American Association of Veterinary Laboratory Diagnosticians

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

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

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Acknowledgments

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Thermo SCIENTIFIC

Bacteriology 1

Saturday, October 19, 2013

Pacific Salon 3

Sponsor: Thermo Scientific

Moderators: Doreene Hyatt, Karen W. Post

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Saturday, October 19, 2013
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Moderators: Ashley Hill, François Elvinger

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Saturday, October 19, 2013

Pacific Salon 1

Sponsor: Biocare Medical

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Toxicology

Saturday, October 19, 2013
Pacific Salon 2

Moderators: Michelle Mostrom, Deon Van der Merwe

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Saturday, October 19, 2013
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Sponsor: ECL2

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Bacteriology 2

Sunday, October 20, 2013
Pacific Salon 3

Moderators: Durda Slavic, Deepanker Tewari

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Epidemiology 2

Sunday, October 20, 2013

Pacific Salon 2

Moderators: Marie Culhane, Amar Patil

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Pathology 2

Sunday, October 20, 2013

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Moderators: Arthur (Bill) Layton, Francisco Uzal

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Virology 2

Sunday, October 20, 2013

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Moderators: Amy Glaser, Binu Velayudhan

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Saturday, 19 October – Sunday, 20 October

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AAVLD Plenary Session
Getting Ahead of the Ball: Diagnostic Adaptation in a Global Health Community
Saturday, October 19, 2013
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Interlaboratory Comparison of Equine Herpesvirus type 1 Polymerase Chain Reaction Techniques Utilized in North American Diagnostic Facilities

Tim Baszler[1], Eileen Ostlund[2], Beate Crossley[3], Udeni Balasuriya[4], Dan Bradway[1], Erdal Erol[5], Donna Johnson[2], Dianne Rodman[2], Steven Sells[5]

[1]Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA; [2]Diagnostic Virology Laboratory, USDA-APHIS National Veterinary Services Laboratories, Ames, IA; [3]California Animal Health and Food Safety Laboratory, University of California-Davis, Davis, CA; [4]Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY; [5]University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY

Narrative: In 2013, the USDA-APHIS National Veterinary Services Laboratory (NVSL) and American Association of Veterinary Laboratory Diagnosticians (AAVLD) conducted a joint interlaboratory comparison (ring trial) of neuropathogenic equine herpesvirus type 1 (nEHV-1) polymerase chain reaction (PCR) techniques in an effort to standardize testing methodology for equine herpesvirus myeloencephalopathy (EHM) carried out at state/university/provincial diagnostic facilities in North America. A total of 28 state diagnostic facilities from the USA and Canada evaluated a ring test "panel" of field EHV isolates. Reference materials for all test panels consisted of EHV-2, EHV-4, EHV-5, wild-type EHV-1, and three strains of nEHV-1. The 28 participating laboratories used 38 different procedures (some laboratories tested multiple procedures) based upon modifications of 10 peer-reviewed published methods for EHV-1 PCR. Two genes were utilized as PCR targets, the EHV-1 glycoprotein B gene, and the EHV-1 ORF 30, viral DNA polymerase gene which also is the gene including the neuropathogenic marker that has been associated with large outbreaks of EHM. Glycoprotein B gene-based PCR assays, which are fundamentally designed as screening assays that detect wt-EHV-1 and nEHV-1, were used by 15 participating laboratories and had excellent diagnostic sensitivity for both wt-EHV-1 (100%; 30/30 samples identified correctly), and nEHV-1 (98.8%; 89/90 samples identified correctly), as well as excellent diagnostic specificity (98.3%; 59/60 non-EHV-1 samples identified correctly). As predicted, none of the glycoprotein B gene-based assays differentiated wt-EHV-1 from nEHV-1 and as such serve as excellent diagnostic tools to identify EHV-1 infected horses from non-EHV-1 infected horses but do not identify nEHV-1 specifically. ORF 30 (viral DNA polymerase) gene-based PCR assay had more variable results from testing of the ring trial samples. Three published ORF 30 A/G $_{2254}$ assays: 1) Allen et al, 2007, 2) Pusterla et al, 2009, and 3) Smith et al, 2012), which differentiate wt-EHV-1 from nEHV-1 by detecting the A_{2254} (wt-EHV-1) or G_{2254} (nEHV-1) polymorphism, were used by 21 participating laboratories. The three assays had diagnostic sensitivity (based upon correct identification of nEHV-1 samples) of 93.1% (67/72 samples, Allen 2007), 100% (36/36 samples, Pusterla 2009) and 94.4% (17/18 samples, Smith 2013). The diagnostic specificity (based upon correct identification of non-nEHV-1 samples) was 88.9% (64/72 samples, Allen 2007), 72.2% (26/36 samples, Pusterla 2009), and 100% (18/18 samples, Smith 2013). Nearly all (17/18) of the "false positive" results for ORF 30 A/G assays 1 & 2 resulted from the nEHV-1 specific ORF 30G assay identifying wt-EHV-1 as nEHV-1.

Speaker Biography: Tim Baszler, DVM, PhD, is Professor in the Department of Microbiology and Pathology, and Director of the Washington Animal Disease Diagnostic Laboratory in the College of Veterinary Medicine, Washington State University. He received his B.S. in Microbiology from North Dakota State University in 1978, D.V.M. from Iowa State University in 1982, PhD in virus pathobiology from the University of Illinois in 1990, and is a certified Diplomate of the American College of Veterinary Pathologists. He is immediate Past President and Accreditation Committee member for the American Association of Veterinary Laboratory Diagnosticians. He has 10 years experience directing diagnostic laboratory operations, policies and procedures for full participation in multiple national animal and public health laboratory networks including: 1) National Animal Health Laboratory Network (USDA-APHIS); 2) Veterinary Laboratory Investigation and Response Network (FDA-CVM); and 3) Laboratory Response Network for Biological Terrorism (CDC). His research has covered a range of infectious diseases caused by neuropathic retroviruses, pestiviruses, herpesviruses, protozoan parasites, and prions. His primary research and collaborative research developed and/or validated several tests for veterinary diagnostic medicine including antibody ELISA and PCR tests for bovine neosporosis, antibody ELISA test for bighorn sheep mycoplasmosis, immunohistochemistry test for bovine virus diarrhea, and live-animal immunohistochemistry tests for sheep scrapie. He has authored over 70 scientific papers in peer-reviewed journals.

H7N9 Emergence, Response, and Projections – Response in the Diagnostic Community

Mia Kim Torchetti

Diagnostic Virology Laboratory, USDA-APHIS-NVSL, Ames, IA

Narrative: The recent event of a low pathogenic avian influenza A(H7N9) causing significant morbidity and mortality in humans from China prompted national veterinary laboratories worldwide to ensure ability to detect this emergent strain. While the specific reservoir remains uncertain, the virus has been recovered from chickens as well as a handful of other species and environmental samples from live bird markets. The virus has been confirmed to be of low pathogenicity for poultry per OIE guidelines, and experimentally infected avian species including chickens, quail, turkeys, ducks, and geese show no clinical signs. Diversity long recognized in the H7 lineage has resulted in use of assays targeting viruses found in specific regions around the world. Rapid evaluation of available molecular assays was made possible thanks to the timely sequence data shared by the Chinese Authorities to the public database at the Global Initiative on Sharing All Influenza Data (GISAID, www.gisaid.org). Eurasian H7 PCR protocols predicted to detect the virus were compiled and shared by the World Organisation for Animal Health/ Food and Agriculture Organization of the United Nations (OIE/FAO) Network of Expertise on Animal Influenza (OFFLU, www.offlu.net), which further expedited the process needed to evaluate and update assays. Coordination and cooperation across public health and animal health facilitated the timely receipt of virus material from China for pathogenicity and transmission studies, as well as assay confirmation. The National Animal Health Laboratory Network (NAHLN) avian influenza testing algorithm used in 52 NAHLN laboratories and the National Veterinary Services Laboratories (NVSL) was reviewed for preparedness; the network was engaged to implement interim actions, informed of next steps, and specific partners involved in assisting with assay validation. The NVSL joined in a cooperative effort working with national partners such as Southeast Poultry Research Laboratory as well as international influenza reference laboratories to develop and incorporate an updated H7 assay. This event has highlighted the strides made in cooperation between public and animal health regarding influenza, and emphasizes the need to continue that dialogue. Surveillance in poultry must cast a broad net to capture the many subtypes that may be present, while human surveillance typically takes a more targeted approach. Renewed discussions are needed to ensure appropriate strategies are in place to address the concern of viruses that are of low pathogenicity in poultry but have the potential to cause significant disease in humans.

Speaker Biography: Dr. Mia Kim Torchetti joined NVSL in 2013 as the Avian Viruses Section Head in the Diagnostic Virology Laboratory at the NVSL. She has a broad background in laboratory networking, research and diagnostics, epidemiology, and biosecurity; earning her veterinary degree and master's at Colorado State University, and subsequently joining ARS in Athens, Georgia for her PhD and postdoctoral work. While at the Southeast Poultry Research Laboratory, her initial focus was on rapid diagnostics for Newcastle disease, moving to influenza as the issues of highly pathogenic avian influenza emerged. Before coming to NVSL, she served as the deputy coordinator and South/Southeast Asia laboratory liaison for the Food and Agriculture Organization of the United Nations, Emergency Prevention Systems Lab Unit based in Rome, Italy. In that role, she worked closely with national laboratory authorities, and coordinated laboratory networking and capacity building primarily in Southeast Asia to address testing needs for control of H5N1 highly pathogenic avian influenza and other priority diseases.

Porcine Epidemic Diarrhea Virus's Emergence in the US: Events, Observations and Opportunities

Rodger Main, Eric Burrough, Vickie Cooper, Phillip Gauger, Karen Harmon, Hai T. Hoang, Darin Madson, Kate Mueller, Angela Pillatzki, Kent Schwartz, Wendy Stensland, Greg Stevenson, Dong Sun, Kyoung-Jin Yoon, Jianqiang Zhang

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

Narrative: Porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) are corona viruses that cause atrophic enteritis and malabsorptive diarrhea in pigs. PED and TGE are clinically indistinguishable from one another. While TGE has long been endemic in North America, PED had not been diagnosed in US swine prior to May 2013. PEDV was identified in a series of diagnostic submissions originating from sow farms experiencing epizootic TGE-like illness that were testing negative for TGEV at the Iowa State Veterinary Diagnostic Laboratory. The initial case was received on April 29, 2013. By the end of the following week four additional breeding herds in three states (IA, IN, and CO) were presenting with similar clinical histories and lesions. Testing for TGEV continued to be negative. Samples from index cases were evaluated by electron microscopy and pan-corona virus PCR. Results indicated the presence of a corona virus in the feces of affected pigs. Clinical samples forwarded to the NVSL were confirmed positive for PEDV. Pan-corona virus PCR positive samples were submitted for sequencing and found to be genetically similar to a cluster of 2011-2012 PEDVs reported from China. The ISU-VDL developed and implemented PEDV PCR and IHC assays for diagnostic use within days of the initial PED confirmation. Retrospective testing of more than 800 fecal samples previously tested at the ISU VDL for TEGV dating back to December 2012 did not identify the presence of PEDV in samples submitted prior to April 16, 2013. As of August 1st, PEDV has spread to 15 states and affected more than 350 swine farms. PEDV has demonstrated its ability to be readily transmitted across vast geographic regions in a short period of time through the movement of infected pigs and transport vehicles. The collective response to the emergence of PEDV as a veterinary diagnostic community (producers, practitioners, diagnosticians, microbiologists, animal health officials, epidemiologists and well equipped state and federal laboratories) speaks well of our capabilities. However, the PEDV experience has reinforced and exposed a long list of vulnerabilities. PEDV's emergence in the US may represent an opportunity to stimulate changes that are needed to better serve and prepare US animal agriculture to meet the challenges ahead in the 21st century.

Speaker Biography: Rodger Main is the Director of the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). The ISU VDL is a full-service and fully accredited veterinary diagnostic laboratory providing comprehensive diagnostic services to protect animal health, human health, and the vitality of the Iowa and US animal agriculture economy. ISU VDL's team of 120 faculty and staff play an active role on the front-lines of animal agriculture processing approximately 50,000 diagnostic case submissions and conducting more than 1,000,000 diagnostic assays each year for livestock producers. Although the ISU VDL takes great pride in providing its state of the art diagnostic and health assurance testing services to all species groups and livestock industries, the ISU VDL is most widely recognized for delivering timely, world-class, and customer-centric diagnostic services to swine practitioners and production systems throughout the US. Rodger received his BS (agriculture) and DVM from Iowa State University in 1991 and 1996, respectively, and a PhD in Clinical Swine Nutrition and Production from Kansas State University in 2005. Prior to coming to ISU VDL in 2009, Dr. Main served as the Director of Production Systems for Murphy-Brown's Western Operations (a division of Smithfield Foods based in Ames, IA) where he had worked since graduating veterinary school.

It Takes a Diagnostic Village…

Beth Lautner

National Veterinary Services Laboratory, APHIS, USDA, Ames, IA

Narrative: The veterinary diagnostic community is expected to be actively engaged in the detection of and responses to emerging diseases whether they are a newly identified pathogen or strain, a known pathogen in a new location, or a new presentation of a known pathogen. With this engagement are new challenges and opportunities. When addressing diagnostic needs and reporting for regulatory program diseases or high-consequence foreign animal diseases, the roles of Veterinary Services (VS) and its diagnostic partners have been clear and described in specific regulations or memorandums. In contrast, development and standardization of diagnostics for domestic animal diseases in general have been viewed as outside the bailiwick of VS. When it comes to the detection of an emerging disease and addressing diagnostic needs and information sharing, there is not specific guidance regarding roles, responsibilities and expectations for those in the diagnostic community. Technological advances now allow public and private laboratories, as well as entrepreneurs from non-traditional sectors, to cost effectively develop assays for disease agents of domestic and international concern and potentially develop tests without the need for the live agent. Concurrently, the decreased costs and reduced difficulty of test development and need to generate funds are creating incentives for university and/or private laboratories to retain isolates of new emerging agents or new variations of known agents for future commercialization. As a result, there is the potential for less information sharing and interactions regarding an emerging disease detection, the quality of the testing being conducted, and the timely reporting of test results. Recently, the National Animal Health Laboratory Network (NAHLN) Coordinating Council requested that the NAHLN Methods Technical Working Group outline a strategy including the potential barriers for the identification of an unknown disease agent in two weeks and deployment of a diagnostic test in another week through diagnostic partnerships. While all in general would agree that cooperation and collaboration would be in the best interest of the animal industries and state and federal government, there are challenges to implementing the most effective diagnostic approach. The recent emergence of avian influenza A (H7N9), neurologic equine herpes virus-1 and porcine epidemic diarrhea virus have provided opportunities for the diagnostic community to work together in new ways.

Speaker Biography: Dr. Lautner was named the Director of USDA's National Veterinary Services Laboratories (NVSL) in Ames, Iowa, in May 2006. In this position, she is responsible for the operations and programs of NVSL which is the only Federal facility engaged in the diagnosis of both domestic and foreign animal diseases with locations in Ames, Iowa, and Plum Island, New York. NVSL serves as the national reference laboratory for the National Animal Health Laboratory Network and as an international reference laboratory for the World Organization for Animal Health and the Food and Agriculture Organization of the United Nations. Previously, Dr. Lautner served as Center Director, Plum Island Animal Disease Center (PIADC) within the Science and Technology Directorate of the Department of Homeland Security (DHS). Prior to joining DHS, Dr. Lautner served as Vice President for Science and Technology at the National Pork Board where she oversaw the research and development programs in animal health, the environment, human nutrition, and animal science. In addition, Dr. Lautner was a practicing veterinarian for more than 12 years including her own swine practice in LeMars, IA. Dr. Lautner received a BS degree and a Doctor of Veterinary Medicine degree from Michigan State University and a MS degree from the University of Minnesota. In 1994, she received the Howard W. Dunne Memorial Award for outstanding service to AASV and the pork industry. Dr. Lautner also received the APHIS Administrator's Award in 1997 in recognition of her contributions to the advancement of animal health. In 2002, she received the Meritorious Service Award from the National Institute for Animal Agriculture. In 2005, she received a DHS Under Secretary Award in recognition of her accomplishments in Program Management at PIADC.

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Bacteriology 1

Saturday, October 19, 2013
Pacific Salon 3

Sponsor: Thermo Scientific

Moderators: Doreene Hyatt, Karen W. Post

Symbols at the end of titles indicate the following designations:

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

Poultry Check MP® MS-MG, a Multiplexed Fluorometric Immunoassay (MFIA) for the Detection of Antibodies to *Mycoplasma synoviae* and *Mycoplasma gallisepticum*

Martine Bertrand, Isabelle Caya, Andre Ch. Broes

Biovet Inc., Saint-Hyacinthe, QC, Canada

Narrative: *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG) are two major pathogens for poultry flocks. The surveillance of poultry flocks for MS and MG relies on regular testing of serum samples for the presence of specific antibodies. Various serological assays may be used such as the serum agglutination test, the haemagglutination inhibition (HI) test, and the enzyme-linked immunosorbent assay (ELISA). Recently a new kind of immunoassay called multiplexed fluorometric immunoassay (MFIA) based on Luminex xMAP technology has been developed. MFIA allows a single small volume of serum to be used to screen for antibodies to many antigens at one time in a single well. The MFIA uses suspensions of microspheres (beads) with unique internal fluorescent dyes. These beads are coupled to their surface with unique antigens. Bead sets and sera are added to 96-well microtitre plates. Antigen-antibody complexes formed during incubation are then detected through successive incubations with biotinylated species-specific anti-immunoglobulins (Ig) followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubations are followed by wash steps to remove unbound serum constituents and reagents. In addition two internal controls consisting in a species-specific Ig bead set and an anti-species Ig bead are incorporated into the assay to evaluate sample suitability and assay function respectively. MFIA plates are read and analyzed using a microtiter plate suspension microarray fluorescence analyser. Beads are exposed to a red laser which excites the internal dyes that identify the bead's color set corresponding to a particular antigen and a green laser which excites the phycoerythrin reporter dye captured during the assay. The intensity of R-phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI) which denotes how much reporter fluorescence of a given microsphere set carries. An S/P ratio of the SA-PE on the antigen-coated microspheres above provided threshold indicates that antibodies to the corresponding antigen are present in the sample. We have developed an MFIA using magnetic beads (Magplex®, Luminex) to detect MS and MG antibodies in chicken serum samples. The assay was compared to reference assays (commercial ELISA, and HI). It demonstrated excellent sensitivity, specificity, repeatability and reproducibility (ruggedness).

Comparative Evaluation of the TF-Transit Tubes (Biomed) vs. InPouch-TF (Biomed) for the Detection of *Tritrichomonas foetus* DNA from Bovine Preputial Samples by Real Time PCR

Feng (Julie) Sun[1], Amy Swinford[1], Pamela J. Ferro[1], Debabrata Mahapatra[2], Alesia Reinisch[2], Anthony Smith[3], Hemant Naikare[2]

[1]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX; [2]Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX; [3]Biomed Diagnostics, Inc, White City, OR

Narrative: Trichomoniasis is a sexually transmitted reproductive disease of cattle caused by the protozoan parasite *Tritrichomonas foetus*. Trichomoniasis is a reportable disease in Texas and many other states within the US. The disease can be found worldwide and causes significant economic losses to cattle breeders. Currently, there is no effective treatment thus infected bulls are culled from the herd and generally sold for slaughter. Recently, Biomed Diagnostics introduced the TF-Transit Tube system for sample collection and transport to the laboratory, specifically for the *T. foetus* PCR test only. TF-Transit Tube system is a field-oriented sample collection device that retains the InPouch-TF liquid medium, but consists of a test tube rather than a pouch which allows for easier handling in the field as well as the laboratory. The objective of the present study was to compare the performance of the *T. foetus* PCR assay on samples collected using the two collection and transport systems: TF-Transit Tube and InPouch-TF. Twenty-five positive (culture and PCR) field samples were used to spike both TF-Transit Tubes and InPouch-TF. These were incubated at 37°C, and aliquots were collected at 0, 24, and 48 hours. The nucleic acid from the samples was extracted using magnetic beads [KingFisher-96 and MagMax™ AM1836 nucleic acid extraction kit (Life Technologies)], QIAamp DNA Mini Kit (QIAGEN), and the boiling method. Following nucleic acid extraction, the samples were tested using real-time PCR with previously published primers and probe [McMillen L, Lew AE: 2006, Improved detection of *Tritrichomonas foetus* in bovine diagnostic specimens using a novel probe-based real time PCR assay. Vet Parasitol 141(3-4):204-215.]. Real-time PCR results indicated a 100% agreement between the TF-Transit Tube and InPouch-TF sample collection systems. All spiked samples using both collection systems were real-time PCR positive with all extraction methods. In addition to the evaluation of the spiked samples, 65 field samples were tested using both collection systems. Five samples were found to be positive for *T. foetus* at all-time points of incubation for both collection systems. No difference was observed between the two collection systems for samples tested at time zero (no incubation). However, the number of cycles required for the fluorescent signal to cross the threshold (Ct) was approximately 1.5-2 cycles lower in samples collected using the InPouch-TF versus the TF-transit tube following 24 or 48 hours incubation. These results indicate that whereas the InPouch-TF collection method allows for the growth of *T. foetus*, no significant growth of the organism occurs in the tube. Further testing of field- collected samples is needed in order to determine the effect of using the TF-Transit Tubes vs. InPouch-TF on diagnostic sensitivity of the real-time PCR assay.

Detection of Bovine Tuberculosis Antibody Response in Sensitized Cattle using the IDEXX *M. bovis* Antibody Test ◊

Jeffrey T. Nelson

USDA, APHIS, VS, NVSL, Ames, IA

Narrative: The Veterinary Services bovine tuberculosis (TB) program staff is in the process of evaluating new serologic tests that detect antibodies specific to *Mycobacterium bovis* in cattle. Several studies in cattle have shown that levels of antibodies developed during bovine TB infection increase after stimulation by the tuberculin injection administered as part of routine TB skin testing. Collecting information on the formation and decline of this antibody response over time will be helpful to identify a "best time to collect" serum samples in order to detect cattle that may be truly infected with *M. bovis* but have a negative skin test or negative gamma interferon response. Heparinized blood and serum samples were collected from cattle sensitized to bovine TB and avian TB prior to any tuberculin stimulation and then at 9, 16, and 23 days after injection. Samples were also collected at 4 week intervals after tuberculin injection for 6 months to determine the length of the stimulation effect. It was noted that antibody levels increased as measured on the IDEXX *M. bovis* Ab test on both bovine TB and avian TB sensitized cattle and peaked between 9 and 16 days after tuberculin injection. This time period may be optimal to collect blood samples after tuberculin injection to detect cattle that may truly be infected with *M. bovis*.

◊ USAHA Paper

Impact of Blood Sample Storage Time and Temperature on Detection of Bovine Tuberculosis Antibodies using the IDEXX *M. bovis* Antibody Test ◊

Jeffrey T. Nelson

National Veterinary Services Laboratory, USDA, Ames, IA

Narrative: Rapid sample submission of blood or serum samples after collection is thought to be critical to obtain results that are minimally affected by degradation during storage and shipping to the laboratory for testing. It is also theorized that once blood is collected from an animal, antibodies will degrade if the serum remains on the clot for an extended time period. Blood was collected in red top blood tubes from cattle sensitized to bovine or avian tuberculosis. Serum was harvested from tubes the day it was collected and subsequently at 1, 2, 3, 4, 7, and 14 days after collection and storage at 4°C, 23°C, and 37°C on the clot. The serum samples harvested from the various tubes were analyzed using the IDEXX *M. bovis* Ab Test. The results of the project demonstrated that the antibody levels that the IDEXX *M. bovis* Ab test detected remained consistent even when the serum was harvested after an extended storage time or when the sample was subjected to different storage temperatures. These data suggest that sample handling or shipping issues should not affect the results of blood samples being analyzed by the IDEXX *M. bovis* Ab test if they are tested within 2 weeks of being collected.

◊ USAHA Paper

A Four Plex Real-Time PCR Assay for the Detection and Quantification of *Escherichia coli* O157 in Cattle Feces * †

Lance W. Noll, Pragathi Belagola Shridhar, Xiaorong Shi, Baoyan An, Tiruvoor Nagaraja, Jianfa Bai

Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS

Narrative: Cattle are asymptomatic reservoirs for Shiga toxin-producing *Escherichia coli* O157:H7, a major food borne pathogen. Typically, the organism colonizes the hindgut and is shed in the feces, which serves as a source of contamination of water and food products. Culture-based methods of detection and quantification of *E. coli* O157 in cattle feces have low-throughput, and are labor-intensive and time-consuming. Although several real-time PCR procedures have been developed, none has targeted four major genes of *E. coli* O157 to quantify *E. coli* O157. Our objective was to develop a multiplex, real-time quantitative PCR (mqPCR) assay for the detection and quantification of *E. coli* O157 in cattle feces based on genes that code for the serogroup specific O157 antigen (*rfbE* O157) and three major virulence factors, Shiga toxins 1 and 2 (*stx*1 and *stx*2) and intimin (*eae*). Primers and probes were selected based on optimum number of matched sequences in the GenBank database. Concentrations of each primer and probe were optimized with extracted DNA from a strain of O157 (ATCC 43894) containing all four genes. The sensitivity of the assay was determined with extracted DNA from serial ten-fold dilutions of *E. coli* O157 ATCC 43894 cultured. Broth culture was spread-plated onto blood agar plates to determine viable cell counts (CFU/mL). The pure culture sensitivity assay was also performed on *E. coli* O157 strains with variable target genes. Cycle threshold values of target genes were compared among O157 strains with different combinations of the target genes to analyze detection sensitivity and reproducibility of the assay. To further assess the specificity of the mqPCR assay on isolates with variable target genes, *E. coli* non-O157 (O26, O45, O103, O111, O121, and O145) strains were subjected to the pure culture sensitivity assay. Serial dilutions of pure cultures of *E. coli* O157 strains (ATCC43889 and ATCC 43894) spiked in cattle feces were prepared to determine applicability of the assay to quantify the organism. Sensitivity of the mqPCR assay from spiked fecal samples before and after six-hour enrichment was then determined. Extracted DNA from cattle fecal samples before and after six-hour enrichment was used for detecting target genes with the mqPCR assay and 4-plex conventional PCR assay. Culture data was compared to PCR results to determine sensitivity between PCR assays. The assay targeting the four genes has the potential to be a high-throughput method for detecting and quantifying *E. coli* O157 in cattle feces.

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

Validation of a Broth Microdilution Method and Investigation of *in vitro* Antimicrobial Susceptibilities of Porcine *Brachyspira* Species # * †

Nandita Mirajkar[1], Connie Gebhart[1, 2]

[1]Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN;
[2]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN

Narrative: *Brachyspira hyodysenteriae* is the etiological agent of swine dysentery, an economically significant disease occurring in grower-finisher pigs worldwide. Having disappeared in the 1990s, swine dysentery and other *Brachyspira*-associated diarrheal diseases have re-emerged in the US in the last few years. Low susceptibility to commonly used antimicrobials may play a role in this re-emergence; however, no routine diagnostic techniques for *in vitro* antimicrobial susceptibility testing of *Brachyspira* species currently exist in the US. Therefore, our aim was to validate a broth microdilution method for routine antimicrobial susceptibility testing (by comparison of a standard agar dilution method with two broth microdilution methods) and to investigate the antimicrobial susceptibilities of pathogenic and commensal *Brachyspira* species. We determined the minimum inhibitory concentration (MIC) for 40 isolates (10 *B. hyodysenteriae*, 10 "*B. hampsonii*", 10 *B. pilosicoli* and 10 *B. murdochii*), originating from a variety of sites and sources across the US in 2011 to 2012. The antimicrobial susceptibilities were evaluated for a range of two-fold dilutions of tiamulin, valnemulin, lincomycin, tylosin and tylvalosin by both agar and broth dilution methods, and for carbadox by agar dilution method only. The MICs for all antimicrobials obtained by the broth dilution method were generally comparable with those obtained by the agar dilution method. Approximately 80% of MIC values obtained by the broth dilution method agreed with or were one or two dilutions lower than those obtained by the agar dilution method. By broth dilution, most isolates of all species exhibited low susceptibility to lincomycin (MIC90 ≥32μg/ml), tylosin (MIC90 ≥128μg/ml) and tylvalosin (MIC90 ≥8μg/ml), and high susceptibility to carbadox (MIC90 ≤0.0156μg/ml). Most isolates of all species showed high susceptibility to tiamulin (MIC90 ≤0.5μg/ml) and valnemulin (MIC90 ≤0.5μg/ml), though several *B. pilosicoli* isolates with unexpected poor susceptibilities to tiamulin (MIC90 >8μg/ml) and valnemulin (MIC90 1μg/ml) were identified. This study determined the antimicrobial susceptibility patterns of *Brachyspira* species currently circulating in swine herds across the US and highlights the importance of surveillance to detect the potential emergence of isolates with decreased susceptibility towards commonly used antimicrobials. This is the first study to validate the use of a broth dilution technique for routine antimicrobial susceptibility testing of *Brachyspira* species in the US.

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

Evaluation of MALDI TOF Mass Spectrometry to Identify *Brachyspira* from Swine Samples

Hallie Warneke, Timothy Frana, Joann M. Kinyon, Eric Burrough, Leslie Bower

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

Narrative: *Brachyspira* are agents of swine dysentery (SD) and other swine diarrheal diseases. The main species causing disease are *Brachyspira hyodysenteriae*, *Brachyspira hampsonii*, *Brachyspira pilosicoli*, and *Brachyspira murdochii*. Other species of *Brachyspira* may be found in samples and can complicate a diagnosis of SD. Common methods used to identify the species of *Brachyspira* include phenotypic and biochemical analysis, targeted PCR, and nox gene sequencing. Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI TOF MS) bacterial identification systems are increasingly being used in veterinary diagnostic laboratories. These systems have the ability to identify bacterial isolates quickly, accurately and inexpensively. In this study we compared MALDI TOF MS identification of standard strains and field isolates of various *Brachyspira* to *nox* gene sequencing. To increase the range of mass spectral profiles available for matching, strains of *B. hyodysenteriae*, *B. hampsonii*, *B. pilosicoli*, *B. murdochii*, *B. intermedia*, and *B. innocens* were added to a MALDI TOF MS user database. All strains were well-characterized, and had previously been confirmed using *nox* gene sequencing. After additions to the user database, cultured field isolates of *Brachyspira* were identified by MALDI TOF MS and *nox* sequencing. Comparative results of more than 18 *Brachyspira* field isolates recovered from feces, rectal swabs, and colon contents indicates that MALDI TOF MS identification matched *nox* gene sequencing in every case. The field isolates identified included *B. hyodysenteriae*, *B. hampsonii*, *B. murdochii*, and *B. pilosicoli*. Evaluation of further field isolates is ongoing. The validation of MALDI TOF MS for important animal pathogens increases its role as a first-line bacterial identification system with the accuracy of molecular methods with less cost and faster results.

Detection of *Brachyspira* in Swine Oral Fluids

Hallie Warneke, Timothy Frana, Joann M. Kinyon, Eric Burrough, Bailey L. Wilberts

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

Narrative: Currently ante mortem detection of brachyspiral pathogens in swine is heavily reliant on testing feces or rectal swabs. Due particularly to the ease of collection, oral fluids are becoming increasingly popular for diagnostic testing. In this study, detection of *Brachyspira* from feces and oral fluids was compared. Two trials were conducted to assess oral fluids as a sample matrix for *Brachyspira* culture. In trial 1, oral fluids, solid feces, and watery feces were spiked with different levels of standard strains of *Brachyspira hyodysenteriae* and *B. hampsonii*. Culture was performed immediately and also at refrigerated holding times of 24, 48, and 72 hrs. Additionally, three media (CVS, BJ, BAM-SR) were inoculated to assess differences in recovery on selective media. In the second trial, oral fluids and individual rectal swabs were collected daily from groups of 10 pigs following challenge with *B. hyodysenteriae* or *B. hampsonii*. The results from the first trail indicated that *B. hyodysenteriae* and *B. hampsonii* were recovered in higher numbers from oral fluid versus either watery or solid feces at all time points. The difference in level of recovery was more pronounced as the holding time increased. Statistically significant ($P<0.05$) differences in *Brachyspira* recovery were seen between oral fluids and either solid or watery feces at 24, 48, and 72 hrs. However, the level of recovered *Brachyspira* from watery feces was closer to levels found in oral fluids. There was no difference in recovery from oral fluids based on media type. In the second trial *B. hyodysenteriae* was detected in oral fluids if at least one pig in the group was positive by rectal swab culture. *B. hampsonii* was consistently detected in oral fluids when more than one pig in the groups was positive by rectal swab culture. These studies indicate that oral fluids may be a suitable alternative to feces or rectal swabs for detection of *Brachyspira* in swine herds thought to be affected by brachyspiral colitis.

Epidemiology 1

Saturday, October 19, 2013
Pacific Salon 6,7

Moderators: Ashley Hill, François Elvinger

Symbols at the end of titles indicate the following designations:

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

Zinc Toxicity in Dairy Cattle

Alan F. Julian

Gribbles Veterinary, Hamilton, New Zealand

Narrative: Zinc toxicity is diagnosed sporadically in dairy cattle in New Zealand as a result of acute or chronic over supplementation of zinc salts for the prevention of facial eczema in the late summer and autumn. In August (winter) 2012, two farms had chronic zinc toxicity of cows outside of the facial eczema risk period due to zinc added to a commercially prepared meal for the purposes of trace element supplementation. On both farms the cows presented with lethargy, anorexia, hematuria, low milk volume and diarrhea. Hematology on affected cows revealed a hemolytic anemia with Heinz body formation. Post mortem examination of severely affected cows on both farms revealed pale carcasses with watery blood. The reserves of fat were severely depleted. The pancreas of one cow appeared grossly normal while the other was firm and nodular. Histology of the pancreas of both cows showed marked interstitial fibrosis. The exocrine acini had scattered degenerate and necrotic cells. This was consistent with chronic zinc toxicity. Analysis of serum zinc in affected cows on both farms revealed elevated levels, with a range of 175-385 umol/l (reference 11-20). The wheat/barley/canola/mineral pellet mix had 34,973 ppm of zinc. It was estimated that the overall daily zinc content of the diet was around 3,900 ppm.

Copper Overload in Wisconsin Holsteins

Doug Lyman

Wisconsin Veterinary Diagnostic Laboratory, Madison, WI

Narrative: Copper is a trace element required by all animals. Its unique redox activity renders it simultaneously essential and potentially toxic. It can become toxic by virtue of its ability to participate in reactions that generate harmful reactive oxygen species, including the highly toxic hydroxyl radical. The purposes of this study are to document its ongoing accumulation in Wisconsin Holstein cattle and to demonstrate its toxicity using light microscopy and immunohistochemical staining for 4-hydroxynonenal (4-HNE), a well characterized marker of oxidative damage that is generated by free radical damage to cell membrane lipids. A review of 225 Wisconsin Veterinary Diagnostic Laboratory accessions that included ICP-MS analysis of liver copper from Holsteins of all ages revealed a mean concentration of 145 ppm wet weight (normal, 25-100 ppm wet weight). It is thought that, in several species including cattle, the threshold for subclinical hepatotoxicity is around 150 ppm wet weight. A subsequent analysis of 30 livers randomly obtained from cull dairy cows at a slaughter facility revealed a mean liver copper concentration of 163 ppm wet weight. Many of these livers (from both groups) had rhodanine stainable copper along with histologic evidence of hepatopathy. Immunostaining for 4-HNE revealed oxidative damage to hepatocytes, particularly in centrilobular zones, corresponding to the zone in which rhodanine staining was most prominent. The results of this study indicate that Wisconsin Holsteins are accumulating large amounts of hepatic copper with concomitant, oxidative liver damage. The effects of this accumulation, involving potentially both health and productivity, are unknown.

Evaluation of a Commercially-Available ELISA for Detection of ≥500 ppt Aflatoxin M1 in Milk

Ashley Hill, Linda Aston, Elizabeth R. Tor, Robert H. Poppenga, Hailu Kinde

California Animal Health and Food Safety Laboratory System, University of California, Davis, CA

Narrative: Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* can cause liver damage and interfere with nutrient absorption and immune system function in humans. Dairy cows consuming high-aflatoxin feeds can excrete aflatoxin M1 (AFM1) in milk. The FDA maximum milk AFM1 concentration is 500 ppt. The objectives of this study were to compare a commercially-available ELISA with HPLC for detecting AFM1, and to identify an ELISA cutoff that had a sensitivity of >90% at detection of AFM1 levels of ≥500 ppt with confidence of 95%. A single bulk-tank milk sample from a mixed-breed dairy was spiked with AFM1 from 0-1000 ppt, 150 aliquots were blindly tested using ELISA and HPLC methods, and results were compared using multiple methods. Another bulk-tank milk sample was spiked with 500 ppt AFM1, and 25 aliquots were blindly tested using 3 different ELISA kits. Overall and kit-specific sensitivities were calculated for a range of cutoff values. For a given ELISA cutoff value, a 95% CI whose lower bound was greater than 0.90 was considered to demonstrate more than 95% confidence of test sensitivity >0.90. In the 400-600 ppt range, correlation between ELISA and HPLC was poor, and ELISA demonstrated poor sensitivity at an ELISA cutoff of 500 ppt. ELISA-based estimates of AFM1 concentration (ppt) were strongly affected by kit. Overall and kit-specific sensitivity estimates increased as the ELISA cutoff for classifying a sample as positive decreased. An ELISA cutoff was identified for which the sensitivity was >90% with more than 95% confidence for detection of milk with ≥500 ppt AFM1.

