body condition and often had fresh grass in the stomachs and/or esophagus. Grain was observed in the proventriculus of some of the 2008 mortalities. Gross lesions consisted of pulmonary congestion and edema, sometimes with concurrent mild renal pallor. Histopathology failed to reveal changes in the brain, spinal cord or peripheral nerves. Pulmonary congestion and edema was generally present microscopically but in many carcasses was confounded by post mortem change and freezing artifact. Renal changes were inconsistently present and were limited to mild tubular nephrosis, sometimes complicated by mild renal coccidiosis. Bacteriologic culture of viscera of several birds was unrewarding, as were tests for decreased cholinesterase activity in brain tissue. Hepatic lead levels were not elevated in the birds tested. Mouse inoculation assays for botulinum toxin in the blood of clinically ill geese (2005 outbreak) were negative. Initial GC/MS assays of liver and stomach contents in the 2004/2005 outbreaks were negative. **GC/MS confirmation of the presence of phosphide required acid extraction of the ingesta collected from the upper alimentary tract, plus the use a separation column capable of detecting the phosphine gas that is released following exposure to acid.**

These epizootics were due to the use of zinc phosphide-based rodenticides, in both granular and grain-baited forms**. Geese may be unusually susceptible to this compound. Diagnosis requires a high index of suspicion and appropriate handling of this labile and potentially hazardous toxicant.**

Arsenic intoxication with sheep dipping powder in a calf

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A 3-month-old black Angus bull calf was found dead without preceding clinical signs and submitted to the Animal Disease Diagnostic Laboratory for post-mortem examination. No other calves or cows within the paddock were affected. Gross findings included focally diffuse abomasal mucosal hemorrhage, pink fluid contents throughout the intestine, and dehydration. Relevant histopathologic findings included hemorrhage in the abomasal mucosa, adrenal cortices, mesenteric lymph nodes, myocardium, and cerebrum. Following the post-mortem exam, the farmer submitted some yellow powder that was found in an old shed in the pasture where the calf died. The lid to one of two wooden boxes marked "Poison" had been overturned, and yellow powder from the box had been scattered on the ground. The yellow powder contained 84% sulfur, 9.3% arsenic, and trace amounts of DDT and lindane. Samples of rumen contents and kidney were submitted for quantitative analysis. Rumen contents from the calf contained 397 ppm arsenic and kidney contained 27 ppm arsenic, consistent with arsenic intoxication. DDT and lindane were not detected in the rumen contents. Based on the gross and histologic lesions, and toxicologic analysis of rumen contents and kidney, arsenic intoxication was the most likely cause of death in this case.

Because both arsenic and organochlorines were detected in the submitted powder, we speculate that this particular sample was manufactured in the late 1940's or early 1950's after organochlorine insecticides were made available for the public, but before arsenic use was discontinued. **Although this product is no longer commercially available, exposure to out-dated stores of arsenic-containing pesticides poses a risk to livestock.**

The use of intravenous lipid solution therapy in the treatment of Moxidectin overdose in a dog

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Two and one half milliliters of a topical parasite control solution containing 10% imidacloprid and 2.5% moxidectin were mistakenly administered orally to an 11.8 kilogram, 9 month old, intact female mixed-breed dog. Realizing her mistake, the dog's owner contacted an animal poison control center for assistance, where it was determined that the moxidectin dosage the dog ingested (5.3 mg/kg) was sufficient to cause significant and serious central nervous system effects (the dosage of imidacloprid was not expected to cause significant clinical effects). Although emesis was successfully induced at home by the owner, within 4 hours of ingestion the dog developed severe ataxia, lethargy, tremors, hyperthermia and clinical blindness. The dog was treated with intravenous fluids and activated charcoal, but her neurological status continued to deteriorate. The animal poison control center recommended the use of intravenous lipid infusion. An 18 mL bolus of 20% lipid was administered followed by an infusion of 180 mL/hr until a total volume of 250 mL had been administered. Within 2.5 hours following the onset of lipid therapy, the dog's ataxia and mentation were markedly improved and only mild facial tremors were present. By 12 hours of receiving the intravenous lipids, the dog had made a full recovery.

This is the first report of an intravenous lipid solution (ILS) being successfully used in the management of a moxidectin overdose. Originally utilized as a component of parenteral nutrition, ILS therapy has recently been proposed as a means of managing severe systemic toxicoses. The initial reports using ILS involved 'rescue' of patients with cardiovascular compromise or collapse secondary to local anesthetic toxicosis. The potential use of ILS for toxicoses has expanded to include a variety of other lipophilic agents including verapamil (dogs) and bupropion (humans). Although the exact mechanism of action of the ILS is not fully understood, it is thought that the lipid compartment formed in the blood by the ILS acts as a 'sink' for lipophilic drugs, making them unavailable for action on their target receptors. **In this case, rapid improvement in the dog's neurological status following the administration of ILS demonstrated the promising potential of this modality in the management of selected toxicoses.**

Rapid screening of samples for Avitrol by LC-MS/MS

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Introduction. Avitrol, 4-aminopyridine, is one of the most frequently used avicides and is available as grain baits or as a powder concentrate. It is registered with the EPA for use against various pest birds such as red-winged blackbirds, grackles, pigeons and sparrows. Avitrol repels birds by poisoning a few members of a flock, causing them to become hyperactive and voice distress calls which signal other birds to leave the site. The central nervous system is strongly excited by 4-aminopyridine as it is a potassium channel blocker. While intended strictly for use as a bird repellant, accidental poisonings of non-target species have been reported. Analytical methods have been published for determination of Avitrol in biological samples including the techniques of gas chromatography-mass spectrometry (GC/MS), capillary electrophoresis and ion-pairing high performance liquid chromatography with UV detection (LC-UV). In recent years, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has become the technique of choice for rapid, unambiguous screening of samples for Avitrol.

Materials and Methods. A rapid, sensitive and selective analytical method for detection of Avitrol in bait and GI content samples using liquid chromatography coupled with triple quadrupole/linear ion trap mass spectrometry was developed. One gram of sample was weighed into a 15 mL screw-cap, disposable tube, the pH was adjusted to above 10 using ammonium hydroxide, 10 mL of methylene chloride was added, and the sample was rotated at medium speed for 30 min. The mixture was centrifuged at 260g for 5 min. An aliquot (8 mL) of the methylene chloride extract was evaporated to dryness, using an N-Evap nitrogen evaporator (Analytical Evaporator, Organomation Assoc. Inc., Berlin, MA) set at 50^{o}C. The extract was redissolved in 400 µL of methanol:water (1:1, v/v) and filtered through a 0.45 µm HPLC filter (Millipore Corp., Milford, MA) into a small volume autosampler vial. All control and fortified samples were prepared in the same manner. An Agilent Model 1100 (binary) 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 mm x 4.6 mm Synergi Polar-RP (Phenomenex^{R}). The mobile phase consisted of: (A) 0.01 M ammonium acetate in 0.1% formic acid in water (v/v); (B) 0.01 M ammonium acetate in 0.1% formic acid in methanol (v/v) at a flow rate of 500 µL/min under a linear gradient of 60% B to 95% B over 15 min. MS data for this screen were acquired in the positive ion electrospray ionization (ESI) mode, using the following TurboIonSpray source conditions: temperature = 550^{o}C, curtain gas = 30 (arbitrary units), GS1 = 60, GS2 = 60, CAD gas pressure high, ion spray voltage = 5500, declustering potential (DP) = 49. The MS/MS acquisition consisted of full scan MS/MS (Enhanced Product Ion) mode using the following parameters: precursor ion = *m/z* 95, collision energy (CE) = 49, product ion scan range = *m/z* 50-100. Twenty µl of standards in methanol:water (1:1, v/v) or sample extracts were injected into the system described above. Each set of samples contained a reagent blank, control and fortified samples.

Discussion. This LC-MS/MS method is suitable for rapid analyses of bait and GI contents for Avitrol. The method offers great advantages over other conventional methods including: two orders of magnitude better sensitivity, reduced length of sample preparation and a guarantee of unambiguous results.

Conclusion. The method has been successfully applied to analyses of many diagnostic samples for Avitrol. Starlicide (another avicide) can also be analyzed by this method.

Development of a GC/MS mulit-residue screen for insecticides in diagnostic samples using multi-layer solid phase extraction technology

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Introduction. Analyte ion suppression and undesirable signal enhancement caused by lipid and fatty acid components in complex biological samples can compromise GC/MS detection of insecticides and other toxicants. As a result, a variety of sample extraction techniques have been established including liquid-liquid extraction (sample preconcentration), gel permeation chromatography, and solid phase extraction (SPE) procedures. Unfortunately, most of these methods are time consuming, utilize copious amounts of solvents, and/or require the use of more than one solid phase extraction technique. In order to alleviate these issues, our laboratory investigated the use of a multi-layer SPE technique to enhance GC/MS detection of insecticides in complex biological samples.

Materials and Methods. Biological samples (e.g., rumen contents, stomach contents, and milk), including positive and negative controls, were homogenized in acetonitrile and NaCl (10:1, v/w) and the filtrate dried over Na_2SO_4. The filtrate was then evaporated to dryness and reconstituted with 1 mL acetonitrile for SPE cleanup. Prior to sample cleanup, an ENVI-Carb™-II/PSA SPE cartridge was conditioned and equilibrated with acetonitrile:toluene (3:1). The reconstituted acetonitrile filtrate was loaded onto the SPE cartridge and the eluate, containing the co-extractive interferences, was discarded. Insecticide analytes were eluted from the SPE cartridge with 20 mL acetonitrile:toluene (3:1) into a 50 mL glass test tube and evaporated to dryness. The residue was then reconstituted with 1.0 mL acetonitrile: toluene (3:1) for GC/MS analysis.

Results. The results of this investigation indicate that the use of ENVI-Carb™-II/PSA SPE technology for sample extraction 1) reduces matrix interferences by removing fatty acid and lipid components, 2) extracts multi-residue insecticides from a variety of sample matrices, and 3) enhances the GC/MS detection of carbamate, organochlorine and organophosphate insecticides.

Conclusion. Using multi-layer SPE chemistries, such as the ENVI-Carb™-II/PSA SPE technology, provides a simple, rapid means to enhance the GC/MS detection of insecticides from a variety of sample matrices.

Raccoon variant rabies research in Ohio: current work and future directions

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Raccoon variant rabies occurs throughout the eastern and northeastern United States. Since the mid 1990's an oral rabies vaccination (ORV) program has been in place to prevent the westward spread of the disease. In 2004 raccoon variant rabies was found in northeastern Ohio, representing a localized breach in the ORV barrier. Previous modeling research suggests the topography of Ohio could lend itself to rapid westward spread of raccoon variant rabies if unimpeded by vaccinations or physical barriers. We are radio tracking raccoons in rural and suburban areas of Cleveland, Ohio to evaluate whether barriers or corridors to raccoon movements exist. **Preliminary results suggest that raccoons are remaining within their home ranges within greenbelts, although some have moved up to 2 km, crossing major highways, before returning.** In addition, we are conducting genetic analysis on tissue samples from 180 raccoons in seven counties. The degree of relatedness and distance between raccoon populations will allow us to estimate movement rates, and thus the potential for rabies spread. A more complete understanding of raccoon movements in rural, suburban and urban environments will allow researchers to make recommendations to Wildlife Services Operations as to the location of ORV bait distribution and trap-vaccinate-release (TVR) strategies. The locations of barriers or corridors to raccoon movement may provide focal points for rabies management. The current study has been extended through September, 2009 with expanded objectives to include abdominal VHF transmitter implants in juveniles and GPS collars on adult raccoons in heavily urbanized areas. Furthermore, genetic sampling will be expanded to include raccoons from counties outside the initial 7-county sampling zone.

Real-time RT-PCR training on high throughput platforms for the National Animal Health Laboratory Network

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Introduction. One of the primary missions of the National Animal Health Laboratory Network (NAHLN) is to conduct surveillance testing for early detection of animal disease, such as avian influenza (AI), Newcastle disease (ND), classical swine fever (CSF) or foot-and-mouth-disease (FMD.) The development of a large scale surveillance program has led to the need for adequate capacity to respond to diagnostic testing needs during surveillance and in the instance of an animal disease outbreak. To meet this goal, high-throughput equipment has been purchased and distributed to thirty-one of the NAHLN member laboratories. The next step in this process is to ensure that NAHLN laboratory personnel have been properly trained in the use of the equipment. Members of National Veterinary Service Laboratories (NVSL) and the NAHLN office, hosted by Kansas State University, provided high-throughput equipment training for members of the NAHLN during the months of May, June and July 2008.

Materials and Methods. Training sessions were conducted at the new Biosecurity Research Institute (BRI) on the Kansas State University campus in Manhattan, KS. The facility boasts an integrated training suite that includes a classroom with computer access adjoining a mock laboratory. The laboratory allows for the use of demonstration machines from vendors in a simulated classroom environment. Manuals with the key strokes needed to program the machines were produced for the training sessions. Training consisted of interactive computer training and hands-on laboratory training with each of the instruments. Participants worked in pairs with NVSL or KSU trainers. Using the same mechanism as "Train the trainer", each participating laboratory was allowed to send one person to attend the session. After training, this individual was proficiency tested and, once passed, able to train the rest of their laboratory personnel on the the high-throughput equipment.

Results. The exercise allowed participants to practice nucleic acid extraction of AI or CSF virus using magnetic particle processor platforms, liquid handlers and high-throughput thermal cyclers with inactivated AI panels or armored RNA CSF panels. Data from the teams was collected for presentation to AAVLD and NAHLN. Concepts of reagent aliquoting and storage, and handling a large number of samples were discussed and practiced in the training classroom or laboratory. Additionally, representatives from the major vendors of this equipment were available to answer questions from all participants on the mechanics and possibilities of the equipment.

Discussion/ Conclusion. Training manuals and the ability to practice the steps involved in using the platforms in a laboratory setting resulted in efficient, effective training of participants on new equipment as determined by performance on proficiency tests. Participation in the training allowed NAHLN laboratories to understand the capabilities of the equipment and the potential of each laboratory as they pursue a standardization of methods within a flexible framework of laboratories and abilities.

A new data analysis tool for the National Animal Health Laboratory Network

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The National Animal Health Laboratory Network (NAHLN) was formed to provide a national response laboratory network to support surveillance, early detection, and assist in response and recovery of high consequence animal disease outbreaks including avian influenza, Newcastle disease, classical swine fever, and foot and mouth disease. Standardized diagnostic methods and ongoing proficiency testing provided by the Proficiency and Validation Services Section (PVSS) of the Foreign Animal Disease Diagnostic Laboratory (FADDL) on Plum Island, are critical to the readiness of the NAHLN. **Manual methods of recording and evaluating NAHLN assay control data, assay performance, and proficiency test results have been utilized to date; however, with the introduction of new diagnostic assays, high-throughput methods, and growth of the NAHLN, such manual methods of data collection have proven to be inefficient and inadequate.** To address this gap, we have developed a rational database and data mining tool to track the reference standards, assay controls, and proficiency test results of the NAHLN.

The new database was designed and implemented with FileMaker Pro v9.0 and uses two modules, a System-module and Lab-module. The System-module stays with PVSS and is used to organize data and data entry settings such as allowable commercial kits, reagents, and experimental settings that will be automatically updated by the second module. The second module, the Lab-module, is intended to be distributed to the NAHLN where it will be utilized to collect proficiency test data, assay and surveillance control data. The data file in the Lab-module can be compressed, encrypted and emailed back to PVSS. After importing the data, the System module can perform the data mining and analysis, and generate a final report.

The database has been designed to: 1) remind testees to follow the correct experimental settings; 2) prevent usage of expired kits and reagents; 3) highlight some errors during data entry; and 4) keep data secure with privilege-ranked user accounts and passwords. No personal private information is required, reducing security concerns, and the system is compatible with Mac and PC operating systems. The Lab-module will be able to generate Ct distribution charts and linear regression charts in real-time to allow member labs to instantly verify the performance of assays and new reagents. The new database reduces the time and errors inherent in manual data entry, and turns data analysis, graphing and report generation into a set of button-clicks. As a demonstration of the utility of data mining, the database has been used to generate the official 2007 NAHLN Proficiency Test Report. Current and future efforts will involve collaboration with the NAHLN and the NVSL and NAHLN information technology teams to implement the database in the NAHLN.

Comparison of automated vRNA purification methods for different throughputs: Application in outbreaks, eradication programs and surveillance

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Introduction. Fast and reproducible automated isolation and purification of viral nucleic acids is one of the key success factors for amplification and detection of nucleic acids of pathogens, such as BVD virus, in eradication programs or in situations such as the Bluetongue virus outbreak in Europe.

Methods. Three automated systems allowing low (QIAcube) and high throughput (BioRobot Universal System, BioSprint 96) sample preparations were compared in combination with appropriate silica spin column (QIAcube, BioRobot Universal System) and silica bead (BioSprint 96) based technologies.
Various animal sample materials such as whole blood, ear notches, or skin samples were processed using the different automated sample preparation systems. Depending on sample material and platform different commercially available kits have been used (e.g. MagAttract RNA Tissue M48 Kit, QIAamp MinElute Virus Kit). Quality of the isolated nucleic acid was confirmed by real-time PCR as downstream application (e.g. *cador*® BVDV RT PCR Reagent).

Results. Viral nucleic acid preparation methods were successfully established on all three instrument platforms covering different throughput needs. An equivalency study was performed with bovine whole blood and has shown comparable performance with all systems.
All three automated sample preparation methods are currently in routine use of customer labs depending on their individual throughput needs. For example the BioRobot Universal System is used in the BVD virus eradication program in Switzerland with whole blood as starting material whereas the QIAcube and the BioRobot Universal System serve as a reliable tool for detection of bluetongue virus in the outbreak in France.

In addition the method based on magnetic silica beads has been compared to other commercial methods available in the veterinary market. Depending on the starting material the nucleic acid preparation on the BioSprint 96 system shows comparable or even better performance.

Conclusions. Diagnostic tests using PCR are replacing traditional ELISA or culture-based methods due to higher sensitivity and speed. This detection method depends on easy and standardized applications for nucleic acid isolation of pathogens that gives high-quality nucleic acids for various throughputs.

Virology Scientific Session

Sunday, October 26, 2008

Guilford-D

Moderator: Beate Crossley
Roman Pogranichniy

* Graduate student presentation

Comparative study of four commercial BVDV antigen ELISAs and five commercial real-time polymerase chain reactions (RT-PCRs) for detection of different European BVDV strains and isolates

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Introduction. The purpose of this study was to compare commercial BVDV tests for detection of different European BVDV strains and isolates.

Materials and Methods. Four commercial ELISA Ag kits where used for this study: HerdChek BVDV Ag/Serum Plus, IDEXX; Priocheck BVDV Ag PIplus, Prionics; Serelisa p80 Ag mono indirect, Synbiotics and Ingezim BVD, Ingenasa.

Five commercial RT-PCR kits were also tested: BoVir-SL® BVDV TaqMan RT-PCR Kit, AnDiaTec GmbH; Cador BVDV RT-PCR, Qiagen; Kit TaqVetTM, LSI; Virotype® BVDV, LDL and Adiavet® BVD Realtime, Adiagène

In total, 178 BVDV strains and isolates and two other pestiviruses were used.

Results. Whereas the E^{rns} Ag capture ELISA of IDEXX clearly detected all strains and isolates (n=180), ELISA 1, 2 and 3 (all NS2/3 Ag capture ELISAs) missed 14, 7 and 7 strains and isolates respectively. All strains and isolates tested except nine scored S/P values five times higher than cut-off with the IDEXX ELISA.

Three commercial real time RT-PCRs scored all strains and isolates positive. However, RT-PCR 2, 3, 4 and 5 showed Ct-values of greater than 35 with 3, 8, 1 and 12 samples, respectively, indicating a putative lower analytical sensitivity. The RT-PCR 4 missed one and RT-PCR 2 missed 18 out of 180 strains and isolates.

Conclusion. **IDEXX BVDV Ag/Serum Plus is the only commercial ELISA detecting all strains and isolates tested in this study and therefore a highly suitable tool for successful use in BVDV eradication programs.** ELISA 1, 2 and 3 missed mostly the same set of strains and isolates. BVDV NS 2/3 antigen detection used in these commercial kits may be reduced to a certain spectrum of BVDV strains.

Three commercial RT-PCR detected all strains and isolates. Two of them showed 3 and 8 results, respectively, above Ct value of 35. In the course of planning BVDV eradication programs with sample pooling, these findings should be considered and further discussed. Two RT-PCRs missed 1 and 18 strains respectively. **These results do not meet the requirements of a BVDV eradication program because not all strains present in the field are detected.**

Testing of cattle ear notch samples using a *Bovine Virus Diarrhea Virus* NS3 antigen ELISA for detection of PI Animals

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Introduction. Identification and elimination of Persistently Infected (PI) cattle is important to controlling Bovine Viral Diarrhea Virus (BVDV) in herds. Compared to many diagnostic laboratory techniques, ELISA (Enzyme Linked ImmunoSorbent Assay) is convenient due to the ability to test large number of samples rapidly and consistently. The objective of this study was to compare the performance of a current commercial ELISA which specifically detects the NS3 antigen on ear notches from cattle to other tests utilized in diagnostic laboratories.