Resources for Collecting and Accessioning Single Bulk Milk Samples from Every Commercial Dairy Farm in New York State ◊

Belinda S. Thompson[1], Paul Virkler[1], Elizabeth A. Lussier[2], David Smith[3]

[1]Population Medicine and Diagnostic Sciences, Cornell University - Animal Health Diagnostic Center, Ithaca, NY; [2]AHPIS - VS, United States Department of Agriculture, Albany, NY; [3]Division of Animal Industry, NYS Department of Agriculture and Markets, Albany, NY

Narrative: The control of contagious diseases of high consequence such as foot and mouth disease requires accurate classification of herds as either infected or uninfected. In the dairy industry in the US, it has been proposed that bulk tank milk sample screening by regulatory agencies for either pathogens or antibodies may be a useful tool for accurately classifying herd infection status. Additionally, this classification of herds of animals has been included in planning for the maintenance of movement of perishable dairy products for human consumption in the cooperative industry initiative called the Secure Milk Supply. It will be important for state and federal animal health officials and industry planners to understand the effort required to collect and handle appropriate specimens to achieve the goal of classifying herds accurately. We solicited, collected, transported and accurately accessioned individual bulk milk samples from approximately 5100 commercial dairy herds in New York State for a disease prevalence study during the period from January 15, 2013 to May 30, 2013. Samples were obtained by a trained technician visiting the laboratories of the milk processors where individual bulk tank samples are subjected to milk quality testing. Cooperating processors were notified in advance and agreed to participate with sample collection. While the time frame for collection was over several months, the intent was to actually measure the number of visits and the time required to collect a single sample from every bulk milk tank. Since repeat sampling was not the intent of the project, repeat visits to processing plants represent the effort to follow up and collect samples not available during prior visits. A total of approximately 215 hours and 32 trips were required to communicate with processing plants, visit them, collect samples, and deliver them to the diagnostic laboratory. Samples representing 4,896 herds (95%) out of a reported 5,152 total New York commercial dairy herds were collected. Problems associated with sample collection and matching with herds included herds with multiple bulk milk tanks not being consistently identified with bar coding conventions between the various processors, not all samples being bar coded, no single central list of producers and the codes used to identify their bulk tank samples, hard copy rather than electronic lists of producers to determine which herd or tank samples were missing. In addition, samples crossing state lines complicated sample collection and contributed to the failure to sample every herd.

◊ USAHA Paper

Salmonella Dublin Herd Bulk Tank Seroprevalence of New York Dairy Farms ◊

Belinda S. Thompson[1], Paul Virkler[1], Elizabeth A. Lussier[2], Diane Kilts[1], David Smith[3], Bettina Wagner[1]

[1]Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center, Cornell University, Ithaca, NY; [2]APHIS-VS, United States Department of Agriculture, Albany, NY; [3]Division of Animal Industry, New York State Department of Agriculture and, Albany, NY

Narrative: *Salmonella* Dublin (SD) is a serotype of *Salmonella* that is host-adapted to develop persistent carrier infections in subclinical bovines. It can cause serious disease outbreaks with high morbidity and mortality in youngstock, and abortions or morbidity and mortality in older cattle. There are also food safety concerns related to SD, a zoonotic pathogen that can cause serious human illness or death. A high morbidity and mortality outbreak has been associated with people consuming unpasteurized milk, and it is identified as one of the top three *Salmonella* serotypes found in beef products, notably ground beef. It also has the potential to infect and cause serious illness in cattle care workers exposed to infectious excretions of SD-infected cattle. Prevalence data at the individual cow level or at the herd level is not available for dairy or beef cattle in the US. A commercial SD ELISA test was validated for testing serum, individual milk samples or bulk tank milk for the presence of anti-SD antibodies in bovine samples. Identity blinded bulk tank samples from a single milk pick-up were solicited from milk processors for all NY State bovine dairy herds licensed to sell milk commercially. Samples were collected between January 15 and May 30, 2013. The samples were tested using the SD ELISA assay. Preliminary analysis of the data for SD seropositivity indicates a single-sample seropositive herd prevalence of less than 1% with close to 100% of herds represented.

◊ USAHA Paper

Beta-hydroxybutyrate Diagnosis of Ketosis in Periparturient Dairy Cattle: Comparison of Blood and Multiple Milk Test Methods for Concordance and Prevalence Estimates in the Same Population of Cows

David J. Wilson[1], Gregory M. Goodell[2]

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Narrative: Measurement of the ketone beta-hydroxybutyrate (BHB) in blood or milk of recently calved dairy cattle is a common test for ketosis. BHB measurement was compared among blood test strips (tested in Precision XTRA® meter), milk test strips (Keto-Test®), DHIA milk meter collected samples and hand stripped milk samples, both latter milks tested with Fossomatic milk analyzer, including classification of cows as ketotic or non-ketotic. Preliminary study found few cows ketotic after 14 days in milk (DIM), therefore 446 Holstein cows from 1 – 14 DIM in 5 herds were blood and milk sampled for BHB testing on the same day (108 cows from 2 herds had no stripped samples). Cows were tail bled from the coccygeal vein in lock-up stalls and then sampled during the next milking; some milk samples had insufficient volume for all milk testing to be done. Tests produced continuous results except one (milk strip) had categorical results (0, 50, 100, 200, 500, 1000 μmol BHB /L); analysis compared whether or not each pair of BHB tests categorized the same cows as ketotic or non-ketotic (concordant, C) or disagreed (discordant, D). Agreement between test methods was tested by kappa value. Test results: blood strips 53 ketotic (K) (> 1200 μmol BHB/L), 393 non-ketotic (N); milk strips 34 K (> 200 μmol BHB/L category on strip), 408 N; milk meter Foss 29 K (> 200 μmol/L), 399 N; stripped milk Foss 41 K (> 200 μmol/L), 297 N. Method comparisons: blood/milk strips 15 K/K, 371 N/N (87% C), 37 K/N, 19 N/K (13% D); blood/milk meter Foss 10 K/K, 357 N/N (86% C), 42 K/N, 19 N/K (14% D); blood/stripped milk Foss 10 K/K, 266 N/N (82% C), 31 K/N, 31 N/K (18% D); milk strips/milk meter Foss 7 K/K, 372 N/N (89% C), 27 K/N, 22 N/K (11% D); milk strips/stripped milk Foss 16 K/K, 287 N/N (90% C), 10 K/N, 25 N/K (10% D); milk meter Foss/stripped milk Foss 5 K/K, 272 N/N (84% C), 20 K/N, 33 N/K (16% D). Kappa evaluation of test pair agreements was mostly “fair” (K = 0.28) or “poor” (K < 0.18). BHB test methods agreed well for most non-ketotic cows, but did not agree well on which cows were ketotic. Milk tests for BHB did not classify as many periparturient cows as ketotic as did blood tests. Calibration improvements are indicated for Fossomatic testing of BHB in milk.

Comparison of the Ability of a Novel Umbilical Dip, Super7+™ Navel Dip, Verses that of 7% Tincture of Iodine to Desiccate the Umbilical Remnant in Neonatal Holstein Dairy Calves ◊

Julie A. Gard[1], Soren P. Rodning[2], Debra Taylor[1], Sue Duran[1], Brad Fields[3], Robin Farrell[4], Misty Edmondson[1], Megan Schnuelle[1], Teri Hathcock[1], Elizabeth Reed[1], Rebecca Woodall[1], Alfred Bearden[1]

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Narrative: Reduction of naval infections through appropriate management including naval dipping is beneficial to the calf and the producer and is reported to decrease naval infection rates from 20-28% to 5-14%. Obviously, naval dipping is of great importance but obtaining tincture of iodine has become problematic. It has become necessary to develop other products that can be utilized to dry the umbilicus and assist in the prevention of navel infections. Therefore, the aim of this study was to evaluate an alternative to 7% tincture of iodine, Super7+™ Navel Dip. A total of a 100 neonatal Holstein heifers were utilized in this study. Fifty calves were dipped with Super7+™ Navel Dip immediately following calving and 50 were dipped with 7% tincture of iodine. The umbilicus and the umbilical remnant of all calves were evaluated 48 hours following dipping and at least a 1 cm segment of umbilical remnant was removed and placed in a labeled airtight container. All samples were analyzed within 6 hours of sampling and in 12 hour increments until the samples contained less than 10% moisture. A serum sample was collected from each calf within 48 hours of birth and tested for total protein and specific gravity. Upon evaluation, the umbilical remnants of all calves were completely desiccated by 60 hours following calving. Of the remnants dipped with 7% tincture of iodine 58% and 42% were desiccated by 48 hours and 60 hours, respectively. Of the remnants dipped with Super7+™ Navel Dip 88% and 12% were desiccated within 48 hours and 60 hours, respectively. Dipping with Super7+™ Navel Dip increased the percentage of calves having a desiccated umbilicus by 30% over that of tincture of iodine. Additionally, the umbilical remnants of all calves utilized in this study had no evidence of infection in the calves having a low total protein (less than 5.0 g/dL). The range of total protein was 4.5 to 7.2 g/dL and the range of specific gravity was 1.032 to 1.048. Additionally, there was no evidence of dermal irritation around the skin surrounding the umbilicus or any other area that may have contacted Super7+™ Navel Dip. Super7+™ Navel Dip appears to be superior to tincture of iodine in its ability to more quickly desiccate the umbilical remnant. Hence, Super7+™ Navel Dip appears to function competently as a navel dip and is a viable alternative to 7% tincture of iodine.

◊ USAHA Paper

Designing a Mobile Application for Field Use on Dairies Experiencing a Toxicologic Event # † ◊

Steven Gallego[1], Hailu Kinde[2]

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[2]California Animal Health and Food Safety Laboratory System, University of California, San Bernardino, CA

Narrative: Large scale toxic events on dairies can be difficult emotionally and financially. Many of the pathologies that affect animals manifest themselves over predicable time frames; conversely, toxic events can appear quite suddenly and unexpectedly. Severity of toxic signs can range from nonclinical to clinical. Mortality rates can vary, but in today's concentrated animal feeding units, high mortalities can be devastating. In addition to the potential disruption of milk shipped and cows lost, a toxic event also presents a possible food safety and public health issue that potentially erodes consumer confidence. Quick and accurate assessment of a toxic event is crucial for the health of both the animals and people consuming products derived from affected animals. The objective of this paper is to describe a smartphone or tablet downloadable app designed to assist field personnel encountering a toxic event. Currently, there are no apps available to veterinarians for food animal diagnostics. Using data from the California Animal Health and Food Safety (CAHFS) Laboratory System a mobile app was designed to assist veterinarians to diagnose the most common causes of large scale toxic events on California dairies. This app provides practitioners an opportunity to harness the power of the web to address a toxic event. Using the app design program Mobione from Genuitec, an app was constructed with "list" menus linked to abridged descriptions of the most common toxins and toxicants encountered by California's dairy cattle, guidance for sample collection and tests selection, shipping information, critical food safety information, carcass disposal information, relevant literature about the suspected toxic agent and a quiz section provided for self-assessment. Additionally, this app provides direct phone contact with expert advice from CAHFS, university and California Department of Food and Agriculture personnel for real time field support. Such an app can help resolve a toxic event rapidly and confidently, minimize suffering or losses of animals, mitigate risk of public health/food safety issues, and bolster consumer confidence in California's dairy industry.

AAVLD Trainee Travel Awardee (Toxicology)
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

BIOCARE MEDICAL

Pathology 1

Saturday, October 19, 2013

Pacific Salon 1

Sponsor: Biocare Medical

Moderators: Gary Mason, Maria Spinato

Symbols at the end of titles indicate the following designations:

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

Dilated Cardiomyopathy in Rio Grande Subspecies of Wild Turkey (*Meleagris gallopavo*)

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[1]Central Utah Veterinary Diagnostic Lab, Utah State University, Nephi, UT; [2]Utah Division of Wildlife Resources, Salt Lake City, UT

Narrative: A mature male Rio Grande wild turkey was submitted for necropsy to the Central Utah Veterinary Diagnostic Laboratory (CUVDL) in March, 2013 by personnel of the Utah Division of Wildlife Resources (DWR). The bird had been living in semi-domestication and there was concern that it may have died from infectious causes that may have threatened the nearby free-living flock. The turkey was emaciated. The most striking gross abnormality was a greatly enlarged heart with flaccid thin-walled ventricles. In addition, yellow, clear gelatinous fluid filled the abdominal cavity, the spleen was enlarged, and the lung was fluid-filled and multifocally dark in color and resilient. Dilated cardiomyopathy (DCM), formally known as spontaneous cardiomyopthy of turkeys, occurs with some frequency in commercial domestic turkey flocks. It has been reported to be more prevalent in flocks raised at moderate to high elevations. Dietary manipulations of the sodium and chloride concentrations in young turkeys may decrease the incidence of DCM. Rapid growth in commercial turkeys contributes to the problem and there are likely other factors involved. To the authors' knowledge, this is the first reported case describing dilated cardiomyopathy in the wild turkey and the prevalence is not known, though it is likely lower than in domestic turkeys. However, it is also likely that wild turkeys are necropsied less frequently than their domestic counterparts. The cause of the disease in this wild turkey is not known. The diet of this semi-domesticated bird was probably different than that of wild flocks and this may have been a contributing factor in the pathogenesis in this case.

Outbreak of Foot and Mouth Disease in Black Bears (*Ursus thibetanus*) and a Sun Bear (*Helarctos malayanus*) at a Bear Rescue Facility in Vietnam #

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Narrative: Foot and mouth disease (FMD) is an acute febrile viral disease typically affecting cloven hoofed animals. FMD has a wide host range, high excretion levels, rapid replication rate, and multiple transmission modes, making it one of the most highly contagious and financially important diseases. FMD is endemic in Vietnam and outbreaks regularly occur in livestock. This paper describes an FMD outbreak in 2011 in a group of bears housed at a rescue facility in northern Vietnam. To our knowledge, this is the first confirmed FMD case in a bear species, and the first report of clinical signs of FMD in a sun bear. Sixteen Asiatic black bears (ABB) and one sun bear were housed in the affected bear house. All had been transferred to the facility from private owners, or illegal bear bile extraction facilities. On August 28, 2011, an adult male ABB was reluctant to walk or eat, and developed large blisters on the foot pads. Over the following 15 days, 14/17 bears developed similar signs; the remaining 3 co-housed bears and an additional 57 bears resident at the facility, in separate enclosures, did not. On day 6, the house was placed under strict quarantine. All affected bears developed vesicles on all foot pads, more severe in the younger bears. Crusty scabbing of the nares was noticed in 6, and very subtle oral mucosal lesions were noted in 3 cases. Bears were lethargic for 24-48 h, apart from the two youngest cubs. Within 1 month the pads of all bears looked normal. RT-PCR was performed on samples from 3 bears using FMDV specific primer sets. All bears were positive and all positive samples were serotype O. Vesicle fluid from one bear was tested and was positive for FMDV antibodies by ELISA. Sequence alignment of 3 virus bear isolates with 3 Vietnamese porcine isolates revealed several nucleotide differences. Phylogenetic analysis demonstrated clustering of one of 3 bear FMDV isolates with a Vietnamese porcine FMDV isolate, in a branch in close proximity but distinct from the two other bear isolates. This outbreak likely occurred due to the high density of the bears in captivity, and possible indirect contact they had with livestock. The findings are significant for captive bear facilities worldwide; they showed that bears are capable of contracting FMD virus and showing clinical disease, and that the virus spreads easily between bears in close contact and between bears of two different species.

AAVLD Trainee Travel Awardee (Pathology)

Comparison of the Gross and Histological Heart Lesions and Heart Weights Between Market Hogs that Died in Transit to the Abattoir and Control Hearts from the Processing Line

Tony van Dreumel, Kathy Zurbrigg

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Narrative: The cause of death for in-transit losses (ITL) of swine has traditionally been labelled as hyperthermia. The objective of this study funded by Ontario Pork was to determine if underlying pathologic factors predispose hogs to ITL. Post mortems on 47 ITL hogs were completed at the Animal Health Laboratory, University of Guelph. Twenty-eight hearts were collected from hogs off the processing line (controls). Total heart (HW), left ventricle and septum (LV+S) and right ventricle (RV) weights for hogs that died in transit were compared to the controls. Forty-five of 47 ITL hogs (96%) had gross heart lesions, while all 28 non-ITL cases appeared grossly normal ($p<0.05$). Left ventricular hypertrophy (LVH) was observed in 41 of 47 (87%) ITL cases. In addition, some of the ITL cases had endocardiosis, dilation of the right ventricle, pulmonary artery and aorta and subaortic fibrosis. Incidental gross findings included bronchopneumonia, pleuritis, tracheitis, septicaemia and traumatic lesions. All histological lesions were scored from 0-3 representing none, mild, moderate or severe, respectively. The 45 ITL cases with gross heart lesions had combinations of myocardial cell disarray, medial hyperplasia of intramural coronary arteries, AV valvular fibrosis and atrophy, fibrosis and fatty replacement of myocardial fibres. Using the Wilcoxon Rank Sum test scores for myocardial fibrosis and valvular fibrosis were greater for ITL cases than non-ITL cases ($p<0.05$). Eight of 28 (29%) non-ITL cases had no histological lesions. The remaining 20 non-ITL cases had similar but milder lesions than ILT cases. Weights, i.e. HW(448.6 gm ± 10.0 vs 372.8 gm ± 8.2), LV+S (278.5 ± 46.8 vs 248.3 ± 27.7), RV (97.8 ± 23.7 vs 78.4 ± 15.3) and heart weight to body weight ratio (0.38 ± 0.01 vs 0.32 ± 0.01) were greater for the ITL group ($p<0.05$). To conclude, cause of death for the majority of ITL hogs appears to be heart failure due to pre-existing heart lesions, not hyperthermia. The heart lesions observed in many of the hogs resemble those found in hypertrophic cardiomyopathy (HCM) in humans and HCM-like lesions observed in pigs [Liu S, Chiu Y, Shyu J, et al.: 1994, Hypertrophic cardiomyopathy in pigs: Quantitative pathologic features in 55 cases. Cardiovascular Pathology 3:261-268].

Immune System Development in Captive Alaskan Reindeer (*Rangifer tarandus*) ◊

Zoe Purtzer[1], Gregory L. Finstad[2], Carla Willetto[3], Sophia Papageorgiou[4], Antony C. Bakke[5]

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Narrative: Reindeer (*Rangifer tarandus*) have evolved in high latitude ecosystems and their immune systems may differ from species that have evolved in more temperate climates. In order to understand the impact of new and emerging diseases on an individual species, it is important to characterize their immune systems to establish baseline numbers for calves and adult reindeer. The purpose of this study was to illustrate how the immune system develops, functions and modulates over time. Our study consisted of calves (n=47) and adult groups ranging from 1-12 years old (n=84). Blood was collected by venipuncture; mononuclear cells isolated, incubated with fluorescent monoclonal antibodies and analyzed by flow cytometry. The monoclonal antibodies used to identify cell subpopulations based on plasma membrane proteins included: CD45 (all white blood cells), CD4 (T-helper), CD8 (T-cytotoxic), CD45RO (memory), CD62L (lymph node homing), IgM (B-cells) and MHCII (activated T-cells and monocytes). Our study revealed multiple changes in lymphocyte subpopulations during maturation of the immune system. The fraction of CD4+ T cells increases with age until the animals are >2 years old (calves = 28.5% vs. 2 yo = 34.1%, $p<0.001$). The fraction of CD8+ T cells dips slightly at one year of age, but then returns to the previous level (calves = 19.6% vs. yearling = 16.5%, $p=0.02$). In calves, 69.6% the CD4+ T cells are naïve, but this drops to 48.7% in 6-12 yo adults. Conversely, the effector and senescent T cell groups increase significantly with age (calf = 13.2% vs. 6-12 yo = 47.6%, $p<0.001$). B-cells produce antibody in response to infections and increase with age, possibly due to antigen exposure (calves = 12.1% vs. 6-12 year old = 18.1%, $p=0.01$). Monocytes are important early responders to infection acting as antigen presenting cells. They were relatively higher in calves (44.8%), and decrease with age to a level of 32.8% in 6-12 year old adults ($p=0.01$). To further characterize response to infection, we studied animals before and during episodes of clinical infection. In the majority of cases, CD8+ T-cells increased during infection leading to an inverted CD4/CD8 ratio. The fraction of B-cells also increases significantly. Understanding the maturation and function of lymphocyte subpopulations may assist future research to elucidate how reindeer will respond to new and emerging diseases.

◊ USAHA Paper

Pathology of *Coccidioides* spp. Infections in Alpacas and Llamas

Francisco Uzal, Jorge P. Garcia, Federico Giannitti, Santiago Diab

California Animal Health and Food Safety Laboratory, University of California, Davis, San Bernardino, CA

Narrative: Coccidioidomycosis is a fungal disease caused by either *Coccidioides immitis* or *Coccidioides posadasii*. South American camelids seem to be particularly susceptible to infection by this microorganism and anecdotal evidence suggests that a relatively large percentage of pneumonias in camelids are due to *Coccidioides* spp. To investigate this possibility and to further characterize the pathology of coccidioidomycosis in camelids, the records of the past 21 years (1992-2013) of the California Animal Health and Food Safety Laboratory were searched for cases of South American camelids submitted for necropsy that had a diagnosis of pneumonia and/or coccidioidomycosis. The organs affected in each case of coccidioidomycosis were recorded and the gross and histological findings were reviewed. The disease was considered to be disseminated when more than one organ was affected. A total of 80 cases of coccidiodomycosis were diagnosed during that period in camelids, of which 53 (66%) had disseminated coccidioidomycosis. The organs most frequently affected were lung (64%) and liver (54%). Other organs affected included lymph nodes (40%), spleen and kidney (25% each), heart (21%), skeletal muscle (10%), skin (8%) and serosas, including pleura, peritoneum and pericardial sac (4%). Three cases of abortion were recorded; these cases presented with lesions in placenta, dam uterus and multiple fetal organs. When all cases of pneumonia in camelids were evaluated, it was found that 329 carcasses (176 alpacas and 153 llamas) had a diagnosis of pneumonia, from which coccidiodomycosis was diagnosed in 51 animals (15%). Other causes of pneumonia included bacterial and other fungal infections, viruses and undetermined causes. The gross and histological lesions of coccidioidomycosis were similar in all animals and organs studied but varied in extension and severity. The lesions consisted of multifocal to coalescing pyogranulomas, mineralized or not, which in most cases were visible grossly, although occasionally histological lesions were detected in tissues in which no gross lesions had been observed. Multifocally, within these pyogranulomas, there were variable numbers of round, 60-100 um fungal spherules (sporangia) with a 4-5 um thick refractile and hyaline double wall. Sporangia contained flocculent basophilic to amphophilic material and, rarely, multiple 5-7 μm endospores. Frequently, the spherules were seen within the cytoplasm of giant cells. Occasionally, no spherules were observed in some pyogranulomas; in these case, because fungal elements were seen in other granulomas within the same organ, it was assumed that the lesions with no spherules were burnt out lesions from which the fungal elements were gone by the time of necropsy. Coccidioidomycosis seems to be very prevalent in camelids and although pneumonia was the most frequent presentation in our study, only 15% of the cases of pneumonia in camelids were due to infection by *Coccidioides* spp.

Public Health Implications of *Echinococcus* in Wolves and Elk in Idaho #

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Narrative: *Echinococcus* is a significant public health concern as humans can serve as an aberrant intermediate host. The severity of disease in humans is associated with a multitude of factors including the species and genotype involved. The phylogeny of *Echinococcus granulosus* has recently been under consideration for reclassification based on sequencing of the ten different genotypes of the cestode, genotypes 1 through 10. The new classification system proposes that *E. granulosus* be divided into at least four different species including *Echinococcus canadensis*, which is comprised of genotypes 6 through 10. Recently, *Echinococcus granulosus* has been documented in wolf populations in Idaho and Montana but the genotype, and species based on genotype classification, has not been confirmed. In order to characterize *Echinococcus* present in this region, lung tissue from two elk and the small intestinal tracts of three wolves legally harvested in Idaho were sent to Colorado State University Veterinary Diagnostic Laboratory. Gross and microscopic examination of the small intestines and multiplex PCR of proglottides confirmed presence of *Echinococcus* in all three wolves and *Taenia* spp. in two. Hydatid cysts were identified on histologic examination of lung tissue from both elk. Sequence analysis of the NAD gene confirmed both hydatid cysts and intestinal tapeworms as *Echinococcus canadensis*, genotypes 8 and 10. Genotype 10 was confirmed in one wolf and both elk, demonstrating infection in a definitive and intermediate host; thus, completing the natural life cycle for *E. canadensis*. These results suggest that *E. canadensis* is endemic in Idaho. With the establishment of this organism in wildlife populations there is a potential of spillover into domestic populations, specifically dogs, as domestic livestock serve as poor intermediate hosts. Through either direct contact with wildlife or through increase prevalence in domestic canids, humans may be at risk of increased exposure to this zoonotic cestode.

AAVLD Trainee Travel Awardee (Parasitology)

Merogonic Stages of *Theileria cervi* in Mule and White-Tailed Deer #

Jason F. Wood[1], Eileen M. Johnson[1], Kelly E. Allen[1], Gregory Campbell[2], Grant Rezabek[2], Daniel S. Bradway[3], Louis L. Pittman[4], Susan E. Little[1], Roger J. Panciera[1]

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Narrative: In February 2012, twelve farmed mule deer were moved from a facility in southwestern Oklahoma to a facility in southeastern Oklahoma that housed 100 farmed white-tailed deer. Between the third and fifth weeks, nine of the 12 mule deer had succumbed, four of which were submitted for necropsy. The deer were heavily infested with lone star ticks (*Amblyomma americanum*). Hematologic data from one deer revealed severe anemia, leukocytosis and intraerythrocytic hemoparasites consistent with *Theileria* spp. Microscopically the liver, lymph nodes and spleen contained multifocally distributed, enlarged monocytic cells whose cytoplasm was replaced by developing meronts in various stages of merogony. In addition, there were renal tubular hemoglobin casts and uremic abomasitis. We hypothesize that, upon arrival, the *T. cervi*-naïve mule deer became infested with large numbers of *Theileria*-infected lone star ticks leading to massive exposure of the mule deer to sporozoites of the protozoan resulting in an acute hemolytic crisis and the fatalities. Further, we describe merogonic stages of *T. cervi* that were previously unreported. We attribute the lack of earlier reports of merogony to our presumption that only a single, short-lived, merogonic cycle follows exposure to sporozoites and thus merogonic stages are demonstrable for only a short period. PCR results of paraffin-embedded tissue show that the 507 bp amplicon sequence was 100% identical with the sequence of *T. cervi* previously reported from white-tailed deer in Oklahoma and from elk in Wisconsin and Indiana.

AAVLD Trainee Travel Awardee (Pathology, Parasitology)

Study on the Prevalence, Risk Indicators and Chemotherapeutic Control of Tick Infestation in Cattle

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Narrative: To determine the prevalence and related risk indicators of tick infestation in ruminants and to assess the efficacy of three different types of acaricides against tick infestation under field conditions, an epidemiological study was carried out from July 2012 to March 2013 in Beni-suef district. A total of 1082 animals of different species (540 cattle, 230 buffaloes, 108 each of sheep & goats and 96 camels) were randomly selected and examined carefully for tick infestation. About 30.1% of all observed animals were found infested with ticks with highest proportion in cattle (60.5%) followed by goats (25.9%), buffaloes (17.8%) and sheep (14.8 %). The most common tick species affecting ruminants in investigated locality was *Boophilus annulatus* (26.5%) followed by *Hyalomma anatolicum* (6.1%), and *Rhipicephalus turnicus* (3.4%). Tick infestation was higher ($P<0.05$) in Friesians (77.5 %), associated with age (>3 years, 78.8%; ≤ 2 months 57.8%) and higher during summer months in cattle, goats and sheep (76.5 %, 33.3%, 22.9 %, respectively) when compared with winter records, while no risk factors were associated with buffaloes. The preferred attachment sites of ticks were udders and external genitalia (70.7%, each), neck and chest (63.0%, each), inner thighs (61.1%), perineum (41.7%), ears (14.6%), around eyes (11.7%). Poor husbandry practices of small holder farmers may be a determinant making the animals more prone to tick infestation in this district. A combination of Ivermectin (0.2 mg/kg body weight subcutaneously) two doses, adjunct to Deltamethrin to the surrounding environment twice at a 10-day interval is advisable for tick control under field conditions.These observations provided insights into what determinants might impact tick infestation in ruminants, and laid a foundation for planning of future control programs.

Micropolymer Detection Permits Rapid Multiplex IHC™ Stains on Animal Tissues

Brenda Karim

Biocare Medical, LLC, Concord, CO

Narrative: Polymer detections are exceedingly sensitive, eliminate the need to biotinylate an antibody and require no avidin-biotin blocking. Biocare Medical's PromARK™ pre-adsorbed, enzyme-conjugated polymers demonstrated minimal cross-reactivity to endogenous IgGs in all tissues. Excellent staining was achieved in a wide variety of clinical primary antibodies on various normal and neoplastic tissues. Double and triple staining techniques on animal tissues were also developed using antibody and detection cocktails and done manually and on an automated IHC stainer. These biotin-free pre-adsorbed polymer detection assays may provide a valuable diagnostic or research tool for the veterinary pathologist.

Toxicology

Saturday, October 19, 2013
Pacific Salon 2

Moderators: Michelle Mostrom, Deon Van der Merwe

Symbols at the end of titles indicate the following designations:

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

An Atypical Case of Anatoxin-A Intoxication in a Dog and Quantitative Analysis of Biomarkers of Exposure

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Narrative: Harmful algal blooms (cyanobacteria) are increasing in frequency and intensity worldwide. Cyanobacteria are bacteria with photosynthetic abilities and they produced a wide variety of environmental toxins which affect wildlife, animals, and humans. Among these are potent neurotoxic bicyclic alkaloids anatoxin-A, homo-, dihydro-, and epoxy-anatoxin-A. Anatoxin-A is at least 10 times more potent than nicotine in stimulating nicotinic acetylcholine receptors, causing a depolarizing blockade of neuromuscular junctions. These alkaloids are produced by a wide range of fresh water cyanobacteria, including *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, and *Oscillatoria*. Anatoxin-A, formerly called "Fast Death Factor", causes acute neurological signs in animals including rigidity, muscle tremors, paralysis, and death from respiratory paralysis. Typically, anatoxin-A intoxication is not associated with pathological lesions. Diagnostic confirmation has also been limited to testing suspect water and gastric contents. Here we present an atypical case of anatoxin-A poisoning in a canine with morphological changes and with the diagnostic approach extended to include quantitative liver analysis of the toxin. A 4-mo old 35 Lb female Laborador Retriever was presented in excellent body condition for necropsy in August 2010. Major gross necropsy findings were severe multifocal cerebellar hemorrhage and gastric serosal hemorrhages. Stomach contents and the liver sample were submitted for cyanotoxin analysis given a history of possible exposure to cyanobacteria. For analysis, 5 g of gastrointestinal contents were added to 10 mL of double distilled deionized water and subjected to 5 freeze/thaw cycles with 10 minutes sonication between cycles. The extract was diluted 1:1 with methanol and a fraction of the diluent was injected onto an LC/MS/MS. For the liver sample, 5 g was added to 2 mL of water, subjected to 5 freeze/thaw cycles with 10 minutes sonication between cycles, and the extract was diluted 1:1 with methanol before injection onto an LC/MS/MS. All samples were analyzed in duplicate. Anatoxin-A concentration was 146 + 26 ppb in the stomach contents, and 0.8 ppb in the liver. In summary, atypical features of this case included acute cerebellar and gastric serosal hemorrhages. The reason for these finding is unclear, but one possibly is the presence of other biotoxins in the water. Results have shown that highly sensitive equipment can be used to quantify anatoxin-A concentration in the liver. Future diagnostic workup will include identification and quantitation of anatoxin-A congeners in biological samples and development of cheaper and rapid diagnostic tests for anatoxin-A in biological matrices.

Investigation into Veterinary Diagnostic Approaches for Cyanobacterial Intoxications # †

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Narrative: In the Midwestern US, eutrophic water conditions have led to proliferation of cyanobacterial harmful algal blooms. Cyanobacteria produce potent cyanotoxins which negatively impact livestock health. Diagnostic laboratories are interested in quick diagnostic procedures for confirmation of livestock intoxications by cyanobacteria. Several diagnostic laboratories rely on enumeration of toxicogenic cyanobacterial species. The reliability of this method as a predictor of risk is questionable. The objectives of this study were to investigate whether biomass correlates with toxin concentration in water and investigate whether toxin concentration in water as measured by two quick ELISA tests correlates well with the triple quadrupole LCMS. Water samples were collected into individualized opaque sterile containers from randomized distributed open water samples. The samples were transported on ice and stored at either -80°C for toxin analysis or 4°C in Lugol's iodine solution for enumeration. Subsequently, split samples were analyzed with two commercially-available kits and LCMS. All lake water samples were positive for *Microcystis* species - accounting for 1 to 100% of the total cyanobacteria population. *Planktothrix* and *Anabaena* species prevalence were 0 to 63% and 0 to 19%, respectively. The range of microcystin concentrations detected by LCMS was 0 to 2.9, 0 to 5.2, 0 to 2.3, and 0 to 1.4 ppb for microcystin LA, LR, RR, and YR, respectively. Microcystin concentration determined by ELISA test kits ranged from 0 to >10 ppb and 0 to >3 ppb for Abraxis and Envirologix kits, respectively. From this observational data set, there is no evidence ($p>0.10$) microcystin-producing cyanobacteria percentage is associated with the presence of microcystin toxin. There is no evidence ($p>0.10$) biomass measurement of *Microcystis* species nor concentration of chlorophyll a is associated with microcystin toxin presence. The commercially-available test kits were considered to have a sensitivity of 100% and 100% and specificity of 83.3% and 87.5% for Abraxis and Envirologix, respectively. The commercially-available microcystin toxin test kits are suitable screening tools for use with diagnostic submissions. Test kits may be easily employed with or without additional limnological evaluation of surface water. Neither biomass nor percentage of *Microcystis* species are strong diagnostic indicators of presence of microcystins in water.

AAVLD Trainee Travel Awardee (Toxicology)
† Graduate Student Oral Presentation Award Applicant

Bromethalin Poisoning in a Raccoon: A Diagnostic Challenge # †

Adrienne C. Bautista, Birgit Puschner, Michael Filigenzi, Leslie Woods

California Animal Health and Food Safety Laboratory System, University of California, Davis, CA

Narrative: Bromethalin, a rodenticide targeting the nervous system, has been available for use since 1985 in bait pellets, bars and place packs. Visually, it is indistinguishable from many other rodenticides, making identification of poisonings by appearance of the substance alone impossible. Following ingestion, bromethalin is metabolized to its active metabolite desmethylbromethalin which uncouples oxidative phosphorylation. Ultimately, the inability to maintain normal electrolyte gradients within the CNS leads to fluid buildup and the development of cerebral edema. Typical clinical signs of intoxication include muscle tremors, hyperthermia, hyperexcitability and seizures which usually develop within 24 hours after ingestion. Characteristic post mortem findings are diffuse spongy degeneration and glial cell hypertrophy within the white matter. Submission of a male, juvenile raccoon for necropsy to the California Animal Health and Food Safety Laboratory following exhaustion at a local wildlife care center after being found in a semi-comatose state revealed minimal gross pathologic changes and only mild vacuolar changes in the white matter of the brain. Turquoise granular material was noted in the gastrointestinal tract and was submitted for toxicological testing along with portions of the brain, liver, kidney, mesenteric fat, and perirenal fat. Testing of the turquoise material from the GI content for seven anticoagulant rodenticides, strychnine, 4-aminopyridine, starlicide and minerals that may suggest ingestion of fertilizer revealed none of these compounds. Desmethylbromethalin, however, was detected in the GI contents by LC-MS/MS. Subsequently, the other tissues were analyzed. Desmethylbromethalin was not detected in the brain, liver or kidney. However, it was detected in the mesenteric and peri-renal fat. Based on the toxicological findings, a diagnosis of bromethalin toxicosis was established. In cases of wildlife species with unknown deaths or inconsistent clinical signs with normal or minimal histological findings, bromethalin toxicosis should be considered as a differential. Adipose tissue (mesenteric or perirenal) is the tissue of choice and can be easily harvested from a live or deceased animal to help confirm or rule out bromethalin exposure or intoxication.

AAVLD Trainee Travel Awardee (Toxicology)
† Graduate Student Oral Presentation Award Applicant

The Detection of Bromethalin and Desmethylbromethalin in Tissue Samples: An Analytical Challenge

Michael Filigenzi, Adrienne C. Bautista, Birgit Puschner

California Animal Health and Food Safety Lab, University of California, Davis, CA

Narrative: Bromethalin, a commonly available rodenticide, is both chemically and functionally dissimilar from other compounds commercially available for this purpose. Heightened concern over increased use and associated poisonings in animals requires an analytical method for reliable diagnostic testing. Thus, a method utilizing high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) was developed and validated. Electrospray ionization of certified bromethalin standards showed a strong signal for an ion consistent with desmethylbromethalin (DMB), the active metabolite of bromethalin. This same signal was also produced via analysis of commercial bromethalin bait and in tissue samples from animals suspected to have been poisoned by bromethalin. Of concern was whether DMB was actually present in the bromethalin standard or if DMB was being formed from breakdown of bromethalin in the ion source of the mass spectrometer. Recently, a DMB standard became commercially available and chromatographic data demonstrated that it was DMB rather than bromethalin itself which was being detected in the various matrices. Use of the DMB standard has allowed for improvement and refinement of the analytical method. Analyses of certified standard material and commercial bromethalin bait have allowed for improvement and refinement of the analytical methods for tissue analysis using HPLC-MS/MS and HPLC combined with high resolution mass spectrometry.

Iatrogenic Chlorate Poisoning in Three Beef Cows ◊

Steven Gallego[1], John Tahara[1], Bruce Hoar[3], Asli Mete[1], Birgit Puschner[1, 2]

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Narrative: Ingestion of experimental sodium chlorate in food animal species as a pre-slaughter feed supplement for reducing fecal *Enterobacteriaceae* shedding has become the focus of food safety investigations. Poultry, swine, large and small ruminant chlorate feeding trials have shown 100 to 1000 fold reductions of *Escherichia coli* 0157:H7 and *Salmonella typhimurium*. However, ingestion of chlorate in excess of 1 g/kg bodyweight can cause death in cattle. Ironically, ruminants appear to crave the taste of chlorate salts when given access. A group of ten 2-3 year old Angus cows previously pastured were moved into a pen and offered grass hay and water *ad libitum*. Sodium chlorate was added to two water tanks at an intended concentration of 10.6 g/L (equivalent to 8.3 g sodium chlorate/L water). Three of the 10 cows, weighing 390, 485, and 526 kg, were found dead approximately 18 hours later. The remaining 7 cows appeared clinically unaffected. All 3 cows, and hay and water samples, were submitted to the California Animal Health and Food Safety Laboratory for diagnostic work-up. Gross findings included firm gas distention and severe bloat, brown colored blood, and brown discoloration of all organs. Other than moderate erythrophagocytosis, no histological lesions were present. A sensitive ion chromatography method was developed for the simultaneous detection of chlorate, chloride and nitrate in gastrointestinal contents, feed, liver, kidney and ocular fluid. Chlorate concentrations were as follows: 360, 280, and 330 mg/L in aqueous humor; 720, 630, and 790 mg/kg (wet weight) in rumen contents; 130, 76, and 140 mg/kg (wet weight) in kidneys; 900 mg/kg (as fed) in hay; and 8,600 and 28,000 mg/L in two separate water samples. The livers contained no chlorate at the method detection limit of 30 mg/kg. The chlorate concentrations in the three cows were consistent with overexposure to chlorate. Assuming a water consumption of 19 L/day (5 gallons), the cows ingested 1.4, 1.1, and 1.1 g/kg of chlorate respectively from the high chlorate-containing water source. The reported oral lethal dose of chlorate in cattle is 1 g/kg body weight. While the use of sodium chlorate has great promise to decrease the number of pathogens in the slaughtering environment, caution must be taken to avoid potentially lethal overexposure. Results from this case investigation provide critical data for future monitoring and interpretation of tissue chloride, and chlorate concentrations.