Materials and Methods. The expected prevalence of PI cattle is estimated between 0.2 and 2%. The limiting factor for a reference population of known PI and non-PI cattle is therefore the recruitment of these PI cattle. Positive samples used in this study were obtained from samples submitted and confirmed positive at the veterinary diagnostic laboratory (VDL) of Kansas State University (KSU). Negative samples were collected from the field. All samples were tested with at least the ImmunoHistoChemistry (IHC) or the Erns antigen ELISA (IDEXX®) used routinely at KSU VDL. Ear notch samples from 245 PI positive and 501 PI negative cattle were tested with the specific NS3 Ag ELISA (SERELISA® BVD p80 Ag Mono Indirect, SYNBIOTICS® Europe, Lyon, France). The NS3 Ag ELISA test was used following directions in the kit insert except for the sample preparation and the results expression sections. Ear notches were stored frozen (-20 C) in PBS. Then 100ul of PBS solution from the ear notch samples were added to the wells for the sample incubation. Optical densities were interpreted as sample to positive ratio corrected by the negative control.

Results. The SP range for the 501 negative samples was -0.06 to 0.60 with a mean of 0.06. The 245 positive samples ranged from 0.33 to 3.04 with a mean of 2.19. The positive population exhibited a tail in its distribution. The ROC curve analysis provides a significant separation between the populations with several possible positions for the cut off with optimum performances. The elected cut off of 0.50 leads to an observed sensitivity of 98.8% (95CI=[96.5%-99.6%]) and an observed specificity of 99.8% (95CI=[98.9%-100%]). This cutoff was located at 6.8 standard deviations of the mean of the negative population and at 3.6 standard deviations of the positive population.

Discussion/Conclusion. An excellent agreement was observed between ear notch NS3 Ag ELISA test results and the reference methods used in this study. Test results demonstrated good sensitivity for this method despite the broad distribution observed with the positive population. The specificity observed was high and the distance between the negative population and the proposed cut off indicates a good positive predictive value. The positive predictive value is a key issue when using a diagnostic test in a population where the condition or disease is observed with a low to very low prevalence. The high positive predictive value was achieved by the positioning of the cut off and its distance to the negative population.

This study demonstrates the suitability of a NS3 Ag ELISA for the detection of PI cattle in a population with low prevalence.

BVDV eradication program in Switzerland: Requirements for a viral RNA assay

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Introduction. Bovine Viral Diarrhea virus (BVDV) is an economically important cattle pathogen. Switzerland started an official governmentally supported program with the aim to eradicate all persistently infected (PI) animals. The program was launched in spring 2008 with the testing of all heifers before transhumance in the alpine region. From October to December 2008 all remaining bovines will be tested for virus, with the aim of removing all PI animals. As from 1st January 2009 until 31st December 2010, all newborn calves will be tested for virus. In addition to the logistic challenge of taking samples from over 1.5 million animals and performing laboratory tests on this scale in a relatively short time period, testing for BVD virus with the aim of eradicating infection represents a biological challenge. Genetically, BVD viruses are highly diverse, with two genotypes of virus and numerous subgroups within the genotypes, especially in genotype-1 BVD viruses. In addition, certain pestiviruses have been shown to be able to cross the species barrier and establish persistent infection in species not normally considered to be target species. The objective of this project has been to develop a BVDV assay for an eradication program that will detect all pestiviruses currently known to infect cattle.

Materials and Methods. When developing a real-time RT-PCR assay for detecting BVD viral RNA, we used sequence information from some 250 virus isolates obtained from cattle in Switzerland. In addition, we included sequence information BVD virus-1 subgroups and BVDV-2 strains that have not been detected in Switzerland. All available sequence information was integrated in the design of the assay which is in the meantime commercially available (QIAGEN *cador* BVDV RT-PCR Reagent). The performance of the assay was checked using different samples including blood and milk samples. For RNA isolation, the QIAamp Virus BioRobot MDx kit or corresponding manual spin column kits have been used. Real time RT-PCR was performed with the QIAGEN *cador* BVDV RT-PCR Reagent.

Results. **Since Spring 2008, over 70,000 animals have been tested for BVD viral RNA using the assay described. The *cador* BVDV RT-PCR Reagent detected the RNA of all strains of BVDV-1 and Border Disease viruses** demonstrated to be present in the Swiss cattle and sheep population, respectively. In addition, it also detected the RNA of representatives of BVDV-1 subgroups not present in Switzerland, as well as of BVDV-2. In addition, also RNA of "exotic" pestiviruses, like the strain HoBi, Chamois and others was detected. **The analytical sensitivity of the assay was determined to be at 1.35 copies/µl for BVDV 1 RNA and 2.52 copies/µl for BVDV 2 RNA. We did not find a detection gap in young animals**. The assay also detected viral RNA in transiently infected animals. The sensitivity of the real time assay exceeded that of a commercial E^{rns} Elisa.

Discussion/Conclusion. "High specificity" of tests may be detrimental in eradication programs because it may select for detection of some, but not all, viruses infecting a host population. The *cador* BVDV RT-PCR Reagent combines detection of viral RNA of all pestiviruses currently known to infect cattle with a high sensitivity that allows pooling of diverse types of sample. It is suitable for mass screening also in young animals which are known to have a low virus load.

Prevalence and antigenic differences of *Bovine viral diarrhea virus* subgenotypes isolated from cattle in the U.S. and Australia

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Bovine viral diarrhea viruses are segregated into two different species within the pestivirus genus, bovine viral diarrhea viruses type 1 (BVDV1) and bovine viral diarrhea viruses type 2 (BVDV2). While this segregation was first based on phylogenetic analysis, subsequent characterization of viral strains from the two species demonstrated antigenic differences. The practical significance of antigenic differences was evidenced by the failure of vaccines and diagnostics based on BVDV1 strains to control and detect, respectively, BVDV2 strains. Further phylogenetic analysis has revealed subgenotype groupings within the BVDV1 and BVDV2 species. Thus far twelve BVDV1 subgenotypes (BVDV1a, BVDV1b, BVDV1c, BVDV1d, BVDV1e, BVDV1f, BVDV1g, BVDV1h, BVDV1i, BVDV1j, BVDV1k, BVDV1l) and two BVDV2 subgenotypes (BVDV2a and BVDV2b) have been identified. The practical significance of segregation into subgenotypes is still a matter of discussion as to the impact on conferring cross protection to heterologous challenge and the ability of reagents in diagnostic tests to detect the broad range of subgenotypes. Different BVDV1 subgenotypes predominate in different countries**.**

Phylogenetic analysis of 298 pestivirus isolates of bovine origin archived at the Elizabeth Macarthur Agriculture Institute in New South Wales, Australia revealed that 15 isolates (5.0 %) belonged to the BVDV1a subgenotype, 3 (1.0 %) belonged to the BVDV1b subgenotype, 272 (91.3%) belonged to the BVDV1c subgenotype, 4 (1.3%) belonged to the BVDV2a subgenotypes and 4 (1.3%) were shown to be isolates of border disease virus (BDV). In contrast phylogenetic analysis 514 bovine pestiviruses isolated from U.S. between June 2007 and June 2008 revealed 62 (12.1%) BVDV1a strains, 387 (75.3%) BVDV1b strains, no BVDV1c strains, 65 (12.6%) BVDV2a strains and no BDV strains. There is a least one commercial vaccine line available in Australia that is based on viruses belonging to the most prevalent subgenotype present in that country, BVDV1c. While the BVDV1b subgenotype predominates in the U.S., vaccine lines are based on BVDV1a and BVDV2a strains. **Goats hyperimmunized with either 3 different BVDV1a, BVDV1b, BVDV1c, BVDV2a or BDV strains mounted serological responses with comparative antibody levels that correlated with subgenotype used as immunogen.** That is, serum from goats hyperimmunized with strains from one subgenotype was better able to neutralize other strains from that same subgenotype compared to strains from a different subgenotype. **Similarly, serological immune responses mounted by 40 co-housed feedlot cattle persistently infected with different BVDV strains suggested differential responses to strains from the same subgenotypes as the persistent virus compared to strains belonging to other subgenotypes.** These studies suggest that there are antigenic differences between BVDV strains belonging to different subgenotypes. Further studies need to be conducted to determine if vaccine protection can be improved by basing vaccines on the BVDV subgenotypes that are prevalent in the region in which the vaccine is to be used.

Genotyping and phylogenetic analysis of *Bovine viral diarrhea virus* isolates from persistently infected alpacas in North America

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Over a three-year period, 2004-2007, greater than 12,000 alpacas from 9 different states of the United States and Canada were screened by real-time RT-PCR to identify persistently infected (PI) alpacas with bovine viral diarrhea virus (BVDV). A total of 44 BVD viruses were isolated from PI animals and diagnostic samples from alpacas. Forty-one U.S. alpaca BVDV isolates and 3 Canadian isolates were compared based on nucleotide sequences of two viral genomic regions, the highly conserved 5'-untranslated region (UTR) and the less conserved N^{pro} gene. Sequences of the PCR products of the two regions were analyzed to determine their genetic relatedness.

Phylogenetic analysis of the 290 base 5'-UTR region of 44 alpaca BVDV isolates was performed. In the phylogenetic analysis, in addition to reference strains of types 1a, 1b, and 2, 21 bovine BVDV field isolates which were collected during this study period were included. Sequences were compiled using the SeqMan II program and compared using CLUSTAL W. Nucleotide percentage similarities were calculated with MegAlign. All 44 alpaca BVDV isolates were type 1b; in contrast, 21 bovine field isolates were grouped into the typical 3 genotypes, 4 (19%) 1a, 13 (62%) 1b, and 4 (19%) 2, respectively. Forty-three alpaca BVDV isolates formed a distinctive cluster separated from closely related bovine genotype 1b isolates; one Canadian isolate, Hercules, was more closely related to the recent bovine 1b isolates. Phylogenetic analysis of the 504 bases N^{pro} gene of 30 alpaca isolates produced a similar observation showing all alpaca isolates were type 1b and forming a separate cluster among 29 isolates distinctive from bovine type 1b strains except for the Canadian strain, Hercules, which was more closely related to bovine type 1b.

The results of this study indicated that the establishment of persistent infection with BVDV in alpacas was confirmed from various parts of the U.S. and Canada. The major source of transmission of BVDV among alpacas appeared to be from PI alpacas in a similar way as observed in bovine BVD, and all alpaca BVDV isolates in this study were genetically closely related type 1b. Based on these observations, we hypothesize that a single clone or closely related isolates of bovine BVDV 1b infected and colonized alpacas in North America. Further studies are needed to address why alpacas are predominantly infected with genotype 1b BVDV isolates and how bovine BVD viruses have evolved to infect and colonize in alpacas.

Bench validation of a realtime PCR assay for surveillance and diagnostic detection of *Bluetongue virus*

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The introduction of bluetongue virus serotype 8 into the European Union in August 2006, followed by the subsequent and largely unexpected spread of the virus has raised awareness concerning changes in bluetongue virus transmission, associated vector-competence, and increased viral pathogenicity. The reports of clinical disease in cattle, including congenital defects during the EU outbreak has additionally alerted the U.S. to possible economic impacts should a virulent bluetongue virus emerge or be introduced to U.S. livestock and wildlife populations. Though virus isolation, PCR, and realtime PCR assays are currently available as diagnostic tools for bluetongue virus detection in the U.S., the assays have not been formatted or used to date for large-scale surveillance or disease response efforts. The current study reports evaluation and modification to a non-robotic high-throughput format of a European realtime PCR assay (*Orrù et al*. J Virol Meth 137, 34-42. 2006) for detection of bluetongue viruses. The assay was evaluated in both single tube spin-column and in 96-well magnetic bead format. The PCR primers and probe target gene segment 10, also known as NS3, which encodes for conserved non-structural proteins allowing detection of all serotypes of bluetongue virus, including U.S endemic and exotic serotypes. In the current evaluation, reference strains of bluetongue virus serotypes 10, 11, 13, and 17 as well as clinical material from sheep, cattle, and deer, and whole blood from experimental infections with bluetongue serotype 17 were used (n=16 sheep). Bench validation performed using whole blood demonstrated a comparable limit of detection between single tube spin-column and 96-well magnetic bead extraction, for both cattle and sheep. The limits of detection estimated assay analytic sensitivity between 1 and 10 infectious viral particles. The ability to detect viral RNA was not affected by the presence or absence of bluetongue-specific antibody in blood specimens tested. Assay linearity ranged from 5 logs (r^2 = 0.9949) for bluetongue serotype 10 to 7 logs (r^2 = 0.9976) for bluetongue virus serotype 17. **The preliminary bench validation, though limited to a subset of U.S. endemic serotypes of bluetongue virus, demonstrates future potential for use in both routine surveillance and diagnostic applications.**

Development of a quantification method to specific anti-NS3 antibodies against BVDV using a blocking ELISA

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Introduction. Numerous epidemiological models published in Europe concern control of the bovine viral diarrhea virus (BVDV) and specific identification and removal of persistently infected (PI) animals from infected herds. In the US, these models are not easily adapted due to the use of BVD vaccine in calves. Vaccination interferes with previous models when measuring total or seroneutralizing antibodies. An alternative approach is to measure a subpopulation such as Ab targeting non structural proteins that can be found only during virus replication.

The objective of the study was to develop a quantitative serum antibody test for BVD with a commercially available test (SERELISA® BVD Ab Mono Blocking, Synbiotics Corporation) which allows specific detection of anti-NS3 (or p80 in previous nomenclature) antibodies against the BVDV.

Materials and Methods. Results of the test are expressed as sample to negative control optical density (OD) ratio corrected by the positive control OD and referred as s/n ratio. The linear range of the bELISA was determined by conducting the assay with the s/n ratio of a positive reference sample at different dilutions. After graphical analysis, determination coefficients (r2) were calculated for eight different models with variable transformations for s/n ratio and the dilution of titer. Transformations were analyzed for the relationship between titer (T), 1/T, and log T and s/n ratio (sn), 1/sn, log sn and logit sn.

Results. Linear s/n ratio values ranging from 0.13 to 0.90 were determined using the reference BVDV positive serum sample at different dilutions.

Comparing eight different regression models correlating different s/n ratio and titer functions, the best model was achieved utilizing the Log of titer and the logit of s/n ratio. This model was linear with an r2 of 0.983, a slope of β=-0.737 and an intersection of α=1.661.

This linear model covered at least a range of a log (base 10) of titer. In order to have a quantitative method valid for serum ranging from negative status to high positive titer, an interpolation using different wells is needed. Inside the linear range, titers range from 10 to 200 for s/n ratio respectively ranging from 0.13 to 0.90. An arbitrary decision was made to apply this result to the 1/100 final dilution in the well. Derived from this decision, interpolation was calculated for the two next dilutions of 1:10 and 1:1000. A correcting factor (multiply by 10 or divide by 10) was applied to each of the titer results obtained within these wells and therefore linearity is respected between the three dilution wells. The final model was not limited on the lower bound of the 1:100 well and an arbitrary limit was fixed on the upper bound of the 1:10000 well. This limit corresponds to the very limit of linearity of the regression model (s/n=0.107). Titers obtained are expressed in ELISA units (EU).

Discussion/Conclusion: This innovative method provides a quantitative method for a blocking BVD ELISA and therefore allows the detection of specific anti-NS3 antibody subpopulation. The linearity and the robustness have been proven to be effective using three wells in a blocking ELISA. This method is independent of the seroneutralizing properties of the targeted antibody subpopulation and therefore a nice alternative to seroneutralizing tests.

This standardized quantitative test is a tool that will lead to a breakthrough in the understanding of the subpopulation of antibodies against non-structural proteins and be utilized to adapt European epidemiological models for BVDV control despite the uses of vaccination.

Occurrence of Wildebeest-associated Malignant Catarrhal Fever in domestic cattle

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A pregnant Brangus heifer in Georgia, purchased from a Texas sale, presented with clinical signs of malignant catarrhal fever (MCF). The animal was reported as healthy when loaded for shipment, but was depressed on arrival and developed bilateral keratitis, nasal discharge and fever (103-104F) the following day. She was treated with antibiotics, non-steriodal anti inflammatory drugs, intravenous fluids and vitamins. A blood sample was sent to Washington Animal Disease Diagnostic Laboratory to test for ovine herpesvirus 2 (OHV-2), the causative agent of sheep-associated MCF. Two other animals purchased during the same sale also presented with clinical MCF upon arrival at their new locations in Alabama and Louisiana.

A foreign animal disease investigation was initiated. The Georgia animal was euthanized seven days after the start of clinical symptoms. Fresh tissue samples were collected from these three animals and additionally, whole blood and fixed tissue from the Alabama animal were sent to the NVSL for testing. The three animals were traced to a ranch in Texas that held cattle and exotic ruminants including wildebeest. Subsequently, two cows and a bull on the ranch presented with clinical MCF. Tissue and whole blood from these animals were sent to the NVSL for testing.

The tissue and blood samples were tested by nested polymerase chain reaction (PCR) for the presence of alcelaphine herpesvirus 1 (AlHV-1) and OHV-2 DNA, two of the viruses causing MCF. All tissue and blood samples tested produced amplicons for two different gene segments of the AlHV-1 genome. The PCR amplicons were sequenced and found to be homologous to AlHV-1. The pathology post-mortem reports from the Alabama and the Georgia heifers indicated gross and microscopic lesions consistent with MCF. Fluorescent antibody test was positive for MCF viral antigens on tissues from the Georgia heifer. In addition, the blood sample from the Georgia heifer was found positive for OHV-2 DNA by real time and nested PCR in an apparent dual infection.

Transmission of AlHV-1 from wildebeest to domestic cattle occurs around the time of wildebeest calving and shortly after. The clinical animals were thought to have had fence line contact with wildebeest that calved in December. The contact animals from the sale and other animals from the ranch were quarantined or restricted with infection status yet to be determined. Precautions must be taken when housing exotic and domestic species in close proximity in zoos, exotic game parks and ranches.

Development and use of indirect ELISA tests detecting antibodies to *Bovine Coronavirus* and *Bovine viral diarrhea virus* in acute and convalescent serums of cattle

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Viral infections have a significant impact on the cattle population and production. Serological assays for viral infections have traditionally used viral neutralization assays in 96-well tissue cultures. These tests while quantifying antibody levels require several days for endpoint determination. For some viruses, ELISA assays have been developed that permit more rapid determination of seropositive status. Bovine viral diarrhea virus (BVDV) and bovine coronavirus (BCV) are two viruses under investigation in our research on respiratory tract infections, and serotesting permits identification of infections by these viruses in cattle.

The objectives of this study included: (1) development of an indirect ELISA test for antibodies to BVDV and BCV; (2) use of the indirect ELISA to test for antibodies in acute and convalescent sera in cattle with known vaccination status for BVDV or exposure to PI cattle for the BVDV; and (3) the correlation of active infection with BCV with increasing antibodies to BCV. BVDV antigens used in the ELISA were prepared from infected MDBK monolayers using BVDV1b CP strain (Oklahoma isolate) and BVDV2a CP strain 125-C. The BCV antigen was prepared from MDBK monolayers infected with the USDA NVSL BCV enteric strain. Uninfected MDBK monolayers were used as control antigens. Sera were diluted 1:100 and tested with both viral and control antigens. Sera included in the study were BVDV antibody positive samples from cattle receiving MLV vaccine and/or exposed to PI BVDV cattle. Endpoint VNT titers of these sera for BVDV1b, 1a, and 2a antibodies were determined for comparison to the ELISA results. The BCV status of calves was determined by PCR on nasal swabs at day 0 and 35 days later for comparison with ELISA results.

The ELISA testing results indicated that for the BVDV testing, the sera seronegative by the VNT were negative by the ELISA, and VNT positive animals were ELISA positive. Seroconversions to BVDV were detected by ELISA in both vaccinated animals and those exposed to PI cattle. Auction market cattle that were BCV PCR positive in the nasal secretions at day 0 and day 7 with clearance by day 35 were ELISA negative at day 0 and positive at day 35. These results indicate that the ELISA test can be used to rapidly detect antibodies using a single serum dilution. Another finding was that the BCV antigen prepared from an enteric origin BCV strain detected antibodies induced by BCV strains found in nasal secretions of infected cattle.

In summary, the BVDV and BCV ELISAs were able to detect antibodies to BVDV and BCV. This ELISA permits evaluation of BVDV vaccination or PI animal exposure. Likewise the BCV ELISA can be used to monitor for BCV active infections in cattle held over time in feedlots or potentially for vaccination response.

Application of real time PCR and ELISA technology during the equine influenza outbreak in Australia

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In late August 2007, Australia experienced its first occurrence of influenza virus infection in horses. A disease control program was quickly established in New South Wales with the ultimate goal of eradication of EIV. The state was divided into infected and virus free zones to control the movement of horses, personnel and vehicles and to aid the regulation of various equestrian activities. Movements between zones were not permitted without strict quarantine and testing procedures. After a short period, very limited racing was permitted within a zone but all horses were tested with 24 hours of the event. Vaccination with a canary-pox vectored vaccine was conducted in buffer zones surrounding the infected zone and in racing thoroughbred populations. The outbreak was brought under control and no new cases of EI were detected after 3 months. Testing to prove freedom commenced soon after and was completed within 6 weeks, with NSW being declared provisionally free of EI in less than 6 months from the start of the outbreak, despite infection of more than 50,000 horses on 8500 farms.