◊ USAHA Paper

A Method Adapted for Extraction and ELISA Detection of the β-Agonist Ractopamine in Retinal Tissue

Christina Wilson[1, 2], Mary Mengel[1], Kendel Weger[1], William Wigle[1, 2], Stephen B. Hooser[1, 2]

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Narrative: Ractopamine is a β-adrenergic agonist marketed under the trade names Paylean™ and Optaflexx™. Approved for use in pig and cattle feed, it acts as an anabolic agent increasing muscle mass, lipolysis, and feed efficiency. Although proper use of this drug in approved animals can be beneficial to livestock owners, it can be used inappropriately as a performance enhancing drug in livestock show and racing animals. Urine or plasma/serum samples are commonly tested in order to monitor abuse of this drug in livestock and racing animals; however, ractopamine has a short plasma/serum half-life and a rapid rate of elimination. Therefore, methods to detect ractopamine in other biological samples, such as retinal tissue, for which the drug remains for a longer period of time, would beneficial in monitoring abuse of the drug. In this study, a method has been adapted for the extraction of ractopamine from retinal tissue. Detection of ractopamine was accomplished using an ELISA traditionally used for urine samples. Twenty-seven eyeballs from 4 different animal species were harvested at necropsy. Excision of the retina/choroid pigmented epithelial tissue from the eyeball and the extraction protocol utilized included a USDA extraction procedure (CLG-AGON1.02) for β-adrenergic agonists. Briefly, the extraction protocol involves: 1) homogenizing approximately 0.40 g retinal tissue with acetonitrile:isopropanol (4:1) and 0.24 g NaCl, 2) drying the extract with 0.10 g $MgSO_4$ and 0.80 g Na_2SO_4, 3) N_2 evaporating the extract to dryness, reconstituting with 0.4 mL PBS, and 4) analyzing 20 µL of the reconstituted sample by an ELISA. Of the 27 samples, 7 pigs tested positive for ractopamine. The remaining animal species, 7 pigs, 6 sheep, 6 cows, and 1 horse all tested negative. Retinal tissue samples that had optical densities ≤ 0.233 were considered positive. This study shows that ractopamine can be extracted from retinal tissue and detected using an ELISA traditionally used to detect the drug in urine. Although this method is limited to animals at necropsy/slaughter, it provides a means by which the presence of ractopamine can be monitored in animals for which the drug is being used off-label.

Accidental Contamination of Equine Feed with Zilpaterol Resulting in Widespread Detection of the Drug in Urine Samples from Performance Horses

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Narrative: Beginning in March of 2013, a large number of routinely collected post-race urine samples from performance horses tested positive for the banned drug zilpaterol by liquid chromatography – mass spectrometry (LC-MS/MS). Zilpaterol is a beta-adrenergic agonist sold under the trade name Zilmax®; it is approved for use to increase weight gain, improve feed efficiency, and increase carcass leanness in cattle fed in confinement for slaughter. The Association of Racing Commissioners International classifies zilpaterol as a "Class 3" substance; its detection at any concentration in performance horses can result in a one-year suspension and fines in excess of $10,000. The large number of positive samples, spread over a wide geographic region, suggested possible feed contamination and prompted a multi-agency investigation into the incident. A rapid method for the confirmation and identification of zilpaterol in equine feed by LC-MS/MS was developed with a reporting limit of 5 ppb. Zilpaterol was detected in five of sixteen feed samples at concentrations ranging from 8 ppb to 41 ppb. This prompted an investigation of the horse feed manufacturing facility. The drug was not intentionally used to manufacture any feed at the site and it was not present at the facility. Further investigation indicated that molasses was a common ingredient in all positive feed samples. The firm supplying the molasses was inspected and it was determined that a molasses-based premix containing zilpaterol had been manufactured for a beef feedlot at a calculated concentration of 266 grams of drug per ton (293 ppm) of molasses. The next use of the mixer was for the formulation of non-medicated molasses which was subsequently supplied to the horse feed manufacturer. A sample of molasses submitted for analysis detected zilpaterol at 600 ppb. The horse feed manufacturer voluntarily recalled all horse feed and grain mixes containing molasses. Twelve of 22 feed samples collected by the feed manufacture and submitted to the California Animal Health and Food Safety Laboratory were positive for zilpaterol at concentrations ranging from 6 ppb to 25 ppb. An additional 54 feed samples were subsequently tested negative in response to the efforts of the feed manufacturer to end the voluntary feed recall. Fortunately, no horses were adversely affected following consumption of the contaminated feed. However, exposure to low concentrations of the drug resulted in violative concentrations in performance horses with potentially serious consequences. Close cooperation between a number of state agencies and university laboratories resulted quick identification of the problem and regulatory action. Timely follow-up feed analyses allowed the manufacturer to quickly resume product sales.

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Moderators: Hong Li, Kyoung-Jin Yoon

1:00 PM **Automating Training to Meet AAVLD Requirements**
Andrew Havens, Keith Ernst *72*

1:15 PM ***Equine Herpesvirus-1*** **(Neuropathic) Mapping in Llama Brain**
Beate M. Crossley, Udeni BR Balasuriya, Gabi Maier, Mark Anderson, Robert Moeller *73*

1:30 PM ***Bovine Viral Diarrhea Virus*** **(BVDV) in Postweaned Calves in a Feedlot after Vaccination and from Fatal Respiratory Cases: Isolation and Differentiation of Modified Live Viral (MLV) BVDV and Field Strains ◊**
Robert W. Fulton, Julia F. Ridpath, John Neill, Lurinda J. Burge, Blake Wilson, Casey Maxwell, D. L. Step *74*

1:45 PM **Malignant Catarrhal Fever in American Bison following Challenge with** ***Alcelaphine herpesvirus-2***
Naomi S. Taus, Hong Li, Bruce S. Seal, Janet V. Warg, Donal O'Toole *75*

2:00 PM **Epidemiology of Re-emerging** ***Feline Panleukopenia Virus*** **in Vaccinated and Unvaccinated Felids in South Africa #**
Helene Brettschneider, Emily Lane, Peter Calwell, Desire Dalton, Antoinette Kotze *76*

2:15 PM **Comparison of Stability of Viral Nucleic Acid in Different Tissues and under Different Conditions in Samples Collected from Fetuses Infected with** ***Bovine Viral Diarrhea Virus***
Julia F. Ridpath, Yu-Wei Chiang, Jill Waldbillig, John Neill *77*

2:30 PM **Development of a Competitive ELISA for the Detection of Antibodies to** ***Rift Valley Fever Virus***
Sandy Rodgers, Alfonso Clavijo, Garry Adams, Sierra Popp, Feng (Julie) Sun, Tammy Beckham . . . *78*

2:45 PM **Translation of a Clinical Laboratory-Validated** ***Equine Herpes Virus-1*** **Real-Time PCR Assay into POCKIT™ - a Novel and Easy-to-Use Field-Deployable PCR System**
Yan Zhang, Jason Herr, Kerri Lawrence, Yun-Lung Tsai, Jian-Hau Chiou, Fu-Chun Lee, Pei-Yu Lee, Simon Chung, Hsiao Fen Grace Chang, Thomas Wang *79*

Symbols at the end of titles indicate the following designations:

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

Automating Training to Meet AAVLD Requirements

Andrew Havens, Keith Ernst

ECL2 - QPulse, Grapevine, TX

Narrative: Keeping up with training can often be a big challenge. Several veterinary diagnostic laboratories have utilized ECL2's application and expertise to overcome this sometimes daunting task. We will introduce you to an application that allows you to develop Job Roles that may be linked to Competencies and Requirements (Training Events, Professional Requirements, etc.). Once linked, the Job Roles can then be applied to a Person's record automatically creating a Training Plan. The application will send out reminders as well as escalations letting you know when competencies and requirements are coming due or are overdue. As Job Roles change, previously completed requirements can be applied to the new Role within the Training Plan. Having a plan allows you to keep track of training, whether it is initial training, renewal or demonstrating proficiency that has been achieved. Through the establishment of Training Plans, training becomes an automatic process that will assist you in accreditation and beyond.

Equine Herpesvirus-1 (Neuropathic) Mapping in Llama Brain

Beate M. Crossley[1], Udeni BR Balasuriya[2], Gabi Maier[3], Mark Anderson[1], Robert Moeller[1]

[1]California Animal Health and Food Safety Laboratory System, University of California, Davis, CA; [2]Gluck Equine Research Center, University of Kentucky, Lexington, KY; [3]Animal Health Branch, California Department of Food and Agriculture, Davis, CA

Narrative: A disease investigation was initiated after the referring veterinarian contacted state animal health officials about a herd of 40 llamas which had four llamas develop acute onset of blindness, circling and hypermetric gait. These animals were co-housed with zebras on a privately owned 200 acres property. The property had fence contact to neighboring ranch horses. Two blind adult llamas were euthanized and submitted for necropsy. Both animals were received for necropsy in good post mortem condition. Llama #1 was 10 years old and in her third trimester of pregnancy. The animal had diffusely congested lungs with abundant edema, and the trachea was filled with foam. The meninges over the cerebellum was cloudy. Llama #2, a 4 year old neutered male, was submitted 6 days after Llama #1. Gross observations identified congested meninges (clear CSF fluid) and nasal pathways. Internal organs including lungs appeared normal. Histological examination confirmed significant pulmonary edema in both animals. In the brain of both animals, there was significant inflammation consisting of prominent multifocal lymphocytic perivascular cuffing. In addition to the lung and brain lesions observed, additional lesions noted only in Llama #1 were a mild necrotizing myocarditis and fibrin thrombi in the sinusoids of the liver. Llama #2 had significant histopathological findings only associated with the brain. In Llama #1 a routine brain pool tested positive for the neuropathogenic *Equine herpesvirus-1* (NEHV-1) by real-time PCR. In Llama #2, multiple tissues taken from different areas of the brain tested positive by real-time PCR for NEHV-1 and an attempt was made to 'map' the viral pathway. The virus was detected in the pyriform lobe, hippocampus, olfactory bulb, posterior colliculus, medulla, cerebellum, olfactory nucleus, cervical spinal cord and basal ganglia of both llamas. Nasal swabs, whole blood and cerebral spinal fluid tested negative in both animals. Due to the history of cohousing with zebras, an infection with EHV-9 was considered. Sequence analysis of large fragments of the polymerase gene, glycoprotein B and glycoprotein D was performed and the presence of EHV-1 in both animals was confirmed. This case report suggests that a) NEHV-1 can cause blindness in affected llamas, b) NEHV-1 was not detectable in nasal secretions, demonstrating a nasal swab is not a reliable sample type for detecting NEHV-1 in llamas showing neurological signs, and c) viral distribution was not uniform indicating that more than one area in the brain should be evaluated by NEHV-1 PCR.

Bovine Viral Diarrhea Virus (BVDV) in Postweaned Calves in a Feedlot after Vaccination and from Fatal Respiratory Cases: Isolation and Differentiation of Modified Live Viral (MLV) BVDV and Field Strains ◊

Robert W. Fulton[1], Julia F. Ridpath[4], John Neill[4], Lurinda J. Burge[1], Blake Wilson[3], Casey Maxwell[3], D. L. Step[2]

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Narrative: Viral infections are important etiologies in bovine respiratory disease cases. Calves at stocker/feedlot entry usually receive modified live viral (MLV) vaccines containing *Bovine herpesvirus-1* (BoHV-1), *Parainfluenza-3 virus* (PI3V), *Bovine viral diarrhea virus* (BVDV), and *Bovine respiratory syncytial virus* (BRSV). In a 2012 study, 516 head arrived over a one-week interval from six sale barns in the state by a single buyer. Calves received a five-way BRD MLV BoHV-1, PI3V, noncytopathic (NCP) BVDV1a and 2a, BRSV vaccine at processing. There were 343 calves treated for BRD (66.5%) and 332 head treated in the first 21 days. Of 516 calves, 72 died with 68 respiratory cases (12.2% of 516). There were 15 sentinel calves monitored for viruses with nasal swabs (NS) and serologic testing after arrival. Nasal swabs and respiratory tissue homogenates were inoculated onto MDBK and HRT monolayers. Viruses were confirmed in cultures by PCR for BVDV, PI3V, BRSV, and *Bovine coronavirus* (BoCV). BVDV isolates were subtyped and positives compared to MLV strains. BVDV and BoCV serology was performed using paired samples. There were 12 of 15 sentinels (80%) between days (d) 9-11 after vaccination with MLV NCP BVDV1a in NS. One calf's NS was positive for PI3V and BRSV. All sentinels seroconverted to BVDV1a and 7 of 15 (46.7%) to BoCV. There were 37 tissue sets available for virus isolation from calves dying from d 8 to d 47. Lungs from nine calves were positive for NCP BVDV1: 7 MLV NCP BVDV1a and 2 BVDV1b. Lungs from four calves were PI3V positive, one BRSV, and one BoCV. The shipments had one persistently infected BVDV calf. This study found multiple viruses from postweaned calves within 1-2 weeks after MLV vaccination. MLV vaccine strains should be differentiated from field strains. Viral genome sequences of MLV vaccines should be available permitting PCR and sequencing. In this study MLV NCP BVDV1a vaccine strain was recovered indicating viral shed in nasal swabs and tissues from dying calves.

◊ USAHA Paper

Malignant Catarrhal Fever in American Bison following Challenge with *Alcelaphine herpesvirus-2*

Naomi S. Taus[2], Hong Li[2], Bruce S. Seal[3], Janet V. Warg[4], Donal O'Toole[1]

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Narrative: Malignant catarrhal fever (MCF) caused by *Ovine herpesvirus-2* (OvHV-2) is a major problem for producers of American bison (*Bison bison*) in the US and Canada. Our group is interested in developing a vaccine that could protect MCF-susceptible ungulate species from disease. *Alcelaphine herpesvirus 2* (AlHV-2), which is another member of the MCF virus group, has not been reported to cause disease. It has the single advantage that, unlike OvHV-2, it can be grown in cell culture. In order to evaluate whether AlHV-2 provides a practical backbone to express relevant genes of OvHV-2 as a vaccine strain, we inoculated yearling American bison intranasally (IN) (n=4) or intramuscularly (IM) (n=3) with AlHV-2 and monitored for infection status. This was assessed using AlHV-2-specific real time PCR and competitive inhibition ELISA, and disease development. The AlHV-2 isolate (strain AHV-2 (840412) was obtained from a Jimela topi (*Damaliscus tunatus jimela*)(Seal et al: 1989, Arch Virol 106:301-320) and passaged in lamb kidney primary cells. An additional 2 bison served as in-contact controls. Five bison, 2 IN challenged and 3 IM challenged, became infected with AlHV-2. One of 3 IM challenged bison developed clinical signs of MCF at 24 days post inoculation (DPI) and was euthanized at 26 DPI. One of 4 IN challenged bison developed clinical signs at 33 DPI and died at 38 DPI. The remaining 5 challenged bison were either euthanized at the end of the study at 71-74 DPI (n = 4), or died of intercurrent disease (n = 1; pulmonary abscesses). AlHV-2 is not a good choice at this time for use as a vector for OvHV-2 vaccine.

Epidemiology of Re-emerging *Feline Panleukopenia Virus* in Vaccinated and Unvaccinated Felids in South Africa #

Helene Brettschneider[1], Emily Lane[1], Peter Calwell[3], Desire Dalton[1, 2], Antoinette Kotze[1, 2]

[1]National Zoological Gardens of South Africa, Pretoria, South Africa; [2]Genetics Department, University of the Free State, Bloemfontein, South Africa; [3]Old Chapel Animal Clinic, Pretoria, South Africa

Narrative: With the increased incidence of *Canine parvovirus* infecting cats in the 1980 - 1990's, there was a general perception that *Feline panleukopenia virus* (FPLV) disappeared from domestic cats in South Africa. However in 1999, molecular diagnosis from a diseased domestic cat and two diseased captive born cheetahs, confirmed the presence of FPLV. Currently cub mortality in captive cheetahs is minimized by vaccination of dams in late pregnancy. This paper reports the epidemiological investigation into a recent re-emergence of FPLV in captive and wild felids in South Africa. In November 2012, eleven vaccinated, 6-8 month old captive bred cheetah cubs, vaccinated with the live and inactivated FPLV vaccines, Felocell® and Fel-o-vax®, developed severe hemorrhagic diarrhea, vomiting and inappetance, which progressed rapidly to pyrexia, hemorrhagic diathesis and marked aggression. Necropsy and histopathological investigations of five cubs revealed necrohemorrhagic enteritis and lymphoid depletion with secondary bacterial enteritis, consistent with FPLV infection. Pathology findings in four subsequent cubs were similar with variable multiple organ hemorrhages, necrohemorrhagic gastritis, esophageal and gastric candidiasis, hydropericardium and hydrothorax, renal tubular and pancreatic necrosis and interstitial pneumonia. In March-May 2013, two unvaccinated adult cheetahs with similar lesions as well as one unvaccinated captive serval with *Salmonella* enteritis, were received for testing. These, along with ten historical samples of suspected and confirmed cases of FPLV in felids (2002-2011) obtained from the National Biobank, were included in molecular analyses. In total, for 2012-2013, a 53% case fatality rate due to FPLV was observed. Molecular detection, identification and analyses of FPLV strains based on a partial fragment of the viral capsid protein (VP2) gene, showed cases from 2002-2013 forming a distinct and novel South African clade, most closely related to a strain recorded in raccoons in the US in 2010. It is also similar to but distinct from both strains used in the administered vaccine; and distinct from the 1999 FPLV strains. Evidence suggests a continuously present FPLV strain in South African felids, potentially persisting in low copy numbers, or in wild reservoir hosts. Animals under stress or immune suppression seem to be at risk, irrespective of vaccination status. Although vaccine viability was not tested, failure of the live vaccine to induce immunity is a possibility. Information gathered in this study serves veterinarians and managers on the movement and vaccination protocols of felids in South Africa.

AAVLD Trainee Travel Awardee (Epidemiology, Virology)

Comparison of Stability of Viral Nucleic Acid in Different Tissues and under Different Conditions in Samples Collected from Fetuses Infected with *Bovine Viral Diarrhea Virus*

Julia F. Ridpath[1], Yu-Wei Chiang[2], Jill Waldbillig[3], John Neill[1]

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Narrative: Accurate diagnosis of *Bovine viral diarrhea virus* (BVDV) induced reproductive disease is important to herd health management and BVDV control programs. Diagnosing BVDV, by polymerase chain reaction (PCR), as a cause of reproductive disease may be problematic because viral nucleic acid may be degraded in aborted or stillborn fetuses due to exposure to the autolytic microenvironment in the time between fetal death and expulsion and/or exposure to heat, light and tissue breakdown in the time between expulsion and discovery in pens and pastures. The purpose of this study was to examine the stability of viral nucleic acid in fetal tissues exposed to different conditions. To this end,seven first calf heifers, six naïve and one vaccinated, were exposed to BVDV1b strain CA0401186a at 84 to 86 days gestation. Fetuses were harvested by cesarean section at 115 -117 days of gestation. The six fetuses removed from the non-vaccinated heifers were BVDV positive based on virus isolation, while the one fetus from the vaccinated animal was negative. The following samples were collected: brain, organ pool A (heart, lungs, thymus), organ pool B (spleen, kidney, intestines), ears, muscle and skin from back leg. These samples were processed as follows: a. No storage – processed immediately; b. No storage, inoculated with fecal matter and processed immediately; c. 7 days, -80°C; d. 7 days, -80°C inoculated with fecal matter; e. 7 days, 4°C; f. 7 days, 4°C inoculated with fecal matter; g. 7 days, room temperature (RMT); h. 7 days, RMT inoculated with fecal matter; i. 7 days, 37°C; j. 7 days, 37°C inoculated with fecal matter. A PCR test based on amplification of sequences from the 5' UTR was used for detection. Fecal contamination did not appear to decrease detection. No difference in detection by PCR was observed in samples stored under conditions a through f. Detection under storage conditions g through j varied by tissue sample with organ pool B samples showing the most variation. These results indicate that BVDV nucleic acid is stable under a wide range of conditions and that PCR-based tests can be reliable for detection of BVDV from abortion and stillbirth cases.

Development of a Competitive ELISA for the Detection of Antibodies to *Rift Valley Fever Virus*

Sandy Rodgers[1], Alfonso Clavijo[2], Garry Adams[3], Sierra Popp[1], Feng (Julie) Sun[1], Tammy Beckham[1, 4]

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Narrative: Rift Valley fever (RVF) is an arthropod-borne viral disease that poses a significant threat to domestic ruminants and human health. The disease has expanded its range from sub-Saharan Africa and the Middle East to also include Saudi Arabia, Kenya, Somalia and Zimbabwe. RVF virus (RVFV) can cause explosive epizootics of abortion and high mortality in sheep, goats and cattle; and thus, is a significant veterinary and human pathogen. Some cattle breeds and exotic ruminants demonstrate varying clinical signs but remain infected and are a source of the virus. While infection in humans most often results in a benign febrile illness, the virus has also been associated with large scale fatal outbreaks of hemorrhagic fever and encephalitis in Egypt (1977-1978) and Kenya (1998) [Meegan JM: 1979, The Rift Valley fever epizootic in Egypt 1977-78. 1. Description of the epizootic and virological studies. Trans. R. Soc. Trop. Med. Hyg. 73:618–623; and Woods CW, Karpati AN, Grein T, et al.: 2002, An outbreak of Rift Valley fever in Northeastern Kenya, 1997–98. Emerging Infectious Diseases 8:138–144]. RVFV is considered a Category A agent, capable of being utilized as a biological agent and poses a high risk to national security and public health. Competent mosquito vectors for RVFV are found in the US, and potential propagation and transmission of the virus is a threat to US livestock populations if introduced domestically. Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL), in collaboration with the DHS National Center for Foreign Animal and Zoonotic Disease Defense (FAZD Center) and Texas A&M College of Veterinary Medicine and Biomedical Sciences, has developed a competitive ELISA (cELISA) for the serological diagnosis of RVFV in multiple species. A cELISA was developed using a recombinant MP-12 nucleoprotein (NP) expressed in *E. coli*. It is anticipated that the cELISA will be less expensive than other techniques by allowing multi-species testing, is rapid and well-suited to large sample numbers, and can also be well standardized, quality controlled and automated. Data from the cELISA was generated using sera collected from two RVFV MP-12 vaccinated sheep on days -1 through 69 post-vaccination [Morrill JC, Laughlin RC, Lokugamage N, et al: 2012, Safety and immunogenicity of recombinant Rift Valley fever MP-12 vaccine candidates in sheep. Vaccine 10.1016/j.vaccine.2012.10.118, 2012]. The vaccination study samples were used to determine the feasibility of the cELISA to detect antibody positive animals. Samples from days 8 through 69 were determined as positive for RVFV antibody using the cELISA. Additionally, 154 field sera collected from routine submissions to the TVMDL were used to ascertain the specificity of the cELISA. The specificity of the cELISA was determined to be 100%.

Translation of a Clinical Laboratory-Validated *Equine Herpes Virus-1* Real-Time PCR Assay into POCKIT™ - a Novel and Easy-to-Use Field-Deployable PCR System

Yan Zhang[1], Jason Herr[1], Kerri Lawrence[1], Yun-Lung Tsai[2], Jian-Hau Chiou[2], Fu-Chun Lee[2], Pei-Yu Lee[2], Simon Chung[2], Hsiao Fen Grace Chang[2], Thomas Wang[2]

[1]Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH; [2]GeneReach USA, Lexington, MA

Narrative: *Equine herpesvirus-1* (EHV-1) is an important pathogen in horse populations worldwide that causes abortion, respiratory and neurologic diseases. Point-of-need (PON) detection of EHV-1 could provide timely and cost-effective diagnosis to facilitate disease management. Although real-time polymerase chain reaction (qPCR) has become an important tool for detecting EHV-1 DNA during an outbreak, the PON application has been limited due to requirement of expensive equipment and reagent. A novel, inexpensive and user-friendly system based on insulated isothermal PCR (iiPCR) method on the POCKIT™, a field-deployable device, has been developed for PON pathogen detection. Signal detection relies on the same principle of fluorescent probe hydrolysis for qPCR and iiPCR on POCKIT™. Here, we report the translation of a validated EHV-1 qPCR assay into an iiPCR on the POCKIT™ system. The original primer/probe set was tested in three assays, namely 1) qPCR assay using original reagents (buffer, salts, and enzyme), 2) qPCR using iiPCR reagents on a real-time machine and 3) iiPCR using iiPCR reagents on a POCKIT™. Limit of detection of these three assays was compared using a known concentration of EHV-1 cultured in rabbit kidney (RK-13) cell line. All three assays were found to have equal analytical sensitivity. In conclusion, the translation of a qPCR into iiPCR assay using the POCKIT™ instrument was successful. The new iiPCR assay is easy to use for field deployment to provide point of need detection for EHV-1.

Bacteriology 2

Sunday, October 20, 2013
Pacific Salon 3

Moderators: Durda Slavic, Deepanker Tewari

11:30 AM **Isolation of *Aurantimonas altamirensis*, a *Brucella canis*-like Bacterium from a Canine Testicle ◊**
Thomas J. Reilly, Michael J. Calcutt, Laura Wennerdahl, Fred Williams, Timothy Evans, Irene K. Ganjam, William H. Fales *94*

11:45 AM **Serodiagnosis of Equine Leptospirosis by ELISA using Four Biomarkers ◊**
Cuilian Ye, WEiwei Yan, Patrick L. McDonough, Sean P. McDonough, Yung-Fu Chang *95*

Symbols at the end of titles indicate the following designations:

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

Brucella spp-Associated Placentitis in a Boer Goat during an Abortion Outbreak

Dodd Sledge, Ailam Lim, Steven R. Bolin

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

Narrative: A herd of Boer goats in West Virginia experienced an outbreak of abortion in fall of 2012. Three quarters of the does in this herd aborted at about 3 months of gestation. The does never appeared ill. Fresh and fixed tissues from a single aborted fetus were submitted to the Diagnostic Center for Population Animal Health, Lansing, MI, for diagnostic evaluation. On histologic examination, there was acute necrosuppurative cotyledonary placentitis with myriads of small, Gram negative, intra and extracellular bacterial rods. No significant lesions were noted in other organs. PCR for the insertion sequence IS711 of *Brucella* spp. was positive using multiple separate DNA extractions from fresh frozen and formalin-fixed, paraffin-embedded placenta. PCR on fresh samples of placenta was negative for *Coxiella burnetii* and no organisms were identified with immunohistochemisty for *C. burnetii*. Bacterial culture of placenta yielded mixed populations of bacteria suggestive of environmental contamination. Blood samples taken from the dam of the aborted fetus, other goats in the herd, and two domestic dogs on the farm were negative for *Brucella* spp. by PCR preformed at the Center for Disease Control (CDC), Atlanta, GA. Serologic testing on similar blood samples preformed at the National Veterinary Services Laboratory, Ames, IA, was not supportive of exposure to rough or smooth strains of *Brucella abortus*. Overall, this case presents a diagnostic dilemma made particularly intriguing due to the regulatory and human health implications inherent in dealing with cases of Brucellosis. Examination and thorough testing of the original specimens support a diagnosis of *Brucella* spp.-associated placentitis, at least in an individual animal. Subsequent testing of other animals failed to support *Brucella* spp. as a cause of disease at the herd level. It is possible that the failure to demonstrate infection or exposure to *Brucella* spp. in the other animals was due to variance in bacterial strain or the testing methods or treatment of affected animals. Another potential abortigenic agent, *C. burnetii*, was identified in few does in the herd by PCR on blood preformed at the CDC; however, it cannot be definitively proven that *C. burnetii* was a cause of the abortion outbreak as the organism was not demonstrated in aborted tissues.

Swine Zoonosis Risk Assessment with New Herd Seroprofiling Assays from QIAGEN

Carsten Schroeder, Christine Gaunitz, Susann Kantiz, Claudia Engemann

QIAGEN Leipzig GmbH, Leipzig, Germany

Narrative: In 2003, the European Parliament issued Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents. The purpose of this directive is to ensure that zoonoses are properly monitored, and that food-borne outbreaks receive proper epidemiological investigation. The collection of this information enables the evaluation of relevant trends and sources which therefore permits the development of effective/dedicated strategies to improve the herd health level and to contribute to consumer protection. QIAGEN Leipzig developed the Pigtype product line of ELISA tests for screening for swine zoonosis based on the Salmotype ELISA. This product line now includes ELISA for detection of *Salmonella*, *Yersinia*, *Trichinella* and *Toxoplasma* antibodies in swine. These Pigtype assays are validated for serum and meat juice samples, and are officially approved by the German Friedrich-Loeffler-Institut. In order to follow the seroprofiling concept, the Pigtype ELISA reagents and assay protocols are standardized. This product concept allows combining serological *Salmonella* monitoring with serological testing for other zoonosis. A set of well characterized serum samples (n=8) was sent to 6 laboratories in Germany and Switzerland. In addition, the participating labs tested serum and meat juice samples from swine and wild boar samples from their routine testing. In this study, there was a ≥99% agreement of the test panel results for the participating labs. Overall variation of sample to positive (S/P) signal of positive samples was low, between 8.8 and 13.5%. Field sample tests scored as expected. Further assay performance data like sensitivity, specificity, and cross reactivity will be presented. Our data suggest the suitability of the Pigtype assays for easy-to-use and cost efficient serological monitoring for zoonotic diseases in swine herds. This could be an effective tool to use, under the European Directive on zoonoses, and could bring improvements to herd risk assessment and risk-oriented meat control.

Toxin Genotypes and Antimicrobial Susceptibility Patterns of *Clostridium perfringens* Isolates Recovered from Horses

Erdal Erol, Alan Loynachan, Steve Locke, Naomi Kelly, Craig N. Carter

Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY

Narrative: This study was performed to assess the incidence of *Clostridium perfringens* genotypes and susceptibility patterns of isolates recovered from equine necropsies. A total of 2,245 horses were necropsied at the University of Kentucky Veterinary Diagnostic Laboratory between November 11, 2011 and May 10, 2013. Anaerobic culture was requested by pathologists in 102 of these cases based on the clinical history and gross pathologic findings. *Clostridium perfringens* were isolated from the small intestine and/or colon of 27 horses. Toxin genotypes and antimicrobial susceptibility patterns of 27 isolates were determined by multiplex PCR and broth micro dilution methods, respectively. Toxin genotype A was most prevalent and was identified in 23 of 27 cases (85.2%). Genotype A/β-2 toxigenic and genotype A/β-2/enterotoxigenic isolates were each identified in 2 (7.4%) foals younger than 5 days of age. All 27 isolates were resistant to amikacin and gentamicin, and were susceptible to cefazolin, ceftazidim, chloramphenicol, enrofloxacin, imipenem, rifampicin, ticarcillin and ticarcillin/clavulanic acid. Twenty-four of 27 (88.9%) isolates were susceptible to penicillin and ampicillin. Additionally, each isolate's contribution to death or euthanasia was speculated upon based on gross and histopathologic findings. *Clostridium perfringens* was the only pathogen identified in 13 cases (48.1%) of enteritis, typhlitis, colitis, or a combination thereof. *Clostridium perfringens* likely caused or contributed to enteric disease in 10 cases (37.0%) in which death or euthanasia ensued due to a combination of disease processes. The significance of *C. perfringens* isolates was considered unknown in 4 horses (14.8%) that lacked enteric lesions.

Isolation, Identification, and Antimicrobial Susceptibility Testing of *Moraxella* spp. Associated with Cases of Infectious Bovine Keratoconjunctivitis in Cattle

John D. Loy[1], Eric Moore[2], John Mayer[2], Bruce W. Brodersen[1]

[1]Veterinary Medicine and Biomedical Sciences, University of Nebraska, Lincoln, NE; [2]Merck Animal Health, Desoto, KS

Narrative: Infectious bovine keratoconjunctivitis (IBK), also known as pinkeye, is the most common and significant eye disease of domestic cattle in both the United States and abroad. Cattle with IBK present with a variety of clinical signs, including ocular pain, increased tear production, photophobia, and ultimately corneal swelling that progresses to corneal ulceration and possibly blindness. IBK is an intensely painful and irritating process, and thus it has significant production implications, especially in young calves. The principal infectious agent of IBK has traditionally been the gram negative bacteria, *Moraxella bovis*. However, recent reports of an association of IBK with a newly described organism, named *Moraxella bovoculi*, have been reported in the absence of *M. bovis*. Evidence of the role of *M. bovoculi* in the causation of IBK includes anecdotal reports of increased autogenous vaccine efficacy when *M. bovoculi* was included in the vaccine. Given a lack of information regarding the overall prevalence and antimicrobial susceptibility of these organisms in large numbers of field cases, a retrospective study was conducted to evaluate the prevalence of *Moraxella* spp., including *M. bovis*, *M. bovoculi*, and *M. ovis* isolated from conjunctival swabs with a history of infectious bovine keratoconjunctivitis in case submissions to the Nebraska Veterinary Diagnostic Center from July 1, 2010 through December 31, 2012. Over 800 isolates from clinical cases with characteristics consistent with members of the genus *Moraxella* (colony morphology and gram stain) were further speciated using a molecular restriction fragment length polymorphism (RFLP) test. Isolates were then subjected to antimicrobial susceptibility testing using minimum inhibitory concentration testing on a microdilution plate. Detected species included *M. bovis*, *M. bovoculi*, and *M. ovis*, with the role of *M. bovoculi* in the pathogenesis of IBK not clearly understood.

Simultaneous Detection of Anti-Apx Toxins Antibodies of *Actinobacillus pleuropneumoniae* (APP) in Pigs with Known and Unknown Exposure using a Multiplexing Liquid Array Platform

Luis G. Gimenez-Lirola, Michelle Hemann, Kevin C. O'Neill, Kyoung-Jin Yoon, Patrick G. Halbur, Tanja Opriessnig

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

Narrative: Industry trends indicate that average swine herd size is increasing and, with that, the risk of economic damage caused by infectious swine diseases like swine pleuropneumonia, caused by *Actinobacillus pleuropneumoniae* (APP). The surveillance of APP infections plays a central role in the control of the disease. We described here a new 4-plex fluorescent microbead-based immunoassay for simultaneous detection of antibodies to RTX toxins ApxI, ApxII, ApxIII and ApxIV of APP. The 4-plex Apx FMIA was evaluated using: (A) sera from pigs experimentally infected with each of the 15 know APP serovars, or with *Actinobacillus suis*, (B) sera from pig vaccinated with a bacterin containing APP serovar 1, 3, 5, or 7, and (C) sera with unknown APP exposure. The results were compared to a dual-plate complement fixation (CF) assay and three commercially available enzyme-linked immunosorbent assays (ELISAs) evaluated previously. On experimental samples, the 4-plex Apx FMIA was able to detect specific seroconversion as early as seven days post-infection in a total of 29 pigs inoculated with all 14 APP serovars included in the study, while the combination of both, the three different commercial ELISAs and the dual-CFT, were able to detect seroconversion in 22 pigs inoculated with 13 APP serovars. Seroconversion against ApxII and ApxIII was detected by FMIA in pigs inoculated with *A. suis*. Vaccinated pigs showed a poor humoral response against ApxII, ApxIII and ApxIV a no response against Apx I. In field samples, APP seroprevalence increased with age, ranging from 18% in suckling pigs to 100% in adults. This new tetra-plex Apx FMIA was found to be more sensitive, accurate and precise than current reference tests, showing a great potential for natural history infection studies, virulence studies, and for monitoring the efficacy of prospective vaccines.

Improving Ante Mortem Diagnosis of *Erysipelothrix rhusiopathiae* Infection by Use of Oral Fluids for Bacterial, Nucleic Acid, and Antibody Detection

Luis G. Gimenez-Lirola, Chaoting Xiao, Marissa Zavala, Patrick G. Halbur, Tanja Opriessnig

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

Narrative: Swine erysipelas is an economically important disease caused by *Erysipelothrix rhusiopathiae*. Pen-based collection of oral fluids has recently been utilized for monitoring infection dynamics in swine operations. The diagnostic performance of bacterial isolation, real-time PCR, and antibody detection by enzyme-linked immunosorbent assay (ELISA) and fluorescent microbead-based immunoassay (FMIA) methods were evaluated on pen-based oral fluid samples from pigs experimentally infected with *E. rhusiopathiae* (n=112) and from negative controls (n=32). While real-time PCR was a sensitive method with an overall detection rate of 100% (7/7 pens) one day post inoculation (dpi), *E. rhusiopathiae* was successfully isolated in only 28.6% (2/7 pens). Anti-*Erysipelothrix* IgM and IgG antibodies in pen-based oral fluids was detected at 4 to 5 dpi by FMIA and at 5 and 8 dpi by ELISA. The number of infected animals per pen, and in particular the timing of antimicrobial treatment administration impacted bacterial isolation and ELISA results. In oral fluid field samples, *E. rhusiopathiae* DNA was found in 23.3% of the samples while anti-*E. rhusiopathiae* IgG and IgM antibodies were found in 59.6% and 5.5% of the samples, respectively. The results suggest that an algorithm integrating oral fluids as specimen and real-time PCR and FMIA as detection methods is effective for earlier detection of an erysipelas outbreak thereby allowing for a more effective treatment outcome.

bactotype® Mg/Ms Real Time PCR Reagent Trial in Three US Laboratories

Mary Jones Dukes, Nevena Djuranovic

QIAGEN, Germantown, MD

Narrative: Avian mycoplasmosis is caused by several pathogenic mycoplasmas of which *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the most important. MG is believed to cost the worldwide poultry industry over $780 million every year. In the US it is believed to cost over $120 million on egg production alone. The bactotype® Mg/Ms PCR reagent is a real-time multiplex PCR, which allows the simultaneous detection of DNA from both MG and MS. An internal control that detects chicken beta actin is also included. The bactotype® Mg/Ms PCR reagent includes reagents necessary for the detection of DNA from MG and MS, as well as positive control. The samples extracted were obtained from swabbing chicken tracheas known to be both positive and negative for MG and MS. The swabs were extracted using the QIAGEN DNeasy® Blood and Tissue Mini Kit. The Ct values were then utilized to create a test panel of 125 "blind" study samples. Each test sample was prepared to allow for triplicate samples to be generated and submitted to the three test locations. The control method selected was an in-house real-time PCR developed with the National Poultry Improvement Plan (NPIP) approved primer sequence. Field test lab observations are similar to in-house developmental validation data. MG detection was down to ~1 copy/sample. MS detection was down to ~10 copies/sample. Similar diagnostic sensitivity and specificity were found as compared to development data with 288 true positive samples (pools included) and 1098 of true negative samples resulting in diagnostic sensitivity of 99.7% and diagnostic specificity of 99.6% as compared to the NPIP approved method. Unexpected positive or negative samples were never repeated in duplicate. All positive and negative DNA test samples tested with the NPIP approved test method yielded results that 100% agreed with QIAGEN's bactotype® Mg/Ms detection method. All positive and negative controls performed as expected. The assay demonstrated outstanding reproducibility with CVs of 4% or less, site depending. The bactotype® Mg/Ms detection method demonstrated high degree of specificity, even in mixed samples, linear and sensitive detection of Mg and Ms DNA and a highly reproducible methodology. Additionally, this method was amenable to pooling and results were concordant with a NPIP-approved method of detection. The IC can be used both as an effective extraction and amplification control and the reagents exhibited overall lab robustness for an effective avian *Mycoplasma* detection.

Performance Evaluation and Validation of Quantitative Real-Time PCR and Competitive ELISA for Detection of *Anaplasma marginale* in Texas Cattle

Megan Schroeder[1], Thomas Hairgrove[1], Sandy Rodgers[1], Chungwon Chung[2], Mangkey A. Bounpheng[1]

[1]Texas A&M Veterinary Medical Diagnostic Lab, College Station, TX; [2]Veterinary Medical Research and Development, Pullman, WA

Narrative: Bovine anaplasmosis is an infectious disease with worldwide distribution caused by *Anaplasma marginale*. *A. marginale* infects erythrocytes causing extravascular destruction and anti-inflammatory reactions resulting in anemia, jaundice, fever, and other acute clinical signs. Animals of any age can be infected, however, disease is most severe in adult cattle (>3 yrs). *A. marginale* is transmitted by transfer of infected erythrocytes through biological (ticks) and mechanical (e.g., flies, contaminated needles, ear taggers, dehorners) methods. Cattle that survive initial infection become lifetime carriers and are important in disease epidemiology. Carriers can cause production problems during restocking when they are introduced into naïve herds or when naïve cattle are introduced into endemic herds. Thus surveillance may be beneficial in disease risk and herd health management. Currently, there is limited data on the prevalence and spatial distribution of *A. marginale* infection in Texas. In order to determine prevalence of infection, robust diagnostic tools that provide accurate and reliable detection are required. Currently, a commercial competitive enzyme-linked immunosorbent assay (cELISA) is widely used for identification of infected cattle. However cross-reactivity among *Anaplasma* species has been reported. In addition, subacute or early infections may be missed due to the delayed antibody response. To address these deficiencies, a quantitative real-time PCR (qPCR) assay for detection of *A. marginale* was developed and performance was compared to the cELISA. Using cELISA determined positive and suspect carriers (n=102) and negative (n=88) reference samples, qPCR diagnostic sensitivity and specificity were 100%. The qPCR assay performance was also compared to cELISA using field samples consisting of 449 samples collected during December, 2012 to April, 2013, from 13 herds located in different geographical regions of Texas. Using this sample population and cELISA as the reference test, the qPCR diagnostic sensitivity was 78% and diagnostic specificity was 100%. Kappa analysis was 0.82 (almost perfect agreement). This sample set was also used to assess the infection prevalence based on geographical region by qPCR and cELISA. cELISA determined prevalence range was 0-89% for the 13 herds; qPCR determined prevalence range was 0-84%; agreement between methods range was 80-97% for respective herds. Both methods identified highest prevalence in the Rolling Plains and Chihuahuan Desert of Texas, which are inhabited by the vector, *Dermacentor albipictus*, and lowest prevalence in Central and East Texas. This result supports the validity of good performance characteristics of the qPCR assay and its ability to identify infection for disease control and management.

Functional Characterization of the Antibiotic Resistance Reservoir in the Chicken Gut Microbiota

Narasimha Hegde, Subhashinie Kariyawasam, Chitrita DebRoy

Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA

Narrative: The emergence and dissemination of antibiotic resistance in bacterial pathogens is a serious emerging problem worldwide. Microorganisms acquire new genes as a result of inter-species (horizontal) transfer of antimicrobial resistance determinants. A potential important factor in the development of antimicrobial resistance stems from animal husbandry practices, in which antimicrobial agents not only are used for treating and preventing animal diseases, but are also administered at sub therapeutic levels to promote faster growth and to improve efficiency of feed conversion into meat. This routine practice may be the driving force in accelerating the emergence of antibiotic resistant bacteria that are subsequently transferred from animals to humans through the food chain, including meat products and fresh produce. The objectives of the present study were to determine the differences in the gut microbiomes of chicken grown under regular condition with application of antimicrobials and those that are grown organically without antibiotics and to determine the prevalence of antimicrobial resistance genes in these two types of chicken. DNA from gut microbiome was extracted directly from fecal samples. Phylogenetic profiling was performed by amplification and sequencing of the 16S ribosomal DNA using universal bacterial 16S primers. Phylogeny was determined using SeqMatch from the Ribosomal Database. Chickens raised on organic diet had *Firmicutes* (34.4%), *Fusobacteria* (40.5%), *Proteobacteria* (22.4%), *Bacteriodetes* (0.4%), and *Actinobacteria* (0.3%) while chickens raised on conventional farm had *Firmicutes* (31.4%), *Fusobacteria* (12.1%), *Proteobacteria* (38.1%), *Bacteriodetes* (10.3%), and *Actinobacteria* (1.3%) in their gut. The results of the investigation reflect the influence of diet on the gut microbiota. Genomic libraries of microbiome are being analyzed to examine the prevalence and variability of the antimicrobial resistance genes that will shed light on the effect of organic diet on the genes conferring antimicrobial resistance in chicken.

An Emerging Strain of *Pasteurella multocida* Capsular type B Associated with Outbreaks of Pleuritis and Peritonitis in New Zealand Calves

Richard Spence

Animal Health Laboratory, Ministry for Primary Industries, Upper Hutt, New Zealand

Narrative: *Pasteurella multocida* has caused at least 19 outbreaks of pleuritis and peritonitis in New Zealand calves since early 2009. On some affected farms significant mortality has been observed (for example 60 dead and 200 treated on one farm). In all outbreaks, *P. multocida* capsular type B was isolated from affected organs; however, hemorrhagic septicemia (HS) was excluded as a cause of disease based on clinical presentation and laboratory test results. Nevertheless, *P. multocida* appeared to be acting as a primary agent and caused an acute septicemia. Molecular typing of outbreak isolates using MLST identified two novel sequence types, ST62 and ST64, that do not cluster with HS-causing strains. Subsequently, random amplification of polymorphic DNA (RAPD), repetitive extragenic palindromic-PCR (REP-PCR) and enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) have provided greater discrimination between isolates. Overall there appears to be one strain (ST62) responsible for the majority of the outbreaks observed to date. There is published evidence that presence of specific virulence genes in *P. multocida* isolates is associated with disease status in bovines. Therefore PCR testing for a range of virulence genes was performed on outbreak isolates. Furthermore, whole genome sequencing was performed on a New Zealand outbreak isolate to allow comparative genomic analysis against HS and non-HS isolates. Increased virulence of this strain may have a genetic basis that may have implications for treatment and control.

Comparison of Culture, PCR, and Fluorescent *In Situ* Hybridization for Detection of *Brachyspira hyodysenteriae* and "*Brachyspira hampsonii*" in Pig Feces # * † +

Bailey L. Wilberts[1], Hallie Warneke[2], Leslie Bower[2], Joann M. Kinyon[2], Eric Burrough[2]

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

Narrative: Swine dysentery associated with infection by either *Brachyspira hyodysenteriae* or "*Brachyspira hampsonii*" has been diagnosed with increasing frequency in grow-finish swine in North America during the last several years. A definitive diagnosis is commonly based on the isolation of strongly beta-hemolytic, ring phenomenon positive spirochetes from culture of mucohemorrhagic feces or colonic tissue or from species-specific PCR assays run on DNA from such samples. While *Brachyspira* culture using selective agars is a highly sensitive assay, it often requires 6 days or longer to complete, resulting in a delay in disease diagnosis. PCR assays, while rapid, are often limited by fecal inhibition. Fluorescent *in situ* hybridization (FISH) assays have been described for the identification of *B. hyodysenteriae* and "*B. hampsonii*" in formalin-fixed tissues; however, these assays require approximately two days or more to complete following fresh tissue collection. Due to the time constraints of currently available assays, a same-day FISH assay was developed to detect *B. hyodysenteriae* and "*B. hampsonii*" in pig feces using previously described oligonucleotide probes (Hyo1210 and Hamp1210) targeting 23S rRNA of *B. hyodysenteriae* and "*B. hampsonii*", respectively. This FISH assay was simultaneously compared to both culture and PCR on pig feces spiked with progressive dilutions of the two target spirochete species to determine the threshold of detection for each assay at 0 and 48 hours after sample preparation. PCR on fresh feces and FISH on formalin-fixed feces had similar levels of detection at both time points for both target spirochete species. Culture was the most sensitive method consistently detecting the two target spirochete species at a minimum difference of three log-dilutions when compared to FISH. Neither PCR nor FISH detected either target species on formalin-fixed or fresh feces, respectively. Additionally, FISH readily detected both target *Brachyspira* spp. in formalin-fixed feces from infected pigs collected as part of a previous experiment. Accordingly, FISH performed on formalin-fixed feces from clinically affected pigs will allow for same-day identification and preliminary speciation of spirochetes commonly associated with swine dysentery in North America.

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

Isolation of *Aurantimonas altamirensis*, a *Brucella canis*-like Bacterium from a Canine Testicle ◊

Thomas J. Reilly, Michael J. Calcutt, Laura Wennerdahl, Fred Williams, Timothy Evans, Irene K. Ganjam, William H. Fales

Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO

Narrative: *Aurantimonas altamirensis* is a Gram-negative coccobacilliary organism recovered from aquatic environments and infrequently reported as a clinical isolate in human medicine. Since the first description of the species in 2006, a limited number of case reports have been published, including isolation from blood, pleural fluid, contact lenses, lens cleansing solution, and trauma-induced corneal ulcers; the clinical-relevance, however, remains uncertain. Here we report the isolation of *A. altamirensis* from testicular tissue (obtained at time of castration) of a two year old Border Collie presenting with fever, sore toes, and scrotal swelling. The bacteriologic culture, plated on Blood Agar MacConkey, and *Brucella* agar (incubated in the presence of 5% CO_2) resulted in light bacterial growth on the latter medium after ~2-3 days incubation. The smooth 2-3 mm diameter colonies were nonhemolytic, KOH and oxidase positive, Gram-negative coccobacilli and were weakly agglutinated when tested with anti-*Brucella canis* antiserum. Like *B. canis*, the isolate was also found to be urease positive though significantly delayed (~60 min) compared to those of the urease-enriched (~1-5 min) *Brucellae* species (*canis* and *suis*). When subjected to the Trek Diagnostic Gram-negative AP80/Sensititre Aris 2X automated microbial identification system, the isolate was identified as *Psychrobacter phenylpyruvicus*. While the tissue was also found upon histopathological examination to harbor *Blastomyces dermatitidis*, it was deemed essential, given the presumptive isolation and identification of *B. canis* from the patient to empirically exclude this canine pathogen from further diagnostic consideration. The isolate was subjected to 16S rRNA sequencing and found to be 100% identical over 1152 base pairs with *Aurantimonas altamirensis*, an organism originally discovered in the microbial wall growth in the Spanish cave of Altamira. The rRNA sequence was 91% identical to those derived from *B. canis*. Although previously reported as an environmental and human isolate, this communication represents the first reported isolation from a veterinary specimen and while of unknown clinical significance with respect to the etiology of the infection, it highlights the critical importance in this case to unambiguously identify the microbe for diagnostic, epidemiological, infection control and public health purposes.

◊ USAHA Paper

Serodiagnosis of Equine Leptospirosis by ELISA using Four Biomarkers ◊

Cuilian Ye[1], WEiwei Yan[1], Patrick L. McDonough[1], Sean P. McDonough[2], Yung-Fu Chang[1]

[1]Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY; [2]Biomedical Sciences, Cornell University, Ithaca, NY

Narrative: Leptospirosis caused by *Leptospira* spp. is one of the most common zoonosis in the world. In a previous study, we have developed an indirect ELISA for the diagnosis of equine leptospirosis (Development of an ELISA using recombinant LigACon4-7.5 as antigen for the diagnosis of equine leptospirosis, CVI, in Press). In this study, we have applied four outer membrane proteins, LipL21, LoaL22, LipL32 and LigACon4-8 of *L. interrogans* to evaluate their potential to be used as antigens for the diagnosis of equine leptospirosis. We have evaluated with equine sera (n= 130) that were microscopic agglutination test (MAT)-negative and sera (n=176) that were MAT-positive to the 5 serovars that most commonly cause equine leptospirosis. The sensitivity and specificity of ELISA were 84.09% and 70.45% when using LipL21; 78.41% and 56.82% when using LoaL22; 77.84% and 64.02% when using LipL32, and 82.39% and 64.77% when using LigACon4-8, respectively compared to MAT. In conclusion, we have developed an indirect ELISA utilizing a recombinant LipL21, LoaL22, LipL32 and LigACon4-7.5 as diagnostic antigens for equine leptospirosis. This ELISA assay was found to be sensitive, specific and concurred with the results of the standard MAT.

◊ USAHA Paper

Epidemiology 2

Sunday, October 20, 2013

Pacific Salon 2

Moderators: Marie Culhane, Amar Patil

8:00 AM **Analysis of Breath Volatile Organic Compounds as a Screening Tool for Disease Detection: Preliminary Studies ◊**
Jack C. Rhyan, Pauline Nol, Randal S. Stahl, Christine K. Ellis, Matthew McCollum, Hossam Haick, Kurt VerCauteren, M.D. Salman *99*

8:15 AM **Sow Serology Reflected in Piglet Litter Oral Fluids: A Field Study for Sow Farm Surveillance**
Apisit Kittawornrat, Ian Levis, Christa K. Goodell, Luc Dufresne, Jeff Zimmerman *100*

8:30 AM **Rapid, Sensitive and Specific Point of Need Detection of Feline Upper Respiratory Disease Complex Associated Pathogens (*Feline herpesvirus*,*Chlamydophila felis*, *Mycoplasma felis*, and *Bordetella bronchiseptica*) using POCKIT™ a Deployable PCR-based Detection System**
Jessie D. Trujillo, Uri Donnett, Cheng-Han Ho, Pin-Hsing Chou, Fu-Chun Lee, Li-Juan Ma, Pei-Yu Lee, Jian-Hau Chiou, Yu-Chun Lin, Yu-Han Shen, Chen-Yi Weng, Shih-Han Weng, Adam Herrick, Peter Nara, Hsiao Fen Grace Chang, Thomas Wang *101*

8:45 AM **Surviving the Transition of a Government Funded Laboratory to a Private Laboratory**
Alan F. Julian *102*

9:00 AM **Stars in our Eyes: Integration of Laboratory Data Across the Australian Veterinary Laboratory Network**
James Watson *103*

9:15 AM **Successful and Unsuccessful Strategies in the Establishment of a Novel Laboratory Information Management System (LIMS)**
Paula Krimer, Matthew A. Blankenship *104*

9:30 AM **Break (45 min)**

9:45 AM **Characterization of H3N2 Swine Influenza Viruses with Genes from 2009 Pandemic H1N1 Virus # †**
Jingjiao Ma, Huigang Shen, Qinfang Liu, Bhupinder Bawa, Michael A. Duff, Richard Hesse, Jianfa Bai, Steven C. Henry, Juergen Richt, Wenjun Ma *105*

10:00 AM **Molecular Characterization and Epidemiology of *Brachyspira hyodysenteriae* in US Swine Herds # * †**
Nandita Mirajkar, Connie Gebhart *106*

10:15 AM **The Trends of Hazardous Substances Exposures in Dogs and Cats, Reported to the Kansas State Veterinary Diagnostic Laboratory: 2009-2013 # †**
Ali Mahdi, Deon Van der Merwe *107*

10:30 AM **Whole Genome Sequencing Reveals the Diversity of Group B and Group C Porcine Rotaviruses**
Elizabeth G. Poulsen, Qing Sun, Chester McDowell, Richard Hesse, Juergen Richt, Gary A. Anderson, Jianfa Bai *108*

10:45 AM **Impact of the Threshold on the Performance of the Interferon-Gamma Detection Assay for Diagnosis of Bovine Tuberculosis**
Julio Alvarez, Maria Luisa De la Cruz, Andres Perez, Sergio Marques, Enrique Pages, Carmen Casal, Yolanda Soriano, Anna Grau, Rosa Diaz, Olga Minguez, Lucas Dominguez, Lucia de Juan 109

11:00 AM **An Assessment of the Likelihood of the Introduction of New Strains of *Foot and Mouth Disease Virus* to Egypt**
Mohamed A. El Bably, N. M. Asma 110

Symbols at the end of titles indicate the following designations:

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

Analysis of Breath Volatile Organic Compounds as a Screening Tool for Disease Detection: Preliminary Studies ◊

Jack C. Rhyan[1], Pauline Nol[1], Randal S. Stahl[1], Christine K. Ellis[1], Matthew McCollum[1], Hossam Haick[2], Kurt VerCauteren[1], M.D. Salman[3]

[1]National Wildlife Research Center, United States Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, CO; [2]Department of Chemical Engineering and Russell Berrie Nanotechnology Institute, Technion – Israel Institute of Technology, Haifa, Israel; [3]Animal Population Health Institute, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO

Narrative: Unique volatile organic compounds (VOCs) or unique patterns of VOCs in the breath have been associated with certain neoplasms and diseases in humans. Additionally, sera from animals with brucellosis and Johnes disease have been found to have distinguishing VOCs. We conducted two limited pilot experiments to examine the breath of animals exposed to or infected with *Mycobacterium bovis* and one study of animals exposed to or infected with *Brucella abortus*. In all studies, samples were collected in a sorbent (Tenax®) and analyzed with gas chromatography/mass spectrometry (GC/MS) and, in two studies, an electronic nose that utilizes a series of nanoparticle sensors (NaNose™). The first experiment involved the collection of breath samples from ten culture or PCR positive cattle and four negative cattle from a dairy naturally infected with *M. bovis* in southern Colorado and from 13 negative cattle from two dairies in northern Colorado. GC/MS analysis identified two VOCs associated with infection and two with health. The NaNose™ identified all infected cattle with 21% false positives in the controls. The second experiment sampled the breath of 16 animals experimentally-infected with *M. bovis* (eight with strain 97-1315 and eight with strain 10-7483) and seven controls. GC/MS analysis differentiated infected from control animals and distinguished between animals infected with the different strains. In the third experiment, we collected breath samples from 20 *B. abortus* seropositive and 17 seronegative bison. GC/MS detected different patterns of VOCs in seropositive and negative bison. The NaNose™ identified all but two seropositive animals with no false positives. Results of these pilot studies are promising. If this technology proves successful, potential applications include screening of individual animals, and wild populations for the presence of specific diseases.

◊ USAHA Paper

Sow Serology Reflected in Piglet Litter Oral Fluids: A Field Study for Sow Farm Surveillance

Apisit Kittawornrat[1], Ian Levis[2], Christa K. Goodell[3], Luc Dufresne[2], Jeff Zimmerman[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Seaboard Farms Inc., Guymon, OK; [3]Livestock, Poultry and Dairy, IDEXX Laboratories, Westbrook, ME

Narrative: Surveillance for *Porcine reproductive and respiratory syndrome virus* (PRRSV) can be done easily, effectively, and inexpensively using pre-weaning oral fluid samples from litters of piglets. Hypothetically, the oral fluid anti-PRRSV antibodies from litters of pigs reflect the PRRSV exposure of both the litter and their dam. Similarly, PRRSV viremia detected from oral fluid of the piglets reflects the status of PRRSV circulation in the sow herd. The objective of this research was to demonstrate an easier, cheaper, and more effective method to detect and monitor PRRSV circulation by use of litter oral fluids. This study was performed in four ~12,500 sow breeding herds. All four herds were considered endemically infected with PRRSV on the basis of routine diagnostics (rRT-PCR and PRRSV antibody ELISA) from growing pigs. Commercial PRRSV vaccines were used intermittently to control clinical losses over the 12 months prior to sampling, with minor differences in vaccination protocols among sites. Gilts entering the sow farms were routinely vaccinated with either PRRS MLV or ATP commercial vaccines. Oral fluid samples were collected from a total of 600 litters of pigs prior to weaning. Serum samples from their dams were collected 3 days after weaning. Samples were frozen (-80°C), randomized and submitted collectively for detection of PRRSV antibodies (PRRS Oral Fluids Ab Test, IDEXX Laboratories, Westbrook, ME, USA; PRRS X3 Ab Test, IDEXX Laboratories, Westbrook, ME, USA) and PRRSV nucleic acid (qRT-PCR and sequencing). Results were analyzed for associations with sow parity, litter size, and farm by analysis of variance (ANOVA) and correlation analyses. Isotype results from both sera and oral fluids sample types showed both PRRS X3 ELISA and PRRS Oral Fluids ELISA corresponded well with the IgG isotype. Over all farms and samples, mean sow sera ELISA S/P ratios were approximately 1.00, while corresponding mean litter oral fluid ELISA S/P ratios were approximately 4.62. Eight of 600 piglet oral fluid samples were confirmed positive by rRT-PCR at two laboratories, representing 3 of the 4 sow farms. Two of these were sequenced (Orf 5) and reported as wild type PRRSV. No sow serum samples were positive for PRRSV by rRT-PCR. Of the 8 litter oral fluids, ELISA S/P ratios ranged from 1.92 to 8.83. Respective dam sera ELISA S/P ratio results ranged from 0.92 to 2.91. These results support that herd PRRSV monitoring programs can be improved by evaluating the dynamics of PRRSV transmission using oral fluid samples collected from litters of pigs prior to weaning. In endemically infected and/or vaccinated herds, piglet oral fluids are a useful tool for surveillance of both PRRSV exposure and shedding in sow herds.

Rapid, Sensitive and Specific Point of Need Detection of Feline Upper Respiratory Disease Complex Associated Pathogens (*Feline herpesvirus*,*Chlamydophila felis*, *Mycoplasma felis*, and *Bordetella bronchiseptica*) using POCKIT™ a Deployable PCR-based Detection System

Jessie D. Trujillo[1], Uri Donnett[1], Cheng-Han Ho[2], Pin-Hsing Chou[2], Fu-Chun Lee[2], Li-Juan Ma[2], Pei-Yu Lee[2], Jian-Hau Chiou[2], Yu-Chun Lin[2], Yu-Han Shen[2], Chen-Yi Weng[2], Shih-Han Weng[2], Adam Herrick[1], Peter Nara[1], Hsiao Fen Grace Chang[2], Thomas Wang[2]

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

Narrative: *Feline herpesvirus* (FHV),*Chlamydophila felis*, *Mycoplasma felis*, and *Bordetella bronchiseptica*, are pathogens associated with Feline Upper Respiratory Disease Complex (FURDC). Proper diagnosis of FURDC pathogens is important for patient care, population medicine and biosecurity. Point-of-need detection of FURD pathogens can provide timely diagnosis, the initiation of appropriate therapy and reduce cost and loss of life associated with infections. Pathogen-specific insulated isothermal polymerase chain reaction (iiPCR) assays in the field deployable device, POCKIT™ were developed for these FURD pathogens and evaluated using clinical samples. Published real-time PCR (qPCR) assays for the detection of FURD pathogens were validated as laboratory reference assays on the BioRad CFX96 thermocycler and used in comparative side-by-side performance evaluation study iiPCR pathogen detection assays on POCKIT™. Limits of detection (LOD) for both platforms were determined using pathogen controls. Randomized feline clinical samples (30 positive and 30 negative) were blind tested in triplicate for FHV, *M. felis,* and *B. bronchiseptica* on POCKIT™. For *C. felis* detection assay evaluation, due to the lack of sufficient positive clinical samples, surrogate positives (dilutions of the pathogen), were utilized. The LOD for reference assays are 1.25 $TCID_{50}$ for FHV, 1 infectious unit (IU) for *M. felis* and *B. bronchiseptica*, and 0.025 IU for *C. felis*. The LOD of iiPCR assays are 1.25 $TCID_{50}$ for FHV, 10 IU for *M. felis* and *B. bronchiseptica*, and 0.25 IU for *C. felis*. Pathogen exclusivity testing performed using nucleic acid concentrations at four logs above assay LOD yielded no false positives. The clinical sensitivity of iiPCR assays for detection of FHV and for both *M. felis* and *B. bronchiseptica* are 96.67% and 90% with a specificity of 96.7% for FHV and 100% for *M. felis*, and *B. bronchiseptica* assays. Kappa values are equal or greater than 0.9. Results demonstrate exceptional performance of point of need iiPCR on POCKIT™, a field deployable device for detection of multiple pathogens associated with FURDC. The iiPCR on POCKIT™ can serve as a user-friendly, rapid, sensitive and specific point of need detection platform for companion animal pathogens.

Surviving the Transition of a Government Funded Laboratory to a Private Laboratory

Alan F. Julian

Gribbles Veterinary, Hamilton, New Zealand

Narrative: New Zealand had a network of government owned and financed laboratories primarily servicing the livestock industry and supporting the disease control schemes. In 1969, five Animal Health Laboratories were consolidated into a unified service called the Veterinary Investigation Service. In 1973, the Central Animal Health Reference Laboratory was established at Wallaceville and the routine diagnostic functions were transferred to the regional laboratories. Wallaceville had the national responsibility for specialized investigations and testing for export certification of animals. In the mid 1980s, the Government required the laboratories to implement cost recovery for services. This allowed them to develop services for the equine industry and for companion animals. Marked development of clinical pathology services then occurred. The first private diagnostic laboratory was established in Auckland in 1986. The regional Animal Health Laboratories were then moved into a State Owned Enterprise: AgriQuality, while the Central Animal Health Laboratory remained part of The Ministry of Agriculture. By 1998, other private laboratories had been established in Hamilton and Wellington. In 2002, AgriQuality sold the Animal Health Laboratories to Gribbles Veterinary and they become a private enterprise. Currently there are two Major private laboratory networks in New Zealand: Gribbles Veterinary and New Zealand Veterinary Pathology, competing for business. The changes in the structure and functions of the laboratories; the marketing strategies that worked or did not; the relationships with veterinary clients; the value of accreditation (IANZ and GLP certification); and the move into non-traditional areas of laboratory work (research trials, drug trials, food testing) will be discussed.

Stars in our Eyes: Integration of Laboratory Data Across the Australian veterinary Laboratory Network

James Watson

Australian Animal Health Laboratory, CSIRO, Geelong, VIC, Australia

Narrative: In common with many countries, integration and exchange of laboratory data amongst veterinary laboratories has been a long held goal in Australia. A recent project titled "STARS" (Sample Tracking and Reporting System) has made significant progress in advancing this goal. Automated exchange of case data between Laboratory Information Systems in different jurisdictions is now occurring routinely in Australian government veterinary laboratories. The concept has also been extended to support networked quality control and proficiency testing for the national veterinary laboratory network, which operates a system of nationally harmonized decentralized testing for key diseases. The project presented a number of challenges and a variety of strategies were employed to engage the participants and ensure delivery of project outcomes. These ranged from technical engagement with data managers to advocacy with senior management across different government agencies. Data standardization was recognized as a key requirement for the project and also as a major risk to its success. Getting broad agreement to, and implementing, data standards is a complex and time-consuming process that presents a considerable up-front barrier to successful integration. Recognizing the challenges in establishing data standards across a heterogeneous range of data sources and owners, the project took a flexible approach to the challenge, working to 1) establish standards by, as appropriate, adopting or adapting existing standards, codify existing agreements as standards and establish new standards by consensus from existing systems; and 2) simultaneously providing a translation layer to enable systems that have varying levels of compliance to the standards to communicate effectively. This approach provided a rapid path to implementation so that the system could achieve key functional goals early, while the details of the data standards can evolve towards true integration over time.

Successful and Unsuccessful Strategies in the Establishment of a Novel Laboratory Information Management System (LIMS)

Paula Krimer[1], Matthew A. Blankenship[2]

[1]Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, Athens, GA;
[2]Information Technology Services, College of Veterinary Medicine, University of Georgia, Athens, GA

Narrative: Recently, the Athens Veterinary Diagnostic Laboratory (AVDL) tested and implemented a new laboratory information management system (LIMS), VetView®, designed by an information technology (IT) development team at the University of Georgia. The process of software testing, personnel training, implementation strategy, solution launch, and post-conversion issue management required intense focus and buy-in at all levels. Key success factors included establishing a multi-tiered team approach with central project management, active rapid communication, weekly meetings with section leaders, integrating testing with training, central structured problem reporting, synchronizing time commitments, adequate IT resources, and upper management commitment. Unsuccessful strategies, attempted then abandoned, included relying solely on IT and "super-users" for testing, a structured internal ticketing system to report/track issues, passive communication, and infrequent informal meetings. Implementation phase was crafted around structured technical steps and laboratory business process workflows in accession receiving, sample analysis, and the billing cycle. Staff availability and caseload were utilized to estimate probable durations of tasks. During and immediately following implementation, increased availability of IT and VetView personnel was critical for effective response to unanticipated issues, most of which were related to legacy hospital information system (UVIS) data conversion and integration via the Rhapsody message broker, and a client facing web portal. Small scale case and simulated load testing did not reveal performance inefficiencies and dynamics that became challenge points for staff during routine daily use. Care in accounting for testing fatigue and morale deficits was vital to success. Ongoing refinement of VetView was most successful when implementation strategies were maintained.

Characterization of H3N2 Swine Influenza Viruses with Genes from 2009 Pandemic H1N1 Virus # †

Jingjiao Ma[1], Huigang Shen[1], Qinfang Liu[1], Bhupinder Bawa[1], Michael A. Duff[1], Richard Hesse[1], Jianfa Bai[1], Steven C. Henry[2], Juergen Richt[1], Wenjun Ma[1]

[1]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS; [2]Abilene Animal Hospital PA, Abilene, KS

Narrative: Swine influenza is a contagious and zoonotic disease that causes economic losses to pork producer and poses threat to public health. The predominant subtypes of *Swine influenza virus* (SIV) circulating in pigs are H1N1, H1N2 and H3N2 subtypes worldwide. Since the introduction of 2009 pandemic H1N1 (pH1N1) into swine, reassortant viruses between endemic SIVs and pH1N1 have been found in pigs worldwide including the US. In this present study, 60 SIVs (including 18 H1N1, 23 H1N2 and 19 H3N2 isolates) have been isolated from diseased pigs from US Midwest swine farms. The HA, NA and M genes of all the isolates were sequenced. Furthermore, the full genome was sequenced for isolates that contain M gene from the pH1N1. Phylogenetic analysis showed that only 1 out of 18 H1N1 viruses has M gene from pH1N1 and 15 out of 23 H1N2 subtype viruses contain one to six genes from pH1N1; 10 out of 19 H3N2 viruses are novel reassortant viruses harboring at least M gene from the pH1N1. Herein, we characterized 3 novel reassortant H3N2 viruses in vitro and in pigs using an endemic H3N2 SIV as a control. These 3 novel reassortant H3N2 viruses contain 3 (NP, M and NS) or 5 genes (PB2, PA, NP, M and NS) from pH1N1 and the remaining genes from endemic SIVs. All 3 novel reassortant H3N2 viruses grew to higher titers than the control endemic H3N2 SIV in canine, swine and human cell lines. In the pig study, all 3 novel reassortant viruses replicated efficiently in lungs and transmitted to sentinel animals, similar to the control endemic H3N2 virus. The novel reassortant viruses with 3 genes (NP, M and NS) from pH1N1 were more transmissible when compared to the reassortant virus with 5 genes (PA, PB2, NP, M and NS) from pH1N1. Furthermore, concurrent molecular surveillance showed that the novel H3N2 virus with 3 genes from pH1N1 is continually isolated from swine herds. All these results indicate that novel reassortant H3N2 virus with 3 genes from pH1N1 may be maintained and become a dominant virus circulating in swine populations in North America.

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

Molecular Characterization and Epidemiology of *Brachyspira hyodysenteriae* in US Swine Herds # * †

Nandita Mirajkar[1], Connie Gebhart[1, 2]

[1]Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN; [2]Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, MN

Narrative: Swine dysentery, caused by *Brachyspira hyodysenteriae*, is a mucohemorrhagic diarrheal disease commonly seen in grower-finisher pigs and is responsible for significant economic losses to swine-rearing countries worldwide. It was largely eliminated from the US in the 1990s, although it continued to cause significant economic losses globally. Recently, however, this disease has re-emerged in US swine herds. Currently, no information exists for strains of *B. hyodysenteriae* circulating in the US and therefore their potential epidemiological significance is unknown. To characterize *B. hyodysenteriae* strains and investigate their distribution, phylogeny and epidemiology, we conducted multi-locus sequence typing (MLST) analyses. Fifty-eight *B. hyodysenteriae* isolates, originating from 42 sites, 17 swine systems and nine states across the US, were genotyped and analyzed on three levels (intra-site, intra-system and international) using an established MLST scheme based on seven housekeeping genes. Isolates were characterized by their sequence types (allelic profiles), and each unique sequence type (ST) represented a different strain. Sequence type information for 226 global isolates (PubMLST *Brachyspira* database) was utilized for international comparisons. All identified US *B. hyodysenteriae* STs were unique (13 novel alleles and 12 novel STs assigned) as compared to those previously reported for the 226 global isolates. The US *B. hyodysenteriae* isolates represented 13 different STs and a predominant ST was identified from 40% of the sites analyzed. With one exception, each site had only one ST and in general, a common ST was found in sites within a system. Isolates representing ST 56 (same ST as that of the *B. hyodysenteriae* type strain ATCC® 27164TM isolated from the US in the 1970s) were identified, and they were found to be closely related to isolates representing ST 94. Preliminary data from another study conducted on a subset of these isolates (antimicrobial susceptibility testing of *B. hyodysenteriae* isolates) was used to identify potential association of antimicrobial susceptibility patterns with STs; however, no obvious association was found. In fact, STs were generally associated with the site, system and location of sample isolation. This is the first study to characterize the strains of *B. hyodysenteriae* currently circulating in swine herds across the US and to elucidate their diversity, distribution and epidemiology. It also highlights the utility and significance of MLST in further molecular epidemiological investigations, dissemination studies and surveillance.

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

The Trends of Hazardous Substances Exposures in Dogs and Cats, Reported to the Kansas State Veterinary Diagnostic Laboratory: 2009-2013 # †

Ali Mahdi, Deon Van der Merwe

Kansas State Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS

Narrative: Pet dogs and cats in the US are commonly exposed to potentially hazardous substances found in domestic environments. The Kansas State Veterinary Diagnostic Laboratory (KSVDL) provides free telephone consultation services to the general public. Requests for assistance and advice regarding exposures in pet dogs and cats to substances, perceived by their caretakers to be potentially harmful, included 2182 phone calls over a four-year period from 2009 to 2013. Calls were received from all states of the US. California, Florida and Kansas were the three states that generated the highest numbers of calls. Inquiries occurred more often during summer (June, July, and August). Dogs were involved in 83.06% of calls, and cats in 16.94%. Routes of exposure were oral (95.16%), dermal (3.94%), inhalation (0.7%), and parenteral (0.2%). Therapeutic drugs (89.9% human drugs and 10.1% veterinary drugs) were the most frequently reported substances, accounting for 33.49% of calls (NSAIDs and acetaminophen were the largest group involved), followed by household products (16.72%; cleaner and detergent were the most frequent); foods (13.89%; cocoa-based products were the most common); pesticides (14.45%; insecticides were the highest); plants (13.91%); industrial chemicals and fertilizers (3.53%); personal care and cosmetics products (2.55%); and animal, insect or microorganism-derived toxins (1.46%). Requests for information or assistance are not a direct measure of poisoning incidence, but it can provide insight regarding relative exposure rates, help to identify changing exposure trends and emerging exposures, and reflect the public concern regarding actual or apparent harmful exposures in pets.

AAVLD Trainee Travel Awardee (Toxicology)
† Graduate Student Oral Presentation Award Applicant

Whole Genome Sequencing Reveals the Diversity of Group B and Group C Porcine Rotaviruses

Elizabeth G. Poulsen[1], Qing Sun[1], Chester McDowell[2], Richard Hesse[1], Juergen Richt[2], Gary A. Anderson[1], Jianfa Bai[1]

[1]Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; [2]Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS

Narrative: *Rotavirus* infections cause severe gastroenteritis in both humans and animals and are responsible for approximately 600,000 human deaths per year worldwide, mostly in children in developing nations. While *Rotavirus A* is the most common, groups B and C also cause devastating infections in production animals. The different groups of *Rotavirus* are morphologically indistinguishable, and their genomes comprise the same number of segments. However, the sequences are drastically different, challenging new vaccine and diagnostic method development. Sequences of porcine rotavirus groups B and C are poorly documented, with only a handful of sequences published for only a handful of the eleven genome segments. Porcine fecal samples of suspected non-group A *Rotavirus* were received by Kansas State University Veterinary Diagnostic Laboratory over a six month period, and RNA was isolated using Trizol reagent (Ambion) and DirectZol filter columns (Zymo Research). Full-length cDNA was produced by the single primer amplification technique and sequencing was performed on a next-generation sequencing platform, MiSeq (Illumina). Assembled sequences were classified by *Rotavirus* genotype and by gene segment. Two subtypes of group B VP4 were found to have 60% sequence identity to each other, and 61% identity to their closest match on NCBI, GQ358712. All group B VP7 segments were nearly identical to each other, and showed the highest identity with published sequences out of all segments, at 95% identical to their closest BLAST hit, JQ043814 (segment VP7 has the largest number of published sequences of all porcine group B rotavirus segments). Two subtypes of group B NSP4 were 94% identical to each other, but only 59% identical to their closest BLAST hit, AY539864. All group B NSP3 genes were nearly identical to each other, but the best match found on NCBI had only 55% identity (L09722). Two subtypes were detected for group C in all eleven gene segments. The identity between subtypes of each segment ranged from 75% to 97% (median 85%), and the range of similarity to published group C segments was between 74 and 94% identity (median 88%). One particular sample yielded eleven group A segment sequences in addition to eleven group B segment sequences, indicating a mixed infection. The results of this study will provide a framework for further research into the genes and genomes of porcine rotavirus groups B and C, and will ultimately provide guidance for new vaccine candidate selection and better diagnostic procedure development for non-group A rotaviruses.