Diagnosis of the index case of equine influenza (EI) and confirmation of subsequent clinical cases utilised an Influenza A real time reverse transcriptase PCR (qRT-PCR) directed at a highly conserved sequence of the matrix gene. This assay was also employed for the extensive testing program that was undertaken during surveillance in free zones, in buffer zones at the time of vaccination, prior to the release of infected properties from quarantine and for movement purposes or to participate in a race. **Testing could be completed in less than 3 hours of receipt of samples and a high throughput capability allowed more than 70,000 samples to be tested, with 30,000 tested in a 4 week period during the 'Proof of Freedom" stage**. **An Influenza A pan-reactive blocking ELISA (bELISA) was used routinely for serology** to confirm freedom in the free zones and to confirm that all horses in large populations had been infected. **More than 65,000 assays were completed during the outbreak.**

While qRT-PCR and bELISA had previously been used for testing of avian samples, neither of these diagnostic tests had been used to test samples from horses infected with EIV. The use of qRT-PCR, with very high sensitivity and ability to detect non-infectious virus, posed a number of questions including the duration of shedding of virus. With the bELISA, there was interest in establishing the time at which antibodies could be first detected and whether this assay could differentiate between a response to vaccination and natural infection.

Establishing the pattern and duration of viral RNA shedding and the serological response to infection using these tests was given a high priority. **An intensive study was initiated using a group of 36 horses that showed clinical signs of EI infection during the first week of the outbreak. Nasal swabs and blood samples were collected from these horses daily for 42 days** and subsequently a serum sample has been collected monthly to a point 12 months after the initial infection.

Comparative levels of virus excretion during infection, the time of onset for sero-conversion and a profile of the serological response were recorded for each of the horses. These data established benchmarks for the diagnostic tests undertaken during the outbreak. **It is noteworthy that Influenza RNA could be detected up to 35 days after the onset of clinical signs.** These periods for the detection of virus are substantially longer than those obtained by virus isolation or antigen capture ELISA. Nevertheless, it could be shown that each horse was free of evidence of active infection within 40 days of the onset of clinical signs. Seroconversion was observed in the bELISA between 5-7 days post infection and **the bELISA readily distinguished between infected and vaccinated horses. This is believed to be the first large scale application of these assays to testing for EIV.**

Increased pathogenicity and virogenesis within avian hosts infected with a *West Nile virus* containing a single helicase point mutation

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Introduction. Previous studies have demonstrated higher mortality and viremia in American crows (AMCRs:Corvus brachyrhynchos) infected with the New York 99 (NY99) strain of West Nile Virus (WNV) as compared to an Old World strain from Kenya (KEN). A single point mutation incorporated within the helicase gene of the NS3 protein (NS3-T249P) of the KEN virus increased serum titers and mortality to that of the NY99 virus. The pathogenic mechanism for the increased virulence of the NY99 virus and the NS3-T249P viruses has not been determined. The purpose of this study was to investigate differential tissue and cell tropism and pathogeneicity of the two WNV strains, as well as the NS3-T249P mutant virus in AMCRs.

Materials and Methods. Field collected AMCRs were randomly divided into three groups and crows in each group were inoculated with one of three viral strains (KEN, NY99 or NS3-T249P) and up to three AMCRs were sacrificed 1-5, 7 and 9 days post-inoculation (dpi) and necropsied. Virus titration, histopathology and immunohistochemistry were performed on tissues from infected crows at each time point.

Results. Crows inoculated with the NY99 strain developed extremely high viremias that peaked at 4 dpi with a mean of 8.2 $\log_{10}$ PFU/mL sera. Crows inoculated with NS3-T249P virus developed slightly lower viremia, 7.3 $\log_{10}$ PFU/mL sera, that also peaked 4 dpi. In contrast, KEN-inoculated AMCRs had a delayed onset of viremia until 3 dpi in 6 out of 9 AMCRs and peaked viremia at 5 dpi at a much-reduced magnitude (5 $\log_{10}$ PFU/mL sera). Lesions were most severe in the crows inoculated with the NY99 virus but were closely followed by crows inoculated with NS3-T249P. Crows inoculated with the KEN strain had relatively mild lesions as compared to the AMCRs inoculated with NY99 and NS3-T249P viruses. Immunohistochemistry demonstrated that the NS3-T249P approximated the same number of infected cells and tissues at 1-2 dpi as the NY99 virus. In contrast, the KEN strain lagged the NY99 virus by 4-5 days and never reached the number of infected cells as the NY99 or the NS3-T249P strains. Intravascular and infiltrating inflammatory leukocytes preceded infected resident cells in most tissues.

Conclusions. Increased viremia, tissue and cellular infection and higher mortality rates were demonstrated in AMCRs infected with a virus incorporating the Pro residue of the NY99 virus at NS3-249 within the helicase domain.

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Comparison of three extraction methods for the detection of PCV2 and PRRSV in semen

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Porcine circovirus type 2 (PCV2) and Porcine Reproductive and Respiratory Syndrome virus (PRRSV) are two major viruses that cause significant economic losses to the swine industry. Since both viruses are shed in semen for a long period of time after infection and artificial insemination is a common practice in the field, semen is an important source of spreading these two viruses to naïve populations. Therefore, rapid and accurate detection of PCV2 or PRRSV in semen is critical from the standpoint of disease prevention and control. Even though molecular assays have been commonly used to meet such goals, semen is notorious for having inhibitory substances that affect the test outcome. In this study, the performance of three different extraction methods on semen samples was compared to determine the optimal extraction method for semen samples to detect PCV2 or PRRSV.

Seven and 115 semen samples were collected from the boars experimentally challenged with PRRSV or PCV2, respectively. These two sets of the semen samples were processed by three different extraction methods: 1) Ambion® High-Throughput Total Nucleic Acids isolation kit (HT-TNA), 2) Ambion® High-Throughput Viral RNA isolation kit with modified procedure (mHT-VR), and 3) Qiagen® 96-well DNeasy kit (DNeasy for PCV2) or Qiagen® RNeasy kit (RNeasy for PRRSV). Then, the efficiency of the three extraction methods were compared by conducting the same real-time PCR for each virus.

HT-TNA and mHT-VR kits were much more convenient to process semen samples for both viruses since these kits can simultaneously extract both DNA (i.e., PCV2) and RNA (i.e., PRRSV) from a sample. Moreover, HT-TNA and mHT-VR could process 96 semen samples within 90 minutes, whereas it took 3 and 5 hours to process the same number of samples with RNeasy for PRRSV and DNeasy for PCV2, respectively. The HT-TNA kit showed higher sensitivity compared to the other two methods while all three methods demonstrated 100% specificity. In conclusion, the Ambion® High-Throughput Total Nucleic Acids isolation kit can significantly reduce testing time and effort to process semen samples with better sensitivity for detection of PCV2 and PRRSV in the samples.

Practical disease surveillance in growing pig populations

John Prickett[1], Pat Hoffmann[1], W Stensland[1], K-J Yoon[1], Jeff Zimmerman[1]*

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Introduction. In the current economic climate, rising feed costs and declining pork prices have threatened the economic viability of the industry. To remain economically viable, pork producers must reduce costs and increase efficiency. Despite advances in vaccine development, antibiotic treatments, and implementation of biosecurity, swine pathogens continue to cost producers millions of dollars as a result of their effect on pig performance.[1] Likewise, surveys show that the cost of diagnostic testing comprises a significant portion of overhead in an operation.[2] Thus, disease prevention and control, an area directly related to profitability, is one area in which gains in efficiency are attainable. The premise of the current study is that accurate, real-time disease monitoring of commercial swine populations will provide producers the information essential to effectively plan and implement disease control interventions, e.g., time vaccinations, manage pig flow, etc. Historically, systematic disease surveillance is cost-prohibitive, labor-intensive, and rarely done. Previously, we reported the detection of PRRSV and anti-PRRSV antibody in pen-based oral fluid samples collected under both experimental and field conditions.[3,4] We have reported similar findings for PCV2 in oral fluid samples.[4] The objective of the current research was to evaluate the feasibility of implementing systematic surveillance for PRRSV, PCV2, SIV (swine influenza virus), and *Mycoplasma hyopneumoniae* in a large, commercial swine production system using pen-based oral fluid samples. **Experimental design:** Oral fluid samples were collected from one wean-to-finish barn on each of 10 sites across the Midwest. At each site (i.e., barn), oral fluid samples were collected from 6 pens containing 25-30 animals at 2-week intervals from placement to slaughter. In addition, serum samples were collected from 5 pigs in each of the 6 pens on the first and last oral fluid sampling days. Oral fluids samples were collected by farm personnel after a brief training session with the investigators. This included sample collection, processing, packaging, and mailing samples. A poster describing and illustrating this process was placed in each barn. Upon the completion of the project, oral fluid samples were randomized and tested by PCR for the presence of PRRSV, PCV2, SIV, and *M. hyo*. Control samples, i.e., 20 samples positive for all 4 pathogens and 4 negative samples, were randomly distributed throughout the sample set. The reference serum samples collected at the initiation and termination of the project were tested for antibodies to the same panel of pathogens by ELISA. **Preliminary results:** Samples of adequate volume and quality were successfully collected by farm personal and shipped to the VDL according to protocol. Preliminary analyses (Figure 1) showed detectable levels of PRRSV, PCV2, and SIV in oral fluids (*M. hyop* PCR is in progress). **Conclusions: The pathogens targeted in this study were successfully detected using an oral fluid surveillance system in combination with PCR-based assays.** Although these data must be considered preliminary, oral fluid sampling offers promise for cost-effective disease surveillance in populations of swine.

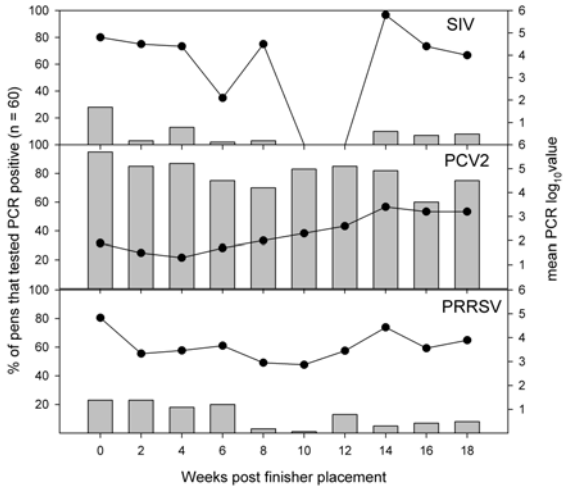

Figure 1. Summary of PCR results (SIV, PCV2, PRRSV)

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PRRSV surveillance: Stability of diagnostic targets in oral fluid: sample storage and critical techniques for testing

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Introduction. Several studies have demonstrated the utility of pen-based oral fluids for the surveillance of PRRSV, PCV2, and other swine pathogens.[1,2] The objective of this research was to evaluate the effect of time and temperature on the stability of PRRSV and anti-PRRSV antibodies in oral fluid with the specific objective of developing handling and storage guidelines for oral fluid samples.

Experimental design. A 4-liter pool of swine oral fluid was collected from 12-week old commercial finisher pigs prior to the start of the experiment. Virus and antibody were added to the oral fluid to ensure adequate and uniform levels for the observation period. The pool was "spiked" with 4 ml of PRRSV isolate ISU-P containing 1 x $10^{11.7}$ RNA copies/ml and 10 ml of concentrated hyper-immune anti-PRRSV antibodies (kindly provided by Dr. Fernando Osorio, UNL). After addition of virus and antibody, the pool was divided into 3 equal portions: One portion received no treatment, one portion received a biguanide antimicrobial, chlorhexidine digluconate, at 0.01% by volume, and one received an isothiazolone, Kathon CG, at 3 parts per million. All transferring and mixing was done in a 4°C incubator on magnetic stir plates. Each treatment was run in triplicate at each of five temperatures (-20°, 4°, 10°, 20°, 30°C). Samples were removed over time (0h, 12h, 24h, 48h, 72h, 144h, 216h, and 288h), stored at -80º C, and then assayed for: (1) total detectable PRRSV RNA; (2) total IgM, IgA, and IgG; (3) PRRS ELISA-detectable antibody; (4) and total bacteria per ml. The loss of qRT-PCR-detectable PRRSV, decline in PRRSV-specific antibody levels, decline in swine immunoglobulin (by class: IgA, IgG, and IgM) and proliferation of bacteria was evaluated in the context of the diagnostic stability of oral fluid samples and the effects of anti-microbial treatment, temperature, and time.

Results. Initial PCR results produced a high rate of PCR negative results distributed in random order. To investigate the cause of possible false negative results, 20 oral fluid samples were processed using 5 different nucleic acid extraction procedures. After selection of an appropriate extraction protocol, the entire samples set was re-tested. These data showed a temperature-dependent degradation of PRRSV RNA.

Antibody was remarkably stable at ≤ 10ºC, i.e., no change in antibody level was seen over 12 days of storage. Antibody declined slowly over time at 20ºC. At 30ºC, the effects of temperature, time, and treatment were more marked, i.e., by 72 hours, ELISA-detectable antibody had disappeared from untreated and Kathon treated samples.

This research constitutes one area of on-going investigations into the use of swine oral fluid samples for infectious disease surveillance in swine populations. Specifically, this research has provided core information regarding the stability of antibody or pathogen targets in oral fluid sample. This information is critical to developing sample handling and processing guidelines**. The data presented here suggest that typical serum storage protocols (freezing or refrigeration at 4°C) are adequate for protecting the integrity of the oral fluid sample.**

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USAHA /AAVLD Plenary Session

Monday, October 27, 2008
Guilford-ABC

Co-Chairs: David Steffen
Don Hoenig
Moderator: Alfonso Torres

Foot-and-Mouth Disease: If "When" Happened

07:30 AM **Welcome and announcements**
Dr. Alfonso Torres

07:35 AM **Status of the national FMD response plan: Coordination within the animal health community** – Jose R. Diez, Bob Hooks 142

08:20 AM **The National Animal Health Laboratory Network (NAHLN): Laboratory response and surge capacity** – Barbara Martin 143

08:35 AM **An overview of the diagnostic and molecular epidemiology data from the 2007 outbreaks of Foot-and-Mouth disease in the UK** – Donald P. King, Eleanor M. Cottam, Scott M. Reid, Katja Ebert, Jemma Wadsworth 144

09:05 AM **New vaccine research, countermeasures, commercialization prospects, allocation of vaccine in an outbreak, NVS** – Tam Garland and Luis Rodriguez 145

10:00 AM **FMD eradication efforts in South America, current status of the disease. Vaccination strategies in countries that are vaccinating** – David Ashford 146

10:45 AM **Issues for the dairy industry in an FMD outbreak**- Speaker to be determined 147

11:00 AM **Issues for the beef industry in an FMD outbreak** – Elizabeth Parker 148

11:15 AM **Foot-and-Mouth disease: If "When" happened-issues facing the swine industry** – Patrick Webb 149

11:30 AM **Consumers are always right (even when they're wrong)** – Dick Crawford 150

11:45 AM **Panel discussion** – Alfonso Torres

12 Noon **Summation**

12:15 PM **Adjournment**

Status of the national FMD response plan: Coordination within the animal health community

José R. Díez, DVM [1], and Bob Hooks[2]

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Foot-and-mouth disease (FMD) is an acute infectious viral disease that causes blisters, fever, and lameness in cloven-hoofed animals such as cattle and swine. It spreads rapidly and can cause tremendous production losses. In responding to an FMD outbreak in the U.S., both USDA and DHS will have important roles and responsibilities. A clear understanding of these roles and responsibilities will promote an effective and coordinated emergency response. The National Response Framework (NRF) is the primary mechanism for coordination of the U.S. Government (USG) roles in support of State level responses to terrorist attacks, major disasters, and other emergencies including outbreaks of agriculturally significant diseases like FMD. DHS and USDA have been working to clearly define the roles and responsibilities of each agency in the event of an outbreak of FMD. The results of these efforts will be presented as will an overview of the (draft) National FMD Response Plan.

The National Animal Health Laboratory Network (NAHLN):
Laboratory response and surge capacity

Barbara M. Martin

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The National Animal Health Laboratory Network (NAHLN) was established in 2002 to enhance the early detection of, response to, and recovery from animal health emergencies, including bioterrorist events, newly emerging diseases, and foreign animal disease outbreaks that threaten the Nation's food supply and public health. The NAHLN is a collaborative effort between the United States Department of Agriculture (USDA) and the American Association of Veterinary Laboratory Diagnosticians. From an initial group of 12 laboratories the NAHLN has expanded to 54 laboratories in 45 states.

To ensure that the NAHLN mission can be achieved, there must be a high level of confidence in the quality of NAHLN laboratories and associated test results. The NAHLN founding principles were established to provide the guidelines necessary to accomplish the mission and include:

- Standardized, rapid diagnostic techniques
- A secure communications, alert, and reporting system
- Modern equipment and trained personnel
- Training, proficiency testing, and quality assurance programs
- Facilities that meet biocontainment and security requirements
- Scenario testing in support of regional and national training exercises

The NAHLN has strategically combined the infrastructure and expertise in the state and university veterinary diagnostic laboratories and the National Veterinary Services Laboratories to establish the animal health laboratory backbone of the United States emergency response and recovery program.

The presentation will focus on how NAHLN resources will be utilized in foreign animal disease investigations and during an outbreak of a foreign animal disease. Updates on the changes to VS Memo 580.4 (Procedures for the Investigation of Potential Foreign Animal Disease/Emerging Disease Incidents (FAD/EDI)), scenario testing, and modeling will be provided.

An overview of the diagnostic and molecular epidemiology data from the 2007 outbreaks of Foot-and-Mouth disease in the UK

Donald P, King[1], Eleanor M. Cottam[1, 2], Scott M. Reid[1], Katja Ebert[1], Jemma Wadsworth[1], Daniel T. Haydon[2], Nigel P. Ferris, Nick J. Knowles[1] and David. J. Paton[1]

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This presentation outlines the role played by molecular assays (for detection and characterization of foot-and-mouth disease virus: FMDV) to test samples arising from the outbreaks of FMD that occurred in the United Kingdom (UK) during 2007. These outbreaks were in two distinct phases (August and September) affecting eight separate premises, situated in the north and west of the county of Surrey in southeast England.

In terms of virus detection, a laboratory-based real-time RT-PCR (rRT-PCR) was used to test of a total of 3216 samples, including clinical material from all eight infected premises. Using a 96-well automated system to prepare nucleic acid template, it was possible to process up to 84 samples within 5 hours of submission, and up to 269 samples were tested per working day. A conservative cut-off was used to designate positive samples: during the outbreaks, the specificity of the assay was estimated to be 99.9% or 100% using negative control material or samples collected from negative premises respectively. For the first time, rRT-PCR results were used to recognize preclinical FMD in a cattle herd. Furthermore, during the later stages of the outbreaks, this rRT-PCR assay also supported an active surveillance program within high-risk cattle herds.

Nucleotide sequencing of viruses recovered from field cases also played an important role during these outbreaks. Within 24 hours of the first case, FMDV sequence data were obtained showing that the outbreak virus had a VP1 gene-identity of 99.8% to FMDV O_1 British Field Sample 1860; a widely used reference and vaccine strain, originally derived from an outbreak of FMD in the UK in 1967. RT-PCR protocols were developed to allow the complete genomes of FMDV to be sequenced within 24-48 hours of sample receipt for the subsequent cases. These data were used to determine the most likely source of the outbreak and to track FMDV movement from farm-to-farm in real-time, assisting the field epidemiological investigations.

Key conclusions:

- **In contrast to the 2001 epidemic, diagnostic support for the 2007 outbreaks was characterized by the use of rRT-PCR as a principal tool for decision making.**
- **Full-genome sequencing conducted in real-time identified the initial and intermediate sources of these outbreaks, providing valuable support to field epidemiological investigations.**

New vaccine research, countermeasurers, commercialization prospects, allocation of vaccine in an outbreak, NVS

Drs. Tam Garland and Luis Rodriguez

FMD eradication efforts in South America, current status of the disease: Vaccination strategies in countries that are vaccinating

Dr. David Ashford
USDA, APHIS, Internation Services, Sao Paulo, Brazil

Issues for the dairy industry in an FMD outbreak

Jaime Jonker, NMPF

Issues for the beef industry in an FMD Outbreak

Dr. Elizabeth Parker, NCBA

Issues Facing the Swine Industry

Patrick Webb DVM
Director, Swine Health Programs
National Pork Board

Introduction

The introduction of Foot and Mouth Disease (FMD) into the U.S. swine herd will have an immediate and drastic effect due to the loss of all export markets for pork and pork products. In addition, aggressive disease control measures enacted to prevent further spread of the disease will affect the movement of live pigs, fresh pork and pork products within the United States.