Impact of the Threshold on the Performance of the Interferon-Gamma Detection Assay for Diagnosis of Bovine Tuberculosis

Julio Alvarez[1, 2], Maria Luisa De la Cruz[1], Andres Perez[3], Sergio Marques[4], Enrique Pages[5], Carmen Casal[1], Yolanda Soriano[5], Anna Grau[4], Rosa Diaz[5], Olga Minguez[4], Lucas Dominguez[1], Lucia de Juan[1]

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Narrative: Diagnosis of bovine tuberculosis is the basis for all eradication programs currently in place around the world. Due to the limitations of the tuberculin skin test, typically performed as the first-line screening technique, the interferon-gamma assay has been increasingly applied in several countries for either maximization of diagnostic test sensitivity (parallel use) or specificity (serial use). Alternative protocols with regards to time from blood collection to in-vitro stimulation, antigens used for specific blood stimulation, blood incubation period, and cut-off value (threshold) used for interpretation of results have been applied, potentially leading to important differences in the performance and accuracy of the test. Here, results on the IFN-γ assay performed with bovine and avian purified protein derivative (bPPD and aPPD) from 222 tuberculosis-infected cattle herds (68,166 tests performed on 31,818 animals) and 3 officially tuberculosis free (OTF)-herds (274 tests performed on 176 animals) located in three regions of Spain were analyzed using the alternative cut-off values used in European Member States and in the US (n=9) to evaluate the effect of such variation on test sensitivity and specificity, and determine the most accurate combination for the field application of the test depending on the purpose (i.e., enhanced sensitivity/specificity). Agreement between thresholds was evaluated and post mortem results which were bacteriological analyses of samples collected at slaughterhouses from animals culled from infected herds were used to compute test accuracy. The association between animal-specific factors such as age, production type (dairy, beef or bullfighting) and number of herd-tests performed in infected herds since disclosure of the outbreak, and test accuracy related to each threshold were also computed. Significant differences in the number of reactors detected with each cut-off value were detected. Proportion of animals in which tuberculosis-infection was confirmed by bacteriology also revealed a significant effect of the cut-off associated with the age of the animal and the number of herd tests performed in the herd since disclosure of the outbreak. Results from OTF herds indicated a major impact of the threshold also in terms of specificity: those thresholds performing better in infected herds yielded significantly higher numbers of false positive reactors in OTF herds. Therefore, in order to maximize the performance of the test a feasible option may be the alternative use of different thresholds depending on the epidemiological setting and the purpose of its use, maximize sensitivity in the case of infected settings and/or maximize specificity if the test is going to be used in situations in which the disease is not expected.

An Assessment of the Likelihood of the Introduction of New Strains of *Foot and Mouth Disease Virus* to Egypt

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Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Benisuef University, Benisuef, Egypt

Narrative: Foot and mouth disease (FMD) is endemic in Egypt; widespread outbreaks of clinical disease occur during most years. FMD has been reported in Egypt at numerous occasions between 1950 and 2012.The most recent epizootics were reported by Libya and Egypt in 2012. Several FMD virus types (A, O, SAT2) were associated with FMD outbreaks in Egypt, type O being the most common. Data recorded in recent years, suggest that FMD is becoming enzootic in Mauritania, Egypt and Libya and disease control becomes more complicated because of marked regional differences in the distribution and prevalence of various serotypes and intratypic variants (4–6). During February 2012, a great number of FMD events were reported throughout Egypt despite a nationwide vaccination campaign in January 2012. The analysis of the epidemiological situation and laboratory findings suggested exotic FMDV serotypes or strains might be responsible for the pattern of outbreaks, and actions were agreed to clarify the situation that resulted in confirmation of SAT2 and other serotypes among recent samples from outbreaks. The vaccines currently used in Egypt do not include SAT2. The recent FMD infection is thus apparently sweeping through a wholly susceptible ruminant population. The number of suspected cases is growing at a rate of 5000 per day while the number of deaths is growing at 500 head per day. The mortality rate was increased especially among calves and in small farms, since SAT2 is newly introduced to Egypt and there is no herd immunity or previous vaccination effort. Losses of older cattle will also be significant. FMD impacts both the cattle and buffalo populations, but the effect is normally more severe on cattle. The disease has already been detected in 25 of 27 governorates in Egypt. Regionally, SAT2 first had an outbreak in Libya in 2009 and February, 2012. Movement of animals is considered to be the main risk factor for the introduction of many infectious diseases, including FMD. The estimation of the risk, the spatial variation in the risk, and the factors associated with the risk for FMDV introduction into a country are a prerequisite for the development of differential policies for prevention and eventual control of epidemics. The risk analysis approach gives a country an objective and defensible method of assessing the disease risks associated with different import activities and can allow different risks to be compared more objectively, which should allow more rational decisions to be made to reduce the overall likelihood of disease outbreaks.

Epidemiology 3

Sunday, October 20, 2013

Pacific Salon 6,7

Moderators: M.D. Salman, Belinda S. Thompson

Symbols at the end of titles indicate the following designations:

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

Epizootic Hemorrhagic Disease in Michigan Deer: A Disease on the Increase

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Narrative: Historically epizootic hemorrhagic disease was first reported in the US in 1955. It has truly been an epizootic disease with outbreaks recognized in Michigan deer occurring in 1955, 1974, and 2006; reappearing at approximately 20 year intervals. However, this disease has become a nearly annual occurrence recently, occurring in 2006, 2008, 2009, 2010, 2011, and 2012, and spreading both geographically as well as affecting increasing numbers of wild white-tailed deer. Gross lesions are characterized by widespread hemorrhages, which commonly affect the skin, mucous membranes, skeletal muscles, heart, lungs, and many different abdominal viscera. Subcutaneous edema in the head, neck and limbs is also common. Some affected individuals also exhibit oral ulcerations. Histopathology can be difficult, since wild deer may not be found for hours to days after death, and a significant number are present in natural bodies of water further accelerating decomposition. The lung is frequently the best tissue for histopathology, with lesions including hemorrhage, perivascular edema, intravascular fibrin thrombi, multifocal parenchymal necrosis, and fibrinous pleuritis. Inflammatory cell infiltrates are generally minimal to mild, and predominantly lympho-plasmacytic. Polymerase chain reaction tests are the best microbiologic method of confirming this disease. Our laboratory utilizes lung, liver, spleen, and kidney. For optimal results we routinely dilute the tissues 1:20 or 1:50 to stop non-specific inhibition due to post-mortem autolysis. The orbivirus is closely related to *Bluetongue virus*, and is not associated with any characteristic viral inclusions. This disease appears to be spreading north not only in Michigan, but throughout much of the upper Midwestern and Eastern US. Since the disease is transmitted by *Culicoides* midges, increased ambient temperatures, milder winters, and drought conditions all are believed to encourage the spread of these midges into areas in which they were previously scarce. Whether you believe global warming is a short-term trend or a long-term change, as the temperatures increase we can expect to see changes in disease prevalence or emergence due to changes in vector distribution, newly introduced vectors, and other ecosystem changes. Therefore, it is important for all diagnosticians to remain vigilant and aware of diseases, even those not regularly present in their geographic region.

Equine and Bovine Fluorosis Attributable to High-Fluoride Well Water in Southern California ◊

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Narrative: Dental and skeletal fluorosis adversely impacts health, productivity, and quality of life for millions of people and their livestock throughout the world. However in North America, cases of fluoride (F) toxicosis in horses and cattle are only rarely recognized or described; most published case reports pre-date 1980. Dental lesions are the most sensitive and clinically useful indicator of excess exposure to F. Horses and beef cattle raised on southern California desert ranches were observed to have lesions consistent with dental fluorosis. Nearby municipal drinking water is de-fluoridatated to <4 ppm (mg/L) in compliance with EPA and USPHS safe drinking water standards. Samples obtained from livestock water sources on Ranch X had 12-14 ppm (mg/L) F, more than 3 times the concentration in municipal drinking water. Two locally-raised horses (A, 16 yr. gelding; B, 9 yr mare) and a 4 yr. old beef cow (Cow C) were evaluated for dental fluorosis. Horse A and Cow C had consumed Ranch X water (12-14 ppm F) throughout their lives; Horse B was raised nearby and consumed de-fluoridated municipal water (<4 ppm F). Lesions consistent with published standards for dental fluorosis (pitted, hypoplastic, and stained enamel) affected the incisor teeth of Horse A and Cow C. Post mortem samples of urine, bone, and incisor teeth from all three animals were submitted for toxicological analysis. Tooth samples from Horse A and Cow C measured 1700 and 2400 ppm F, respectively; (normal population reference values, 400-1200 ppm). Analysis of multiple samples of dry fat-free bone measured 2600-4200 ppm F for Horse A, and 5300-5800 ppm F for Cow C. Based on reference values of 3000-5000 ppm (bone) for fluorosis, these results are diagnostic. Analytical results for tooth and bone samples from Horse B (130-920 ppm F) did not exceed published normal values. Other potential sources of excess dietary F which were considered include rock phosphate-based mineral supplements, and forages (e.g., hay) fertilized with high-fluoride rock phosphate. Routine use of commercial mineral supplements was ruled out, but no data was available to evaluate the F content of commercial forages consumed by these animals throughout their lifetimes. As a micronutrient, F has a very narrow margin of safety; the F concentration in Ranch X livestock drinking water is 6-7 times higher than the 2 ppm currently recommended by the National Academy of Science and the US EPA. Lifetime exposure to 12-14 ppm F was sufficient to cause both dental and skeletal fluorosis in the two animals submitted from Ranch X. These findings potentially have broader implications regarding high F in livestock drinking water as a limitation to animal health and livestock production in southern California.

◊ USAHA Paper

Wainaku Grass (*Panicum maxiumus*) Endophyte (*Cladosporium* spp.) Toxicosis in Horses

Hana Van Campen[1], Brady Bergin[2], Terry Spraker[1], Gayle Thompson[1], Anita Holman[3], Morrie Craig[3]

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Narrative: The elaboration of tremogenic mycotoxins by endophyte infested rye grasses, and the resulting intoxication of horses is commonly referred to as "grass staggers" [Osweiler GD: 2001, Mycotoxins. Vet Clin North Am Equine Pract 17:547-566, viii; and Galey FD: 2009, Disorders caused by toxicants. *In*: Smith BP, ed, Large Animal Internal Medicine, 4th edition, Mosby/Elsevier, pp. 1707-1708]. In November 2012, 3 of 4 ranch horses on the island of Hawai'i presented with neurologic signs and dyspnea. The horses were afebrile, bright and alert. Two affected horses had normal CBCs and elevated serum CPK and LDH levels. Nasal swabs and blood samples were negative for EHV-1 DNA by PCR. Other viral encephalitides including West Nile, western and eastern equine encephalitis were excluded as these viruses are not found in the Hawaiian Islands. The horses had been placed in a pasture dominated by *Panicum maximus* for 3 weeks prior to the onset of signs. *Panicum maximus* is related to *Panicum repens* locally known as Wainaku grass [Whitney LD, Hosaka EY, Ripperton JC: 1939, Grasses of the Hawaiian Ranges. Hawaii Agricultural Experiment Station Bulletin 82. University of Hawaii, pp.85-87, Fig. on p. 84]. Dried samples of the grass were analyzed by HPLC with fluorescence detection followed by time-of-flight (TOF) MSMS-MSMS. The chromatogram revealed a unique compound distinct from the lolitrem B controls. Enlarged kidneys and discolored lungs were the only grossly observed changes on necropsy. Histopathologic findings included severe cerebral edema with widespread neuronal necrosis and microvascular hemorrhage, acute pulmonary edema, pulmonary congestion and hemorrhage with multifocal necrosis of alveolar walls without evidence of inflammation, and mild renal tubular necrosis. A fungus was isolated on potato dextrose agar from seed heads, and was identified as a species of *Cladosporium* based on morphology. Histopathologic sections of black (parasitized) seeds revealed sclerotia which were not present in green seeds. This case of neurologic and respiratory disease in horses is associated with ingestion or exposure to a potential mycotoxin produced by *Cladosporium* spp. infested *P. maximus* seed heads that chromatographs and excites and fluoresces at the same wavelength as lolitrem B [Hovermale JT, Craig AM: 2001, Correlation of ergovaline and Lolitrem B levels in endophyte-infected perennial ryegrass (*Lolium perenne*). J Vet Diagn Invest 13:323-327].

Epidemiology of Intoxication by *Cestrum parqui* in Cattle in the Northwest of Uruguay

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[1]Northwest Regional Laboratory, DILAVE "Miguel C. Rubino", Paysandú, Uruguay; [2]Faculty of Veterinary Medicine, Universidad de la República, Paysandú, Uruguay

Narrative: From 1998 to 2012, the database of the Northwest Regional Laboratory DILAVE Miguel C. Rubino diagnosed 224 outbreaks of diseases in cattle of toxic etiology, 45 of which (20%) were associated with *Cestrum parqui*. This intoxication is endemic in the littoral region of Uruguay, with epidemic peaks related mainly to drought and forage shortages. Most of the outbreaks were observed in areas where soils have good fertility with native forest and/or near to rivers. Cases were presented in autumn (31%), winter (28%) and spring (26%), affecting principally animals under 2 years old (59%).The morbidity and mortality was from 0.67% to 100% and lethality, 60-100%. Sixty per cent of the outbreaks occurred in beef cattle farms, whereas 28.9% were in dairy farms. Although the toxic principle is not fully understood, currently, carboxyatractyloside is considered the cause of the clinical signs, gross and histologic lesions. Several authors indicate that the toxicity of the plant is maintained throughout the year, while other studies indicate it to be present with seasonal variation. For clarification of this question, during winter and spring of 2011, green leaves of *Cestrum parqui* were administered to seven calves at single doses of 10, 20 and 30 grams per kilogram of body weight (g/kg BW). In spring, a calf received 20 g/kg BW at repeated doses (total of 40 g/kg BW). At single dose of 10 g/kg BW in both seasons, no changes were found in plasma levels of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), with similar values to the control calf, showing no hepatotoxicity. At single doses of 20 and 30 g/kg BW in the two seasons, hepatotoxic effect was verified through an increase in plasma levels of hepatic enzymes AST and GGT and clinical signs, but none of the animals died. At these doses were not recorded seasonal variations in toxicity. In repeated doses of 20 g/kg BW the calf dead within 48 hours, showing symptoms, gross and histological lesions compatible with intoxication by *C. parqui*. Bibliographic references that mention seasonal variations in the toxicity are closely related to epidemiological factors. It is necessary to continue studying them, because *Cestrum parqui* is the plant that causes more outbreaks in the northwest of Uruguay.

Fatty Liver Hemorrhagic Syndrome in the Backyard Chicken: A Retrospective Histopathologic Case Series

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Narrative: "Fatty Liver Hemorrhagic Syndrome" (FLHS), characterized by sudden death in over-conditioned hens due to hepatic rupture and suffusive hemorrhage is one of the leading non-infectious idiopathic causes of mortality in backyard chickens. Nutritional, genetic, environmental and hormonal factors, or combinations of these, have been proposed as the underlying cause. However, proof of a direct causal association with any of these factors has not been demonstrated. In an attempt to characterize the hepatic changes leading to the syndrome, 76 backyard chickens that presented for necropsy to the CAHFS, Davis diagnostic laboratory that were diagnosed with FLHS between January, 2007 and September, 2012 were included in this retrospective case study. The sex, age, laying activity and nutritional condition of the birds were recorded when available. A majority of the birds were female (99%), obese (97.5%), and in active lay (69.7%). Livers were examined histologically, and the degree of hepatocellular vacuolation (lipidosis), the reticular stromal architecture, the presence of collagenous connective tissue, and vascular wall changes were evaluated and graded using hematoxylin and eosin, reticulin, oil red O, Masson's trichrome, and Verhoeff-Van Gieson stains. Interestingly, there was no correlation between lipidosis and reticulin grades, and hepatocellular lipidosis was absent in 22% or mild in an additional 26% of the cases. Furthermore, there was evidence of repeat bouts of intraparenchymal hemorrhage and hematoma formation prior to the acute "bleed-out" in 35.5% of the cases. This data is not supportive of the previously proposed causes, and provides a framework for future studies to elucidate the pathogenesis of this highly prevalent condition. In addition, FLHS has been historically used as a descriptive name of the disease since the initial presentation of the entity, and the data shown in this study advocates for "Hemorrhagic Liver Syndrome" as a more accurate name, as hepatic lipidosis is absent in a significant proportion of ruptured livers.

Influenza A virus (IAV) Surveillance on Vaccinated Sow Farms using Piglet Pre-weaning Oral Fluid Samples

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[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Statistics, Iowa State University, Ames, IA; [3]Seaboard Farms, Inc., Guymon, OK; [4]Tetracore, Inc., Rockville, MD

Narrative: *Influenza A virus* (IAV) circulates endemically in all age groups in contemporary swine herds, including suckling pigs. That is, IAV transmission and infection occurs in piglets despite the presence of maternally-derived antibodies. The purpose of this study was to evaluate the feasibility of influenza surveillance using pre-weaning oral fluid samples from litters of piglets. Four commercial swine herds vaccinated with autogenous vaccine participated in the study. Gilts were vaccinated at 9, 12, and ~24 weeks of age and again one week post-farrowing. Oral fluid samples were collected from 600 litters 24 hours prior to weaning and serum samples from their dams post-weaning. Litter oral fluid samples were completely randomized and tested for IAV (virus isolation, RT-qPCR, subtyping and sequencing). Sow serum and litter oral fluid specimens were tested for IAV nucleoprotein (NP) antibody using a commercial NP blocking ELISA (IDEXX Laboratories, Inc.) and NP isotype-specific assays (IgM, IgA, and IgG). All litter oral fluid specimens (n = 600) were negative by virus isolation. Twenty-five oral fluid samples were positive by RT-qPCR, based on screening and then confirmatory testing in 2 diagnostic labs. Dual H1N1 and H3N2 infections were found in one farm. All RT-qPCR-positive oral fluid samples (n=18) submitted for sequencing hemagglutinin (HA), neuraminidase (NA), and matrix (M) genes. Attempts at sequencing of HA and NA genes were unsuccessful, but M gene sequencing was successful. Genetic analysis demonstrated that the M genes were identical and were not closely related to the pandemic M gene. Mean of sample-to-negative (S/N) ratios by commercial NP blocking ELISA were not significantly different between litter oral fluid and sow serum (0.33 vs. 0.28). Sow parity had no significant effect on the level of IgM, IgA, and IgG antibodies detected in both sow serum and litter oral fluid. The circulation of IAV in vaccinated sow herds was detected in oral fluid samples collected from litters of pigs prior to weaning. Thus, influenza surveillance can be done more easily, effectively, and cheaply using pre-weaning oral fluid samples from litters of piglets.

A Triplex Real-Time PCR Assay for the Detection and Differentiation of *Canine parvovirus* 2a, 2b, and 2c Genotypes

Jianfa Bai, Baoyan An, Joe Anderson, Richard Hesse, Richard D. Oberst, William Fortney, Gary Anderson

Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS

Narrative: *Canine parvovirus 2* (CPV2), which causes severe diarrhea with high mortality in dogs, was identified in the 1970s. CPV1, also known as canine minute virus, does not appear to be clinically significant. CPV2 is highly contagious, and can spread through dog-to-dog contact or through contact with virus-containing feces. A new genotype emerged in the 1980s, and was named CPV2b; the previously identified genotype was named CPV2a. The third genotype, CPV2c, has been identified since the beginning of this century. These compose the 3 major genotypes circulating in dog and cat populations. Sequence analysis indicated that there are a few amino acid residue differences in the capsid VP2 protein among these 3 genotypes, and the differences are conserved in the corresponding genotypes. In a previous study (Hong et al., JVDI, 2007), two minor-grove binding probe-based PCR assays targeting nucleotide residues that code for these different amino acid residues were developed to detect all 3 genotypes in 2 real-time PCR reactions: one for the detection and differentiation of 2a/2b strains; the other for 2b/2c strains. We have developed a TaqMan-based, triplex real-time PCR assay that targets on the same region, but is formulated into a single-tube assay that can detect and differentiate all 3 CPV2 genotypes in one PCR reaction. A strong positive sample from each genotype was used for the initial sensitivity analyses with 10-fold serial dilutions. The correlation coefficients for all 3 genotypes were 0.999; the PCR amplification efficiencies were 95.4%, 92.6% and 90.8% for 2a, 2b, and 2c genotypes, respectively. Similar results were obtained when all 3 genotype templates were used in the same reaction, which also served as a positive control for later stages of the test development. Tests on 30 isolates or fecal samples identified distinct, single genotypes; no mixed infections, as defined by the identification of more than one genotypes from the same sample, were observed. To further confirm the test results, amplicons of the first 16 samples were subjected to DNA sequencing. The sequencing data were in accordance with the genotypes identified by the newly developed triplex real-time PCR assay.

Prevalence of *Brucella canis* in Recent Diagnostic Samples Analyzed by a Duplex Real-Time PCR Assay

Jianfa Bai, Baoyan An, Brian Lubbers, William Fortney, Gary Anderson

Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS

Narrative: *Brucella canis* is the primary causal agent of canine brucellosis that causes reproductive disorders. The disease is contagious and the organism persists longer in animals than other *Brucella* species. Although mortality is relatively low, morbidity is high. We have previously developed a duplex real-time PCR assay to detect all *Brucella* species, as well as *B. canis* specifically. The common molecular target was designed from the spacer region between the 16S and 23S rRNA genes that is present in all *Brucella* species. The *B. canis*-specific target was designed flanking a nearly 1 kb deletion that occurs only in *B. canis* strains. Our previous study indicated that this duplex real-time PCR detects approximately 50% more positives than the traditional culture method (3 PCR positives for every 2 culture positives). Domestic dog blood samples submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) for diagnosis were used for this study. A total of 1,347 samples were submitted to KSVDL from February, 2012 through May, 2013. Among these submissions, 78 tested positive for *Brucella canis*, which is 5.8% of the total sample size. During the validation process of this PCR assay, there were 36 positive samples identified from a total of 676 domestic dog samples. The positive rate was 5.3%, which is very similar to the 5.8% positive rate on the diagnostic samples. In comparing 24 h to 48 h incubation times, 48 h enrichment expanded the detection limit of the test by ~10 fold (from 14 CFU/ml to 1.4 CFU/ml), thus 48 h was used in our current diagnostic services. The use of extended incubation time could also explain the slightly increased positive rate for the diagnostic samples. The presence of other *Brucella* species would be indicated by our PCR when the target for all *Brucella* species is positive, but *B. canis* is negative, yet no other *Brucella* species were identified among these diagnostic samples. The *B. canis* prevalence data presented in this study was generated from diagnostic submissions, and not from a well-designed prevalence study. However, it provided useful information and may closely represent the percentage of domestic dogs that carry this pathogen in the US.

Pathology 2

Sunday, October 20, 2013

Pacific Salon 1

Moderators: Arthur (Bill) Layton, Francisco Uzal

Symbols at the end of titles indicate the following designations:

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

Diagnosis and Characterization of *Parelaphostrongylus tenuis* in Mule Deer of Nebraska 2006-2012 # * † +

Elisa Salas, Bruce W. Brodersen, Dave Oates

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

Narrative: *Parelaphostrongylus tenuis* is a nematode of the Metastrongyloidae family that has a complete life cycle in the white-tailed deer. Infection is accidental in many other ruminant and camelid species, and one guinea pig resulting in eosinophilic meningoencephalitis. Mule deer (*Odocoileus hemionus*) were first identified to have clinical signs of circling, emaciation, ataxia, lethargy weakness and lack of fear in 2005. Over a seven-year period (January 1, 2005 to January 30, 2013), 139 deer brains were examined. Retrospective analysis of slides and databases revealed 6/139 had grossly identifiable nematodes and 19/139 had histologic lesions. Histologic lesions consisted of cross sections of lymphoplasmacytic meningoencephalitis (60/139) with rare eosinophils; nematode larvae (17/139); cross sections of adults (6/139); and linear areas of malacia, astrofibrosis, low to moderate spheroids, and mild infiltrate of lymphocytes and plasma cells and very rarely eosinophils. Cut profiles of adults nematodes were characterized by 1-2 μm thick cuticle, polymyarian coelomyarian musculature, lateral chords, a pseudocoelom, an intestinal tract lined by few multinucleated cells, and either ovaries or testes consistent with a Metastrongyloid nematode. Other diagnostic tests including bacteriologic culture of brain specimens, testing for *Bluetongue virus*, enzootic hemorrhagic disease, *West Nile virus*, and *Bovine viral diarrhea virus* were negative. *P. tenuis* was confirmed by digestion of tissue blocks (8 deer, 29 blocks), PCR, and BLASTn analysis (3 deer). These data are supportive of infection of multiple mule deer and the expansion of *P. tenuis* beyond its normal range from the eastern seaboard to Nebraska following migration of their normal hosts, white-tailed deer. This is the first description of the pathology and definitive diagnosis of *P. tenuis* in naturally infected mule deer. [1. Oates DW, Sterner MC, Boyd E: 2000, Meningeal worm in deer from western Nebraska. J Wildl Dis 36:370-373. 2. Tanabe M, Gerhold RW, Beckstead RB, et al.: 2010, Molecular confirmation of *Parelaphostrongylus tenuis* infection in a horse with verminous encephalitis. Veterinary Pathology Online 47:759-759. 3. Thomas LJ, Cahn AR: 1932, A new disease in moose. I. Preliminary report. J Parasit 18:219-231. 4. Tyler GV, Hibler CP, Prestwood AK: 1980, Experimental infection of mule deer with *Parelaphostrongylus tenuis*. J Wildl Dis 16:533-540.]

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

Impact of Silver Nanoparticles on the Serum Enzymes and Liver Pathology of Broiler Chickens During the Starter Period # † +

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[1]Animal Science, Islamic Azad University, Sanandaj, Islamic Republic of Iran; [2]Clinical Pathology, Islamic Azad University, Sanandaj, Islamic Republic of Iran

Narrative: This research was conducted to evaluate the effects of different levels of silver nanoparticles (Ag-NPs) on liver pathology and some related enzymes of broiler chickens. A total of 240 one-day-old male broilers (Ross 308) were used. Birds were randomly divided into 4 experimental groups of 60 birds and 15 birds in each pen. Trial diets were the following: T1 (control, without Ag-NPs), T2, T3, and T4 basal diet supplemented with 4, 8, and 12 mg Ag-NPs/Kg diet, respectively. The birds were maintained on those treatments from 1 to 21 days of age with free access to feed and water throughout the experimental period. At the end of the trial, four birds (one bird per replicate) with weight closest to mean weight of any treatment were selected. Blood samples were collected from the bronchial vein, centrifuged and removed serum stored at -20°C until analysis. Then birds were slaughtered, liver removed and after cleaning with PBS, stored in 10% formalin until further examination. Results indicated those on the diets supplemented with 8 and 12 mg Ag-NPs (T3 and T4) had significantly increased ($P \leq 0.05$) serum glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, lactate dehydrogenase, and malondialdehyde (MDA) compared to control and T2 treatment. Histopathology examination of liver revealed no pathologic findings in the control (T1) and T2 groups. However, the liver of birds fed the diet supplemented with 8 (T3) or 12 (T4) mg Ag-NPs/kg had hepatocyte necrosis, vacuolation of hepatocyte cytoplasm, and biliary hyperplasia. The liver of birds in T4 (fed the diet with 12 mg Ag-NPs) had chronic degree of necrosis and observed local hemorrhage. In conclusion, exposure of birds to Ag-NPs at concentrations >4 mg had an adverse affect on the liver enzymes indices, and microscopic structural liver damage that could decrease growth performance and overall health of broiler chickens.

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

Characterization of Ionized Calcium Binding Adapter Molecule 1 (Iba1) as an Immunohistochemical Marker for Canine Macrophages, Dendritic Cells, and Cutaneous Round Cell Tumors # * † +

Kenneth Kim[1], Christine Zewe[1], Daniel Paulsen[1, 2], Fabio Del Piero[1], Nobuko Wakamatsu[1]

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Narrative: A spectrum of cutaneous and systemic histiocytic diseases is well-recognized in dogs. Biopsies of canine cutaneous round cell tumors also represent a large portion of the veterinary diagnostic pathology caseload. Yet the current standard of macrophage detection requires interpretation of membrane surface marker intensity and multiple immunohistochemistries (CD18, CD11 a-d, lymphocyte), thereby increasing cost and room for technical and interpretive error. Other macrophage markers such as lysozyme and myeloid-histiocytic antigen lack in some combination of sensitivity and specificity. Thus there is justification and need for the development of a single sensitive and specific pan-macrophage marker to answer the simple but essential question, "Are these macrophages or not?" Ionized calcium binding adapter molecule 1 (Iba1) has recently been demonstrated to mark subdural histiocytic sarcomas and microglia in dogs. Brief undocumented mention has also been made of Iba1's utility as a pan-macrophage marker. In the current study we used enzyme immunohistochemistry to morphologically determine that Iba1 (Wako Chemicals USA, Inc.) is a sensitive and specific marker for all cells of the monocyte-macrophage lineage in 5 spleens, 4 lymph nodes, 1 tonsil, 1 thymus, 1 gut associated lymphoid tissue, 4 lungs, 2 livers, and 1 case of granulomatous meningoencephalomyelitis. Staining was also seen in scattered intravascular mononuclear cells (presumed monocytes). All positive and negative controls worked as expected. We determined that Iba1 does not cross react with neutrophils or eosinophils by staining 3 suppurative and 3 eosinophilic lesions. We also found sensitive and specific neoplasm staining in 4 cases of cutaneous histiocytoma. Specificity for cutaneous histiocytoma was confirmed with negative staining of other cutaneous round cell tumors: 2 mast cell tumors, 2 amelanotic melanosarcomas, 1 plasmacytoma, and 2 mycoses fungoides. We conclude that Iba1 is a sensitive and specific marker for all morphologically recognized cells of the monocyte-macrophage lineage in the aforementioned tissues, and that Iba1 is a sensitive and specific marker for cutaneous histiocytoma. Iba1's cytoplasmic localization also allows opportunities for further characterization and investigations including co-staining with most other macrophage markers.

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

Veronaea botryosa: A Potentially Zoonotic, Emerging Fungal Pathogen in Farmed White Sturgeon (*Acipenser transmontanus*) # * † +

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Narrative: A periodic increase in mortality of sub-adult (5-6 year old) white sturgeon (*Acipenser transmontanus*) from an aquaculture facility in California occurred between 2006 and 2012. Affected sturgeon were lethargic and lingered on the bottom of the tank. Bath treatments with salt and hydrogen peroxide yielded no benefits. Multiple sturgeon were submitted for diagnostic necropsy, most recently in 2012. Grossly, the fish had dark brown-black pigmentation of the cranial hematopoietic organ and multiple, coalescing, pale tan to dark pink renal and hepatic nodules that were occasionally delineated by a dark red halo. The gills were grossly unremarkable. Microscopically, the cranial hematopoietic organ, liver, kidney, and gills contained pyogranulocytic to granulomatous inflammatory foci, some of which formed well-defined, coalescing pyogranulomas. There were abundant narrow, intralesional, pigmented fungal hyphae and numerous oval to round yeast-like structures. Fungal cultures of kidney grew dark grey-brown, velvety colonies exhibiting mostly 2-celled, pigmented conidia borne terminally and along the sides of the geniculate conidiogenous portion of long, dark conidiophores. Sequencing of the ITS and D1/D2 rDNA loci demonstrated 99-100% identity with *Veronaea botryosa*. Retrospective examination of the UC Davis hospital case database revealed 3 additional sub-adult white sturgeon with similar lesions from the same facility. *Veronaea botryosa* was cultured and identified via sequencing from 2 of the 3 fish. *Veronaea botryosa* is an environmental black mould within the order Chaetothyriales. This order also includes *Exophiala* spp., which commonly infects aquatic animals. *Veronaea botryosa* has a worldwide distribution and has been cultured from numerous sources including water reservoirs, soil, and plant material. In humans, infections caused by *V. botryosa* are classified as subcutaneous or systemic/disseminated, and can be mutilating and debilitating. Lesions are granulomatous or pyogranulomatous plaques and nodules with microabscessation, exudation, and crusting. To the authors' knowledge, there are 11 published cases of human phaeohyphomycosis due to *V. botryosa*; none have been published in the veterinary literature. The majority of infected humans were immunosuppressed secondary to anti-transplant rejection drugs, prolonged oral corticosteroid use, or numerous comorbidities. However, cases have been described in immunocompetent individuals. The stress of intensive monoculture, including visualization of the gonads via a coelomic incision to determine sex, stage of egg maturity, and the collection of eggs, may result in clinical immunosuppression and subsequent phaeohyphomycosis. As a substantial investment of resources occurs by the time sturgeon reach their 6th year, these cases constitute a financially significant, potentially zoonotic, emerging pathogen of farm-raised sturgeon.

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

Detection and Molecular Characterization of a Novel Type of *Canine papillomavirus* Isolated from a Dog with Multiple Bowenoid *In Situ* Carcinomas with Focal Progression to Squamous Cell Carcinoma # * +

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Narrative: Bowenoid *in situ* carcinoma is best described in humans, but has also rarely been found in dogs and cats. Present as single tumors in humans, they are often multifocal in dogs and cats. Progression to squamous cell carcinoma has been reported in a small fraction of cases. In humans, Bowenoid *in situ* carcinoma is associated with infection with *Human papillomavirus*. There is some evidence that papillomaviruses also play a role in lesion development in dogs. However, only few reports have confirmed papillomavirus infection in canine lesions. Here we describe a 12-year-old, male, Basenji dog with multiple masses over the right lateral digit, left medial forelimb and left lateral thorax. The masses exhibited histopathological features of Bowenoid *in situ* carcinoma with progression to squamous cell carcinoma in the digit and left medial forelimb. To further investigate the potential association with papillomavirus infection, immunohistochemistry, *in situ* hybridization, and polymerase chain reaction for papillomavirus were performed on formalin-fixed, paraffin-embedded samples representing all three biopsied sites. Positive immunoreactivity for papillomavirus was observed focally within each tumor and in the adjacent hyperplastic epidermis. Positive signals for *Canine papillomavirus* DNA were demonstrated within each tumor using *in situ* hybridization. In addition, positive polymerase chain reaction (PCR) results were obtained with consensus primers, MY09 and MY11, amplifying a 450-bp portion of the L1 gene of papillomaviruses. Sequence analysis and alignment of the amplified DNA revealed only 75% nucleotide sequence identity with other published papillomaviruses in the GenBank database. The new sequence was most similar to *Canine papillomavirus 12*, which was previously detected in canine pigmented plaques. In conclusion, a novel type of papillomavirus was detected within multiple Bowenoid *in situ* carcinomas from a single dog with some of the lesions progressing to squamous cell carcinoma. These results suggest that this papillomavirus may play a role in the development of Bowenoid *in situ* carcinomas in dogs and malignant transformation to squamous cell carcinoma.

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

Fatal Intestinal Inflammatory Lesions from Equine Necropsy Cases from California: A Case Series Study from 1990 to 2013 # * † +

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Narrative: Equine inflammatory digestive diseases are any inflammatory diseases that affect the digestive tract of horses. They can be categorized by location within the intestine. The causes could be mechanical, infectious, toxic or immune-mediated. This case series study was performed to 1) determine the frequency of nine fatal intestinal inflammatory lesions from equine necropsy cases that were submitted to the California Animal Health and Food Safety (CAHFS); 2) identify and describe the most common etiology of the three most prevalent fatal intestinal inflammatory lesions from equine necropsy cases that were submitted to the CAHFS; 3) evaluate possible associations between animal signalment and the form of fatal intestinal inflammatory lesion that affect our population of study. Cases considered were equine necropsy cases submitted to any laboratory of CAHFS during the period of January 1, 1990 to April 16, 2013 with diagnoses of colitis, duodenitis, enteritis, enterocolitis, enteropathy, enterotyphlitis, gastritis, gastroenteritis, ileitis, jejunitis, typhlitis, and typhlocolitis. Seven hundred ten equine necropsy cases fitted our case definition. Colitis, enteritis and typhlocolitis were the three most common fatal intestinal inflammatory lesions from our study population and the most common known etiology of the three lesions was bacterial. The bacteria most commonly identified in colitis, enteritis, and typhlocolitis cases were *Clostridium* spp. and *Salmonella* spp. Lastly, there were associations between animal signalment and both etiology and location of the fatal intestinal inflammatory lesion. This study presents a brief description of cases that were submitted to the California Animal Health and Food Safety Laboratory and it could be the basis for further epidemiologic investigation.

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

An Outbreak of Goiter (Thyroid Follicular Hyperplasia) with High Mortality in Young Adult English Budgerigars (*Melopsittacus undulatus*) #

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Narrative: An outbreak of goiter (thyroid follicular hyperplasia) with high mortality and morbidity in a flock of English budgerigars (*Melopsittacus undulatus*) in southern California is described. Thirty out of 300-400 young adult birds (7.5-10%) had died and 15 (11.25-15%) exhibited signs of illness and weight loss in the previous two to three months. Diet consisted of a commercial mixture (~70% of total feed) that did not contain minerals or other supplements, and whole oats, carrot and broccoli. Inbreeding occurred but was comparatively limited. Ten live, approximately one-year-old English budgerigars were submitted for necropsy and diagnostic work up. On clinical examination, the birds appeared moderately depressed, had poor to good nutritional state and had variably enlarged thyroid glands. Four birds were subjected to necropsy immediately following euthanasia. The birds had severely enlarged thyroid glands. Histologically, thyroid follicular hyperplasia was observed in all birds examined, while granulomatous thyroiditis and microfollicular adenoma were observed in one bird each. A large number of thyroid follicles were dilated, lined by hyperplastic and hypertrophic cells that in places formed papillary projections and were filled with blood, aggregates of hemosiderophages, fibrin, sloughed epithelial cells and colloid-like material, while other follicular luminae were collapsed and lacked colloid. The interstitium was multifocally mildly fibrotic. Pharyngeal swab and cloacal swab pool samples were negative for the presence of the *Avian Influenza virus* matrix gene by qRT-PCR. *Gallibacterium anatis* biovar *anatis* and mixed flora were isolated from the liver and lungs of one bird. Mixed flora was also isolated from the kidney of the same bird, and the small intestine, lung, and liver of second bird. No parasite eggs were detected on examination of a fecal pool sample by flotation. Following supplementation of water with iodine and removal of broccoli from the diet, the owner reported weight gain and reduced deaths among birds with goiter; no additional birds were affected. Budgerigars are susceptible to developing goiter when on an iodine-deficient diet or when consuming large quantities of goitrogenic agents including cabbage, broccoli, kale, turnips, rapeseed and soybean. The presence of broccoli and the almost complete lack of minerals in the diet of these animals were thought to be the culprits for goiter in these animals. Inbreeding may have further contributed to the deterioration of the animals' condition. Recognition of this condition, which may have recently re-emerged or be underdiagnosed, may help improve medical, welfare and trade standards concerning this species in practice.

AAVLD Trainee Travel Awardee (Pathology)

Ulcerative Enterocolitis and Typhlocolitis Associated with *Actinobacillus equuli* and *Streptococcus equi* in Horses

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Narrative: Bacterial agents are frequent causes of enterocolitis in horses. The most commonly reported bacterial pathogens associated with equine enterocolitis are *Clostridium perfringens*, *Clostridium difficile* and *Salmonella* spp., but little information is available about enterocolitis produced by other bacteria. This study describes four cases of multifocal ulcerative colitis, typhlocolitis or enterotyphlocolitis associated with *Actinobacillus equuli* and *Streptococcus equi*. Case 1 was an 8-month-old, mini horse gelding with acute depression and inappetence followed by death; case 2 was a 2-year-old Quarter horse colt with acute diarrhea and colitis followed by euthanasia; case 3 was a 6-month-old filly of unknown breed with acute onset of depression followed by death; and case 4 was a 3-year-old Thoroughbred gelding with acute colic followed by euthanasia. The lesions, similar in all cases, affected the large colon of all horses, the cecum of horses 1 and 2, and the small intestine of horse 1. Grossly, large segments of small or large intestine had multifocal to coalescing, approximately 0.3 to 1 cm diameter, discrete, often raised, tan to yellow foci, most of which had a pale or red central depression sometimes overlaid by a thin pseudomembrane. Histologically, these foci were usually associated with the gut associated lymphoid tissue (GALT) and characterized by a focally ulcerated mucosa with neutrophilic infiltration, fibrin deposition, and necrotic cellular debris admixed with moderate to large numbers of Gram positive cocci, pleomorphic Gram negative bacteria (cocci, coccobacilli and bacilli), or a mixture of both. Bacteria isolated on aerobic cultures from the colon of these horses include *Streptococcus equi* subsp. *zooepidemicus* (horses 1 and 2), *Streptococcus equi* subsp. *equi* (horse 1), *Actinobacillus equuli* subsp. *haemolyticus* (horses 3 and 4), and *Actinobacillus equuli* subsp. *equuli* (horse 1). *C. perfringens*, *C. difficile* and *Salmonella* spp. were ruled out in all cases by anaerobic cultures, clostridial toxins ELISA and Salmonella PCR and culture. In addition, all horses had pathological and/or bacteriological evidence of secondary septicemia or toxemia. The multifocal ulcerative nature of these intestinal lesions often associated with GALT and with mixed intralesional bacteria is different from the classic diffuse or regionally extensive necrotizing lesions described for *C. perfringens* type C, *C. difficile* and *Salmonella* spp. *Actinobacillus* sp. and *Streptococcus* sp. may be occasional opportunistic intestinal pathogens of horses and they should be considered as a differential diagnosis for multifocal, ulcerative intestinal lesions.

Pathologic Lesions and Pathogenesis of Percutaneous Infection of CD-1 Mice with *Western Equine Encephalitis Virus* (WEEV) ◊

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Narrative: *Western equine encephalitis* (WEE) *virus* (*Alphavirus; Togaviridae*) is a mosquito-borne virus that is capable of causing severe encephalitis in humans and equids. Although some aspects of the pathogenesis have been elucidated for other alphaviruses, the mechanism of WEE neuroinvasion largely remains elusive. A model describing the pathogenesis of neurovirulent, firefly luciferase (FLUC)-expressing WEE McMillan strain (McM), following respiratory exposure, has been recently developed in outbred CD-1 mice at Colorado State University (CSU). A percutaneous model requiring re-engineering of the viral construct to increase transgene stability has also been successfully produced at CSU. After inoculating 10 outbred CD-1 mice in the foot pad with 104 PFU of WEE.McM.FLUC, daily whole body plus ex vivo imaging at the time of euthanasia of affected mice was conducted. Luciferase activity indicates a consistency of the distribution of the viral antigen in the inoculated leg and CNS of infected mice. Local reaction of multifocal panniculitis, myositis and rare perineuritis is observed in the ipsilateral foot. Neuronal necrosis and multifocal lymphocytic to neutrophilic meningoencephalitis corresponding to the areas of virus bioluminescence is further confirmed via immunohistochemistry using a monoclonal antibody against WEE. The most affected areas are concentrated at the floor of the brain mainly in the mid brain and cerebellar peduncle early in the infection (9 animals) with rare involvement of the olfactory bulb and cerebrum (1 animal) late in the course of the disease. Control animals show neither bioluminescence nor pathology in the inoculated leg or CNS. The data indicate that, following the initial viremia, the virus enters the CNS in 72-108 hours post inoculation (PI) via fenestrated capillaries at sites where blood-brain barrier is naturally absent. These areas are namely the area of postrema, median eminence, neurohypophysis, pineal body, subfornical organ, commissural organ and supraoptic crest. Luciferase activity can persist in some mice up to 25 days PI. Mapping of the CNS lesions in relation to the viral antigen is crucial to understand the pathogenesis of alphaviruses neuroinvasion in general and WEE encephalitis in specific.

◊ USAHA Paper

Use of PCR for *Ureaplasma diversum* in Routine Diagnostics of Bovine Abortion in Wisconsin

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Narrative: *Ureaplasma* vaginitis, described in the 1970s along with early embryonic death, abortion and the birth of weak calves, was thought to be a sexually transmitted disease; yet it persists today despite the nearly exclusive use of AI, and treatment of semen to prevent its spread. Recent studies show that *Ureaplasma* and *Mycoplasma* colonize the vagina of virgin heifers. Because *Ureaplasma diversum* was suspected as a cause of some cases of bovine abortion but rarely cultured at our laboratory, PCR for that mollicute was put in place and used over 2 years. Caseload was then examined to look for an improvement in diagnostic rate of abortions. Out of 147 aborted calves received from 2009-2012, all but 18 were Holstein-Friesians. Gestational age, obtained by measurements and developmental characteristics in whole calves (60% of submissions) and by history in "bottle necropsies", was predominantly 2nd (42%) and 3rd (34%) trimester; a few calves were term (<10%) and fewer yet were in the first trimester (<5%); the remainder were of unknown gestational age. Approximately a third of cases come with placenta: that tissue is almost always inflamed (most with necrotizing placentitis). During 2009-2010 when *Ureaplasma* PCR was not yet on line, diagnoses obtained in 68 cases were as follows: placentitis of unknown etiology 26%, abortion due to unknown cause 20%, bacterial abortion 15%, neosporosis 10%, congenital malformation 9%, IBR 3% and mycotic abortion 1%. In 2011-2012 with the help of *Ureaplasma* PCR, 79 cases were worked up, with the following diagnoses: placentitis of unknown etiology 16%, abortion due to unknown cause 18%, bacterial abortion 16%, neosporosis 10%, *Ureaplasma* abortion 14%, congenital malformation 4%, IBR 6% and mycotic abortion 1%. Some etiologic agents of bacterial abortion were *Trueperella pyogenes*, *Salmonella* (Cerro, Kentucky, Typhimurium), *Listeria monocytogenes*, *Yersinia pseudotuberculosis* and *Coxiella burnettii*. Other, miscellaneous causes of abortion in 1-3 cases each were BVD, dystocia, twinning, hepatic fibrosis, vaccine reaction, vitamin A deficiency, vitamin E deficiency, manganese deficiency, selenium toxicosis, ergot poisoning and adventitious placentation. A handful of cases had more than one diagnosis (for example, Neo/Urea, BVD/salmonella, Q fever/mycosis); for these and all cases, one can question the significance of isolates; taking into account Ct values and correlating isolates with pathologic findings, *Ureaplasma* holds its own as an agent of bovine abortion: placentitis is the most common lesion; amnionitis and textbook lymphoid nodules in fetal lung, conjunctiva and synovium are less commonly found. In conclusion, *Ureaplasma* PCR is a valuable addition to our tools; it may explain some of our cases of placentitis which previously had no etiology; and it may point out the need for more diagnostics aimed at detecting mollicutes and other non-culturable agents of bovine abortion.

Domestic Cattle Resist Chronic Wasting Disease following Oral Challenge or Ten Tears Datural Exposure in Contaminated Environments

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Narrative: A 10 year study was performed to establish whether chronic wasting disease (CWD) was transmissible to domestic cattle (*Bos taurus*) following oral inoculation, or by co-housing cattle with captive cervids in outdoor research facilities where CWD was endemic. Calves (n = 12) were challenged orally on one occasion using a brain homogenate derived from CWD-infected mule deer (*Odocoileus hemionus*). Five uninoculated cattle served as unchallenged controls. Two other groups of cattle were housed outdoors for 10 years in cervid facilities operated by the Wyoming Game and Fish Department (WGFD) (n = 12) or Colorado Division of Wildlife (CDW)(n = 12). The 24 environmentally challenged cattle were exposed to CWD-associated prions through common paddocks, feed, and water. They were in direct daily contact with potentially infected deer or elk throughout the decade long study period. None of the exposed cattle developed neurological disease during the study. The 34 cattle surviving to 10 years post-challenge were euthanized and examined for abnormal prion protein (PrP^{CWD}) by conventional histopathology, immunohistochemistry and Western immunoblot analysis of central nervous system and lymphoid tissue. None had evidence of PrP^{CWD} accumulation. The risks of CWD transmission to cattle following oral inoculation or after prolonged exposure to PrP^{CWD}-contaminated environments are low. [Note: Dr. ES Williams deceased on 29 December 2004.]

Spontaneous Congenital Dystrophic Epidermolysis Bullosa in Sprague Dawley Rats

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Narrative: "Epidermolysis bullosa" encompasses a group of inherited skin disorders characterized by blistering and sloughing of the epidermis at or around the level of the basement membrane due to a defect in collagen VII. Variants of epidermolysis bullosa have been sporadically described in several domestic species, including dogs, cattle, and horses. A naturally occurring model of congenital epidermolysis bullosa in laboratory animals remains elusive; only one previous report of an epidermolysis bullosa-like disease has been reported in a single litter of Sprague Dawley rats with the structural alteration appearing at the level of the hemidesomsomes. Four pups in a litter of Sprague Dawley rats at Texas A&M developed hemorrhagic and blistering skin lesions approximately three days after birth and were euthanized. The pups' skin was diffusely thin and fragile, peeling easily away from the subcutis. Hemorrhagic, blistering areas were most severe around the extremities and oral cavity, and several pups displayed pseudosyndactly of the distal front limbs. All other organ systems were within normal limits. Upon histologic examination, there was distinct separation of the epidermis from the dermis at the level of the basement membrane. Clefting and bulla formation with varying degrees of inflammation and hemorrhage were particularly severe around the forelimbs, shoulders, oral cavity, and extremities. Age-matched controls from the same litter were normal. Several unaffected siblings were separated and bred in an attempt to reproduce the disease. Four more litters were produced, with approximately one quarter to one third of the pups displaying similar lesions. Examination of the skin via electron microscopy demonstrated a diffuse, marked loss of anchoring fibrils and clefting directly under the basement membrane. This type of clefting is consistent with dystrophic epidermolysis bullosa, one of the most destructive forms of this family of disorders. This case represents a novel and spontaneous disease in Sprague Dawley rats that can be reliably reproduced through natural means.

Virology 2

Sunday, October 20, 2013

Pacific Salon 4,5

Moderators: Amy Glaser, Binu Velayudhan

Symbols at the end of titles indicate the following designations:

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

Optimization and Addition of beta-Actin Gene Detection for Two *Capripoxvirus* Real-Time Polymerase Chain Reaction Assays

Amaresh Das, Ming Yi Deng, Michael T. McIntosh

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Narrative: Capripoxviruses (CaPVs) including *Sheeppox virus*, *Goatpox virus* and *Lumpy skin disease virus* cause serious diseases of livestock that are reportable to the World Organization for Animal Health (OIE). We further optimized two previously published CaPV real-time quantitative PCR (qPCR) diagnostic tests, referred to as Balinsky [Balinsky CA, et al: 2008, Rapid preclinical detection of *Sheeppox virus* by a real-time PCR assay. J. Clin. Microbiol 46:438-442] and Bowden [Bowden TR, et al: 2008, *Capripoxvirus* tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. Virology 371:380-393] assays by testing different commercial qPCR kits and by multiplexing the assays for detection of the ACTB gene encoding Beta-actin as an internal control for sample and reaction quality. The Balinsky assay using the GenAmp® EZ rTth RNA PCR kit (Kit-GEZ) and the Bowden assay using TaqMan® Universal PCR Master Mix (Kit-TU) were tested using two new chemistries, the Path-ID™ Multiplex One-Step RT-PCR (Kit-PIM) and the TaqMan Fast Virus 1-Step Master Mix (Kit-TFV). Regardless of the chemistry employed, the Balinsky and Bowden assays displayed similar analytical sensitivities with no apparent cross-reactivity to other pox viruses. The Balinsky assay detected CaPV in 80% (Kit-GEZ), 75% (Kit-PIM) and 89% (Kit-TFV) of samples, while the Bowden assay detected CaPV in 47% (Kit-TU), 72% (Kit-PIM), and 75% (Kit-TFV) of clinical samples from infected animals suggesting Kit-TFV to be a preferable chemistry. Using Kit-TFV, the tests were further multiplexed for detection of ACTB revealing 100% detection for ACTB in clinical samples with no apparent effects on CaPV detection in either the Balinsky or Bowden modified multiplex assays as compared to the singleplex assays without internal control detection.

Development and Evaluation of a Blocking Enzyme-Linked Immunosorbent Assay and Virus Neutralization Assay to Detect Antibodies to *Viral Hemorrhagic Septicemia Virus* (VHSV) ◊

Anna E. Wilson[1], Tony L. Goldberg[1], Susan Marcquenski[2], Wendy Olson[3], Frederick Goetz[4], Paul Hershberger[5], Lucas Hart[5], Kathy L. Toohey-Kurth[6]

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Narrative: *Viral hemorrhagic septicemia virus* (VHSV) is the target of surveillance by many state and federal agencies in the US. Currently, detection of VHSV relies on virus isolation and only indicates current infection status. A serological method is required to ascertain prior exposure; however, no blocking ELISAs for detection of antibodies to VHSV have been reported. Here, we report the development of two serologic tests for VHSV that are non-lethal, rapid, and species-independent: a virus neutralization assay (VN) and a blocking enzyme-linked immunosorbent assay (ELISA). Serum was collected from 34 uninfected fish (VHS negative group) and 28 fish that survived VHS virus infection (VHS positive group). The VN did not detect neutralizing antibodies in the serum of any of the 34 fish in the VHSV negative group, demonstrating the test specificity was 100%. The VN detected neutralizing antibodies in the serum from 12 of 28 fish in the VHS positive group, indicating the sensitivity of the test was 42.9%. The anti-nucleocapsid blocking ELISA did not detect non-neutralizing VHSV antibodies in the serum of 30 of the 34 fish in the VHS negative group, indicating a specificity of 88.2%. Non-neutralizing antibodies were detected in the serum of 27 of the 28 fish in the VHS positive group, indicating the test sensitivity was 96.4%. Used in parallel, the VN and ELISA correctly identified all survivors of VHSV infection and unexposed fish. Our VN and ELISA are valuable tools for assessing exposure to VHSV in fish and should improve detection and surveillance efforts for both wild and commercial fish populations.

◊ USAHA Paper

Pathogenicity of Highly Pathogenic *Porcine Reproductive and Respiratory Syndrome Virus* in a United States Pig Model

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Narrative: In July 2008, domestic swine in the Philippines experienced multiple, severe outbreaks of respiratory and abortion disease syndrome. Samples from this outbreak were sent to the Foreign Animal Disease Diagnostic Laboratory, United States Department of Agriculture, and highly pathogenic *Porcine reproductive and respiratory syndrome virus* (HP-PRRSV) was isolated along with *Reston ebolavirus* (REBOV). The objective of this study was to evaluate the pathogenicity of this HP-PPRSV isolate in pigs in the US and to establish a working dose of HP-PRRSV for future co-infection studies with REBOV in pigs. The experiment was conducted in a BSL-4 facility at Rocky Mountain Laboratories. Three pigs in each of 4 groups were intranasally challenged with a dose of 10^1 to 10^4 $TCID_{50}$ and sacrificed on day 21 post inoculation (p.i.). Additionally, 6 pigs infected intranasally with a dose of 10^4 $TCID_{50}$ were sacrificed on days 2, 4 and 7 p.i. to examine virus replication and histopathology. Clinical signs and body weight were recorded, and blood and swabs (nasal, oropharyngeal and rectal) were collected each day. All animals exhibited clinical signs of sickness, and weight loss or reduced weight gain was observed in all of the pigs in all groups. Viremia was detected in all pigs until day 21 p.i. Virus was shed by all pigs by the oropharyngeal route until day 21 p.i. and by some pigs until day 21 p.i. by the fecal route and until day 14 p.i. by the nasal route. Two pigs in the 10^4 $TCID_{50}$ and one pig in the 10^2 $TCID_{50}$ group were euthanized at earlier time points for humane reasons. Mild to moderate interstitial pneumonia was observed grossly and histologically in all pigs in the 10^4 $TCID_{50}$ group ; a secondary bacterial bronchopneumonia and/or septicemia was seen in 50% of the pigs in this group. In summary, the HP-PRRSV was very pathogenic in a US pig model, as has been reported during outbreaks in pigs in the Philippines and China. Additionally, we have established a minimum dose of 10^1 $TCID_{50}$ by the intranasal route for future co-infection studies with REBOV.

Rift Valley Fever Virus Gn and Gc Glycoprotein-Based Vaccine Elicits Neutralizing Antibodies in Sheep

Bonto Faburay[1], Alan Young[2], Igor Morozov[1], Juergen Richt[1]

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Narrative: *Rift Valley fever virus* (RVFV), a member of the *Bunyaviridae* family, is a mosquito-borne zoonotic pathogen that causes serious morbidity and mortality in humans and livestock. The recent spread of the virus beyond its traditional endemic boundaries in Africa to the Arabian Peninsula coupled with the presence of susceptible vectors in non-endemic countries, has created increased interest in RVFV vaccines. A major milestone in vaccine development has been the use of specific virus proteins expressed in eukaryotic or prokaryotic expression systems as subunit vaccines. Some of these vaccines have been shown to be efficient at eliciting neutralizing antibodies in host organisms. In this study, we expressed RVFV structural proteins, N-terminus glycoprotein (GN) and C-terminus glycoprotein (GC), using a recombinant baculovirus expression system. The proteins were reconstituted in a subunit vaccine formulation and evaluated for immunogenicity in a natural host model, the sheep. Six sheep were each immunized with a primary vaccine dose of 50 μg of GN and 50 μg of GC. At day 21 post-vaccination, each sheep received a booster of the same vaccine dose. The vaccine induced strong antibody response in sheep as determined by indirect enzyme-linked immunosorbent assay (ELISA). Plaque reduction neutralization test (PRNT80) showed that the primary dose of the subunit vaccine was sufficient to elicit protective virus neutralization titers of 1:40 to 1:160, whereas the second vaccine dose boosted the titer to more than 1:1280. Immunblot analysis showed that the subunit vaccine is DIVA compatible and represents a promising vaccine candidate for RVFV in a natural host species.

Development and Validation of the VetMAXTM-Gold SIV Subtyping Kit

Darcy A. Myers, Angela Burrell, Quoc Hoang, Ivan Leyva Baca, Catherine O'Connell

Life Technologies, Austin, TX

Narrative: *Swine influenza virus* (SIV) is a highly contagious viral infection of pigs, resulting in significant economic losses in the swine industry. SIV subtypes are defined by the surface glycoproteins: hemagglutinin and neuraminidase, with H1N1, H3N2, and H1N2 representing the predominant subtypes in swine. We have validated an SIV typing workflow consisting of high throughput nucleic acid purification, SIV detection, and SIV typing from porcine nasal swab samples. The VetMAXTM-Gold SIV Subtyping Kit is a pair of single-well real-time RT-PCR assays: the SIV H1/H3 Primer Probe Mix for the multiplex real-time RT-PCR amplification and differentiation of RNA from the H1 and H3 alleles, and the SIV N1/N2 Primer Probe Mix for the multiplex real-time RT-PCR amplification and differentiation of RNA from the N1 and N2 alleles. When performed in conjunction with the VetMAX™-Gold SIV Detection Kit to screen samples for SIV as well as monitor sample isolation and inhibition via the Xeno™RNA extraction control, the VetMAXTM-Gold SIV Subtyping Kit provides a robust method for typing the predominant SIV subtypes in swine. The SIV typing workflow was evaluated with >150 SIV-positive and >150 SIV-negative porcine nasal swab field samples and virus isolates originating from diverse geographic regions in the US. The SIV status and subtype of each sample was confirmed prior to the start of the study. The >150 characterized positive samples processed in this study consisted of the H1N1, H3N2, H1N2, and H2N3 genotypes, with the majority of samples representing strains circulating within the swine population in the last five years. Collaborator laboratories purified the viral nucleic acid using the MagMAX™-96 Viral RNA Isolation Kit (AM1836) and MagMAX™ Express instruments. Xeno™RNA was spiked into each nucleic acid isolation to serve as an extraction control and samples were first tested with the VetMAX™-Gold SIV Detection Kit to verify that the Xeno™RNA amplified within the acceptable range, prior to performing the H1/H3 and N1/N2 SIV subtyping reactions on the AB 7500-Fast Real-Time PCR System. RNA isolated from diagnostic nasal swab samples of known SIV status (n=>300) were used to determine diagnostic sensitivity and specificity of VetMAX™-Gold SIV Subtyping Kit. The assays produced >90% sensitivity, >90% specificity for identifying the SIV subtype from nasal swab samples. This study indicates that RNA isolated from diagnostic porcine nasal swab samples, tested with the VetMAX™-Gold SIV Subtyping Kit in conjunction with the VetMAX™-Gold SIV Detection Kit, provides an economical and rapid solution for SIV subtype identification.

Epizootic Hemorrhagic Disease Virus (EHDV) Serotype Identification by One-Step Multiplex RT-PCR

Feng (Julie) Sun, Matthew Cochran, Tammy Beckham, Alfonso Clavijo

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

Narrative: *Epizootic hemorrhagic disease virus* (EHDV) causes a highly infectious noncontagious hemorrhagic disease in wild and captive deer populations in the US. Although rapid and accurate identification of the disease is important, the identification of the serotype is equally as important for understanding the epidemiology of the disease in white-tailed deer populations. As such, a one-step multiplex RT-PCR assay was developed for the rapid differentiation and identification of EHDV serotypes 1, 2 and 6 in cell culture and clinical samples by targeting the viral gene segment 2 (L2). From 2009-2012, 427 clinical samples including tissue and EDTA blood from white-tailed deer from 17 states (primarily Texas and 16 other states), determined to be EHDV positive by real-time PCR, were used to evaluate this subtyping assay. Eighteen percent of the positive samples tested were EHDV-1, 59% were EHDV-2, and 21% were EHDV-6. Interestingly, 2% of the samples tested were positive for more than one subtype, indicating a mixed infection. Results also showed that the proportion of EHDV-1 identified had declined, while the ratio of EHDV-2 infection to total detected EHDV remained relatively stable during 2009 to 2012. Both EHDV-6 and mixed-serotype infections increased significantly from January 2011 to November 2012. Results showed a seasonal variation of EHDV, with highest infection in early summer through late fall, and a low levels of detection in the early months of each year. Based on these results, this assay provides a rapid, sensitive, and specific diagnostic tool for the differentiation and identification of EHDV serotypes in field samples as well as virus isolates. The subtyping data provides useful information on the distribution and ecology of EHDV in deer. Additionally, the PCR based assay enables the detection of mixed infections which may be important not only to continue monitoring the distribution of endemic EHDV subtyping but to assist to identify the origins of some epidemic EHDV serotypes.

A Neutralization Test: Antibody Blocking of *Ovine Herpesvirus 2* Entry in Rabbits

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Narrative: *Ovine herpesvirus 2* (OvHV-2), the causative agent of sheep-associated malignant catarrhal fever (SA-MCF), has never been propagated *in vitro*. Thus, an alternative to *in vitro* virus neutralization is needed to assess neutralizing antibody activity against OvHV-2. An *in vivo* system using rabbits was evaluated to determine whether it could be used to assess the ability of antibodies to block OvHV-2 at the entry site. The experimental procedure included: 1) the 'minimal' infectivity and lethality of OvHV-2 collected from sheep nasal secretions were determined in rabbits; 2) OvHV-2 (10^6 viral DNA copies) was incubated with sheep serum known with or without anti-OvHV-2 antibodies (1:4 final dilution) at 37°C for 1 hr; and 3) the virus and antibody mixture was delivered by intranasal nebulization to rabbits and the animals were monitored for infection and development of SA-MCF. The result showed that all rabbits receiving virus mixed with the serum containing anti-OvHV-2 antibodies were protected from OvHV-2 infection while all rabbits in the control group developed SA-MCF. The data indicate that this *in vivo* system using rabbits can be used to assess antibody's ability to block OvHV-2 entry mimicking a neutralization test, which is a significant tool for the analysis of protective antibody responses to the virus and critical for SA-MCF vaccine development.

Correlations of Various *Swine Influenza Virus* Diagnostic Assays by Specimen Type

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[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Veterinary Diagnostic Laboratory, University of Minnesota, St Paul, MN

Narrative: Detecting *Influenza A virus* (IAV) in swine samples is usually done via IAV screening PCRs targeting the conserved nucleoprotein or matrix genes. Diagnostic samples positive by the screening PCR are subject to multiplex hemagglutinin (HA) and neuraminidase (NA) subtyping PCRs. Often, HA gene sequencing is requested to evaluate the genetic relationships of viruses, and virus isolation (VI) is performed to obtain an isolate for vaccine production or to further characterize the virus. Traditionally, nasal swabs (NS) and lungs have been the primary specimens for IAV testing. However, oral fluids (OF) are becoming more common for IAV prognostic profiling. Understanding the performance and correlation of various IAV diagnostic assays on different specimen types will help swine practitioners and diagnosticians choose appropriate tests and interpret results. Here we summarize the IAV diagnostic data from ISUVDL through its lab-specific testing algorithm (July, 2012-April, 2013) as well as combined data available from both ISUVDL and UMNVDL through the USDA surveillance testing algorithm (November, 2010-May, 2013). To correlate IAV screening PCR and subtyping PCR outcome, 1,562 lungs, 551 NS, and 3,423 OF were analyzed. The success rates of subtyping HA and NA on IAV PCR positive lungs, NS and OF were: 99.4%, 99.6%, and 98.6% (screening PCR Ct<25); 98.7%, 99.4%, and 96.2% (Ct 25-29.9); 73.8%, 91.4%, and 78.6% (Ct 30-34.9); 9.1%, 15.2%, and 8.8% (Ct 35-40). To correlate IAV screening PCR and success of direct HA gene sequencing, 227 lungs, 56 NS, and 134 OF were analyzed. The success rates of HA gene sequencing from lungs, NS and OF were: 90.3%, 83.3%, and 72.1% (Ct<25); 19.2%, 56.3%, and 26.5% (Ct 25-29.9); 0%, 0%, and 0% (Ct>30). To correlate IAV screening PCR and VI outcome, 1,563 lungs, 841 NS, and 947 OF were analyzed. The success rates of isolating a virus from lungs, NS and OF were: 89.6%, 88.5%, and 45.6% (Ct<25); 75.2%, 61.8%, and 17.9% (Ct 25-29.9); 25.8%, 28.8%, and 5.2% (Ct 30-34.9); 3.4%, 0%, and 0% (Ct 35-40). In summary, successful HA and NA subtyping was comparable among lungs, NS and OF at screening PCR Ct values <35. Direct HA gene sequencing success was lower on OF compared to lungs and NS when Ct<25 and failed when the Ct was >30 regardless of the specimen type. The success rates of VI were much lower on OF than on lungs and NS within the same Ct ranges.

Isolation of Two Non-A Genotypes of *Bovine Parainfluenza Virus* 3 in the US

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Narrative: *Bovine parainfluenza virus* type 3 (BPI3) is a member of the *Paramyxoviridae*, genus *Respirovirus*. BPI3 are pathogens commonly associated with bovine respiratory disease complex. Most infections are subclinical but they can cause acute respiratory disease characterized by cough, fever and nasal discharge. The presence of BPI3 with other respiratory pathogens can result in severe disease. To date, three genotypes of BPI3 have been identified. Genotype A viruses were reported primarily in North America, genotype B was reported in Australia, and genotype C was reported in China and South Korea. The US strains found in GenBank are all genotype A viruses. BPI3 strains characterized in this study were isolated by the Texas A&M Veterinary Medical Diagnostic Laboratory between 2007 and 2011. The near full-length genome sequences of the BPI3 viruses were determined using a next generation, Ion Torrent-based sequencing procedure where multiple viruses were sequenced simultaneously. In this study, BPI3 viruses were sequenced that were not the North American genotype A viruses as expected, but were more closely related to the genotype B and C viruses. The US genotype B and C viruses were only 82-84% similar to genotype A virus sequences found in GenBank. Similarly, genotypes B and C were only 82% similar to each other. Interestingly, the most closely related human PI3 strains were 80% similar. Comparison of the US genotype B viruses with the single full-length Australian virus sequence showed they were 94% similar, indicating some divergence from the original strain. The US genotype C viruses were 98% similar to the strain reported in South Korea. Of the 13 viruses with sequence, 1 was genotype A, 3 were genotype B and 9 were genotype C. This is the first report of the presence of the B and C genotypes of BPI3 in the US. These results indicate that the A genotype may have been replaced by the B and C genotypes as the predominant BPI3 viruses in the US. The implications of these findings will be discussed.

A Sensitive and Rapid Taqman RRT-PCR for the Detection of *Avian bornavirus* (ABV) #

Paulette F. Suchodolski, Jianhua Guo, Donald Brightsmith, Susan Payne, Ian Tizard, Shuping Zhang

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Narrative: *Avian bornavirus* (ABV) is the only known etiologic agent of proventricular dilatation disease (PDD) of birds. PDD is a fatal neurological disease that affects the central and enteric nervous systems. Clinical signs include weight loss, crop stasis, intestinal dilatation, regurgitation, maldigestion, tremors, ataxia, seizures, blindness and eventually starvation and death. Due to the devastating nature of the disease, bird owners, veterinary practitioners and aviary owners need a reliable, sensitive and quick diagnostic test for ABV. ABV infections have been reported in birds from North and South America, Europe, Africa and Japan. ABV can infect a wide range of avian species including psittacines, water fowl and passerines. However, birds infected with ABV may or may not develop clinical disease and infected birds shed virus in their droppings intermittently. Therefore, a reliable diagnostic protocol is needed to determine ABV infectious status. In the present study, we have developed a rapid and sensitive one-step Taqman based real-time reverse transcription-PCR (Taqman RRT-PCR) for the detection of ABV matrix (M) and phosphoprotein (P) RNA from fecal samples and cloacal swabs. Oligonucleotide primers and probes were designed to detect sequences from conserved regions of the M and P genes that recognize several ABV genotypes. The sensitivity of the Taqman RRT-PCR assay was determined using *in vitro* transcribed M and P RNA and 10-fold serial dilutions of titrated ABV M24 RNA. The specificity of the test was validated using RNA isolated from *Avian leukosis virus*, *Newcastle disease virus*, and *Infections bronchitis virus*. The M and P primer/probes performed with high correlation coefficients (R^2 >0.99). The detection limits of the Taqman RRT-PCR assays ranged from 90 and 50 RNA copies for the M and P RNA transcripts respectively, and as little as 5.45 $X10^{-3}$ ABV M24 focus forming units were detected with both M and P primer/probe sets. The Taqman RRT-PCR assays were specific for ABV M and P RNAs only. The assays were validated using archived diagnostic samples from previously confirmed cases of ABV infection, suspected cases of PDD, and cases of unknown status. The results show that our Taqman RRT-PCR assays provide a sensitive, specific and rapid method for the detection of ABV RNA. The Taqman RRT-PCR assay eliminates the need for a separate reverse-transcription step, synthesizing cDNA in 5 minutes, completing the PCR cycles within 45 minutes, while utilizing Taqman probes for high sensitivity. The sensitivity of the Taqman probes and the improved speed of these diagnostics to traditional RT-PCR methods will facilitate the development of a reliable testing protocol for ABV infection.

AAVLD Trainee Travel Awardee (Virology)

Use of a Bovine Viral Diarrhea (BVD) Management Tool: BVD CONSULT ◊

Brad White[1], Robert Larson[1], Dale M. Grotelueschen[2], Sherri Merrill[1], David Smith[3], Dan Givens[4], Richard Randle[5]

[1]Clinical Sciences, Kansas State University, Manhattan, KS; [2]Great Plains Veterinary Educational Center, University of Nebraska, Clay Center, NE; [3]Pathobiology and Population Medicine, Mississippi State University, Starkville, MS; [4]College of Veterinary Medicine, Auburn University, Auburn, AL; [5]Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE

Narrative: *Bovine viral diarrhea* (BVD) *virus* infection is responsible for a variety of economically important syndromes in beef herds. The cattle industry and veterinary profession have made significant efforts in recent years to control BVD based on research that has provided a more complete understanding of the epidemiology of BVD, enhanced availability of diagnostic tests for detecting persistently infected (PI) cattle, and incorporation of biosecurity and biocontainment principles into control strategies. BVD CONSULT (Collaborative, Online, Novel, Science-based, User-friendly, Learning, Tool) is an internet-based decision tool, designed to aid development of BVD control programs for cow-calf herds. The BVD CONSULT organizes available BVD control recommendations based on available research into a user-friendly interactive format to develop BVD prevention and control programs customized for individual herds that emphasizes key management decisions that impact the success of these programs. BVD CONSULT was designed to mimic a conversation between a veterinarian and a producer by asking if the producer is willing and able to perform specific management practices that will aid in prevention or control and eradication of BVD. After clicking on "yes" or "no" to each question, an appropriate response is given based on the choices that have been made, followed by another question. A printable report is available at the end of the tool which records the choices that were made and the responses that were given. BVD CONSULT can be found at the website, www.bvdinfo.org which contains information about BVD from peer-reviewed articles as well as white papers and popular press articles.

◊ USAHA Paper

Validation of a Field-Deployable POCKIT™ Nucleic Acid Detection System for Specific and Sensitive Point-of-Need Detection of *Equine Influenza Virus* (H3N8) ◊

Udeni BR Balasuriya[1], Ashish Tiwari[1], Ashley Skillman[1], Bora Nam[1], Li-Juan Ma[2], Pai-Chun Yang[2], Alison Lee[2], Simon Chung[2], Hsiao Fen Grace Chang[2], Thomas Wang[2]

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Narrative: Equine influenza (EI) is an acute, highly contagious viral respiratory disease of equids. Currently, *Equine influenza virus* (EIV) subtype H3N8 continues to be the most important equine respiratory pathogen of horses in many countries around the world. The need to achieve a rapid diagnosis and to implement effective quarantine and movement restrictions is critical in controlling the spread of EI. Here we describe and validate a novel, inexpensive, user-friendly assay based on insulated isothermal PCR (iiPCR) method on the POCKIT™, a field-deployable device, for point-of-need detection of EIV-H3N8 in clinical samples. Limit of detection with a 95% probability (LoD95%) was determined using in-vitro transcribed (IVT) RNA. A published real-time RT-PCR (rRT-PCR) was used as the reference method. Ten-fold serial dilutions of RNA extracted from the H3N8 strain A/equine/Miami/63 were used to compare the detection limits of the EIV-H3N8 iiPCR on POCKIT™ with the reference rRT-PCR assays [Lu Z, Chambers TM, Boliar S, et al: 2009, Development and Evaluation of One-Step TaqMan Real-Time Reverse Transcription-PCR Assays Targeting Nucleoprotein, Matrix, and Hemagglutinin Genes of Equine Influenza Virus J. Clin. Microbiol. 47:3907-3913]. Equine clinical samples were randomized and tested blinded in the validation study. LoD95% for the EIV-H3N8 iiPCR on POCKIT™ assay was determined to be approximately 10 copies of IVT RNA. An exclusivity study using closely related influenza viruses suggested high pathogen exclusivity for the established assay. A validation study using 72 equine clinical samples (nasal swabs) demonstrated that sensitivity and specificity of EIV-H3N8 iiPCR on POCKIT™ are equivalent to those of the reference method. EIV-H3N8 iiPCR on POCKIT™ assay could serve as an easy field-deployable tool for rapid, specific and sensitive point-of-need detection of EIV-H3N8.