The adage that all disasters start local and end local identifies the starting point for response activities on which the maintenance or rapid resumption of commerce and international trade will be built. The ability for animal health authorities and industry to mitigate an outbreak relates to the robustness of the animal health infrastructure that has been built. This forces the question, is the infrastructure today where it needs to be to support disease response and the resumption of commerce and trade?

Disease Response Issues

Rapid detection and response to an FMD event is critical to limiting the scope of an outbreak and supporting efforts to maintain or rapidly reestablish business continuity of pork producers during an outbreak. During an event, industry must work with animal health authorities to accomplish the following actions to contain, control and eradicate an incursion.

Action #1: Set up the appropriate control area and surveillance zone around the infected farm to define the area in which disease control measures and resources should be targeted.

Action #2: Identify and classify farms with susceptible species and identify industry assets in the control area.

Action #3: Communicate disease control measures, regulatory information, disease identification and reporting pathways, and biological risk management protocols (biosecurity) to producers for implementation.

Action #4: Increase targeted surveillance in affected areas to identify new cases of disease, prove a disease is contained and to prove disease freedom in unaffected areas.

The pork industry's primary concerns in this situation are supporting actions that facilitate producer awareness, rapid disease diagnosis, containment and control in affected areas and stepping up surveillance programming to quickly identify free and affected areas. The ability to facilitate this is directly related to the ability to maintain and/or re-establish business and eventually international trade.

Domestic Commerce Issues

The pork industry today is an industry on wheels. The predominance of multisite production has made moving swine a necessity and those movements are carefully coordinated to insure delivered feed is utilized, facilities are cleaned, disinfected and ready for the next incoming group. Conservative estimates place on average over 624,000 pigs on the road moving through the production chain on any given day. Any event that disrupts that movement for a prolonged period of time can have significant ramifications. An outbreak of FMD would represent such an event.

While it is common to focus on the importance of export markets, the maintenance or resumption of domestic commerce in free areas for swine, pork and pork products represent a crucial first step in that process. If interstate commerce cannot be reestablished then what hope is there for promoting the reestablishment of international trade?

More importantly, intrastate and interstate commerce in free areas are critical to business continuity for pork producers by providing the means to continue to move negative animals through production systems.

International Trade Issues

The pork industry has been experiencing a growing dependence on international trade to increase the value of U.S. hogs. According to a recent Pork Checkoff-funded economic analysis the value of pork and pork byproduct exports has grown from $1.97 per hog harvested in 1986 to $28.91 per head harvested in 2007. From a production standpoint, in 2007, 16 percent of U.S. production went to export markets. In 2008, that percentage is projected to exceed 30 percent. With the current dependence on international trade, the U.S. pork industry is at risk for adverse economic effects with any event that disrupts exports.

Once there is a confirmed case of FMD, it has been estimated by Steve Meyer of Paragon Economics that the price of pigs would drop 80 percent in the United States. This would result from the loss of added value from export markets and from the excess pork and pork product supply suddenly available in the U.S. market, as well as from losses in the commodity markets.

The maintenance or rapid resumption of international trade in pork and pork products during an FMD outbreak remains a priority. Efforts to support market access are best done prior to an event through the process of compartmentalization which requires enhancements to the swine health infrastructure, increased planning efforts with USDA and cooperation with trading partners.

Summary

Issues surrounding infrastructure, response capabilities, and business continuity in regards to commerce and trade are all critical to the swine industry in efforts to adequately prepare for the introduction of FMD into the United States. Of these issues, the robustness of the animal health infrastructure remains the most important issue that should be addressed as it directly influences how the other issues are mitigated.

Consumers are always right (even when they're wrong)

Dick Crawford

Corporate Vice President, Government Relations,
McDonalds Corporation, 2915 Jorie Blvd. (018), Oak Brook, IL

Contingency planning must incorporate insights on consumer behaviors to achieve necessary objectives. The inclusion of retail enterprises in contingency planning and execution is essential as the have resources and expertise to contribute, millions of points of contact with consumers daily and are part of the food chain.

Topics to be discussed are:

What does the American consumer know about animal diseases?
How are they likely to react to an animal disease?
What is necessary to ensure consumer confidence in our food safety regulatory schemes?
Who should participate in contingency planning and what assets do they bring to the table?

AAVLD Poster Session

3 p.m. Friday, October 24, 2008 through
3 p.m. Sunday, October, 26, 2008
Guilford-ABC

**Graduate Student Presentation*

Validation of real-time PCR to Detect *Streptococcus equi* in clinical specimens

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Equine strangles is an economically important disease caused by *S equi*. Strangles is clinically manifested as fever, purulent nasal discharge, and enlargement of lymph nodes. Laboratory detection of the bacterium in nasal discharge and pus is usually achieved by culture and/or end- point PCR. Laboratory culture of the bacterium is often complicated by presence of *S. zooepidemicus* in the sample. The detection of signal in the end-point PCR involves making agarose gels and handling carcinogens like ethidium bromide. Additionally, PCR product may cause contamination of workplace, instruments, and pipettes leading to false positive results. Therefore, it is desirable to detect the bacterium using fluorogenic probe based real time PCR assay.

Recently, a *Sod A* and *See I* based real time PCR (rPCR) was described to detect and differentiate *S. equi* and *S. zooepidemicus* (Vet Microbiol. 124[3-4]:219-29). Authors described validation of this rPCR on the primary streak* of nasal swabs or tracheal washes and on clinical isolates. However, authors did not validate the rPCR on DNA extracted directly from clinical specimens. Therefore, we designed our study to validate this rPCR on DNA extracted directly from clinical specimens. Our study was aimed at comparing rPCR on DNA extracted from clinical sample and from primary streak to conventional culture and identification. A total of 74 nasal swabs submitted to the NJDA Animal Health Diagnostic laboratory for *S. equi* detection were used in this study. *S. equi* was detected in 7 samples using DNA extracted directly from swab and in 9 samples using DNA extracted from primary streak of nasal swabs whereas conventional culture and identification detected *S. equi* in only 6 samples. Thus, rPCR performed on primary streak identified 3 additional samples as positive for *S. equi*.

Conventional culture and identification usually takes 3 to 4 days. During this period horse sheds bacteria and might lead to infection of other horses. Therefore, it is important to detect bacteria in clinical specimens as soon as possible. We have been successful in detecting *S. equi* and reporting results next day using this rPCR. **In conclusion, this rPCR is equally or more sensitive than conventional culture and identification in detecting *S. equi*. Also, rPCR performed on DNA extracted from primary streak of clinical specimen is more sensitive than on DNA extracted directly from clinical specimens in detecting *S. equi*.**

Currently, we are comparing this real time PCR with the end-point PCR for detection of *S. equi*.

*Primary streak: Nasal swab streaked on blood agar and incubated overnight at 37^0C.

Susceptibility of swine pathogens to Ceftiofur, 2002 – 2006

E. S. Portis, C. J. Lindeman, L. Johansen

Pfizer Animal Health, Kalamazoo Michigan

Introduction. Ceftiofur is a late-generation cephalosporin antimicrobial agent that was approved for the treatment of swine respiratory disease (SRD) pathogens in 1992. Pfizer Animal Health initiated an ongoing program to evaluate trends in susceptibility of SRD pathogens to ceftiofur in 1998. The data generated for the period 1998 through 2001 have been presented (1). This poster provides updated susceptibility data for the period 2002 to 2006.

Materials and Methods. Isolates were submitted by veterinary diagnostic laboratories in the United States, Canada, and Australia. From 2002 to 2006, fifteen to seventeen US laboratories and one Canadian laboratory per year participated in the PAH Bovine and Swine Respiratory Pathogen Monitoring Program. In 2003 and 2004, one Australian diagnostic laboratory participated in this program. For each quarter year, all strains of each bacterial species originated from different herds or farms. Minimal Inhibitory Concentrations (MICs) were determined using a broth microdilution system (Sensititre Division, Trek Diagnostic Systems, Inc. Cleveland, OH) which conforms to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method [2].

Results and Discussion. Ceftiofur continues to demonstrate excellent *in vitro* activity against the SRD pathogens *Actinobacillus pleuropneumoniae, Pasteurella multocida*, and *Streptococcus suis*.

Table 1. Ceftiofur Susceptibility Data 2002-2006

Pathogen	MIC Range (□g/mL)	% Susceptible	% Intermediate	% Resistant
A. pleuropneumoniae (n=678)	≤0.03-0.12	100%	0%	0%
P. multocida (n=1186)	≤0.03-0.12	100%	0%	0%
S. suis (n=1253)	≤0.03-4.0	99.9%	0.1%	0%
CLSI Approved Interpretive Criteria		≤2.0 □g/mL	4 □g/mL	≥8 □g/mL

During this five year period, one *S. suis* isolate in 2002 was of 'intermediate' susceptibility using the CLSI approved breakpoint criteria. The remaining 1252 *S. suis* isolates and all of the *A. pleuropneumoniae* and *P. multocida* isolates were classified as 'susceptible'. The ceftiofur MIC_{90} values for isolates tested between 1998 and 2006 range from ≤0.03 to 0.06 □g/mL for *A. pleuropneumoniae* and from ≤0.03 to 0.50 □g/mL for *S. suis*. The MIC_{90} for ceftiofur against *P. multocida* has remained ≤0.03 □g/mL (2).

Conclusion. With over 16 years of ceftiofur use in North America, swine pathogens have remained highly susceptible, with no resistance detected. When comparing the ceftiofur MIC results for the entire nine years of surveillance, MIC_{90} values have remained the same or, in the case of *S. suis*, decreased.

References

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Validation of real-time PCR for targeted surveillance of *Salmonella* in equine colic patients

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Introduction. Subclinical and clinical *Salmonella* infections are common in hospitalized large animals. Outbreaks of nosocomial *Salmonella* infection have been documented at veterinary teaching hospitals, resulting in several closures. Among equine patients, horses with colic are at high risk of both shedding and becoming infected with *Salmonella*. Rapid identification of colics that shed *Salmonella* is essential for effective infection control.

Materials and Methods. Fecal surveillance samples (496 samples collected from 218 equine colic patients at the University of Pennsylvania School of Veterinary Medicine's George D. Widener Hospital for large animals between January 1st and August 31st 2007) were subject to ten procedures for *Salmonella* detection (Table).

Enrichment	**Detection**
None	PCR for *invA* set 1
None	PCR for *invA* set 2
None	PCR for *ttr*
Buffered peptone water (BPW)	PCR for *invA* **set 1**
Buffered peptone water (BPW)	PCR for *ttr*
Tetrathionate broth (TTb)	**PCR for *invA* set 2**
None	Salmonella-Shigella (SS) agar
Tetrathionate broth (TTb)	Salmonella-Shigella (SS) agar
Selenite broth	Xylose-lysine-deoxycholate (XLD) or MacConkey (MAC) agar
International Standards Organization (ISO) method: Buffered peptone water (BPW) overnight, then Rappoport-Vassiliadis broth (RV)	Deoxycholate-citrate agar (DCA), XLD, MAC; **after** 24 and 48 h enrichment **in RV**

Test characteristics (sensitivity [Se], specificity [Sp], positive and negative predictive values and the kappa statistic) were calculated relative to the ISO culture method using standard techniques.

Results and Discussion. Fecal samples require overnight enrichment to yield consistent PCR results. Agreement between PCRs performed on enriched samples was excellent (kappa >0.81). Detection rates for *Salmonella* were poor by direct culture and following selenite or TTb enrichment. ISO was the most sensitive technique (70/496 positive). When other methods were compared relative to ISO as "gold-standard", PCR following overnight enrichment (Se ranged from 52-60% and kappa from 0.63-0.69 for the three methods) was more sensitive than culture after enrichment in selenite (Se 31.4%, kappa 0.44) or TTb (Se 30.8%, kappa 0.42). Specificity for all methods was >99%. **PCR offers better sensitivity than standard culture methods and results are available in 18-24 hours, compared to at least 3-5 days for ISO.**

Conclusion. Real-time PCR is useful for targeted *Salmonella* surveillance in equine colic patients.

Validation of a real-ime PCR assay for the detection of *Taylorella equigenitalis*

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A real-time, TaqMan® PCR for the detection of *Taylorella equigenitalis*, the causative agent of contagious equine metritis (CEM) has recently been commercialised and produced in kit form by QIAGEN. This assay is marketed under the trade name *cador*.

Contagious Equine Metritis (CEM) is a non-systemic, transmissible venereal disease of horses caused by the bacterium *Taylorella equigenitalis.* CEM is a notifiable disease in the United Kingdom and as such isolation of *T. equigenitalis* from horses must be reported to the Department of the Environment, Food and Rural Affairs (DEFRA).

The primers amplify a 112bp fragment of the 16S ribosomal DNA and the target is detected using a specific TaqMan® probe. A second probe detects an internal control amplification product to check for PCR inhibition.

The VLA 'in-house' assay, from which the kit assay was adapted, was used as the 'gold standard' for comparison. All nucleic acid extractions were performed on the QIAGEN QIAcube using the QIAGEN DNA Mini kit.

Areas under investigation included absolute quantitation, analytical sensitivity, analytical specificity, diagnostic specificity and repeatability.

Absolute quantitation. A plasmid containing the a portion of the *T. equigenitalis* 16S rDNA sequence was diluted to extinction and used as a template to determine the sensitivity of the assay tested using the QIAGEN *cador T. equigenitalis* PCR kit. This demonstrated that the assay was able to detect 1 plasmid copy/µl when using a 6µl sample volume and the assay had an apparent dynamic range showing linearity across 5 logs.

Analytical sensitivity. Dilutions of quantified *T. equigenitalis* bacterial culture were spiked onto culture negative swabs in triplicate. The organism was detected down to the 10^{-6} dilution equating to a spiked bacterial load of 122 cfu.

Testing of quantified bacterial cultures demonstrated a detection limit of 10 – 50 cfu *per* reaction.

Diagnostic specificity. A panel of 80 culture negative swabs were tested using the kit assay. 79/80 produced the expected negative result. One swab produced a positive result. Investigations confirmed that the positive was one of the apparently negative cultures from a positive horse (replicate swabbing was positive on culture). Positive field samples (32) as determined by culture have also been tested using the assay and all produced a positive result as expected.

Analytical specificity. A panel of 30 equine commensals and pathogens were tested. No cross-hybridysation was observed.

Repeatability. A positive control template was tested on consecutive runs and produced a variability of 0.99 Cts between runs (%CV = 1.20)

The *cador T.equigenitalis* PCR kit has been compared with respect to performance to the "in house" assay developed by the VLA. The assay is sensitive, specific and is comparable to the published method (Wakeley et al., 2006).

Reference

Wakeley PR, Errington J, Hannon S, Roest HIJ, Carson T, Hunt B, Sawyer J, Heath P. Development of a real time PCR for the detection of *Taylorella equigenitalis* directly from genital swabs and discrimination from *Taylorella asinigenitalis*. *Vet Microbiol*. 2006; **118**: 247-254

Evalulation of polymerase chain reaction using multiple primer sets for the detection of *Leptospira* serovars

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Polymerase chain reaction (PCR) assays have been developed for diagnosis of *Leptospira* infection in animals. The sensitivity and specificity of these tests have not been extensively evaluated. The genetic heterogeneity in the genus *Leptospira* and varying degree of endemicity increases the complexity of interpretation and limits the application of these tests in a universal manner. In this study, fourteen primer sets (sets 1-9, published in literature and sets 10-14, developed in house) were tested against 12 serovars of *Leptospira* belonging to three species. Serovars included seven *L. interrogans* (LI), four *L. borgpetersenii* (LB), and one saprophytic species *L. biflexa* (LBF). Three sets of primers (6, 7, and 10) yielded products with template from all *Leptospira* serovars. Primer sets 8 and 14 amplified only *L. borgpetersenii* spp and primer sets 4 and 5 amplified only *L. interrogans* spp (Table). The results indicate that use of a combination of primer sets may be helpful in differentiating LB, LI and LBF species. However, a preliminary evaluation of these primer sets in cattle urine samples positive for *Leptospira* spp. by fluorescent antibody staining yielded negative results. Further evaluation of these primer sets on a wide variety of clinical samples is needed.

Table: Amplification of DNA from leptospirae by various PCR primer pairs

		PCR Results for Primer sets													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Species	**Serovar/strain**														
LI	autmnalis, AkiyamiA	P	P	P	P	P	P	P	N	N	P	N	P	N	N
LI	bratislava, JezBratislava	P	P	P	P	P	P	P	N	N	P	P	p	N	N
LI	canicola, HondUtrech IV	P	P	P	P	P	P	P	N	N	P	N	p	N	N
LI	grippotyphosa,Andaman	P	P	P	P	P	P	p	N	N	P	P	p	N	N
LI	Hardjo, hardjoprajitino	P	P	P	P	P	P	P	N	N	P	N	p	N	N
LI	icterohemorrhagiaeM20	P	P	P	P	P	P	P	N	N	P	N	p	P	N
LI	pomona, Pomona	P	P	P	P	P	P	P	N	N	P	P	p	N	N
LB	javanica, javanica	P	P	P	N	P	P	P	P	N	P	N	N	P	p
LB	hardjobovis ANVSLS1343	P	P	P	N	N	P	P	P	N	P	N	P	P	P
LB	hardjobovis B NVSLS818	P	P	P	N	N	P	P	P	N	P	N	P	P	P
LB	tarassovi, perepelicin	P	P	P	N	N	P	P	P	N	P	N	N	P	P
LBF	patoc, patoc1	N	N	N	N	N	P	P	N	N	P	N	N	N	N

Utilizing multilocus variable number tandem repeat analysis for the characterization of North American *Mycobacterium bovis* animal isolates

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In 1917, the U.S. Department of Agriculture initiated the Cooperative State-Federal Tuberculosis Eradication Program with the goal of eliminating the risk of zoonotic transmission of bovine tuberculosis. The incidence of infection has been greatly reduced, with identified outbreaks ranging from four to nine affected herds per year in domestic livestock.[1] Potential reservoirs for these outbreaks include infected imported livestock, identified wildlife reservoirs of white-tailed deer (*Odocoileus virginianus)* in Michigan, and other potential residual reservoirs in livestock and wildlife.[2] To successfully accomplish the goal of eradication, robust molecular typing methods are needed to assist the epidemiological detection of reservoirs of infection, establishment of routes of transmission, and definition of at-risk populations of animals.

Multilocus variable number tandem repeat analysis (MLVA) has recently emerged as a genotyping method that is both robust and highly discriminatory for the differentiation of *Mycobacterium tuberculosis* complex (MTBC) strains, including *M. bovis*. However, MLVA assessment of *Mycobacterium bovis* isolates recovered from animals in North America has been limited. We have evaluated MLVA based on 27 published variable number tandem repeat (VNTR) loci using an epidemiologically diverse set of 148 North American *M. bovis* animal isolates. **An informative subset of six VNTR loci differentiated the isolates into genetically related groups that displayed concordance with the epidemiological data gathered via traditional trace back methods. MLVA genotyping of *M. bovis* shows great potential as a molecular typing tool for characterizing the epidemiology of *M. bovis* animal infections in North America.**

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*Graduate student presentation

Characterization of uropathogenic *Escherichia coli* strains from humans, dogs and cats

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Uropathogenic *Escherichia coli* strains belonging to serotype O6:H31 are implicated in urinary tract infection (UTI) in humans, dogs and cats. Genotype and phenotype of 18 strains (13 human, 4 canine, and 1 feline) were determined by biotyping, phylogenetic typing, pulsed field gel electrophoresis (PFGE), PCR detection of virulence genes, and plasmid analysis.

Based on sugar fermentation, the 18 UPEC isolates belonged to 13 biotypes. **Several dog and human UPEC isolates belonged to the same biotype. Phenogram analysis revealed that biotyping of UPEC isolates had limited discriminatory power as 3 of the 5 clusters were shared between dog and human UPEC isolates.** Phylogenetic typing of UPEC isolates showed that all 18 isolates belonged to phylogenetic group B2. PFGE analysis showed that the 18 isolates belonged to 13 PFGE profiles (PFPs), and **inferred that there was considerable similarity between human and dog UPEC isolates.** Twenty-two virulence genes were examined by PCR-based assays, and **the resulting profile of UPEC isolates from dogs, cat and humans are similar, implying that they perhaps originate from a single clone.**

Plasmid analysis revealed that 14 out of 18 isolates harbored one or more plasmids ranging in size from 55 to 125 kb. A plasmid (pUPEC-O6) derived from a strain isolated from a human UTI case was purified and sequenced. Analysis of plasmid sequence revealed that the plasmid had 141 coding regions. Of these genes in coding regions, 13, 9, 4, 3, 2 and 1 shared homologies to transposases, maintenance, immunity related, replication and putative virulence and transport gene sequences. Based upon BLAST results, it was inferred that plasmid pUPEC-O6 was analogous to pUTI89, isolated from UPEC O6:H31 obtained from human urine sample.

In conclusion, considerable similarity was observed among human and dog UPEC isolates, suggesting that the O6:H31 UPEC isolates may be transferred from human to dogs or vice-versa. It could be hypothesized that dogs, humans, or both may serve as potential reservoirs of these organisms. Moreover, both genomic and plasmid DNA play an important role in pathogenesis of UTIs caused by UPEC.