◊ USAHA Paper

AAVLD/USAHA Joint Plenary Session
Vaccines: 100 Years of Virus Serum Toxin Act and Beyond

Monday, October 21, 2013
Towne & Country

8:00 AM **Welcome and Orientation - Ron DeHaven**

8:15 AM **The Virus-Serum-Toxin Act 1913-2013: Intent, Innovations, and Impact**
Richard E. Hill, Linda Schlater, Mark P. Pagala, Javaraiah Srinivas *150*

8:45 AM **The Economics of Vaccination: What is behind the Development and Use of Vaccines?**
John M. Hardham *151*

9:15 AM **Rational Development of Foot-and-Mouth Disease Virus Vaccines**
Bryan Charleston *152*

9:45 AM **Break (30 min)**

10:15 AM **Autogenous Vaccines: Isn't this why VSTA was established?**
John A. Smith *153*

10:45 AM **Using Gap Analysis to Drive the Selection of New Vaccine Technologies for High Consequence Diseases**
Luis L. Rodriguez *154*

11:15 AM **Future of Vaccines in Biodefense and Disease Control Programs**
Cyril G. Gay *155*

Symbols at the end of titles indicate the following designations:

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

The Virus-Serum-Toxin Act 1913-2013: Intent, Innovations, and Impact

Richard E. Hill, Linda Schlater, Mark P. Pagala, Javaraiah Srinivas

Center for Veterinary Biologics, Veterinary Services, APHIS, USDA, Ames, IA

Narrative: "Vaccination" has been spotlighted as one of the top 10 medical discoveries of all time and 2013 marks the 100th year that the USDA has been licensing and permitting quality animal biologics (which includes vaccines). Federal regulation of veterinary biological products in the United States began with the 1913 passage of the Virus-Serum-Toxin Act (VSTA). The law was passed to establish standards and control the importation of products into the U.S. and the interstate distribution of products, while safeguarding against worthless, dangerous, contaminated, and/or harmful veterinary biological products. In the time leading up to the VSTA, Foot and Mouth Disease outbreaks in the northeastern U.S., contaminated and dangerous hog cholera "remedies and cures", and food safety concerns as portrayed in Upton Sinclair's novel The Jungle were the news of the day. Such events led to the passage of consumer protection laws such as the Pure Food and Drug and the Federal Meat Inspection Acts of 1906, as well as legislation for both human and veterinary biological products. August 5, 1913, marked the issuance of the first veterinary biological product license for hog cholera serum; within a year approximately 120 licensed products were available for dogs, horses, cattle and pigs. In comparison, today there are nearly 1900 licensed veterinary biological products for the prevention, diagnosis, or treatment of over 215 different diseases in 38 species of animals. Along the way, innovations in vaccinology and veterinary medicine have led to the availability of a wide variety of different types of products and vaccination technologies (e.g. RNA, DNA, and Chimera vaccines, recombinant vectored vaccines with companion diagnostics, cancer vaccines, plant-cell derived, and needleless administration), as well as production methods (e.g., cell culture, bioreactors/fermenters, hybridomas). This presentation will provide a snapshot of the issues that led to the passage of the VSTA a century ago, and highlight the innovations and impacts that veterinary biological products have had on animal health in the United States and the world.

Speaker Biography: Richard E. Hill, Jr. (Director, Center for Veterinary Biologics) - Dr. Hill received a D.V.M. degree from Michigan State University in 1983 and following graduation, worked in private veterinary practice. In 1985, he joined the USDA and worked as a field Veterinary Medical Officer before joining the Biologics Program in 1986. Rick worked as an Inspector, Epidemiologist, and Team Leader, for the Biologics Program where he was involved in regulatory compliance and coordination of the pharmacovigilance program. In 1990, he received an M.S. degree in Veterinary Preventive Medicine at Iowa State University and is a Diplomate in the American College of Veterinary Preventive Medicine. In 1995, Dr. Hill transferred to the position of Quality Assurance Manager, responsible for overseeing the Quality Assurance Program at the National Veterinary Services Laboratories and Center for Veterinary Biologics Laboratory. In November 1998, he re-joined the Center for Veterinary Biologics as Director of Licensing and Policy Development and then served as the Center Director from 2005 through 2013. Beginning in October 2013, Dr. Hill is expected to transition to the position of Executive Director for Veterinary Services, National Import and Export Services.

The Economics of Vaccination: What is behind the Development and Use of Vaccines?

John M. Hardham

Global Biologicals Research, Zoetis, Kalamazoo, MI

Narrative: There are many complex and inter-related factors that drive the discovery, development, licensure, and use of vaccines in the United States. The veterinary vaccine business in the US is an ~ $1.3 Billion a year business, representing approximately 25% of the global biological market. The USDA-Center for Veterinary Biologicals regulates the development and use of biological products in the US and licenses approximately 60 new vaccine products each year. The process by which animal health companies discover and develop vaccines will be discussed along with the factors considered for new product concept initiation decisions. Animal health companies invest in a broad portfolio of products in an attrition-based fashion in order to meet regulatory requirements for product licensure. The process of bringing a single product to the market often takes 7-10 years, of which more than 50% will fail. The profit realized from a single product on the market supports broad-based research and development on multiple other products that may or may not make it to market. For veterinarians and producers, the drivers of the decision to utilize USDA approved vaccines as part of a herd health program includes an analysis of the health and economic impact of disease on the animal population. The development and utilization of vaccines plays an important role in protecting the health of both humans and animals as well as safeguarding the US food supply.

Speaker Biography: Dr. John Hardham is a Senior Principal Scientist and Technical Lead for the Emerging Infectious Disease Program for Zoetis, Inc. In addition, he is a Commander in the US Navy and specializes in Biological and Chemicals Weapons Detection and Defense and is a Committee member for the National Research Council, National Academies of Science, Standing Committee on Countering Biological Threats. He has over 20 years of experience in the discovery and development of vaccine and biopharmaceutical products for various bacterial, viral, and parasitic diseases and in the detection of and defense against biological and chemical weapons agents. Dr. Hardham was the Medical Director for the Office of the Assistant Secretary of Defense for Nuclear, Chemical, and Biological Defense where he led the Medical Countermeasure Development Program and the Medical Countermeasure Initiative for Advanced Development and Manufacturing. Dr. Hardham received his BS degree from the Pennsylvania State University and his PhD in Microbiology from The University of North Carolina at Chapel Hill. His Postdoctoral fellowship was with the University of Texas Health Science Center at Houston.

Rational Development of Foot-and-Mouth Disease Virus Vaccines

Bryan Charleston

Livestock Viral Diseases, The Pirbright Institute, Surrey, United Kingdom

Narrative: Current FMD virus vaccines are highly effective at inducing protective immunity in cattle. A single low microgram dose in adjuvant can generate protection from disease (though not necessarily infection) within 4-5 days. Vaccination is currently reliant on the use of inactivated virus produced in large-bioreactors in high containment facilities; their set-up and running costs, limit the global production capacity. Storage and supply are further constrained by the poor vaccine stability at ambient temperatures. Thus, on several grounds the current vaccine manufacturing situation is unsatisfactory and developments that increase the options available for FMD vaccine production are urgently required. We have performed proof-of-principle experiments for a vaccine produced from non-infectious cultures. The implementation of methods to produce non-infectious FMDV capsids as vaccines, outside of high containment facilities, would significantly lower costs, improve production capacity and eliminate the risks associated with infectious virus during vaccine production. Also, the absence of non-structural proteins from the vaccine antigen means companion DIVA diagnostic tests will provide greater certainty of discriminating between vaccinated and infected animals. In addition, our initial work has demonstrated that a non-infectious source of virus capsids allows sequence manipulation to address the issue of antigen stability. X-ray crystallography shows the mutant and wild-type capsids to be essentially the same structure as virus. Implementation of improvements in vaccine stability would reduce the quantity of antigen required per vaccine dose, mainly by reducing losses during production and improving the shelf life of the formulated product. Cattle vaccinated with wild-type and stabilised recombinant capsids showed sustained virus neutralisation titres and protection from challenge 34 weeks after immunization. In summary, combined with new tests to facilitate pre-clinical/ pre-transmission diagnosis, these new rapidly deployable recombinant vaccines support a “vaccine to live policy”.

Speaker Biography: Dr Charleston obtained a BVetMed from the Royal Veterinary College, University of London, UK in 1982. After a period of time in Large Animal Practice, studied for a Masters degree in Molecular Biology at University College, London in 1988, then a PhD degree, as a Wellcome Trust Scholar, from the University of London, UK, in 1991. He is currently the Head of the Livestock Viral Diseases programme at the Pirbright Institute, UK. His research group’s efforts are focused on understanding the immune response to Foot-and–Mouth disease virus in cattle to develop novel vaccines.

Autogenous Vaccines: Isn't this why VSTA was established?

John A. Smith

Director of Health Services, Fieldale Farms Corporation, Baldwin, GA

Narrative: Only 0.25% of all doses of animal vaccines, bacterins, and vaccine/bacterin combinations produced in US-licensed establishments in 2012 were classified as autogenous products. Nevertheless, autogenous vaccines and bacterins are increasingly important adjuncts to health maintenance and food safety in the integrated broiler, turkey, and egg industries in the US. Almost 11% of all animal bacterins produced in these establishments in 2012 were classified as autogenous products, confirming the importance of autogenous products in this category. The need for autogenous products seems to be increasing; the corresponding figure for autogenous production 10 years ago (2002) was 0.18% of all production. There are four main factors driving this need: increasingly rapid evolution of the pathogens (possibly driven in part by large-scale industrial production); changes in the host (in which amazing increases in productive capacity have correspondingly increased the impact of infectious diseases); new management priorities (particularly an increased emphasis on reducing antimicrobial usage and on improving food safety on the farm); and changes in the vaccine industry (particularly consolidation and costs of research and development) and regulatory processes themselves. The US regulations governing the manufacture of autogenous vaccines in 9 CFR 113.113 have served the veterinary profession, the livestock industries, and the regulatory agency well, but the needs of the modern commercial poultry industry should prompt a re-examination of these processes. The need to protect practitioners, clients, and the public from worthless or harmful products, the risks associated with "less-than-fully-licensed products", and the considerable benefits to be gained from autogenous products must be carefully balanced. The current structure of the integrated poultry industries has greatly reduced the likelihood of an unscrupulous manufacturer foisting a worthless product on an unsuspecting or uneducated user; the data-driven nature of these businesses has greatly decreased the likelihood that a non-efficacious (or harmful) product would go undetected or be tolerated for long; and the large-scale, integrated nature of the businesses requires that both geographical and temporal limits on application need to be interpreted liberally. Our common goals should be to produce autogenous biologicals that are pure and safe and that meet the challenges of emerging or localized diseases in a modern industry, within a regulatory framework that maintains adequate regulatory control while recognizing the current structure and needs of that industry.

Speaker Biography: John Andrew Smith DVM, MS, MAM Education: DVM 1975, University of Georgia MS in Medical Microbiology 1983, University of Georgia Master of Avian Medicine (MAM) 1991, University of Georgia Specialty Boards: American College of Veterinary Internal Medicine, Large Animal (ACVIM), 1984 American College of Poultry Veterinarians (ACPV), 1994 Employment: Captain, US Army Veterinary Corps, 1975-1977 Intern, Instructor, Large Animal Surgery and Medicine, Auburn University, 1977-1979 Resident, Large Animal Internal Medicine, University of Georgia, 1979-1982 Assistant Professor of Food Animal Internal Medicine, Auburn University, 1982-1984 Assistant, Associate Professor of Food Animal Medicine and Surgery, Colorado State University, 1984-1989 Graduate Assistant, Department of Avian Medicine, University of Georgia, 1989-1991 Director of Health and Hatchery Services, Fieldale Farms Corp., Baldwin, GA 1991-present. Fieldale Farms is the 13th largest broiler producer in the United States, placing approximately 3.2 million broilers per week. Selected Industry Activities: President, Association of Veterinarians in Broiler Production and Chair, National Chicken Council (NCC) Poultry Health Committee, 1996-1997 United States Animal Health Association, Committee on Transmissible Diseases of Poultry and Other Avian Species: Vice Chair 1999-2004; Chair 2004-2009 NCC representative to the AVMA Animal Agriculture Liaison Committee, 2002-2008; Committee Chair 2007-2008 US Poultry and Egg Association Foundation Research Advisory Council, 1999-Present; Chair, 2006-present American Association of Avian Pathologists Scientific Program Committee, 2009-2015; Chair and AAAP representative to AVMA Convention Management and Program Committee, 2012-2015 Selected Awards: US Poultry and Egg Association Lamplighter Award for industry service, 2004 American Association of Avian Pathologists Lasher-Botorff Award 2006

Using Gap Analysis to Drive the Selection of New Vaccine Technologies for High Consequence Diseases

Luis L. Rodriguez

Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Greenport, NY

Narrative: High consequence animal diseases are complex problems with multiple facets that require specific fit-for-purpose control and eradication strategies. These strategies typically include diagnostics, vaccines, surveillance, biosecurity, animal control, disinfection and disposal of animals. In order to devise the appropriate strategies, it is crucial to know the nature of the disease, its ecology, life cycle, biology and pathogen-host interactions. Gap analysis is an important tool to guide the development of disease countermeasures, as the process helps identify and characterize the threat associated with the disease agent, its epidemiology and the host response to infection and vaccination. Importantly this process identifies the existing countermeasures, including available diagnostics and vaccines, and ranks them in terms of their applicability to disease control and eradication. This information helps emergency veterinary services decide what materials they should include in their stockpile. However, one of the most relevant and consequential aspects of gap analysis is to determine what needs are not being fulfilled by current technologies, what knowledge gaps are there preventing the development of more effective countermeasures and finally what countermeasure technologies are at the laboratory bench level or early development level. This allows veterinary authorities to reach back in the developmental pipeline in order to do strategic investment and move forward those promising countermeasures to a level of development that will make them more appealing for industry to invest and convert these technologies into products that could be added to disease control strategic stockpiles. This process also identified products that could be in global control and eradication efforts, with the potential of offsetting R&D investment with access to global markets in endemic settings. We will illustrate how gap analysis has been used to guide research and development efforts toward the development of countermeasures against two diseases: classical swine fever and foot-and-mouth disease in swine. We will discuss the success and challenges encountered in the process of selecting the best available countermeasures, and when these are not available, developing fit-for-purpose countermeasures.

Speaker Biography: Luis L. Rodriguez, DVM, PhD is the Research Leader of the Foreign Animal Disease Research Unit at Plum Island Animal Disease Center, ARS-USDA. During the last 28 years, Dr. Rodriguez has worked on animal virology. Dr. Rodriguez also has worked both in field and BL-4 laboratory conditions with members of the mononegavirales such as hantaviruses (Sin Nombre), filoviruses (Ebola) and nairoviruses (Crimean-Congo Hemorrhagic Fever) at the Centers for Disease Control and Prevention in Atlanta, Georgia. For the last 8 years, Dr. Rodriguez's research has focused on understanding functional genomics and virus-host interactions of foot-and-mouth disease virus and the application of this knowledge to effective vaccine development. Recent accomplishments of his research team include the development of a controlled FMDV aerosol exposure method and the characterization of the primary replication sites of FMDV in cattle; and the development of a novel platform for production of FMD vaccines that are safe to produce and allow differentiation between infected and vaccinated animals (DIVA). Dr. Rodriguez has authored over 100 peer-reviewed scientific publications, two patents, and several scientific reviews and book chapters.

Future of Vaccines in Biodefense and Disease Control Programs

Cyril G. Gay

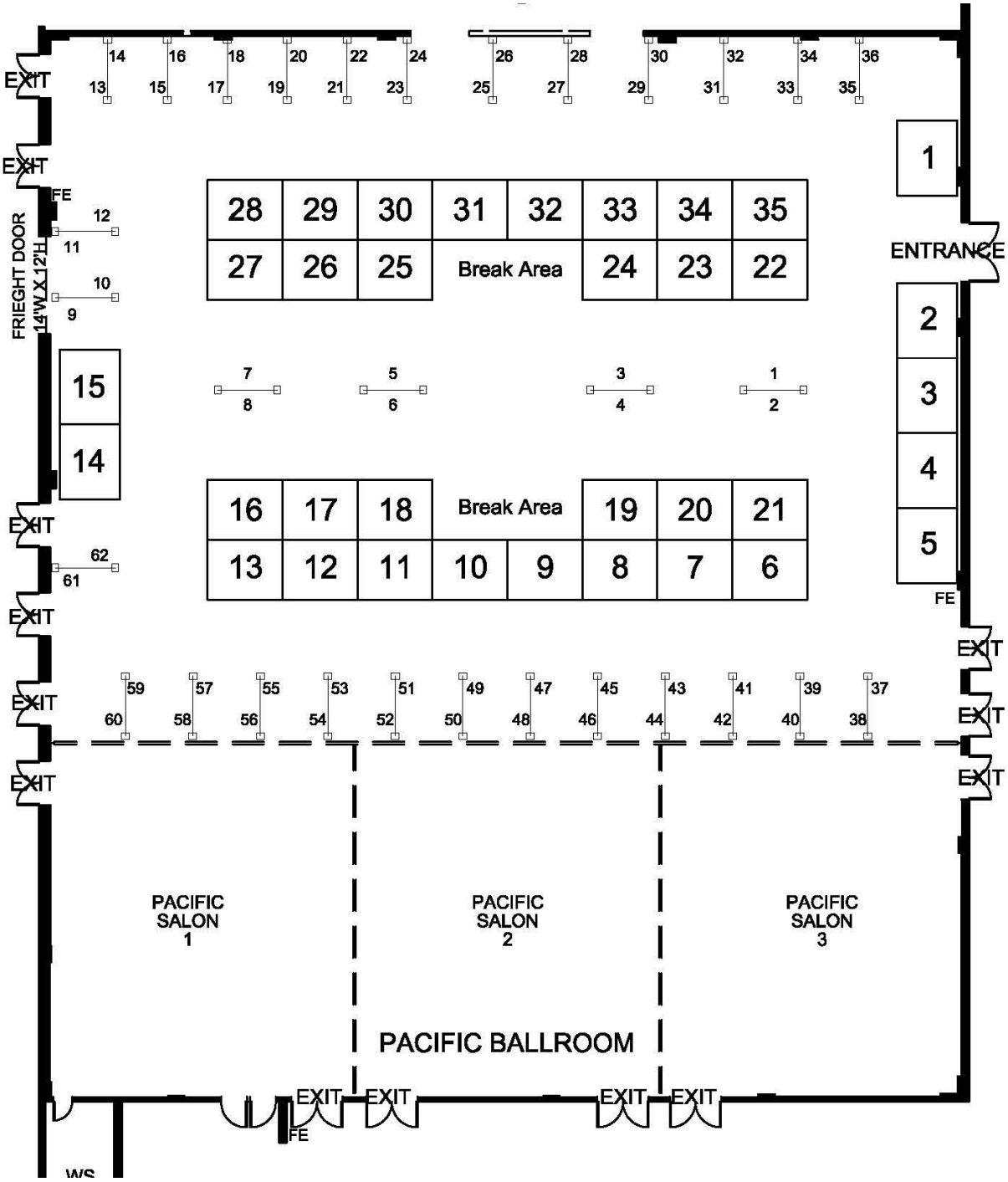
Animal Production and Protection, Office of National Programs, Agricultural Research Service, Beltsville, MD

Narrative: Vaccines represent the single most cost-effective countermeasure to respond and mitigate disease outbreaks. In addition, their effective use in disease control programs is paramount to global food security and the safe production of livestock, poultry, and the rapidly expanding aquaculture sectors. Moreover, the threat of emerging zoonotic diseases has renewed interest in the use of animal vaccines as an integral component of biodefense. But are veterinary vaccines up to the challenge? Have tangible advancements been made in the field of veterinary vaccinology? Are there new technologies driving the discovery of new vaccines that will fundamentally change the way we approach the stockpile of veterinary vaccines, prepare for disease outbreaks, and implement disease control programs? This presentation will outline some of the new technologies in the research pipeline, and provide specific examples of new vaccines under development for some of the most important diseases that threaten animal agriculture and the livelihood of people worldwide.

Speaker Biography: Dr. Gay obtained a B.Sc. in Chemistry and a Doctor of Veterinary Medicine from Auburn University, and a Ph.D. in Microbiology from The George Washington University. Dr. Gay has worked in the animal health research field for the last 25 years holding several positions of increasing responsibility in the federal government and the pharmaceutical industry. As Chief, Biotechnology Section, Center for Veterinary Biologics (CVB), United States Department of Agriculture (USDA), Dr. Gay developed the procedures for licensing molecular vaccines that led to the first license for a live recombinant vectored vaccine. In the pharmaceutical industry (SmithKline Beecham and Pfizer) Dr. Gay led several cross-functional teams that successfully developed and licensed veterinary vaccines for companion animals and livestock. As Director, Global Product Development, Pfizer, Dr. Gay developed strategic and tactical plans that interfaced R&D, clinical development, manufacturing, marketing, and product life-cycle management. Dr. Gay joined Agricultural Research Service (ARS), USDA, in 2002. Dr. Gay currently holds the position of Senior National Program Leader and provides program direction and national coordination for the Department's intramural animal health research program, with focus on eight research laboratories located in Ames, Iowa, East Lansing, Michigan, Clay Center, Nebraska, Athens, Georgia, Orient Point, New York, Beltsville, Maryland, Pullman, Washington, and Manhattan, Kansas. Dr. Gay was the 2010 recipient of the USDA Secretary's Honors Award for interagency response to the pandemic H1N1 influenza outbreak and the ARS Special Administrator's Award for outstanding and rapid research support for pandemic H1N1. Contact Information: Cyril G. Gay, D.V.M., Ph.D Senior National Program Leader, Animal Health Agricultural Research Service Unites States Department of Agriculture 5601 Sunnyside Avenue, Beltsville, Maryland 20705-5138 e-mail: cyril.gay@ars.usda.gov; Telephone: 301 504-4786
http://www.ars.usda.gov/pandp/people/people.htm?personid=32566

Poster Session Abstracts

Floor plan for Exhibits and Posters



Poster Session

Saturday, 19 October – Sunday, 20 October

Golden Ballroom

Symbols at the end of titles indicate the following designations:

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

Poster 01

A Multiplexed Fluorometric Immunoassay for the Detection of Antibodies to *Actinobacillus pleuropneumoniae* 1-9-11 and 2

Andre Ch. Broes[1], Rajeev Dahwan[2], Elena Seletskaia[2], William Shek[2], Isabelle Caya[1], Martine Bertrand[1]

[1]Biovet Inc., Saint-Hyacinthe, QC, Canada; [2]Research Animal Diagnostic Services, Charles River Laboratories, Wilmington, MA

Narrative: *Actinobacillus pleuropneumoniae* (APP) remains an important swine respiratory pathogen in many countries worldwide. Fifteen APP serotypes based on capsular polysaccharide (CPS) antigens have been identified so far. The surveillance of swine herds for APP mostly relies on the detection of serogroup specific serum antibodies. Various serological assays have been developed. The most sensitive and specific is the indirect ELISA which is using highly purified long chain lipopolysaccharides (LC-LPS) as antigen. However in order to detect antibodies to the 15 serotypes, up to nine serogroup specific ELISAs for 1-9-11, 2, 3-6-8-15, 4-7, 5, 10,12, 13, and 14 have to be performed at the same time, which is expensive. Recently a new kind of immunoassay called multiplexed fluorometric immunoassay (MFIA) based on Luminex xMAP technology has been developed. MFIA allows a single small volume of serum to be used to screen for antibodies to many antigens at one time in a single well . The MFIA uses suspensions of microspheres (beads) with unique internal fluorescent dyes. These beads are coupled to their surface with unique antigens. Bead sets and sera are added to 96 well microtitre plates. Antigen-antibody complexes formed during incubation are then detected through successive incubations with biotinylated species-specific anti-immunoglobulins (Ig) followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubations are followed by wash steps to remove unbound serum constituents and reagents. In addition two internal controls consisting in a species-specific Ig bead set and an anti-species Ig bead are incorporated into the assay to evaluate sample suitability and assay function respectively. MFIA plates are read and analyzed using a microtiter plate suspension microarray fluorescence analyser. Beads are exposed to a red laser which excites the internal dyes that identify the bead's color set corresponding to a particular antigen and a green laser which excites the phycoerythrin reporter dye captured during the assay. The intensity of R-phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI) which denotes how much reporter fluorescence of a given microsphere set carries. An S/P ratio of the SA-PE on the antigen-coated microspheres above provided threshold indicates that antibodies to the corresponding antigen are present in the sample. We have developed a MFIA using magnetic beads (Magplex®, Luminex) and LC-LPS as antigen to detect antibodies to APP1-9-11 and APP2 in swine serum samples. The assay was compared to the LC-LPS ELISA. It demonstrated excellent sensitivity, specificity, repeatability and reproducibility (ruggedness). A MFIA which will allow detecting all the nine APP serogroups (9-plex MFIA) is in development.

Poster 02

Bovicheck BoHV-1 gB Antibody Test: A Blocking ELISA to Detect Antibodies to *Bovine Herpes Virus 1* in Bovine Serum

Martine Bertrand, Isabelle Caya, Andre Ch. Broes

Biovet Inc., Saint-Hyacinthe, QC, Canada

Narrative: Infectious bovine rhinotracheitis/infectious pustular vaginitis (IBR/IPV) is a highly contagious disease caused by the *Bovine herpes virus 1* (BoHV-1). The virus affects domestic and wild cattle and is present worldwide. However several countries, especially in Europe, have implemented control programs and the virus has even been eradicated from some of them. Moreover, breeding bulls have to be tested negative for BoHV-1 prior to entrance in artificial insemination centers in most countries. Testing for BoHV-1 antibodies is thus regularly conducted in these countries either to monitor BoHV-1 infection in the bovine population or prior to moving cattle between herds. Various serological assays have been developed to detect BoHV-1 antibodies in serum or milk samples. The virus neutralization test (VNT) and various enzyme immunosorbent assays (ELISA; indirect or blocking) are the most widely used assays on serum samples. The ELISAs are more convenient than VNT for large scale testing as they can be easily automated. However the performances of the various ELISA may vary. For regulatory purposes it is important to use assays with very high sensitivity and specificity. We have recently developed a blocking ELISA using an anti-gB monoclonal antibody for detecting gB antibodies in bovine serum samples. The assay has been compared to an indirect in house ELISA and a commercial blocking ELISA. It has demonstrated excellent sensitivity, specificity, and repeatability.

Poster 03

A New *Bovine Viral Diarrhea Virus* (BVDV) Antigen Capture ELISA Test Kit for the Detection of BVDV in Ear Notches

Martine Bertrand, Isabelle Caya, Andre Ch. Broes

Biovet Inc., Saint-Hyacinthe, QC, Canada

Narrative: *Bovine viral diarrhea virus* (BVDV) is a pestivirus that is widely spread into cattle populations and is responsible for a variety of economically important disorders. Two major antigenically distinct genotypes of BVDV exist, type 1 and 2. Fetal infections with non-cytopathic BVDV during the first trimester of pregnancy can result in the birth of persistently infected (PI) calves. PI animals are mainly responsible for maintenance of the virus in cattle populations and their elimination is the corner stone to efficiently control BVDV infections. Persistently infected animals may be identified by detecting the virus in various clinical samples including blood, serum, or skin biopsies and using a variety of techniques such as virus isolation, polymerase chain reaction (PCR), immunohistochemistry (IHC), and antigen capture enzyme linked immunosorbent assay (ACE). Compared to blood samples, ear notches are especially convenient as they are easy to collect, to store and to ship. ACE are widely used as they are easy to perform and inexpensive. We have recently developed an ACE using bovine polyclonal antibodies as capture and detection antibodies. These antibodies are produced by experimentally infecting BVDV naïve pregnant heifers in late pregnancy and collecting the serum of their foetus after they have seroconverted. The assay has been compared to commercial ACE and PCR. It has demonstrated excellent sensitivity, specificity, and repeatability.

Poster 04

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

Andriy Tkachenko[1], James Clark[2], Natalie Knutson[2], Betzy Wallace[3], Malgorzata Bomba[3], Michele Yacopucci[3], Nina French[4], Sarah Nemser[1], Renate Reimschuessel[1]

[1]Office of Research, FDA, Center for Veterinary Medicine, Laurel, MD; [2]Arizona State Public Health Laboratory, Phoenix, AZ; [3]New Hampshire State Public Health Laboratory, Concord, NH; [4]Washington State Public Health Laboratory, Shoreline, WA

Narrative: Two major melamine adulteration events, US pet food in 2007 and China infant formula in 2008, demonstrated the need to increase capability to rapidly analyze large numbers of samples using reliable methods. To address this need, a collaborative project between four laboratories was established with two major goals: (i) to develop LC-MS/MS methods to quantify melamine (MEL) and cyanuric acid (CYA) in various pig tissues at or above level of concern (2.5 mg/kg) and (ii) to analyze MEL and CYA in various tissues from pigs that had been exposed to these chemicals alone and/or in combination for 7 or 28 days. Pigs treated with 200 mg/kg CYA for 7 days did not accumulate significant residue concentrations in muscle, liver and kidney. Treatment with 200 mg/kg MEL for 7 or 28 days increased residues of MEL in muscles (3-13 ppm), liver (2.8-14.1 ppm) and kidney (9.4-27.2 ppm). Treatment with a combination of MEL and CYA at 100 mg/kg each for 7 days significantly increased residues of both MEL (26-59 ppm in muscle, 30-49 ppm in liver and 367-6,300 ppm in kidney) and CYA (1.8-5.8 ppm in muscle, 2.6-6.5 ppm in liver and 303-7,100 ppm in kidney). In summary, residues of MEL and CYA in tissues depend on type of pig tissue (muscle, liver or kidney) and type of administration of MEL and CYA (alone or in combination).

Poster 05

Localization of Influenza A Antigen in Formalin Fixed Avian Tissue Specimens by Immunohistochemistry

Arach J. Wilson[1], Mia Torchetti[2], Rebecca Madison[3], Arthur J. Davis[1], S. Mark Hall[1], Janice C. Pedersen[1], Mary Killian[2], Nichole Hines[2], Nadine Beckwith[1]

[1]Pathobiology Lab, USDA, APHIS, VS, NCAH, Ames, IA; [2]Diagnostic Virology Lab, USDA, APHIS, VS, NCAH, Ames, IA; [3]Animal Resources, National Centers for Animal Health, Ames, IA

Narrative: Five-week old chickens were intravenously inoculated with avian influenza A(H7N9) virus [A/Anhui/1/2013] obtained from CDC as part of a One Health Diagnostic Collaboration on avian influenza A(H7N9) between USDA & CDC, and were humanely euthanized on day 3 post-inoculation. Samples were processed for virus isolation, PCR testing, histology and immunohistochemistry (IHC). Gross necropsy lesions in the two A(H7N9) infected birds included pulmonary vascular congestion and splenomegaly as compared to the uninoculated control. Influenza A viral antigen was localized in renal epithelial cells, pancreatic parenchymal cells and lungs of both inoculated chickens by IHC.

Poster 06

Genomic Comparison of the Novel Papillomavirus Isolated from the North American Beaver (CcanPV1) to Other Rodent Papillomaviruses # * †

Artem S. Rogovskyy[1, 2], Zigui Chen[3], Robert Burk[3, 4], Troy Bankhead[1, 5]

[1]Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA; [2]Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA; [3]Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY; [4]Department of Microbiology and Immunology, Epidemiology and Population Health, and Obstetrics, Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, NY; [5]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA

Narrative: The papillomaviruses comprise a large group of DNA viruses that cause proliferations of the stratified squamous epithelium of skin and mucosa in a variety of animals. Our earlier report identified a novel papillomavirus of the North American beaver, *Castor canadensis* (CcanPV1) that was associated with cutaneous exophytic lesions. In the current study, we determined the sequence of the complete 7435 basepair genome of CcanPV1. The genome contains an Upstream Regulatory Region located between the end of L1 and the start of E6, and seven canonical papillomavirus open reading frames encoding five early (E6, E7, E1, E2, and E4) and two late (L2 and L1) proteins. No E5 open reading frame was detected. Phylogenetic analyses of the CcanPV1 genome demonstrate stable placement between the genera *Kappapapillomavirus* and *Mupapillomavirus*. CcanPV1 is one of the few rodent papillomaviruses that have been genetically characterized and isolated from naturally occurring papillomas. The papillomavirus genomes isolated from different species of the order Rodentia do not form a monophyletic clade. Specifically, the papillomaviruses isolated from cutaneous lesions represent all four distinct rodent papillomavirus lineages. The pairwise nucleotide and amino acid sequence alignments between an L1 fragment isolated and sequenced from hair-follicle cells of the European beaver, *Castor fiber* and L1 ORF of CcanPV1 show 57.7% and 72.5% similarities, respectively, suggesting that a putative European beaver PV and CcanPV1 may not belong to the same genus. Overall, our analyses provide additional evidence that does not support the hypothesis of co-divergence between papillomaviruses and their hosts. Further identification and characterization of papillomaviruses from other Rodentia species should contribute to better understanding of papillomavirus evolution, in general, and the relationship of CcanPV1 to other rodent papillomaviruses, in particular.

AAVLD Trainee Travel Awardee (Virology)
* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant

Poster 07

Temporospatial Analysis of Equine Encephalitis Positive Results by IgM ELISA on Equine Sera Submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory over the Last Decade

Binu T. Velayudhan[1], Sandy Rodgers[2], Terry Hensley[2], Robert W. Sprowls[1]

[1]Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX; [2]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX

Narrative: Equine viral encephalitis is a zoonotically significant disease of equids with a world-wide distribution. In the Americas, arthropod-borne viruses such as *West Nile virus* (WNV), *Eastern equine encephalitis virus* (EEEV), *Venezuelan equine encephalitis virus* (VEEV), and *Western equine encephalitis virus* (WEEV) play major roles in the causation of encephalitis in horses and humans. In this retrospective study, equine serum samples submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) were analyzed for the presence of immunoglobulin M antibodies specific to WNV, EEEV, VEEV, and WEEV by enzyme linked immunosorbent assay (IgM ELISA) on suspected cases of encephalitis. A temporospatial analysis was performed on data collected from 2002 to 2012. Preliminary data from 2013 was also included. The numbers of WNV IgM antibody positive samples were high in 2002 and 2003 (62% and 22.9% respectively). The percentage of positive WNV cases declined gradually from 2003 to 2011. The number of positive WNV samples remained below 7% from 2008 to 2011 whereas there was a spike in 2012 with 14.9% of samples giving positive results. Majority of the WNV positive equine samples over the period of this study were submitted from horses in the state of Texas but samples were also received from other states in the US. The numbers of positive WNV cases were generally higher in the months of August and September. The year 2012 also saw an increase in the incidence of human WNV cases in Texas according to the Department of State Health Services. The number of positive samples for VEEV, WEEV and EEEV were nil to very low compared to WNV over the time period of this study. No confirmed positive cases of VEE were reported during the course of this study. The only WEEV positive case was a single sample submitted from Louisiana in 2006. There were fewer than ten positive EEEV cases reported in the years 2005, 2006, 2008, 2009 and 2012. The highest incidence of EEEV was in 2007 with 69 samples (8.6%) showing a positive reaction. No samples were tested for VEEV or WEEV or EEEV in 2002 and 2003 by IgM ELISA. Of the four viruses discussed in the study, WNV continues to be the major cause of encephalitis in equids. A spike in the number of positive WNV cases in 2012 in this retrospective study indicates the need for increased vigilance and improved intervention strategies including vaccination and mosquito control.

Poster 08

Reliable Detection and Typing of *Porcine Reproductive and Respiratory Syndrome* (PRRS) *Virus* using Multiplex Real-Time Reverse Transcriptase Polymerase Chain Reaction Assay Reagents

Christine Gaunitz[1], Carsten Schroeder[1], Marco Labitzke[1], Eva Knoop[1], Nevena Djuranovic[2]

[1]QIAGEN Leipzig GmbH, Leipzig, Germany; [2]QIAGEN, Germantown, MD

Narrative: Infection with the *Porcine reproductive and respiratory syndrome virus* (PRRSV) is economically most important for the swine industry. Clinical signs are respiratory disease in piglets and reproductive failure in pregnant sows. The purpose of this study was to develop a multiplex real-time PCR which allows reliable detection and differentiation of PRRSV strains in one test run. To evaluate analytical sensitivity of virotype® PRRSV, titration studies were performed with in-vitro RNA (PRRSV EU1/EU2/NA [10^8 copies/well to 10 copies/well]), performed in triplicates of ten-fold dilutions. A high correlation between number of copies and amount of amplification product was demonstrated in the range of 10^8 to 10^3 and 10^8 to 10^2 copies, respectively. Analytical specificity was evaluated by testing an RNA-panel of 12 PRRSV reference strains with virotype® PRRSV and two commercial available tests (Test I and Test II). All reference strains were detected well with the virotype® PRRSV. In comparison to Test II, the virotype® PRRSV reagents detected the EU strains around two Ct-values and the NA strains around five Ct-values more sensitively. The results of commercial Test I and virotype® PRRSV were comparable. Diagnostic sensitivity was proven by testing the 2011 EPIZONE Ring Trial panel. Serum, tissue and saliva samples were tested in comparison to other PCR assays. Two commercial PCR kits and virotype® PRRSV reagents (QIAGEN, Hilden, Germany) were used according to the manufacturers recommendations. In comparison to an in-house PCR performed at the Friedrich Loeffler Institute, virotype® PRRSV detected all samples of the 2011 EPIZONE Ring Trial more sensitively. virotype® PRRSV reliably detected the samples of the EU-2, EU-3, EU-4 and the atypical EU genotype1. In conclusion, virotype® PRRSV allows the reliable and simultaneously detection of PRRSV EU and NA genotype from porcine blood, serum, tissue, bronchial swabs, bronchial lavage, saliva, semen and also cell culture samples. The assay internal control guarantees the control of extraction as well as amplification. virotype® PRRSV is easy to use with only one reaction-mix.