Suppression of Gram-negative contaminants by Ceftriaxone in BD BACTEC™ MGIT™ 960 Para TB system Liquid culture medium

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Introduction. Hexadecylpyridinium chloride (HPC) is used to decontaminate bovine feces during processing for liquid culture of the causative agent of Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Antimicrobials are added to the culture medium to inhibit growth of those contaminant organisms capable of surviving HPC treatment. In this study, ceftriaxone was tested against recently recognized Gram-negative contaminants, as well as two modified fecal processing protocols.

Materials and Methods. All 26 of 2008 Johne's Fecal Culture Check Test kit #57 samples were processed for culture in the BD BACTEC™ MGIT™ 960 Para TB System according to manufacturer's instructions (Standard method), which includes addition of 0.05% malachite green (MAG) to the HPC solution. Samples were also processed with the overnight decontamination incubation in HPC-MAG extended from 24 to 72 hours (3-day HPC-MAG method), or storing pellets resuspended in antibiotic brew for six days at 4°C after the initial overnight incubation at 35°C prior to culture tube inoculations (7-day brew method). Three antimicrobial cocktails were tested: the standard amphotericin B-nalidixic acid-vancomycin (ANV) imparting a final supplemented medium concentration of 18-19 µg/ml nalidixic acid (standard ANV), an ANV imparting 200 µg/ml nalidixic acid as is currently recommended for highly contaminated sample sets (NAL_{200}), and an ANV imparting 50 µg/ml nalidixic acid and 8 µg/ml ceftriaxone (NAL_{200}/CTR_8). Two culture tubes were inoculated per condition for a total of 468 cultures from Check Test kit #57. Times-to-detection were analyzed by means of ANOVA-type general linear model, and numbers of positive (P) or contaminated (C) cultures by means of binary linear regression. Also, a Gram-negative laboratory water system contaminant identified as *Methylobacterium extorquens* was seeded into feces-free cultures supplemented with the three different antimicrobial cocktails (N = 10).

Results. Gram-negative contaminants from some liquid cultures were identified from biochemical methods and/or rDNA sequences as *Serratia marcescens*, *Providencia rettgeri*, and *Morganella morganii*. Total positive cultures between the 3-day HPC-MAG (61P), Standard (71P), or 7-day brew (78P) processing methods did not differ significantly ($P < 0.05$). Total cultures lost to contamination were significantly different among the 3-day HPC-MAG (59C), Standard (37C), and 7-day brew (24C) methods ($P < 0.05$). Total positive or contaminated cultures did not differ significantly from NAL_{200} (75P, 19C) and NAL_{50}/CTR_8 (84P, 11C) cocktails, but both metrics differed significantly versus cultures with standard ANV (51P, 90C) ($P < 0.05$). Mean detection times (days) from the Standard fecal processing method were not significantly different with standard ANV (17.80d, 17P, 31C) and NAL_{200} (17,60d, 26P, 4C), but both were significantly shorter versus NAL_{50}/CTR_8 (20.66d, 28P, 2C) ($P < 0.05$). NAL_{200} and NAL_{50}/CTR_8 results only differed by two detections with standard processing, but the consequence was a high shedder detected with NAL_{50}/CTR_8 but lost to contamination with NAL_{200}. Mean detection time differences from the Check Test sample contaminated by *M. avium* were not significant among processing methods, but were significant for results from NAL_{200} (15.25d), NAL_{50}/CTR_8 (8.32d), and standard (5.18d) ANV cocktails ($P < 0.05$). *M. extorquens* overgrew all 10 cultures with standard ANV, three of 10 with NAL_{200}, and none with the NAL_{50}/CTR_8 cocktail.

Discussion/Conclusion. A 3-day HPC-MAG incubation caused increased contamination rates, but the 7-day brew processing method reduced contamination but not MAP recovery versus standard fecal processing. MAP recovery and Gram-negative contamination breakthrough were slightly improved with NAL_{50}/CTR_8 cocktail versus NAL_{200}, and both were superior to the standard ANV, but MAP detection times were increased by NAL_{50}/CTR_8.

Development of a real-time TaqMan RT-PCR assay with an internal amplification control for rapid detection of *Transmissible gastroenteritis virus* in swine fecal samples

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Introduction. Transmissible gastroenteritis (TGE) is an acute enteric viral disease of pigs of all ages. The disease is caused by TGE virus (TGEV), a member of *Coronaviridae*, a group of enveloped viruses with a large, single positive-strand RNA genome. TGEV replicates in intestinal enterocytes and shed in the feces of infected pigs. Natural mutants of TGEV with deletions in spike (S) gene, known as porcine respiratory coronavirus (PRCV), show tropism towards respiratory tissue and cause mild or subclinical respiratory infections. Some pigs infected with PRCV can shed the virus in their feces. TGEV infections, especially in unweaned piglets, can cause severe economic losses to swine industry. Because of the highly contagious nature of TGE, availability of rapid diagnostic methods that are highly specific and sensitive for detection of TGEV in fecal samples is very useful for timely implementation of disease management practices. The objective of the present study was to develop a TaqMan probe-based real-time RT-PCR for rapid, sensitive and reliable detection of TGEV in pig fecal samples.

Materials and Methods. Nucleotide sequences of TGEV S gene available in the databases at GenBank were aligned using a computer program to identify the conserved sequences within the region that is absent in PRCV. A set of primers and a TaqMan probe were designed using Beacon Designer 3 software (PREMIER Biosoft International, Palo Alto, CA) and were custom synthesized at a commercial company. The TaqMan probe contained the fluorescent dye 6-carboxyfluorescein (FAM) at the 5'-end and a non-fluorescent quencher (Black Hole Quencher 1) at the 3'-end. The TaqMan RT-PCR assay was performed using Smart Cyler II instrument (Cepheid, Sunnyvale, CA) and reagents from commercially available One-Step RT-PCR kits. The assay was optimized using RNA extracted from tissue culture grown TGEV. The performance of the assay was determined using in vitro transcribed RNA of the specific region of TGEV S gene. Since nucleic acids extracted from fecal samples may contain unknown PCR inhibitors, an internal amplification control was developed using in vitro transcribed RNA of GFP gene and the corresponding primers and the TaqMan probe. The duplex assay containing the primers and probes for simultaneous detection of TGEV RNA and the spiked GFP RNA was established and used to test the clinical samples received at Purdue Animal Disease Diagnostic Laboratory over a 3 year period. A previously described nested RT-PCR assay (Kim et al., J Vet Diagn Invest 12:385-388) was also used to test the clinical samples for comparison.

Results. The optimized TaqMan assay detected a range of 2.8×10^0–2.8×10^8 target TGEV S gene RNA copies in a linear manner ($R^2 = 0.99$). The minimum concentration of the virus detected by the assay was determined to be 0.1 $TCID_{50}$. The GFP RNA detection in the duplex assay did not affect the performance of TGEV TaqMan assay and served as the control for detection of PCR inhibitors in the extracted RNA samples. The established RNA extraction procedure and the real-time PCR parameters allowed the entire assay to be completed within 2 hours. All the clinical samples which tested positive for TGEV by the nested RT-PCR assay also tested positive by the TaqMan assay. However, approximately 9% of the samples that tested negative by the nested RT-PCR assay tested positive by the TaqMan assay.

Conclusion. The TaqMan RT-PCR assay developed in this study is a highly sensitive diagnostic test for rapid detection of TGEV in pig fecal samples. Availability of this assay should permit diagnostic laboratories to provide the results of TGE suspect cases within a few hours of receiving the samples.

A multiphasic typing approach to subtype *Streptococcus equi* subspecies *equi*

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The objective of this study was to design a typing system to differentiate between strains of *Streptococcus equi* subspecies *equi (S. equi)*. Four hundred and thirty isolates of *S. equi* that included those recovered from clinical specimens associated with equine strangles cases submitted to the Unviersity of Illinois Veterinary Diagnostic Laboratory, as well as those recovered from at least 12 lots of Pinnacle IN (Fort Dodge) vaccine vials were banked for further study. Genotyping techniques evaluated include Random Amplified Polymorphic DNA analysis (REP-, BOX- and ERIC- PCR), Riboprinting®, DNA sequencing of virulence genes and Pulsed Field Electrophoresis (PFGE). Phenotypic evaluations were performed using Biolog® Microphenotypic Array Analysis (Biolog). In cases where Biolog and PFGE results did not coincide, DNA sequence analysis was performed on the upstream regulatory region of *szp* gene (*szp* sequencing) and the presence and nature of a single nucleotide polymorphism (SNP) was utilized to differentiate the *S. equi* strains.

REP-, BOX- and ERIC- PCR, and Riboprinting did not distinguish different wild type *S. equi* strains from each other or wild type strains from vaccine strains. Both PFGE and Biolog successfully differentiated wild type *S. equi* strains isolated from clinical submissions from isolates of the modified live vaccine (MLV). In addition, PFGE genotyping was found to be useful in further sub typing the wild type strains while Biolog combined with *szp* sequencing was useful in differentiating the MLV strain from its progenitor. Of the 403 isolates tested, PFGE analysis identified 179 isolates as wild type and 223 as vaccine type, while Biolog identified 251 isolates as wild type and 151 as vaccine type. The combined sensitivity, specificity, and accuracy of Biolog and PFGE were 69.4%, 97.4%, and 79.9% respectively. A deletion of a G located 85 base pairs upstream of the *szp* start codon appears to be conserved among vaccine isolates, and shows a 100% correlation to Biolog identification. Using *szp* sequencing data obtained from 65 isolates analyzed so far, the combined sensitivity, specificity and accuracy of *szp* sequencing and Biolog were found to be 100%, 93% and 98.5% respectively. It is concluded that this multiphasic approach involving all three methods can be applied to reliably differentiate *S. equi* strains or any one of the three methods can be used to answer specific diagnostic questions pertaining to the source of infection or response to an outbreak.

Serovar distribution and antimicrobial susceptibility of swine *Salmonella* isolates from clinically ill pigs in diagnostic submissions

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Clinical salmonellosis causes septicemia, associated respiratory disease complex, and enteritis in the swine herds, contributing to significant economic loss in the swine industry. The primary approach for treatment and control of porcine salmonellosis is the use of antimicrobial agents; however, swine *Salmonella* isolates are often non-responsive to various antimicrobials used clinically. Difference in antimicrobial resistance affects antimicrobial therapy. The purpose of the present study was to determine serovar distribution and the levels of antimicrobial susceptibility of swine *Salmonella* isolates from clinically ill pigs in diagnostic submissions.

A total of 197 *Salmonella* isolates were obtained by the Indiana Animal Disease Diagnostic Laboratory from 2003 to 2005. Bacterial identification was confirmed by biochemical tests (API 20E system, bioMerieux, Inc., Hazelwood, MO). Serotyping of *Salmonella* isolates was carried out by the National Veterinary Services Laboratory (Ames, IA). Minimal inhibitory concentration (MIC) of 14 antimicrobial agents against each bacterial isolate was determined using the standard microbroth dilution method as described by the Clinical Laboratory Standards Institute (CLSI). The top four serovars identified were *Salmonella enteric* serovar Typhimurium var. Copenhagen, *Salmonella* Derby, *Salmonella* Choleraesuis var. Kunzendorf, and *Salmonella* Typhimurium. All isolates were susceptible to the fluoroquinolones tested except eight isolates that were intermediate to difloxacin. The isolates were highly susceptible to trimethoprim/sulfadiazine, gentamicin, ceftiofur, and cephalothin, with low MIC_{50} value of <=0.5, 0.5, 1, and 4 □g/ml, respectively. They were highly resistant to tetracycline (83.76%) and moderately resistant to ampicillin (55.84%), spectinomycin (42.64%), ticarcillin (41.62%), and florfenicol (41.12%). Among *S. enterica* serovars, *S*. Derby showed little or no resistance to amoxicillin/clavulanic acid, ampicillin, ceftiofur, and cephalothin; however, more than half of *S*. Agona, *S*. Anatum, *S*. Enteritidis, and *S*. Heidelberg isolates were resistant to the same antimicrobials. Seventy eight isolates (39.59%) were resistant to five or more than five antimicrobials in various combinations. Resistance to beta-lactam antimicrobials including amoxicillin/clavulanic acid, ampicillin, ceftiofur, and cephalothin (AxApCfCp) was found in 38 isolates (19.29%). There were more isolates of *S*. Agona and *S*. Typhimurium that possessed multiple antimicrobial resistance to AxApCfCp than the other serovars.

In conclusion, *S. enterica* serovar Typhimurium var. Copenhagen, *S*. Derby, *S*. Choleraesuis var. Kunzendorf, and *S*. Typhimurium were the predominant serovars from clinically ill pigs in Indiana from 2003 to 2005. **The swine *Salmonella* isolates in the present study were susceptible to the fluoroquinolones, trimethoprim/sulfadiazine, gentamicin, ceftiofur, and cephalothin, but resistant to tetracycline.** Such findings provided useful information regarding antimicrobial susceptibility for swine salmonellosis.

Failure to detect *Clostridium septicum* via the direct florescent antibody test

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Clostridium septicum causes malignant edema in animals and humans. Routine diagnosis is based on clinical signs, gross and microscopic examination, fluorescent antibody stain of direct smears, or isolation of the organism from affected tissues. Here, we report two cases with typical clinical signs and lesions caused by *C. septicum*. However, fluorescent antibody stain of direct smears of affected tissues was negative for the organism. Heavy and pure growth of *C. septicum* was isolated from affected tissues in each case. Colony morphology and biochemical reactions of both isolates were typical for *C. septicum*. Surprisingly, both isolates were negative by the fluorescent antibody stain although the fluorescent antibody correctly identified *C. septicum* provided by the manufacturer. DNA sequence analysis of the 16S rDNA indicated that both isolates were *C. septicum*. **These findings suggest that immunofluorescent antibody testing for *C. septicum* alone may not detect all cases of malignant edema, and that other testing such as culture procedures and DNA sequencing may be required for definitive diagnosis.**

Congenital erythropoitic protoporphyria in Limousin calves in the United Kingdom

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Until 2007 this condition had rarely been reported in the United Kingdom. However, following its diagnosis in a suckler calf in the spring of 2007 an increased awareness campaign was started both in my region (Cumbria) and amongst other veterinary colleagues in the Veterinary Laboratories Agency.

During 2007/2008 a further ten cases were confirmed. Where possible, visits were made to the affected calves and EDTA blood samples and digital images were taken. Full case histories, including breeding details where available, were also gathered. Blood samples were submitted to a testing laboratory in south Wales where they were tested for total erthyrocyte porphyrin and plasma porphyrin. All confirmed cases showed dramatically elevated levels.

Clinical histories almost invariably included characteristic signs of photosensitisation but in at least two cases neurological signs were reported and in one case nervous signs were reported in the absence of skin changes.

The true incidence of this condition in the United Kingdom is unknown as no systematic testing programme has been instituted. However, recent experience would suggest that the condition is more common than previously thought. It is assumed that many cases in the past will have been diagnosed as primary or secondary photosensitisation without further recourse to laboratory testing.

Proliferative spinal osteoarthropathy with ankylosis in a Green Iguana (*Iguana iguana*)

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A 9 year-old intact, female, Green Iguana (*Iguana iguana*) was submitted for necropsy following a month long history of decreased mobility of the hindlimbs and tail. Previously taken radiographs demonstrated marked osseous proliferation along the vertebral column affecting the sacral and cranial two-thirds of the coccygeal vertebrae with multiple coccygeal vertebral fractures. Upon necropsy, there was marked osseous proliferation on the surface of the sacral vertebra with circumferential ankylosis of the cranial two-thirds of the coccygeal vertebrae and absence of any discernible intervertebral disks. There was a mid-body transverse, complete fracture of the second coccygeal vertebra which was surrounded by abundant fibrous tissue. Histologically, there was complete absence of intervertebral spaces with ankylosis of adjacent vertebral bodies and dorsal processes. There were multifocal, irregular protruberances of bone into the vertebral canal. The trabeculae of the vertebral bodies varied markedly in thickness and orientation and often contained retained cartilage cores. No significant inflammation was seen. Hematopoetic elements sparsely populated marrow spaces, which were occasionally filled by fibrous connective tissue of low cellularity. There was marked Wallerian degeneration of the spinal cord within all fasciculi. Proliferative spinal osteoarthropathy is common in snakes and lizards, especially green iguanas. These lesions have been well described in the snake and bacterial osteomyelitis is strongly implicated in the pathogenesis. Trauma and metabolic disease are other etiologic possibilities. Studies describing these lesions and potential pathogeneses in the lizard are lacking. **This report describes the histologic lesions present within the spinal column of a Green Iguana and discusses potential etiologies.**

Immunohistochemical characterization of canine testicular tumors with antibody to GATA-4

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The immunoreactivity of an antibody to GATA-4 was examined in 76 testicular tumors including 21 Sertoli cell tumors (SCT), 28 Leydig (interstitial) cell tumors (LCT), 24 seminomas (GCT), and 3 mixed germ cell / sex cord-stromal tumors (MGSCT). Tissues were fixed in 10% buffered neutral formalin and embedded in paraffin. A goat polyclonal anti-GATA-4 (Santa Cruz, sc-1237) was used at a dilution of 1/200, incubated at RT for 90 min. Heat-induced antigen retrieval in a decloaker chamber with citrate buffer, pH 6.0, was used. The immune reaction was detected with a peroxidase-DAB method (LSAB+, Dako Corporation). The hypothesis was that immunohistochemistry for GATA-4 can discriminate between germ cell and sex cord-stromal tumors of the canine testis.

Sertoli cell tumors (21/21) had strong, diffuse nuclear staining. Interstitial cell tumors (27/28) also had strong diffuse nuclear staining and much weaker and granular cytoplasmic staining. Seminomas were negative for this marker; however, individual cells (<10%) in seminomas had strong nuclear (particularly nucleolar) staining. **These results indicate that GATA-4 is mainly expressed in sex cord-stromal tumors and not expressed in germ cell tumors.** The nuclear staining is distinct and background produced by this antibody is minimal.

GATA-4 is a transcription factor that belongs to the family of GATA transcription factors.[3] GATA-4 plays a critical role in regulating embryonic morphogenesis and cellular differentiation. GATA-4 is expressed in human fetal and adult testis.[1] It has been detected in granulosa cell tumors and thecomas of the human ovary.[2] To our knowledge, the expression of GATA-4 in testicular tumors has not been evaluated in any species, except for a single case report in a dog.[4]

In summary, GATA-4 expression in canine testicular tumors is similar to that in the normal human testis and ovarian sex cord-stromal tumors. This confirms the **usefulness of immunohistochemistry for GATA-4 to distinguish canine testicular sex cord-stromal tumors from germ cell tumors.**

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Immunohistochemical application of an antibody specific for human CD1a to the diagnosis of canine mast cell tumor

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In canine mast cell tumors (MCTs), microscopic examination is required to accurately grade neoplasms for prognostic purposes. Most of grade I (well differentiated) and grade II (intermediated differentiated) MCTs are easily recognized by the abundance of cytoplasmic granules. However, the diagnosis of grade III (poorly differentiated) MCTs can be difficult as they frequently have fewer discernable granules or lack sufficient numbers of granules for identification. This study utilized CD1a (anti-human CD1a, clone O10) which easily recognized canine MCTs of all grades. Detection of CD1a clone O10 in canine patients with grade I, II, and III MCTs was evaluated by using immunohistochemistry from 48 individual tissue samples. Concurrently, serial sections from each paraffin block were stained with toluidine blue and safranin O to visually compare diagnostic sensitivity. All 48 MCTs were labelled positively by the CD1a clone O10 antibody, but identification of mast cells was difficult in some cases with toluidine blue or safranin O staining method. To exclude non-specific binding, heparinase-I pre-incubation was performed. The labelling intensity was not reduced with heparinase-I. CD1a clone O10 did not label the tumor cells of histiocytomas, plasmacytomas, and amelanotic melanomas. In additional experiments, neither of the alternative antisera specific for CD1a (clones JMP30 and CTB6) labelled neoplastic or non-neoplastic mast cells. CD1a clone O10 antibody may be used as a valuable marker to diagnose MCTs especially anaplastic grade III MCTs which many times fail to stain using traditional methods.

Diagnostic methods used in the *Tritrichomonas foetus* control program in Wyoming

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A bovine trichomoniasis control program was initiated in Wyoming by the Wyoming Livestock Board (WLB) in the year 2000 in response to requests by livestock production groups within the State. Rules for diagnostic testing and processing of infected animals were established by the Wyoming State Veterinarian, with authorization of the WLB, and approval of the State Legislature.

Testing has involved collection of smegma or vaginal cells/secretions from the prepuce of a bull or vaginal canal of a cow, suspension of specimens in lactated Ringer's solution or Diamond's culture medium and shipment to the Wyoming State Veterinary Diagnostic Laboratory (WSVL) or a private veterinary clinic for diagnostic processing. Bulls entering or leaving Wyoming, or marketed within the State for breeding were required to be tested by culture 3 times, the first sample taken approximately 2 weeks after isolation from cows, and 1 week after the first and second smegma samples were collected, respectively. Under certain other conditions, a single test was required. **Analysis of samples by Polymerase Chain Reaction (PCR) has recently been approved for use by the WLB, as a choice at the discretion of producers and/or their veterinarians.**

Testing at WSVL involves culture of specimens in modified Diamond's medium in tubes or In-Pouch containers at 37 C for 48 to 72 hours at which time(s) microscopic examination is performed. Cultures in which protozoa are observed are passed into fresh medium and re-incubated for re-inspection. Five intestinal flagellates, 1 amoeboflagellate and at least 1 ciliate have been seen occasionally in cultures of smegma samples that contain significant fecal contamination**.** One of the flagellates is an intestinal trichomonad morphologically similar to *T. foetus***. The successive passage and examination of cultures inhabited by protozoa reliably results in dilution/disappearance of fecal debris and the concomitant disappearance of the intestinal protozoa, while enhancing the production of *T. foetus*.** Six bacterial contaminants of smegma samples have been identified, one of which is *Escherichia coli*, all of which appear to be resistant to the Penn-Strep antibiotic in Diamond's medium**. Exposure of *T. foetus* to heavy *E. coli* contamination in Diamond's medium results in death of the protozoan within 8 hours of culture. Culture sensitivity/confidence level in our lab is approximately 86% on 1 sample, 93% on 2 samples and >98% on 3 samples, depending on sample quality, processing and shipping factors.**

Single PCR testing was recently authorized by the WLB as an option to the culture method**. Significant research effort at WSVL indicates that at least 25 trophozoites must be present in a relatively 'clean' smegma sample to give a reliable, positive diagnosis. Fecal and/or bacterial contamination of smegma has been shown to affect the sensitivity of the test. Sensitivity has been enhanced by direct inoculation of smegma into Diamond's culture medium at the time of sample collection, followed by 24 or 48 hr culture after arrival at the testing lab, prior to PCR testing. Our research has shown a sensitivity range of 86 to 90% confidence in PCR analysis of single samples in lactated Ringer's transport medium, and 95 to 98% confidence in samples inoculated into Diamond's medium and incubated for 24-48 hr prior to PCR analysis at the diagnostic lab.** Specificity of identification with the PCR test is technically superior to other known methods.

Economic assessment of bovine trichomoniasis and the effectiveness of the control program, begun in the year 2000, is underway.

Sulfur induced polioencephalomalacia in cattle fed distiller's grains and corn gluten

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In January 2007, a group of 50 feedlot steers developed progressive weakness and depression with 10/50 unable to rise. The steers were fed a ration composed primarily of distiller's grains (approximately 75% by weight) and corn gluten (approximately 20% by weight) with only around 5% clover and grass roughage. The steers had free access to working automatic water dispensers. Over the course of three days, 4 of the 10 down steers became progressively obtunded. On day 2, one steer (steer 1) was treated by the referring veterinarian with Baytril® (enrofloxacin), Banamine® (flunixin meglumine), and dexamethasone with no result. By the third day, the treated steer (steer 1) had died and this steer as well as another affected but untreated live steer (steer 2) were submitted along with samples of feed and drinking water to the University of Illinois Veterinary Diagnostic Laboratory (VDL) for examination. Two additional steers died by the 4th day bringing the mortality to 40% in the down steers and 8% in the entire group.

At gross necropsy the cerebrocortical gyri of both animals were moderately flattened with narrowing of somewhat indistinct sulci consistent with cerebral edema. On cut surface the gray matter was discolored pale yellow to tan and was soft and gelatinous (malacia). Under ultraviolet light (360 nm), the affected gray matter exhibited a bilaterally symmetric laminar pattern of fluorescence that was more prominent in the parieto-occipital lobes. **Based on these characteristic gross findings the steer were diagnosed with marked acute polioencephalomalacia (PEM).** The diagnosis of PEM was confirmed histologically. The neurons of the middle to deep lamina of the cerebral cortex, particularly in the sulci, were necrotic with markedly shrunken, angular, brightly eosinophilic cytoplasm and pyknotic or karyorrhectic nuclei. In affected areas there was moderate edema and local astrocytes were moderately swollen with increased amounts of pale eosinophilic granular cytoplasm and chromatin clearing. Occasionally the Virchow-Robin's space and pia mater were infiltrated by a few Gitter cells.

Toxicological examination of feed and water samples was pursued. Lead toxicosis was ruled out using atomic absorption on samples of fresh frozen liver from both steers. Samples of the drinking water, distiller's grains, corn gluten and roughage were sent to the Iowa State University Veterinary Diagnostic Laboratory for total dietary sulfur analysis. All samples were evaluated on a dry matter basis. **The results of the sulfur testing confirmed total dietary sulfur levels of 7593 ppm or 0.76 % which is almost twice the maximum tolerable concentration of 4000 ppm or 0.4% total dietary sulfur.** The vast majority of the sulfur was derived from the distiller's grains and corn gluten, with no sulfur detected in the water.

Sulfur toxicity causing PEM has been frequently reported in cattle associated with high sulfur containing drinking water. **This case report highlights the dangers of feeding novel diets composed of manufacturing by-products which may contain high sulfur levels.**

Retrospective study of Melamine associated renal failure of dogs in Korea between 2003 and 2004

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In early 2007, the American pet food ingredients leading to nephrotoxic renal failure of dogs and cats raised serious concerns about the safety of pet foods. Major pet food companies have recalled more than 1,000 commercial pet foods in consideration of pet food safety. A similar pet food associated outbreak of nephrotoxic renal failure occurred in Asia, in late 2003 and 2004, resulting in a similar extensive pet food recall. At that time, contamination of ingredients with a nephrotoxin-producing fungus at a pet food production facility was suspected. However, toxicologic evidence to substantiate this proposed mycotoxicosis was lacking. Moreover, the renal lesions were not typical of those reported with fungal nephrotoxins.

During 2003 and 2004, 14 dogs were presented to the Veterinary Medical Teaching Hospital of Konkuk University, Seoul, Korea with renal failure and similar renal pathologic findings. Grossly, the kidneys were greenish in color with greenish uroliths in the renal pelvis and/or bladder. Histologically, characteristic crystals with pinwheel radiating striations were present in distal tubular segments. Immunohistochemistry with cytokeratin antibody confirmed characteristic distal tubular necrosis, and toxicologic analysis identified these crystals as melamine/cyanuric acid. In this study, the histopathological analysis and the toxicologic analysis led to the determination that the large outbreaks of renal diseases leading to death in 2003 and 2004 Korea was due to melamine associated renal failure, as has been reported in the United States in 2007.

Inadvertent Intra-auricular artery injection with ceftiofur crystalline free acid sterile suspension and subsequent cerebral hemorrhage

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A 7-month-old steer suspected of shipping fever was treated with a commercial ceftiofur crystalline free acid sterile suspension (Excede®, Pfizer Animal Health Inc.), which was injected subcutaneously in the base of the ear. Shortly after treatment the steer became somnolent and comatose. The steer was euthanized and submitted for post mortem examination. At necropsy there was focal subcutaneous hemorrhage at the base of the left ear and focal subdural hemorrhage in the left cerebral occipital lobe. The ventral aspect of the right cranial lung lobe was firm. Microscopically, there was a locally extensive region of subcutaneous hemorrhage accompanied by abundant pale blue-gray foreign material within tissue sections from the base of the ear. The left occipital lobe of the cerebrum contained a focal region of hemorrhage and a similar blue-gray foreign material within several blood vessels. An Oil red O stain of the cerebral hemorrhage demonstrated intravascular and extravascular oil droplets. Microscopic examination of the lungs revealed a mild suppurative bronchopneumonia. The acute hemorrhage at the site of ceftiofur injection supports traumatic disruption of the blood vessels and provides evidence for concurrent inadvertent intra-auricular artery injection. Furthermore, **the cerebral intravascular foreign material is identical to that deposited at the injection site and most likely corresponds to cotton seed oil from the ceftiofur suspension.**

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Comparison of three lots of a rapid influenza virus detection kit

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This study was conducted to compare three different lots of a rapid influenza virus detection kit (Binax-now) for testing tracheal swabs from porcine and avian species. A total of 98 tracheal swabs collected from different diagnostic cases from pigs submitted during years 2006 and 2007 were used. These swabs had been stored at -80^{0}C for 1-2 years before use in this comparison. Swabs from avian species were collected from ducks. Of the 98 swabs from pigs, 25 were positive for swine influenza by all three lots. None of the swabs from avian species was positive by any of the three lots. **These results indicated that different lots of kits performed similarly and that there was no batch to batch variation.**

Interlaboratory comparison of serological assays for porcine circovirus type 2

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Introduction. Many serological tests exist for the diagnosis of porcine circovirus type 2 (PCV2) associated disease including indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assays (ELISA). While multiple laboratories offer serological assays for PCV2, there has been no interlaboratory assessment of the diagnostic accuracy of currently utilized commercial and in-house serological assays. The objective of this study was to evaluate the diagnostic accuracy between PCV2 serological results from seven diagnostic laboratories.

Materials and Methods. Serum samples were collected weekly for 49 days from the following seven groups of animals: 1) Negative controls (n=7), 2) PCV2a (n=8; inoculated with PCV2 ISU-40895), 3) PCV2b (n=8; inoculated with PCV2 NC-16845), 4) PCV1 (n=8), 5) PCV2 vaccine A (n=8;vaccinated with Ingelvac® CircoFLEX™), 6) PCV2 vaccine B (n=8; vaccinated with Circumvent® PCV2), and 7) PCV2 vaccine C (n=8; vaccinated with Suvaxyn® PCV2 One Dose). Samples were divided into seven aliquots, re-labeled for blinding purposes, and mailed overnight on ice to each of the diagnostic laboratories. Results from each laboratory were analyzed by receiver operating characteristic (ROC) analysis to determine the area under the ROC curve (AUC) value for each test. For statistical analysis, infected and vaccinated animals were considered as true positives and PCV1 and negative controls were considered as true negatives.

Results. AUC results for the two laboratories offering IFA assays ranged from 0.97 – 1.00 for all trial days (14-49 days post inoculated). AUC results from the three laboratories offering in-house ELISAs ranged from 0.54 to 1.00 for all trial days. AUC results from the three laboratories utilizing commercially available anti-PCV2-IgG ELISAs ranged from 0.54 to 1.00. Only two laboratories had statistically significant differences in AUC values at multiple trial days.

Discussion/Conclusion. ROC analysis revealed that in 5/7 laboratories, the PCV2 assays had high diagnostic sensitivity and specificity as quantified by AUC values greater then 0.85; no statistical differences were noted among AUC values from these five laboratories by trial day 49.

Experimental infection of white-tailed deer (*Odocoileus virginianus*) fawns with *Bovine viral diarrhea virus* type-1 isolated from free-ranging white tailed deer

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Bovine viral diarrhea virus (BVDV) is a pestivirus associated with vast economic losses to the cattle industry in the United States. In November 2006 BVDV type 1 virus was isolated from free ranging deer in Indiana without previous history on this infected animal. However, role of BVDV in pathogenesis and ecology in white tail deer is not clear. The objective of this study was to assess if captive white tailed deer (WTD) can be experimentally infected with a BVDV type 1 virus, and to document whether they develop clinical signs and lesions.

Six BVD-negative fawns (4 treated and 2 controls) were used in this study. Both groups were housed in an isolaton units throughout the study. After a period of adaptation, the experimental group was inoculated intranasally with a BVDV type 1, while the control group was inoculated with virus-free sham inoculum. Blood samples, nasal and rectal swabs were obtained on days 0, 1, 7, 1 post inoculation (PIN) for real time RT-PCR, ELISA and virus neutralization (VN). On day 7, 2 of the 4 experimentally infected fawns and one fawn from the control group were euthanized. At necropsy, multiple tissue samples were obtained for histopathology and *in situ* hybridization (ISH). The same procedure was performed on day 14 for the remaining two experimentally infected fawns and the control fawn.

No clinical signs were observed in any of the animals in this study. The control fawns remained seronegative throughout the study period, and no lesions or virus were found at necropsy. In the inoculated deer, BVDV was confirmed in nasal (2/2) and rectal (1/2) swabs at day 7 by real time RT-PCR only. No gross lesions were observed at necropsy in any of the fawns; however, infected deer had microscopic evidence of lymphoid depletion, apoptosis and lymphoid necrosis (at day 14 PIN) in the Peyer's patches and mesenteric lymph nodes. Virus isolation (VI) and ISH (tissues harvested at necropsy) yielded positive results for BVDV in all inoculated fawns. Control fawns remained negative for BVDV by VI and ISH throughout the study.

The results of this study established that white-tailed deer can be infected with BVD type 1 virus, and that infection results in the development of histological lesions in lymphoid tissues. In addition, virus shedding into the environment may be possible based on positive nasal and rectal swabs. Infected white-tailed deer may be a possible source of BVDV transmission to pastured cattle.

Validation of real-time assays for *Bovine viral diarrhoea virus*

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A real-time PCR assay, developed by the Veterinary Laboratories Agency (VLA), has recently been commercialised and produced in kit form by QIAGEN. The assays are marketed under the trade name *cador*. The single tube, real-time, reverse transcriptase (RT), TaqMan® PCR for detection of ruminant pestiviruses (based on that of McGoldrick *et al.*[1]), allows the rapid and specific detection of viral RNA from clinical specimens. The primers amplify a 288bp fragment of the 5' UTR of the pestiviral genome. The use of multiple probes in the *cador* BVDV type 1/2 RT-PCR "typing" assay allows detection and differentiation of BVDV types 1, 2 and Border disease virus in heparinised blood, milk and post mortem tissue samples. A *cador* BVDV RT-PCR "screening" assay, also developed by QIAGEN, based on that by Peterhans et al. (unpublished), was also assessed by VLA
The VLA 'in-house' assay, from which the typing assay was adapted, was used as the 'gold standard' for comparison. The screening and typing kits both underwent the same validation process. All nucleic acid extractions were performed on the QIAGEN QIAcube using the QIAGEN MinElute Virus Spin kit.
The areas subjected to investigation included analytical sensitivity, analytical specificity, repeatability and reproducibility.

Analytical Sensitivity. 3 different viral strains were tested; C24V (BVDV type 1), Lees (BVDV type 2) and Moredun (BDV). Tissue culture fluid for each of the viruses was diluted 1:5 to extinction and tested in triplicate for each dilution. Both assays proved to be as sensitive as the 'gold standard' but produced higher Ct values.

Analytical Specificity. A panel of 15 BVDV/BDV strains were tested and both assays detected all 15 viruses. The assays were shown not to cross-hybridise with 5 bovine viruses (Parainfluenza 3 Virus, Respiratory Syncytial Virus, Adenovirus, Enterovirus, and Cowpox Virus).

Diagnostic specificity. 100 BVDV positive and 100 BVDV negative heparinised whole blood samples were selected. BVDV infection was previously confirmed using an antigen ELISA assay (Serelisa BVD/MD Ag, Synbiotics, Lyon, France). The samples were tested using both assays.
In the screening assay, 100/100 were positive and 100/100 negative (but 6 internal controls failed). The typing assay produced 100/100 positive; 98/100 negative results but the Ct values for "false" positives were higher (37-38) than would be expected for a real positive sample.

Repeatability. A sample of BVDV type 2 (strain Lees) was tested on 20 consecutive test runs with both assays. The screening assay produced an inter-assay variability of 3.7 Cts (%CV = 4.26) whereas the typing assay had a variability of 2.34 Cts (%CV = 1.81).

Both assays appear to be highly sensitive, specific, produce reproducible results and the screening assay has been used as part of the effort to eradicate BVDV in Switzerland.

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Use of a single ELISA test as a screening tool for the detection of Avian Influenza antibodies in different avian species around the world

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Multiple test methods have traditionally been required for the detection of avian influenza antibodies in serum samples from different avian species. An innovative single ELISA test for the detection of antibodies against avian influenza in multiple avian species of birds was developed. The test uses avian influenza antigen coated on the solid phase, to bind avian serum antibodies against avian influenza virus. A conjugate prepared with monoclonal antibody directed against the conserved influenza A nucleoprotein is then used as the detection reagent. The presence of the avian influenza antibodies in a test sample is determined by the ability of the test sample to inhibit binding of the anti-nucleoprotein conjugate to the plate. Because the basis of the test is the ability of a sample antibody to block a conserved immunodominant epitope on the avian influenza coated plate, the assay format does not require use of any species specific reagents and is therefore broadly applicable for avian species that generate an antibody response to the nucleoprotein of avian influenza.

Based on the reference methods AGID (agar gel immuno diffusion), HI (hemagglutination inhibition) and indirect ELISA (enzyme linked immunosorbent assay) from a total of 5007 negative samples the avian influenza multi-species ELISA had only 15 samples with discrepant results. From a total of 1250 positive samples the avian influenza multi-species had only 58 discrepant results to the traditional tests resulting in a kappa value of 0.963.

Use of infrared thermography to detect signs of Foot and Mouth disease in experimentally infected mule deer (*Odocoileus hemionus*) and other ungulates

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Introduction. Foot and mouth disease (FMD) is an extremely contagious, acute viral disease of all cloven-footed animals and is characterized by fever and vesicular eruption in the mouth and on the feet. FMD was eradicated in the United States in 1929; however, the disease is endemic in many parts of the world and an outbreak in the US could occur. Early detection of the disease may reduce economic loss and loss of susceptible wildlife. We evaluated the use of infrared thermography (IRT) to detect signs of FMD in experimentally infected mule deer (*Odocoileus hemionus*), and pronghorn antelope (*Antilocapra americana*).

Materials and Methods. Animals were either experimentally inoculated intradermally in the tongue with 10,000 lesion forming units of FMD virus (O1 Manisa strain) or exposed to the disease through inter- or intra- species contact. Thermal images were taken of the animals using Forward Looking Infrared camera (FLIR) Thermcam EX360 or E65. Images were taken of the head, rear feet, and front feet. Some of the animals had VHF temperature transmitters surgically implanted into the abdomen to recorded body temperature. The animals were examined daily for lesions.

Results. Early vesicular lesions were observed within 24 hours post-inoculation (PI) for the two inoculated mule deer and for the mule deer exposed through contact (n=12), the first lesions either on the mouth and/or the feet were observed 48-96 hrs PE. On the day before, the day of, and the day after first lesion occurrence, the daily maximum body temperatures were significantly different ($P \leq 0.002$) from the pre-infection maximum body temperatures. Eye thermal temperatures were compared with body temperatures and were found not to be significantly different. For feet thermal images, three methods were examined: 1) visual changes, 2) temperature change in the maximum foot temperature, and 3) temperature difference among the four feet. Visual changes from dark gray (cool) to white (hot) over the course of the infection were observed. The maximum foot temperature rose significantly (P= 0.017) from two days before (27.3°C ±1.9°C SE) to two days after (33.0°C ±2.0°C SE) first foot lesion occurrence. The differences between maximum and minimum foot temperatures were significant from each other on the day of (P=0.002) and one day after (P=0.046) lesion occurrence when they plateaued at a difference of 5.4°C. For pronghorns, increased foot temperatures were observed 22 hours PI, up to 20 hrs before clinical signs were observed.

Discussion/Conclusion. The rise in foot temperature may be a sign attributable to FMD and thus IRT may be a noninvasive method to help detect FMD. The current screening method for FMD is a labor intensive process requiring the restraint of the animals for clinical examination. Depending on the stage of the infection, clinical signs may not yet be apparent and animals may need to be re-screened. This research indicates the potential of IRT as a rapid, remote surveillance technique that can detect suspect animals for clinical testing. This may reduce the number of capture events for wild animals, decrease labor and costs associated with the clinical examinations and allow for more rabid detection to help control the spread of the disease.

Real-time PCR panel for detection of canine enteric and respiratory pathogens

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A real-time PCR panel for the detection of canine enteric and respiratory diseases has been developed at the Wisconsin Veterinary Diagnostic Laboratory. The following organisms are included on the canine enteric panel: canine adenovirus type 1 (infectious canine hepatitis) (CAV-1), canine coronavirus type 1 (CCoV-1) and canine parvovirus type 2 (CPV2) The following pathogens are included on the canine respiratory panel: canine herpesvirus (CHV), canine parainfluenza virus (CPIV), canine adenovirus type 2 (infectious laryngotracheitis) (CAV-2), canine distemper virus (CDV) and canine respiratory corona virus (CRCoV). Real time PCR assays were either developed or adapted from previously published conventional PCR protocols for CAV-1, CHV, CPIV, CAV-2 and CRCoV. Primer and probe sets for CDV, CPV2 and CCoV-1 were used without modification from the original publication; however, cycling parameters were modified to comply with panel conditions.

The genes targeted are: the inverted terminal repeat region responsible for DNA replication initiation of CAV-1 and CAV-2; the gB glycoprotein of CHV; the nucleocapsid gene of CPIV; and the spike protein of CRCoV. Vector NTI Suite 9.0 (Informax, Frederic, MD) was used for sequence alignments and multiple primer sets were generated using the Primer Express program (Applied Biosystems, Foster City, CA). Multiple primer sets were evaluated and the most efficient primer sets and the corresponding probes were selected; the assay was then evaluated for analytical and diagnostic sensitivity and specificity. Efficiency of all reactions was at least 98 %. The assays developed at WVDL showed high sensitivity and specificity for samples known to be positive for a particular agent as determined by fluorescent antibody staining (FA) on tissue sections or by real-time PCR at another laboratory. The panel PCR assays were able to accurately detect 104/104 negative samples and 10/10 positive samples for CAV-1; 54/54 negative samples and 4/4 positive sample for CAV-2; 66/66 negative and 15/15 positive samples for CHV; 18/18 negative and 8/8 positive samples for CPIV and 17/17 negative samples for CRCOV. No positive samples for CRCoV were available for validation. (The sequences encoding the bovine corona virus spike protein are homologous to CRCoV so BCV was used to determine analytical sensitivity.) In summary, the new real-time PCR panels for canine respiratory and enteric disease performed well in this initial validation. Continued validation is underway to collect and test other known positive and negative samples.

High throughput, low volume real-time PCR pathogen detection in a novel nanofluidic platform

Shau Neen Liu-Cordero[1], Andrew Bond[1], Elen Ortenberg[1], D.G.W Roberts[1], Delia Muise[2], K.D. Munnelly[1], Ken Henderson[2]

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Nucleic acid array technology depends on capture oligonucleotides that are bound to solid surfaces or polymer beads and is commonly used for genetics application. The use of capture array technology for the detection of infectious agent nucleic acid has been hampered by poor analytical sensitivity but can be improved by implementing PCR amplification prior to hybridization. However, introducing an initial PCR step necessitates the use of multiplex PCR which can lead to competitive inhibition and cross-assay primer interaction, or PCR product pooling which promotes amplicon contamination and process challenges. A novel nanofluidic platform provides an alternative format that permits the amplification of up to 3072 individual nucleic acid targets within the array. This format eliminates the need to multiplex or pool PCR assays prior to the array step. The platform accommodates the use of TaqMan® PCR technology, which has been documented to provide better analytical sensitivity and specificity over conventional gel-based PCR. We investigated the the platform to determine its utility for the detection of rodent infectious agents. An array was produced which contained primer and probes representing 20 common viruses and bacteria. Each of the 48 64-hole sub-arrays contained each agent assay in triplicate. Ten-fold dilutions of each target nucleic acid were evaluated in both the nanofluidic OpenArray™ plaftorm and a standard 96-well format (duplicate wells). **The analytical sensitivity determined for the OpenArray and 96-well format were similar. The OpenArray was resistant to PCR inhibition associated nucleic acid isolated from feces and also to high concentrations of test nucleic acid (600ng/µl final reaction concentration). Rodent samples, which had previously been determined to be positive for at least one agent by the 96-well format, were positive for the same and sometimes additional agents in the OpenArray platform. Potential applications for the OpenArray platform include virus panels for biologics testing and health monitoring panels for post-quarantine or routine health monitoring of sentinel mice.**

Feasibility of reverse transcriptase loop mediated isothermal amplification (RT-LAMP) for the detection of *Porcine reproductive and respiratory syndrome virus* (PRRSV)

Albert Rovira[1], *Juan Abrahante*[2], *Michael Murtaugh*[2], *Claudia Muñoz-Zanzi*[3]

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Monitoring PRRSV in boar studs is critical because of the devastating consequences of a potential PRRSV introduction and transmission to sow farms through semen. Currently, most boar studs send samples routinely (serum, blood swab and/or semen) to diagnostic laboratories to be tested for PRRSV by PCR. However, this monitoring schedule is expensive and not all boars can be tested. Moreover, the test results are usually reported the next day. The aim of this study was to explore the use of a novel molecular technique to develop a simple, rapid, and accurate on-site diagnostic test for PRRSV that could be routinely used in negative boar studs to detect PRRSV acute infection. A diagnostic test based on the recently described loop mediated isothermal amplification (LAMP) technology was developed. Because of the major genomic differences between European and North American strains of PRRSV, two sets of primers were designed and integrated in a multiplex RT-LAMP test. Primers were designed with LAMP-specific software (Primer Explorer V4).

To evaluate the analytical sensitivity, the RT-LAMP test was run on 10-fold dilutions of pure cultures of the European and North American strains of PRRSV. RNA was extracted from the samples with a commercial kit. The RT-LAMP reaction was performed in a 25 µl reaction mixture containing 1.6 µM of each inner primer, 0.2 µM of each outer primer, 1.4 mM of each dNTP, 0.8 M betaine, 2.5 µl 10x ThermoBuffer, 8 mM $MgSO_4$, 8 units Bst DNA Polymerase, 2 units enhanced avian myeloblastosis virus reverse transcriptase and 2 microliters of template DNA. The reaction was performed in a heat block at a constant temperature of 63 C for 60 minutes. Upon completion of the amplification step, visual inspection was used to determine positivity. Samples showing turbidity were considered positive. The limit of detection of the new RT-LAMP test was 10^2 $TCID_{50}$/ml for the European strain and 10^3 $TCID_{50}$/ml for the North American strain.

To confirm the specificity of the reaction, the products of positive RT-LAMP reactions with the European and North American strains were digested with the restriction enzymes NlaIV and EaeI, respectively. After digestion, the number and molecular weight of the resulting fragments were consistent with the predictions based on the nucleotide sequence of the products, indicating specific reactions. Furthermore, to evaluate analytical specificity, the RT-LAMP test was run on a panel of viral isolates commonly isolated from pigs. The panel included influenza viruses H1N1 and H3N2, PRCV, TGEV, PRV, EMCV, Enterovirus, BVDV1, BVDV2, HEV, PPV, PCMV, PCV1 and PCV2. All of these samples were negative.

The performance of the LAMP test on serum samples was evaluated with 100 samples from PRRSV-negative pigs and 114 samples from 15 boars experimentally inoculated with a North American PRRSV strain. Only one of the 100 negative sera tested positive by RT-LAMP. This sample was negative when the test was repeated in duplicate. Forty-nine of the 114 sera from experimentally inoculated boars tested positive by RT-LAMP. In contrast, 94 of these samples tested positive for PRRSV by a commercially available PRRSV PCR kit. **The feasibility of RT-LAMP to detect PRRSV was demonstrated in this study.** The RT-LAMP reaction can be performed in just 1 hour with a simple and inexpensive heat block. However, the sensitivity of the test needs to be improved before this test can be used in the field.

A newly developed multiplex PCR procedure for PCV2a and PCV2b identifications

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Porcine circle virus type 2 (PCV2) becomes increasingly important as the major player of porcine circle virus disease (PCVD) which includes the poorly understood porcine dermatitis and nephropathy syndrome (PDNS), and porcine multisystemic wasting syndrome (PMWS). It is suggested that PCV2 may also involve in porcine high fever disease (PHFD) that has been causing serious problems in Asia lately.

In recent years, PCV2b, or PCV2 group 1, has emerged into the major subtype as compared to PCV2a, or PCV2 group 2. Development of an accurate diagnostic method becomes more important for early detections, as well as for PCVD and PHFD research in general.

To develop a molecular detection method, 209 PCV2a and 98 PCV2b full-length sequences that were available from the GenBank were analyzed, and a new multiplex real-time PCR procedure was developed for PCV2a and PCV2b identifications. The open-reading frame (ORF) 2 in the PCV2 genome has a variable region that is distinct between PCV2a and PCV2b, and conserved within each subtypes of PCV2. This region was used to design the test. Through analysis of all available full-length PCV2 sequences, two separate pairs of primer sequences were designed to target on PCV2a and PCV2b respectively. Although they cross amplify each other for some sequences, by using slightly different primers, the coverage of the test for PCV2a is increased to 97% and for PCV2b is 98%. Each primer pair was subjected to a primer efficiency test by using a series of template dilutions. Correlation coefficiencies were 0.998 and 0.992, and PCR amplification efficiencies were 100.8% and 106.2% for PCV2a and PCV2b primer pair, respectively. To tackle with the AT-rich, variable region for the probe design, both TaqMan and MGB probes for each subtype were designed and tested. A combination of TaqMan labeled PCV2a, and MGB probe for PCV2b generated the best results. The procedures were first tested on each subtype separately with different primer and probe concentrations. They were then mixed together and applied to PCV2a and PCV2b DNA templates respectively, using the best primer/probe concentrations determined previously. The results show that they were detecting the corresponding subtype only, and they were not cross detecting. To verify if the multiplexing of the two sets of primer/probe together is generating sensitive result as individual test, the individual tests were compared with the multiplex PCR procedure using the same tubes of template samples with five different concentrations. For each concentration, the Ct values were very similar between the single tests and the multiplex PCR tests indicating that the sensitivity of the test is not affected by multiplexing.

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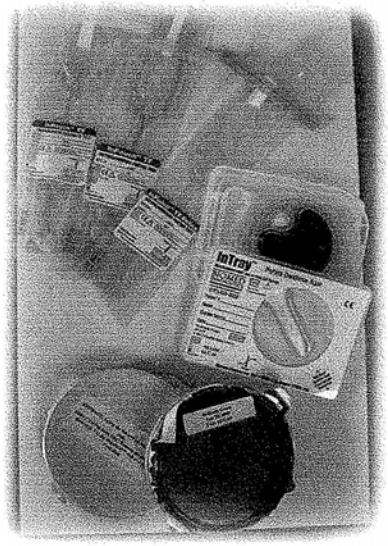

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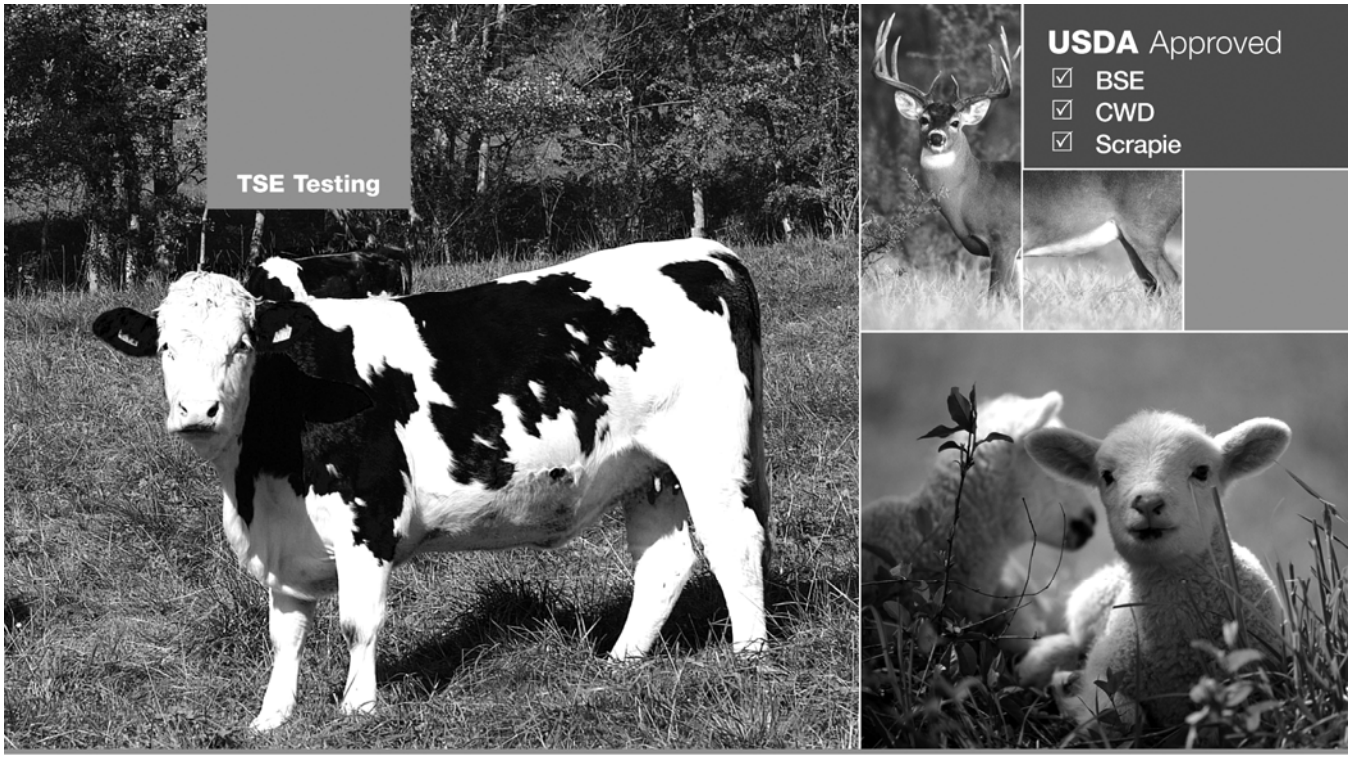

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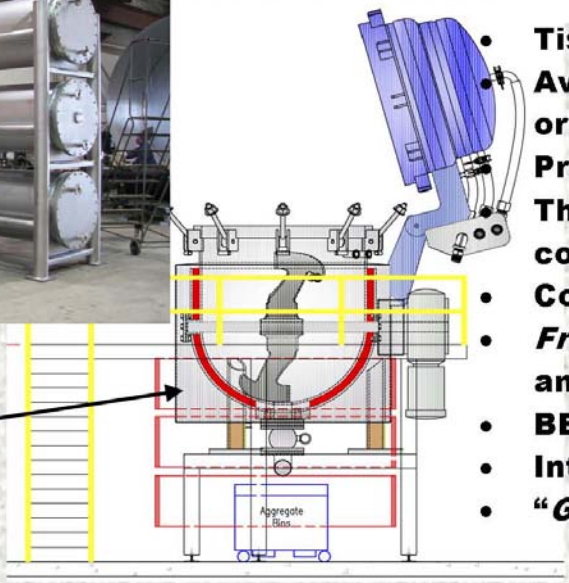

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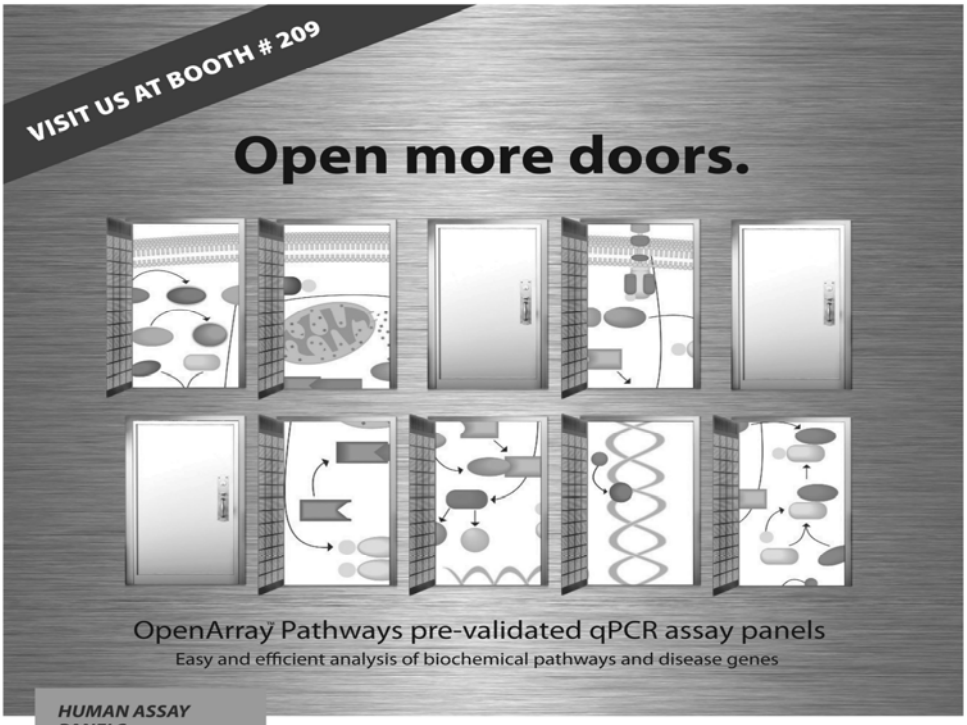

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Enfer Diagnostics (www.enferdiagnostics.com) is a specialized provider of advanced diagnostics to improve animal disease management worldwide. Enfer is committed to delivering only the most accurate diagnostics that industry and government disease 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.

FBI – Laboratory Division

Booth **409**

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

FBI – Agroterrorism Program:
The display describes the FBI response to terrorism against US animal, crop, and food production. The FBI leads the criminal investigation of terrorist attacks and the use of WMD against US infrastructure. USDA and the State Agriculture Departments lead animal disease outbreak response (diagnosis, epidemiology, outbreak management, and attribution).

Agriculture is one of 17 critical infrastructures (crops, livestock, food, forestry - water resources). Terrorists have identified FAD's as weapons against the US economy (livestock and food production).
The FBI has outreach to USDA, State Dept's of Agriculture, CDC, Universities, DoD, and private industry involved in food and agricultural production. Activities include joint agency working groups, MOU's w laboratories, working groups, conferences, and interagency training programs.

FBI Units (Hazardous Materials Response Unit, Chemical-Biological Science Unit, Weapons of Mass Destruction Directorate) are involved in WMD prevention and response, screening, collection, transport, forensic exam, and evaluation of evidence.

Global Vet Link

Booth **307**

2625 N. Loop Dr. #2130
Ames, IA 50010
www.globalvetlink.com
Contact: Kaylen Henry
515.296.0860
khenry@globalvetlink.com

Company Representatives: Kaylen Henry, Kevin Maher, Jill Wagner

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 **202**

37842 Avalon Drive
Zephyrhills, FL 33541
www.hydrolpro.com
Contact: Kim Etherington
813.780.6753
kim@hydrolpro.com

Company Representatives: Kevin Morris, Kim Etherington, Bob Hockman

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 or wet output versions available. A low-cost, effective alternative to incineration. New Bio-effluent De-con systems are also now offered.

ID VET

Booth **101**

570 rue des Bouissettes
Montpellier, France 34070
Contact: Philippe Pourcuier
+38 467 414933
idvet.info@id-vet.com

ID VET proposes innovative diagnostic tools for the detection of infectious veterinary diseases, ID VET's ELISA kit range, ID Screen®, as well as other standard products used in major European surveillance programs.

ID VET distinguishes itself through its innovative strategy in the following areas:

- Progress in the performance of diagnostic tests
- Detection of emerging pathologies.

ID VET's priority is to provide our clients with efficient, professional customer support.

ID Vet is certified ISO 9001:2000.

Its commitment to quality covers all ID VET activities, from Research & Development, to Production, Marketing and Sales. ID VET :

- Requires rigorous quality control and traceability of all products and services.
- Implicates all employees in its assurance quality program.

With extensive expertise in the diagnostic field, ID VET will design, develop and manufacture immunoassays to meet your specific requirements.

Upon request, we propose a collaborative approach for the development of specific tools for antibody or antigen detection.

IDEXX Laboratories

Booths **203**

One Idexx Dr.
Westbrook, ME 04092
www.idexx.com/production
Contact: Mary Spear
207.856.8059 207.838.0792
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

*HerdChek, FlockChek, CHEKIT, Pourquier and xChek are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.

Inverness Medical Professional Diagnostics

Booth **103**

10 Southgate Rd.
Scarborough, ME 04074
www.invernessmedicalpd.com
Contact: Sandra Rodgers
207.730.5723
sandra.rodgers@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.

Jorgensen Laboratories

Booth **100**

1450 N Van Buren Av
Loveland, CO 80538
www.jorvet.com
Contact: Earl Sethre
970.669.2500
earl@jorvet.com

Jorgensen Laboratories has supplied veterinaries with high quality instruments and equipment for over 40 years. This veterinarian owned and family run business has been recognized by the American Veterinary Distributor Association (AVDA) and for two years in a row was given the New Opportunities Award. Jorgensen was chosen by their industry peers to receive this award for their rate of new product introduction, marketing support for new products and demo product availability .The JORVET trademark is synonymous with high quality products for the veterinary profession and the JORVET brand is known throughout the world as the premier source for veterinary instruments and equipment.

Merrick and Company

Booth **407**

2450 S Peoria St
Aurora, CO 80014-5475
www.merrick.com
Contact: Ross Graham, DVM, PhD
703.680.6086
ross.graham@merrick.com

Merrick & Company is an employee-owned, national A/E design firm, with over 400 employees located in Colorado, New Mexico, Georgia and Canada. Founded in 1955, Merrick provides full service architecture and engineering, construction management, and commissioning services to Federal clients including the USDA, DOD, DOE and DHS as well as universities and institutions, international and private clients. We have been a single-source provider of services for analytical, research laboratories and high containment facilities for over 20 years with our area of expertise originally focused on agencies and facilities involved in animal and plant research. Merrick is consistently registered in the top 200 of Engineering News Record's "Top 500 Design Firms" and has received numerous quality achievement awards for outstanding service since its inception. We are committed to sustainable 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 designs.

National Institute for Animal Agriculture (NIAA)

Booth **301**

1910 Lyda Ave.
Bowling Green, KY 42104
www.animalagriculture.org
Contact: Michele Vise-Brown
270.782.9798
mvisebrown@animalagriculture.org

Company Representatives: *Michele Vise-Brown, Teres Lambert, and Gale Johnson*

The National Institute for Animal Agriculture (NIAA) is a forum for building consensus and advancing solutions for animal agriculture and to provide continuing education and communications linkages to animal agriculture professionals. Our mission truly reflects NIAA - where it's been, where it is, and where it is going. NIAA is the only forum where producers/owners (cattle, equine, poultry, small ruminants and swine), scientists (corporate, academia and government), veterinarians (private practice, industrial and government), regulators (state and federal), and business executives (corporate and association) meet in a common effort to deal with shared issues and opportunities. Visit our website at www.animalagriculture.org

Prionics USA, Inc.

Booth **303**

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 **107**

19300 Germantown Road
Germantown, MD 20874
www.qiagen.com
Contact: Pam Daniels
240.686.7688
pam.daniels@qiagen.com

QIAGEN Inc., offering over 500 products, is the worldwide leader of sample and assay technologies for research in life sciences, applied testing and molecular diagnostics.

Rural Technologies, Inc.

Booth **104**

1008 32 Avenue
Brookings, SD 57006
www.ruraltech.inc.com
Contact: Elliot Stevens
605.692.6953
estevens@ruraltechinc.com

Rural Technologies, Inc. (RTI) has over fourteen years of experience in providing the animal health industry with the highest quality products, services, and results that meet or exceed industry standards. Rural Technologies, Inc. offers monoclonal antibodies that are used internationally for bovine, porcine and avian diagnostic purposes. In addition to monoclonal antibodies, we have an in-house milk quality laboratory (Heartland Milk Labs, Inc.), which allows veterinarians to provide their clients with timely and accurate results. We also offer contract research and consulting services to the animal health, pharmaceutical, biomedical and feed additive industries. Services offered to clients include immunology, virology, microbiology and animal nutrition studies. Rural Technologies, Inc. goal is to lead the way in animal health and improvements.

Sherlock Microbial Identification, Inc.

Booth **102**

125 Sandy Drive
Newark, DE 19713
www.midi-inc.com/
Contact: Jeanette Bluman
302.737.4297
jbluman@midi-inc.com

MIDI, Inc. has been developing automated microbial identification solutions for quality control, research and diagnostic purposes since 1985. The Sherlock® Microbial Identification System is used to identify over 1,500 bacterial species (including 6 bioterrorism agents) based on gas chromatographic analysis of cellular fatty acids. This system has both U.S. Department of Homeland Security and U.S. FDA 510(k) clearance for confirmation of the anthrax pathogen. The MIDI System can identify microbes in as little as 15 minutes from 2mg of pure culture using a new procedure called Instant FAME.

Sherlock® DNA is an optional add-on to the Sherlock Microbial Identification System that can identify bacteria, yeast and fungi by rRNA gene sequencing.

MIDI, Inc. is a Premier Channel Partner of Agilent Technologies.

Smiths Detection

Booth **411**

459 Park Avenue, Bushey
Watford, Hertfordshire
United Kingdom WD23 2BW
www.smithsdetection.com
Contact: Carmelo Volpe
(0) 1923 658 338
carmelo.volpe@smithsdetection.com

Disease outbreaks such as Foot-and-Mouth, Avian Influenza and Bluetongue have highlighted the importance of early identification of the disease in limiting the spread. However, efforts to control outbreaks can be complicated by the need to transport all samples to a reference laboratory for ultimate identification. Smiths Detection's Bio-Seeq Portable Veterinary Diagnostic System addresses the issue by avoiding any transportation of samples to a lab. With this system the lab is taken into the field. It has been designed with veterinarians in mind, comprising a portable briefcase-sized instrument and a disposable sample preparation unit. 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, HAZMAT teams and government agencies around the world.
www.smithsdetection.com/vet

Svanova Biotech AB

Booth **408**

Uppsala Science Park
Uppsala, Sweden SE-751 83
www.svanova.se
Contact: Afsaneh Jalali
+46 1865 4915
Afsaneh.Jalali@svanova.com

Svanova Biotech AB was established in the beginning of 2001. The company was founded upon the activities, which have been ongoing since 1988 within the National Veterinary Institute, in Uppsala, Sweden.

Svanova Biotech AB develops, manufactures, markets and sells a wide range of diagnostic products for large-scale laboratories as well as for the veterinarians in the clinic or in the field, mainly for diagnosing infectious diseases in livestock and companion animals.

The majority of our products are based on the ELISA technique and are marketed under the trade name SVANOVIR®. Additionally, Svanova offers a line of pen-side products under the trade name SVANODIP®. All products are manufactured in an ISO 9001:2000 certified facility and are adapted to the needs of the laboratories as well as field conditions.

The company has a good reputation worldwide in the field of veterinary diagnostics, well-established manufacturing and marketing units, good research capacity with in-depth cooperation with specialists and researchers throughout the world.

Synbiotics Corporation

Booth **400**

12200 NW Ambassador, Suite 101
Kansas City, MO 64163
www.synbiotics.com
Contact: John Donovin
800.228.4305
customerservice1@synbiotics.com

Synbiotics Corporation develops, manufactures and markets veterinary diagnostics and related products for the companion animal, large animal and poultry markets worldwide. 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 **402**

9901 Belward Campus Dr. #300
Rockville, MD 20850
www.tetracore.com
Contact: Brian K. Kijowski
240.268.5417
bkijowski@tetracore.com

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

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 **309**

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.

Ventana Medical Systems, Inc.

Booth 308

1910 Innovation Park Dr.
Tucson, AZ 85755
www.ventantamed.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.

VMRD, Inc.

Booth 405

4641 Pullman Albion Rd.
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.

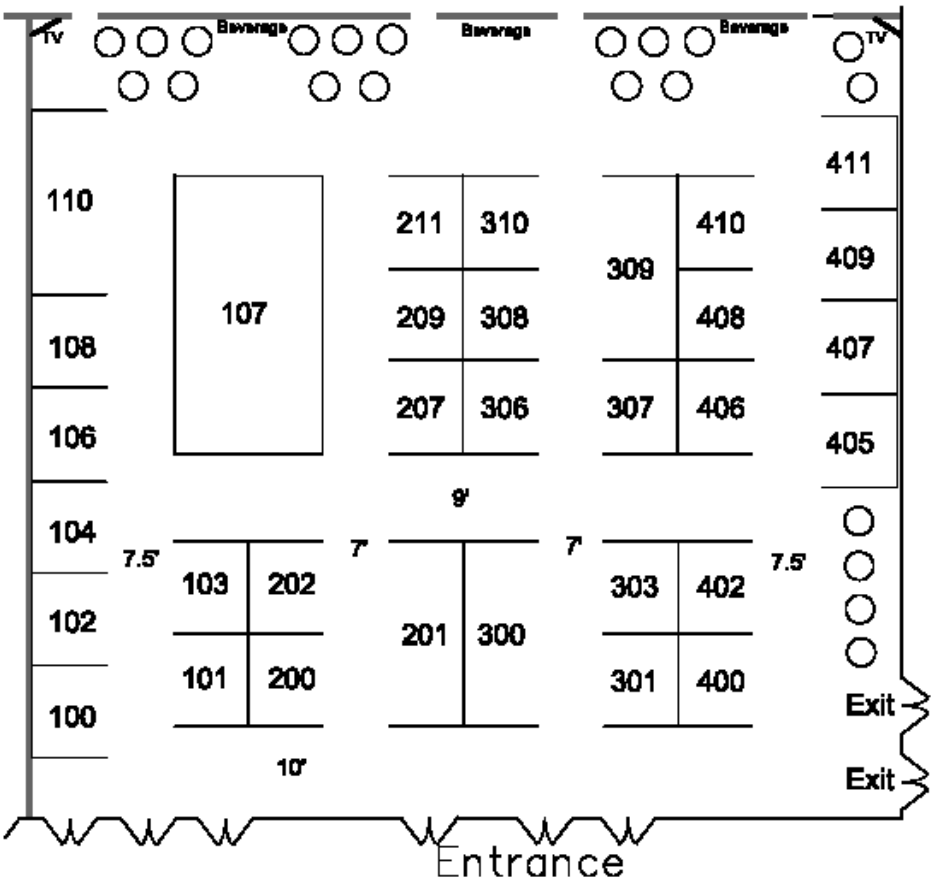
American BioResearch (ABR, Inc.). ABR conducts 9 CFR and EMEA extraneous virus testing on cell lines, virus seeds, cell culture reagents and supplements, and other virology related services. ABR and VMRD have worked together for many years and we are dedicated to continuing to refine ABR's services.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

Upcoming AAVLD/USAHA meetings:

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

2010: November 10-17, Hilton
Minneapolis, Minnesota

AAVLD Exhibit Floor Plan
October 25-26, 2008



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200	Advanced Technology Corp. VADDS
300	Applied Biosystems
306	ATI (Automated Technologies Inc.)
406	Biolog, Inc.
211	Bio-Rad Laboratories
410	BioSAFE Engineering WR^2
106	Biosearch Technologies, Inc.
209	BioTrove
310	Centaur, Inc.
108	Corbett Robotics, Inc.
207	Crawford Industrial Group
110	Enfer Diagnostics
409	FBI, Laboratory Division
307	Global Vet Link
202	Hydrol-Pro Technologies, Inc.
101	ID Vet

BOOTH #	COMPANY
201	IDEXX Laboratories
103	Inverness Medical Professional Diagnostics
100	Jorgensen Labs Inc.
407	Merrick and Company
301	National Institute for Animal Agriculture
303	Prionics
107	Qiagen, Inc.
104	Rural Technologies, Inc.
102	Sherlock Microbial ID Systems MIDI, Inc.
411	Smiths Detection
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400	Synbiotics Corporation
402	Tetracore, Inc.
309	TREK Diagnostic Systems
308	Ventana Medical Systems, Inc.
405	VMRD, Inc.

AAVLD FOUNDATION DONATION FORM

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 (Payments must be made in US dollars and drawn on US Funds.)

Please accept this donation of $_____ as an expression of support in promoting Veterinary Diagnostic Medicine for unrestricted use.

Please send me additional information on charitable gift or deferred gift annuity. _____

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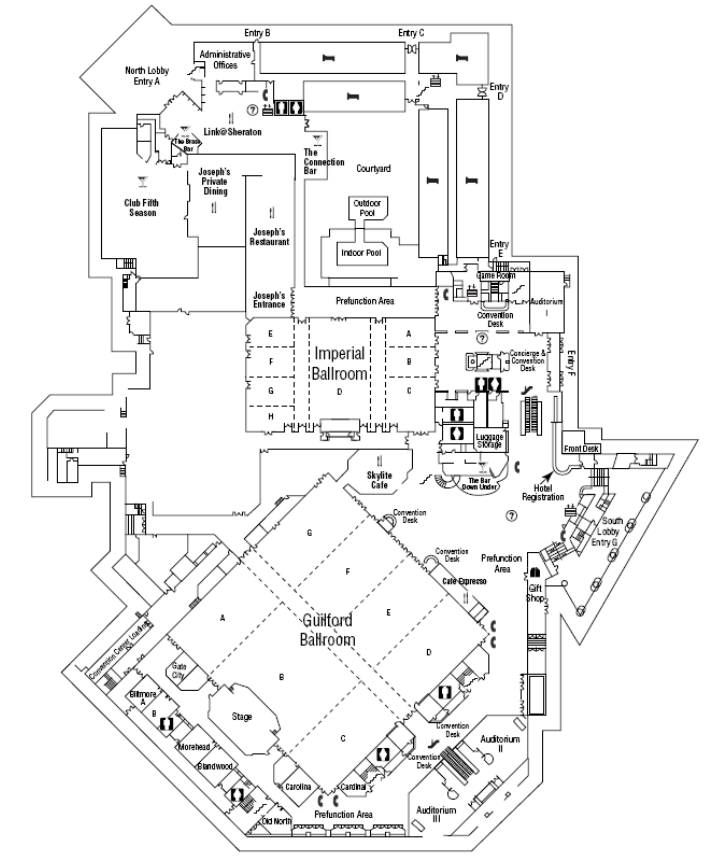
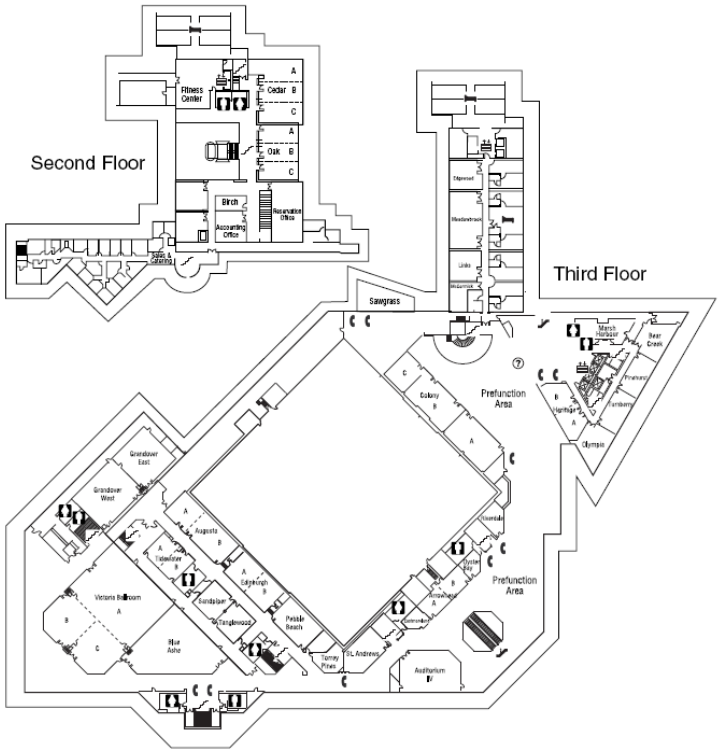




Sheraton Four Seasons

DIRECTORY

Hotel Registration **Convention Desks**
Ballrooms • Meeting Rooms

First Floor			Second Floor
Auditorium I	Cardinal	Imperial Ballroom	Birch
Auditorium II	Carolina	A•B•C•D•E•F•G•H	Cedar A•B•C
Auditorium III	Gate City	Morehead	Oak A•B•C
Biltmore A•B	Guilford Ballroom	Old North	
Blandwood	A•B•C•D•E•F•G		

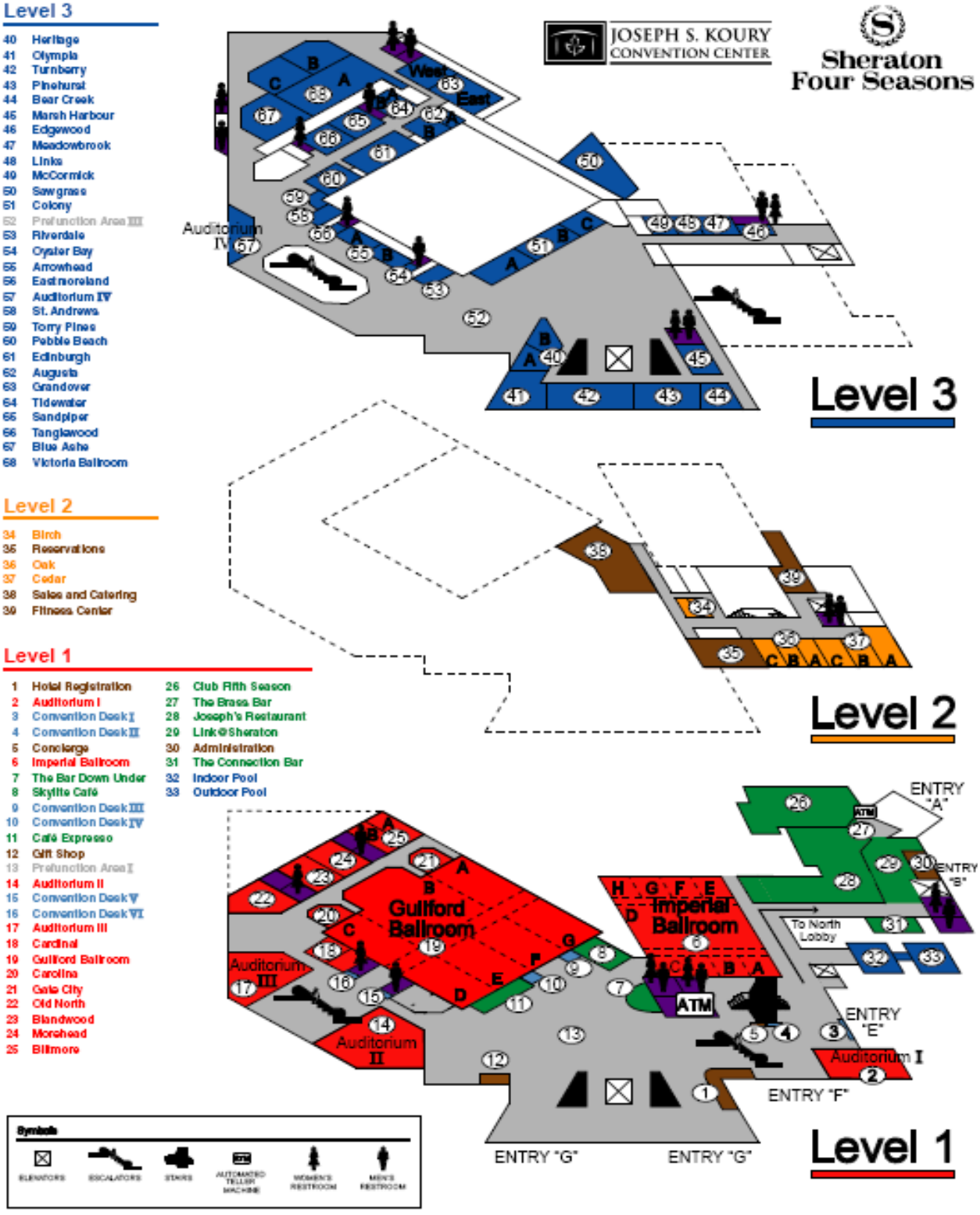
Third Floor			Victoria Wing
Arrowhead A•B	Heritage A•B	Pinehurst	Blue Ashe
Auditorium IV	Links	Riverdale	Grandover East
Augusta A•B	Marsh Harbour	Sandpiper	Grandover West
Bear Creek	McCormick	St. Andrews	Victoria A•B•C
Colony A•B•C	Meadowbrook	Tanglewood	
Eastmoreland	Olympia	Tidewater A•B	
Edgewood	Oyster Bay	Torrey Pines	
Edinburgh A•B	Pebble Beach	Turnberry	

Elevators **Escalators** **Stairs**
Public Telephones **House Telephones**
Information Centers **Restrooms**
Restaurants **Lounges**

- Joseph's Restaurant
- Skylite Café
- Café Expresso
- Link@Sheraton
- Club Fifth Season
- The Brass Bar
- The Connection Bar
- The Bar Down Under

Administrative Offices **Fitness Center**
Sales & Catering **Pools**
Reservations **Game Room**
Guest Rooms **Gift Shop**

JOSEPH S. KOURY CONVENTION CENTER


JOSEPH S. KOURY CONVENTION CENTER
Sheraton Four Seasons
Level 3
40 Heritage
41 Olympia
42 Turnberry
43 Pinehurst
44 Bear Creek
45 Marsh Harbour
46 Edgewood
47 Meadowbrook
48 Links
49 McCormick
50 Sawgrass
51 Colony
52 Prefunction Area III
53 Riverdale
54 Oyster Bay
55 Arrowhead
56 Eastmoreland
57 Auditorium IV
58 St. Andrews
59 Torry Pines
60 Pebble Beach
61 Edinburgh
62 Augusta
63 Grandover
64 Tidewater
65 Sandpiper
66 Tanglewood
67 Blue Ashe
68 Victoria Ballroom
Auditorium IV
Level 3
Level 2
34 Birch
35 Reservations
36 Oak
37 Cedar
38 Sales and Catering
39 Fitness Center
Level 1
1 Hotel Registration
2 Auditorium I
3 Convention Desk I
4 Convention Desk II
5 Concierge
6 Imperial Ballroom
7 The Bar Down Under
8 Skylite Café
9 Convention Desk III
10 Convention Desk IV
11 Café Espresso
12 Gift Shop
13 Prefunction Area I
14 Auditorium II
15 Convention Desk V
16 Convention Desk VI
17 Auditorium III
18 Cardinal
19 Guilford Ballroom
20 Carolina
21 Gate City
22 Old North
23 Blandwood
24 Morehead
25 Biltmore
26 Club Fifth Season
27 The Brass Bar
28 Joseph's Restaurant
29 Link@Sheraton
30 Administration
31 The Connection Bar
32 Indoor Pool
33 Outdoor Pool
Guilford Ballroom
Imperial Ballroom
Auditorium III
Auditorium II
Auditorium I
ATM
To North Lobby
ENTRY "A"
ENTRY "B"
ENTRY "E"
ENTRY "F"
ENTRY "G"
ENTRY "G"
Symbols
ELEVATORS
ESCALATORS
STAIRS
AUTOMATED TELLER MACHINE
WOMEN'S RESTROOM
MEN'S RESTROOM
Level 1