Poster 09

Eradication of *Bovine Viral Diarrhea Virus* from Thuringia's Biggest Heifer Producer with QIAGEN BVDV RT PCR Reagents

Carsten Schroeder[1], Christine Gaunitz[1], Marco Labitzke[1], B. Dirchen[2]

[1]QIAGEN Leipzig GmbH, Leipzig, Germany; [2]Thüringer Zuchtgenossenschaft "Rind" Ernstroda e.G, Ernstoda, Germany

Narrative: Thuringia's (Germany) biggest heifer producer is producing 1,300 heifers annually which are sold pregnant to partner farms. The calves from these heifers are purchased back at 4 to 8 weeks of age. The farm had an ongoing history of BVDV. Calves (n=2,586) were sampled upon arrival at 4 to 8 weeks of age. BVDV RNA was prepared from ear tissue samples using virotype ® Tissue Lysis Reagent (TLR) and testing was performed with QIAGEN BVDV real-time RT-PCR reagents with pools of 10 samples. In case of positive pools - individual ear tissue lysates were tested with the same RT-PCR. Calves tested positive for BVDV from ear tissue were retested from blood after two weeks for up to eight weeks. At 8-9 months, of age all cattle were retested from pooled blood samples in order to check if all PI calves were detected from ear tissue. Twenty-three of 260 pools were positive for BVDV (9%). Thirteen persistently infected (PI) in 12 pools (0.5%). Thirty-four non-persistently infected (NPI) in 11 pools (1.3%). The PI had mean Ct-values of 29.09 or less (SD 2.81-4.53). The NPI had mean Ct-values of 36.5 or more (SD 1.91-2.4). The NPI indicate a PI in the population. Six of 34 calves were most likely transiently infected (TI) animals since BVDV was detected for up to 8 weeks with declining ct-values. Twenty-eight out of 34 calves could be TI, but also BVDV contamination by licking ears can't be excluded. Since current BVDV programs in Switzerland and Germany are based on testing calves at a very early age, before they interact with other calves, and the risk of ear contamination as well as transient infection is low. Testing at the age of 8-9 months for BVDV all remaining animals scored negative, proofing detection of all PI from ear tissue. Since February 2009 no more PI animals were found in Thüringer Zuchtgenossenschaft Ernstroda and their partner farms. Starting a BVDV control program – initially testing pools of 10 ear tissue samples and retesting positive pools - is an economically sound approach as well. Virotype TLR allows simple ear tissue sample preparation. QIAGEN BVDV rRT-PCR allows an assessment of status PI versus non-PI based on the ct-values. Transiently infected animals in the population are an indicator for PI animals. Results of this study support earlier findings that ear notch samples and rRT-PCR are a promising tool for successful BVDV eradication.

Poster 10

Comparative Effects of Vaccination against *Porcine Circovirus* Type 2 (PCV2) and *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) in a PCV2-PRRSV Challenge Model

Changhoon Park, Yeonsu Oh, Hwi Won Seo, Kiwon Han, Chanhee Chae

Seoul National University, Seoul, Republic of Korea

Narrative: The objective of the present study was to determine the effects of the *Porcine circovirus* type 2 (PCV2) and *Porcine reproductive and respiratory syndrome virus* (PRRSV) vaccinations in an experimental PCV2-PRRSV challenge model that was based on virological (viremia), immunological (neutralizing antibodies [NAs], interferon-γ-secreting cells [IFN-γ-SCs], and CD4+CD8+ double positive cells), and pathological (lesions and antigens in lymph nodes and lungs) evaluations. A total of 72 pigs were randomly divided into 9 groups (8 pigs per group): 5 vaccinated and challenged groups, 3 non-vaccinated and challenged groups, and a negative control group. Vaccination against PCV2 induced immunological responses (NAs and PCV2-specific IFN-γ-SCs) and reduced PCV2 viremia, PCV2-induced lesions, and PCV2-antigens in the dual infected pigs. However, vaccination against PCV2 did not affect the immunological responses (NAs and PRRSV-specific IFN-γ-SCs), and PRRSV viremia, PRRSV-induced lesions, and PRRSV antigens in the dual infected pigs. Vaccination against PRRSV did not induce immunological responses (PRRSV-specific IFN-γ-SCs) and reduce PRRSV viremia, PRRSV-induced lesions, and PRRSV antigen in the dual infected pigs. In addition, vaccination against PRRSV increased PCV2 viremia, PCV2-induced lesions, and PCV2-antigens in the dual infected pigs. In summary, vaccination against PCV2 reduced PCV2 viremia, PCV2-induced lesions, and PCV2-antigens in the dual infected pigs. However, vaccination against PRRSV increased PCV2 viremia, PCV2-induced lesions, and PCV2-antigens in the dual infected pigs. Therefore, PCV2 vaccine decreased the potentiation of PCV2-induced lesions by PRRSV in dual infected pigs. In contrary, PRRSV vaccine alone did not decrease the potentiation of PCV2-induced lesions by PRRSV in dual infected pigs.

Poster 11

A Decade of Results from the Marine Mammal Stranding Project in Maryland *

Douglas Snider, Wilson K. Rumbeiha, Steve M. Ensley

Iowa State University, Ames, IA

Narrative: From 2000 to 2009, the Marine Mammal and Sea Turtle Stranding Network (MMSTSN) collected toxicology samples from 36 fresh dead stranded marine mammals in Maryland. Tissues were analyzed for heavy metal and pesticides. Heavy metal contaminants are able to persist in the environment for long periods of time while organochlorine pesticides and polychlorinated bipheyl compounds have been known to lead to bioaccumulation in tissue, particularly blubber. The ecotoxicolocial impacts are considered to be potentially significant despite most of the data being observational only. Due to environmental contamination from anthropogenic sources, marine mammals are being exposed to contaminants that may have deleterious effects on various tissues and lead to stranding. This study is consistent with reported toxin levels revealed in marine mammals throughout recent decades. Here, one hundred two samples were presented for analysis. Thirty-six animals' samples were analyzed for organochlorine compounds and PCB congeners by triple quadrupole liquid chromatography mass spectrometry (LCMSMS). Detectable levels of PCB congeners and multiple organochlorine pesticides were identified in every sample. The organochlorine pesticides found include aldrin, chlordane, PCH, endosulfan, endrin, nonchlor, oxychlor, heptachlor, and DDT congeners. Sixty-six kidney and liver samples were analyzed by inductively-coupled plasma mass spectrometry (ICPMS) for heavy metals. This data has been used to establish a database and calculate range and mean concentration values for marine mammals stranding in Maryland. Maryland concentration levels have been compared to concentrations of heavy metals in marine mammals found elsewhere to determine that Maryland values are comparable to those found by NIST. The following metals were found to fall within the range of the NIST data concentrations for some of the elements analyzed. Cadmium, calcium, iron, mercury, manganese, sodium, selenium, and zinc were found to exceed NIST reference ranges. Iron, mercury, and selenium demonstrate an increasing trend throughout the decade and may be considered to bioaccumulate in some species.

* Graduate Student Poster Presentation Award Applicant

Poster 12

Asymptomatic Bacteriuria in Female Dogs: Prevalence and Microbiologic Findings

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Narrative: Asymptomatic bacteriuria (ABU) is defined as the isolation of a specified quantitative count of bacteria in the urine of an individual without signs or symptoms of a urinary tract infection. In adult nonpregnant women, ABU is recognized as a benign non-progressive condition that should not be treated. Similar studies have not been performed to evaluate the prevalence and natural occurrence of ABU in healthy female dogs. The objectives of this study were to determine the prevalence of ABU in healthy female dogs and follow its natural course over a 3-month period. Healthy client-owned female dogs (n=101) without clinical signs of lower urinary tract disease were evaluated after obtaining client consent. Blood and urine were collected for a CBC, biochemical profile, urinalysis, and urine culture. The diagnosis of ABU was based on isolation of the same bacterial strain in 2 consecutive urine specimens or culture positive from a single cystocentis collected sample. The prevalence of ABU in healthy female dogs was 9% (9/101). Bacteria associated with ABU included *Escherichia coli*, *Enteroccus* spp., *Staphylococcus pseudintermedius*, *Klebsiella* spp., and *Streptococcus canis*. The antibiograms for the isolates demonstrated typical patterns of susceptibility with the exception of an isolate of *Klebsiella oxytoca*. There were no significant differences between antibiograms for subsequent isolates from the same dog. Bacteriuria associated with ABU persisted in 4/9 dogs and resolved in 5/9 dogs after 3 months. None of the dogs diagnosed with ABU developed clinical signs over the 3-moth observation period. In conclusion, ABU appears to be a non-progressive condition that can either be transient or persistent in healthy female dogs.

Poster 13

An Outbreak of *West Nile Virus* and *Salmonella enterica* serovar Typhimurium Co-infection in Chukar Partridge Chicks (*Alectoris chukar*) in California # *

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Narrative: *West Nile virus* (WNV) infection was diagnosed in September 2012 in a flock of 1,000 2-day-old captive chukar partridge chicks (*Alectoris chukar*) enclosed in 4 houses with 250 birds each in California. Over a period of 6 weeks, 200 birds died suddenly or after showing ruffled feathers and anorexia for 24 to 72 hours. Three carcasses (A-C) were submitted to the California Animal Health and Food Safety Laboratory for necropsy and diagnostic work-up. Birds B and C had hemorrhagic tracheitis at necropsy, and all 3 birds had lymphoplasmacytic and histiocytic myocarditis on histopathology. Cardiomyocyte necrosis and coronary arteritis was also noted in bird B. WNV was detected by PCR (kidney) and immunohistochemistry (heart or trachea) in all birds. Additionally *Salmonella* spp. was detected by PCR in intestinal contents of all 3 birds, and the bacterium was isolated from lung and intestine in bird A and liver in bird C, although only bird C had lesions consistent with *Salmonella* spp. septicemia (multifocal random hepatitis with hepatocellular necrosis and intralesional bacterial colonies). The *Salmonella* species isolated from the intestine in bird A was further identified as *Salmonella enterica* serovar Typhimurium by serotyping. Infections with *Avian influenza virus*, *Avian paramyxovirus-1*, *Avian infectious bronchitis virus* (*Coronavirus*), and very virulent *Infectious bursal disease virus* (avibirnavirus) were ruled out by specific PCR testing and Infectious laryngotracheitis virus (*Gallid herpesvirus-1*) was ruled out by direct fluorescent antibody test. WNV infection is reported very rarely in birds of the order Galliformes, and to the best of our knowledge there is only one published report in chukars. The detection of two zoonotic agents in these commercial birds makes this a particularly interesting case from a public health perspective.

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

Poster 14

Aleutian Disease in 4 Free-ranging Striped Skunks (*Mephitis mephitis*) from Northern California # *

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Narrative: Aleutian Disease (AD) was diagnosed in 4 free-ranging skunks (*Mephitis mephitis*, cases A-D) from Alameda and Marin Counties, California, in January 2010, March and October 2011, and May 2012. The carcasses, 2 females (A, D) and 2 males (B, C), were submitted to the California Animal Health and Food Safety Laboratory for necropsy and diagnostic work-up. Three animals were adults (B-D) and the age was unknown in case A. The animals had a spectrum of lesions, including (but not limited to) varying severity of lymphoplasmacytic tubulointerstitial nephritis or glomerulonephritis (4/4) with proliferative arteritis (2/4), lymphoplasmacytic meningoencephalitis (4/4) with extensive encephalomalacia and necrotizing vasculitis (1/4), lymphohistiocytic and plasmacytic myocarditis with segmental proliferative arteritis/vasculitis (3/4) and fibrinoid necrosis of cardiac arterioles (1/4), and portal lymphoplasmacytic and perivascular hepatitis (2/4). Rare *Parvovirus*-like basophilic intranuclear inclusion bodies were seen in the renal tubular epithelial cells in one case (D). The causative *Parvovirus* was detected by PCR in pooled tissues (kidney, liver, lung and spleen or brain) in cases A and B, liver in case C, and kidney, spleen, and urine in case D). Full genomic sequences were obtained from cases C and D. Although Aleutian Disease has been diagnosed in captive skunks in the US, to the best of our knowledge this is the first report of natural occurrence of the disease in wild skunks and the first report in California.

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

Poster 15

Caprine Abortion: Fetal and Placental Lesions attributed to Natural *Chlamydophila pecorum* Infection # *

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Narrative: A 16-month-old female Boer goat grouped with 34 other goats in a flock of 140 animals in California aborted 2 twin fetuses at approximately 130 days of gestation. There was no recent history of infectious abortions in the flock. Both fetuses (A-B) and a piece of placenta corresponding to fetus A were submitted to the California Animal Health and Food Safety Laboratory for post mortem examination and diagnostic work-up. At necropsy fetus A, a 3.2 kg male, had moderate diffuse anasarca and intermuscular (hind quarters) and scrotal edema. The piece of placenta had 4 cotyledons, one of which was covered by a yellowish friable necrotic exudate that extended to the adjacent intercotyledonary placenta. Fetus B, a 2.450 kg female, had moderate diffuse anasarca, brachygnathia superior and inferior, and palatoschisis affecting the hard palate. Histologically in fetus A there was focal marked suppurative and necrotizing placentitis with multifocal vasculitis/arteritis and thrombosis, focal area of cartilaginous metaplasia in the chorioallantoic stroma, and multifocal moderate lymphohistiocytic and neutrophilic hepatitis. In the colon there was moderate fibrinosuppurative exudate distending the lumen. In fetus B there was multifocal marked fibrinosuppurative enteritis with pseudomembrane formation and cryptitis. Brucellosis, toxoplasmosis, leptospirosis, Q fever, campylobacteriosis, *Bluetongue virus*, *Border disease virus/Bovine viral diarrhea virus*, and *Schmallenberg virus/Bunyavirus* infections, and aerobic bacterial infections were ruled out by specific testing. *Chlamydophila* spp. antigen was detected by direct fluorescent antibody test in direct smears of placenta, and intralesionally by immunohistochemistry in the placenta and colon (A), and small intestine (B). A nested PCR which detects *Chlamydophila* spp. DNA performed at Wyoming State Veterinary Laboratory was positive on formalin-fixed paraffin-embedded sections of placenta (A) and small intestine (B). Sequencing of the 16s rRNA gene in both samples yielded a 400 bp sequence that was found to be 99% identical to *Chlamydophila pecorum* by BLAST analysis (94-95% homology with *C. abortus* and *C. pscittaci*). Chlamydial abortion in small ruminants is usually associated with *C. abortus* infection. *C. pecorum* strains have been detected in ovine and caprine abortion cases in Morocco, Tunisia and France, although the pathogenicity is unclear. *C. pecorum* has also been detected in buffalo fetuses in Italy, sometimes in conjunction with *C. abortus*. These findings and epidemiological data suggest that *C. pecorum* may be abortigenic in ruminants. However to our knowledge, descriptions of fetal and placental pathology are not reported and the role of *C. pecorum* in ruminant abortion remains unresolved. Our report describes placental and fetal lesions in aborted goat fetuses naturally infected with *C. pecorum*.

AAVLD Trainee Travel Awardee (Pathology, Bacteriology/Mycology)
* Graduate Student Poster Presentation Award Applicant

Poster 16

Hepatitis Associated with *Reovirus* Infection in a Pigeon in California # *

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Narrative: A 5-month-old female pigeon with a 1-week history of uncharacterized clinical signs was submitted to the California Animal Health and Food Safety Laboratory for post mortem examination on December, 2011. The carcass was in poor body condition with mild lice infestation. Grossly the liver was markedly swollen and had diffuse dark red pinpoint mottling throughout the capsular and cut surfaces. Histologically there was severe multifocal extensive random hepatitis with disruption of the hepatic cord histoarchitecture, hepatocellular degeneration and lipidosis, necrosis and loss. Remaining hepatocytes were either markedly enlarged and swollen (megalocytosis) with large vesicular nuclei (megalokaryosis), or were smaller with increased nuclear: cytoplasmic ratio and increased cytoplasmic basophilia. Hepatocytes and occasionally epithelial cells lining the bile ducts formed large multinucleated syncytial cells (polykaryocytes). Both hepatocytes and syncytial cells contained large numbers of irregularly-shaped 3-8 micron deeply eosinophilic intracytoplasmic viral inclusion bodies and round pale glassy eosinophilic intranuclear pseudo-inclusions with margination of the chromatin. There were scattered parenchymal and portal infiltrates of lymphocytes, plasma cells, histiocytes and hemosiderophages. Numerous 70 nm round viral particles with a moderately electron dense external ring/capsid around an electron dense central nucleus, arranged in paracrystalline arrays consistent with *Reovirus* were found in the cytoplasm of isolated hepatocytes or in syncytial cells by transmission electron microscopy. A pool of intestinal tissue was processed for virus isolation. Cytopathic effect was observed 3 days post-inoculation and the isolated virus identified as *Reovirus* by direct electron microscopy. Additionally the bird had severe fibrinous and caseonecrotic right thoracoabdominal airsacculitis caused by *Escherichia coli* with associated embolic pneumonia; in the bursa of Fabricius the epithelial cells and histiocytes contained myriads of large intracytoplasmic basophilic viral inclusion bodies morphologically resembling *Pigeon circovirus*. *Salmonella* spp. PCR was negative in intestinal contents and *Avian paramyxovirus-1* and *Avian influenza virus* were not detected by specific RT-PCRs in an oral swab. *Avian paramyxovirus* immunohistochemistry in liver and kidney was also negative. *Chlamydophila* spp. fluorescent antibody test was negative on direct smears of liver, spleen, and air sac. A heavy metal screen revealed normal or non-diagnostic hepatic levels of lead, manganese, iron, mercury, arsenic, molybdenum, zinc, copper, and cadmium. *Reovirus* infection has previously been reported in pigeons, and young chickens experimentally infected with *Reovirus* developed hepatic lesions comparable to those described in this pigeon. To the best of our knowledge this is the first written communication of hepatitis associated with this virus in the pigeon. The pathogenic role of *Reovirus* in pigeons needs to be further investigated.

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

Poster 17

A Previously Undescribed Highly Divergent Circular DNA Viral Genome from the Liver of a Horse with Severe Hepatopathy # *

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Narrative: A 9-year-old intact male paint horse presented with a 14-hour history of anorexia, incoordination, ataxia, partial unilateral facial paralysis, yellow discoloration of the sclerae, and terminal labored breathing. The animal had not received any injectable biologics of equine origin within 60 days prior of the onset of the clinical signs. The horse was euthanized due to poor prognosis, and the carcass submitted to the California Animal Health and Food Safety Laboratory for necropsy and diagnostic work-up. Necropsy findings included diffuse icterus, a flabby liver with a dark brown to red capsular and cut surfaces, and diffuse enhancement of the reticular pattern that was more evident on cut sections of the parenchyma. Microscopically the most relevant histologic lesions were present in the liver and consisted of diffuse parenchymal collapse with degeneration, necrosis/apoptosis and loss of approximately 70-80% of the hepatocytes in the centrilobular, midzonal, and periportal areas (panlobular distribution), diffuse sinusoidal congestion and hemorrhage, diffuse marked infiltration of histiocytes, lymphocytes, fewer plasma cells and rare neutrophils particularly in the centrilobular areas, scattered erythrophagocytosis, multifocal moderate perivenous fibrosis in the centrilobular veins, with occasional hepatocellular regeneration (mitosis) and bi-/tri-nucleation. Portal tracts were variably expanded by histiocytes, lymphocytes, plasma cells, and rare neutrophils and increased amounts of collagen (fibrosis). Clinical and pathologic findings were suggestive of, although not pathognomonic for, Theiler's disease and given the historical suspicion for a viral etiology in this condition, liver samples were processed for viral metagenomics at the Blood System Research Institute. A previously undescribed small circular DNA genome distantly related to small circular DNA recently identified in the feces of piglets was characterized. The virus was tentatively named *Equine Kyklovirus-1*. No other viral sequences were identified by Next Gen sequencing including the recently described members of the genus *Pegivirus* (*Flaviviridae*) described in horses, one of which has been clinically associated with Theiler's disease (*Theiler's Disease Associated Virus*= TDAV). A causative relationship between this novel virus and hepatitis in this horse cannot be made based solely on the findings of the present report, although the potential pathogenicity of the virus should be further investigated.

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

Poster 18

Generation of Animals Persistently Infected with HoBi-like Viruses and its Detection using RT-PCR, ELISA and IHC

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Narrative: The emergence of HoBi-like viruses presents problems for diagnosticians because of the similarities in clinical presentation, genetic sequence and antigenicity to *Bovine viral diarrhea virus* (BVDV). Because BVDV persistently infected (PI) calves are major vectors in spread of the virus they are the focus of BVDV detection efforts in the US. HoBi-like virus persistently infected (HoBi PI) calves were generated by the infection of seven pregnant heifers at 75 days of gestation. Four heifers were infected with the Italian HoBi-like strain Italy-1/10-1 and three were infected with the strain HoBi_D32/00. HoBi-like virus was detected by RT-PCR in the buffy coat of all heifers at day 6 post-infection and elevated body temperature was observed in all heifers at least one day between days 3 and 7 post-infection. One heifer aborted at 8 months of gestation, two calves died shortly after birth and four surviving calves appeared clinically normal. HoBi-like virus was isolated from the aborted fetus and the two calves that died. Based on multiple tests on samples collected more than 2 weeks apart, surviving calves were persistently infected with HoBi-like viruses. Ear notches, buffy coats and serum samples were collected from HoBi PI calves at day of birth (DOB), weekly for one month and at 10 weeks of age. Ear notches were tested by ELISA and immunohistochemistry (IHC). Total RNA were extracted from serum, buffy coat and ear notches samples and tested by RT-PCR using panpestiviruses and HoBi-like specific primer pair. IHC detected 100% of samples at all time points, ELISA missed one calf at DOB and at week 1 of age. Panpestivirus primer pairs 323-326 and 90-368 detected respectively, about 45% and 39% of positive samples. Using HoBi-like virus specific primers, 88% of samples were detected as positive. These results indicated that HoBi-like viruses efficiently infected the fetuses and established persistent infection in calves. It also demonstrated that while HoBi-like viruses share similarities with strains of BVDV-1 and BVDV-2, diagnostic tests designed for BVDV detection may fail in detecting HoBi PI calves and that PI detection using samples from calves in the first week of age may not be reliable.

Poster 19

Chronic Wasting Disease Infectivity in Peripheral Tissues of White-Tailed Deer (*Odocoileus virginianus*)

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Narrative: Chronic wasting disease (CWD) continues to emerge as a neurodegenerative prion disease of farmed and free-ranging cervids in North America. Clinical neurological disease manifests following the progressive accumulation of abnormal prions (PrP^{CWD}) within the central nervous system. The presence of PrP^{CWD} in other organ systems and bodily fluids occurs during the course of disease and facilitates the release of prions into the environment where they may persist and remain infectious for a considerable time. Natural transmission between cervids occurs relatively efficiently but a species barrier appears to limit transmission from cervids to domestic livestock and humans. However, it remains unclear if certain circumstances may permit these transmission barriers to be overcome. As the geographical range of CWD expands, the frequency of human and animal contact with CWD-infected tissues and fluids will increase making it important to understand the distribution of infectious prions in CWD-infected animals and define which tissues represent the highest risk of exposure to infectivity. An array of tissues and bodily fluids were harvested from white-tailed deer orally exposed to CWD in an experimental setting. Animals had developed clinical signs consistent with CWD and preliminary testing of brain and lymphoid tissue by ELISA, immunohistochemistry and western blot confirmed the diagnosis. Tissues sampled included spleen, liver, kidney, heart, skeletal muscle, brain, bladder and regions of small intestine with representative tissues both formalin-fixed and frozen. To estimate infectious prion titres using the incubation period assay, homogenates of each organ were subjected to bioassay in mice transgenic for elk (TgElk) prion proteins. Highest levels were identified in the brain and significant levels were found in most other tissues tested with the exception of skeletal muscle. The distribution of PrP^{CWD} within these organs was further investigated using immunohistochemistry, western blot and protein misfolding cyclic amplification (PMCA). These findings corroborate studies identifying widespread infectivity in the peripheral tissues of CWD-infected cervids and suggest a precautionary approach towards the use or consumption of products derived from these tissues.

Poster 20

Phosphide Poisoning in 4 Equids in California # *

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Narrative: Phosphide poisoning was diagnosed in two horses (an 8-year-old mare and a 5-year-old gelding) in September, 2011 and two donkeys (an 11-year-old male and a 14-year-old female) in May, 2013. The animals were kept on two unrelated premises in Amador and Santa Cruz counties, CA. Both horses were found dead overnight in their pen. The donkeys had a clinical history of simultaneous, acute onset of recumbency and convulsions. One died naturally and the other was euthanized due to poor prognosis. All 4 carcasses were submitted to the California Animal Health and Food Safety Laboratory at Davis for post mortem examination and diagnostic work-up. Malicious poisoning was suspected by the owners in both cases. Along with the horses, a sample of grain coated with a fine, grey powder to which the animals had access to, was also submitted. Complete necropsies were done on one of the horses and both donkeys. Pathological findings in the horse included severe, extensive, bilateral, caudo-dorsal, pulmonary congestion and hemorrhage; tracheal submucosal and peritracheal/perilaryngeal hemorrhage; adventitial hemorrhage in the aortic trunk; severe hemorrhage on the thoracic surface of the diaphragm and endo/epicardial ecchymoses. The donkeys had severe, diffuse, pulmonary congestion and petechiae and/or ecchymoses on multiple serosal surfaces, including mesentery, pleura and endo/epicardium. The stomachs of the 4 animals contained abundant green forage and grains admixed with a grey, finely, moist granular material. Phosphide salts (zinc/aluminum) were detected by gas chromatography–mass spectrometry in stomach contents of all 4 animals and the grain sample submitted with the horses. Zinc/aluminum phosphides are widely used as pesticides and rodenticides. Once ingested, the stomach acidity and moisture generate the toxic phosphine gas from the phosphide compound. The mechanism of toxicity is not clearly understood but phosphine gas seems to block cytochrome-C oxidase, impairing the mitochondrial respiratory chain. There is no specific antidote; treatment is palliative and includes early gastric lavage. Release of the gas during necropsy represents a potential risk for human exposure. According to a recent report, eight cases of phosphine gas poisoning were reported in veterinary hospital staff members between 2006 and 2011. Symptoms include nausea, restlessness, abdominal pain, cardiac arrhythmias, pulmonary edema, dyspnea and cyanosis. Phosphide toxicity should be considered as a differential diagnosis in cases of sudden death in equids. Adequate sampling of stomach contents and toxicological tests are crucial for diagnosis. Special care regarding safety of personnel should be considered while handling gastrointestinal contents of potentially exposed animals.

AAVLD Trainee Travel Awardee (Toxicology, Pathology)
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Poster 21

PCR Ribotypes of *Clostridium difficile* Isolates from Pigs

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Narrative: PCR ribotyping is highly discriminative, reproducible and can be used for interlaboratory comparison. It is the primary method used for molecular epidemiological analysis of *Clostridium difficile*. The objective of this study was to investigate the ribotype patterns of *C. difficile* isolated from pigs in Korea. A total of 122 isolates originated from 835 pigs were tested using PCR ribotyping. Isolates were also tested using PCR for the presence of genes encoding toxin A, toxin B, and binary toxin. Among 122 *C. difficile* isolates from pigs, 106 isolates (86.9%) were A+B+CDT+ and 15 isolates (13.1%) were A-B-CDT-. All of the A+B+CDT+ isolates were typed as PCR ribotype 078. On the other hand, all of the A-B-CDT- were types as PCR ribotype 084. In humans, PCR ribotype 078 has been reported to be a new emerging hypervirulent strain that is genetically indistinguishable from porcine isolates. PCR ribotype 078 was dominant among porcine *C. difficile* isolates in Korea.

Poster 22

Clostridium difficile in Diarrheic Korean Native Calves

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Narrative: *Clostridium difficile* is a gram-positive, spore-forming, anaerobic bacterium that has been associated with pseudomembranous colitis in humans. *C. difficile* also appears to be an important cause of enteric disease in pigs, horses, dogs and cattle. Neonatal calf diarrhea (NCD) is usually leading to death in preweaning calves. A variety of enteropathogens have been implicated in NCD. We investigated *C. difficile* in diarrheic Korean native calves and characterized bovine *C. difficile* PCR ribotypes. A total of 232 diarrheic Korean native calves aged from three to 60 days (average 22 days) in Jeonnam province, Korea, were included in the study. Fecal samples were obtained from April to August, 2012, and were cultured for *C. difficile* and tested with an ELISA for *C. difficile* toxins A and B. Toxin gene PCR and PCR ribotyping were performed with the isolates as previously described. Other enteropathogens including *Escherichia coli*, *Salmonella* spp., *Bovine viral diarrhea virus*, *Bovine rhinovirus*, *Bovine coronavirus*, *Eimeria* spp., and *Cryptosporidium parvum* were investigated. *C. difficile* was isolated from 43 (18.5%) of 232 calves; 20 isolates of A+B+CDT+, 19 isolates of A+B+CDT-, one isolate of A-B+CDT- and two isolates of A-B-CDT-. One A+B+ isolate encoded cdtA gene but did not encode cdtB gene. Toxins were detected in calf feces from 8 (3.5%) of 232 calves with diarrhea. *C. difficile* and its toxins were detected concurrently in only 2 (0.9%) of 232 samples. Twenty-six PCR ribotypes were identified among 43 *C. difficile* isolates. Of these, 17 strains (39.5%) were PCR ribotype 078, one strain (2.3%) was ribotype 017, which are emerging types for humans. Of 43 *C. difficile* infected samples, 10 were infected with *C. difficile* only. The others were complex infection of *Eimeria* spp., ETEC, BRV, BCV, and/or BVDV, but no *Salmonella* spp. and *C. parvum*. In conclusion, 41 of 43 (95.3%) *C. difficile* isolates were toxigenic. Some isolates were identified as ribotype 078 and 017. So this pathogen may be associated with calf diarrhea, and cattle could be reservoirs of *C. difficile* for humans.

Poster 23

A Case of Cardiomegaly in Brown Trout

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Narrative: Chronic low to moderate level mortality (1-40 fish per day) was observed in a group of 9,000 brown trout fingerlings 3 weeks after fish were transferred from a hatchery to a small cooperative rearing pond. In the coop pond, sufficient water exchanges with water flow at 100 gallons per minute and good water quality were maintained. The volunteer caretakers observed that significant numbers of fish became lethargic and died shortly after each handling, e.g. for taking weight counts, etc. The mortality rate apparently coincided with the level of handling stress. Salt treatments were applied a few times without improvement. At necropsy, dead fish showed variably mild to severe heart enlargement. No significant bacteria were isolated and no parasites were detected. Severely dilated ventricle with thin outer ventricular myocardium and increased lumen space among trabeculae were noted by the histological examination of the enlarged hearts. We checked the group of fish several times over a nine-month period for overall condition and particularly focused on the hearts. The degree of enlargement was consistent or slightly increased over this time, but did not affect all the fish. The lesions of myofiber hyperplasia, hypertrophy and karyomegaly as well as myofiber degeneration were observed in affected fish. Additionally, during an annual fish health inspection at the source state hatchery, enlarged hearts were observed in about 5-10% of the 60 fish sampled (siblings of the coop pond fish), however, no low level chronic mortality occurred at the state hatchery. This was the first time this type of lesion was observed in Wisconsin hatchery raised fish. The cause of enlarged heart of this group was not identified.

Poster 24

Concurrent Pulmonary Malignant Peripheral Nerve Sheath Tumor and Thyroid Adenocarcinoma in a Dog +

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Narrative: Peripheral nerve sheath tumors (PNSTs) are tumors that originate from the cells that comprise the sheaths of peripheral nerves. PNSTs are relatively common in human beings, but occur infrequently in domestic animals, with most cases recorded in cows and dogs. Tumors of thyroid follicular cells are classified either as adenomas or carcinomas, with various subtypes based on histopathological or behavioral characteristics. Most animals with thyroid tumors are adult or aged. Thyroid carcinomas occur more frequently than adenomas in dogs, whereas adenomas are diagnosed more frequently in cats. Here, we report a case of concurrent thyroid adenocarcinoma and malignant PNST originating from the pulmonary parenchyma with metastasis to the liver and duodenal wall in an unknown aged female mixed dog. An unknown aged female mixed dog was submitted for necropsy. The dog was housed in the animal shelter and showed no apparent clinical signs before death. After necropsy, representative tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. The 4μm sections were stained with hematoxylin and eosin for light microscopic examination. Immunohistochemistry was perfomed on serial sections from the organs to examine the origin of tumor. The antibodies against vimentin, cytokeratin, desmin, S-100, neuron specific enolase(NSE), von Willebrand Factor (vWF), GFAP and thyroglobulin were used as the primary antibodies. Grossly, the right thyroid gland was severely enlarged. In the lung, multiple white to gray, firm, and well demarcated masses of variable diameter were observed. A neoplastic mass was observed in the liver and duodenal serosa. Histopathologically, the normal architecture of thyroid gland was replaced by neoplastic cells. The tumor consisted of follicles of varying size and shape. The neoplastic cells were cuboidal to columnar and had vacuoles in their cytoplasms. Mitotic index was minimal. The tumor cells showed positive reactivity for cytokeratin and thyroglobulin. The masses of lung, liver, and duodenum were different from thyroid gland tumor. The tumors consisted of highly pleomorphic cells arranged in interwoven bundles. The cells were spindle shaped with an abundant eosinophilic cytoplasm. Several tumor emboli were found in the pulmonary blood vessels. Immunohistochemistry revealed that these tumor cells were positive for vimentin, NSE, and S-100, but negative for cytokeratin, vWF, and GFAP. In human beings, pulmonary malignant PNSTs are extremely rare, and metastatic rate is very low. Based on the histological and immunohistochemical features, this case was diagnosed as concurrent thyroid adenocarcinoma and pulmonary malignant PNST with metastasis to the liver and duodenal wall in the dog.

+ AAVLD/ACVP Pathology Award Applicant

Poster 25

Pathology of Natural *Nerium oleander* Intoxication in South American Camelids

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Narrative: *Nerium oleander* (oleander) is an ornamental, evergreen shrub highly toxic to many domestic animal species. The toxicity of oleander results from cardiac glycosides like oleandrin and nerine, and as little as 0.005% of animal body weight of dry leaves may be lethal. In California, oleander is a frequent cause of death among South American camelids. Animals exposed to oleander may have acute respiratory distress, non-specific clinical signs that sometimes resemble colic, or are just found dead without any previous clinical signs. South American camelids with oleander intoxication present with a triad of clinical abnormalities that indicate renal, hepatic, gastrointestinal and/or cardiovascular dysfunction. In this study, we describe the pathological findings of oleander intoxication in 6 alpacas (*Vicugna pacos*) and 4 llamas (*Lama glama*) submitted for necropsy to the California Animal Health and Food Safety laboratory, that was confirmed by detection of oleandrin in the gastrointestinal contents using liquid chromatography-mass spectrometry. Grossly all the animals had multifocal petechial and/or ecchymotic subepicardial or subendocardial hemorrhage (n=6), sometimes associated with poorly demarcated areas of myocardial pallor (3), diffuse reddening or diffuse pink/red mottling of the lungs with moderate to severe pulmonary edema (6), hydropericardium and/or hydrothorax (5), ascites (5), locally extensive, multifocal or diffuse small and/or large intestinal congestion, often with hemorrhagic intestinal contents (5) and sometimes with a thin pseudomembrane lining the intestinal mucosa (2), and diffusely pale kidneys (3). Three animals did not show any gross lesions. Microscopically, the most consistent and severe lesions were observed in the heart. All animals had mild, moderate or severe, multifocal, acute or subacute, myocardial degeneration and necrosis sometimes accompanied by interstitial infiltration of neutrophils and macrophages and subendocardial and subepicardial hemorrhage. In addition, there was diffuse pulmonary congestion and edema (6), mild to moderate multifocal acute renal tubular degeneration and necrosis (3), neutrophilic enteritis with superficial fibrinocellular casts (3), and diffuse marked hepatic congestion (3). This shows that, in natural cases, oleander glycosides cause microscopically detectable damage primarily in the heart, suggesting that the clinical signs and abnormal clinical parameters indicating renal, hepatic and/or gastrointestinal dysfunction may be secondary to a cardiovascular problem. However, as suggested by other authors, a possible direct effect of oleander glycosides in the urinary or gastrointestinal tracts of South American camelids cannot be ruled out.

Poster 26

Adrenal Necrosis and Thrombosis in a Dog Treated with Trilostane # * † +

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Narrative: An adrenal gland from a castrated, male, 12 year-old, Dachshund was received for histopathologic examination at the Oregon State University Veterinary Diagnostic Laboratory. This patient had been diagnosed with hyperadrenocorticism ~ 2.5 months prior and had been treated with trilostane, with good control of Cushing's clinical signs during that time span. However, the patient was recently presented to the referring DVM with a 5 day history of vomiting, inappetence, and listlessness. The owners had discontinued trilostane treatment during this period. The animal was transferred to an emergency clinic for overnight fluid therapy to correct azotemia and permit further investigation of elevated alkaline phosphatase levels (ALP). A brief ultrasound suggested a splenic mass was present. Following stabilization, the patient was discharged with the recommendation of further diagnostics at the Olympia Veterinary Cancer Center (OVCC). Subsequent work-up at the OVCC revealed a mild azotemia, increased ALP, hyponatremia, hyperkalemia and an ACTH stimulation test consistent with Addison's disease. A complete diagnostic ultrasound exam demonstrated an enlarged right adrenal gland and a normal spleen. Thirteen days after initial presentation to the referring veterinary clinic (18 days after stopping trilostane therapy), an exploratory laparoscopic surgery was performed and the excised right adrenal gland was fixed in 10% formalin prior to shipment. Grossly, the gland was diffusely swollen, with a white-grey core and poor corticomedullary distinction visible on cut surface. Microscopically, there was acute coagulation necrosis of all of the medulla and 90% of the cortical tissue, sparing only a thin subcapsular rim. There were multiple fibrin thrombi in large medullary veins. Plump fibroblasts and endothelial cells were visible along the capsular aspect, as well as nodules of regenerative cortical tissue, indicating a subacute to early chronic lesion. Post-surgery the patient improved clinically with increased appetite and energy, but died unexpectedly 7 days after the laparotomy. A necropsy examination was declined. Rare cases of adrenal necrosis have been linked to treatment with trilostane but the mechanism is unknown. Postulated causes of this side effect include: idiosyncratic reaction causing apoptosis due to toxic cellular concentrations, drug induced vascular changes, and hypersecretion of ACTH causing necrosis/apoptosis. Vascular thrombosis has not been identified but was suspected in previous reports of adrenal necrosis with trilostane administration. In this case, the necrosis and vascular thrombi confirm a vascular disturbance and pose questions regarding the influence of trilostane on adrenal vascular anatomy.

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

Poster 27

Evaluation of Effects of Media Conditions on Recovery and Identification of *Tritrichomonas foetus*

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Narrative: *Tritrichomonas foetus* infection in represents a major economic problem to the cattle industry. Due to its insidious nature and lack of clinical signs in infected adult cattle, this pathogen can go unrecognized in a herd for many years. Historically, diagnosis required culture of the agent in specialized media and daily examination to demonstrate motile trichomonads. Further characterization was needed to differentiate *T. foetus* from non-pathogenic trichomonads. Recently, molecular detection techniques have been developed that permit faster and more sensitive detection over culture methods. Sensitivity of culture and PCR methods are maximized by inoculation into and incubation of a growth medium prior to microscopic evaluation or PCR over direct examination of a reproductive sample. However, alteration of media conditions may interfere with trichomonad detection. This study was conducted to evaluate the effects of exposure to a variety of media environments on the survivability and detection of *T. foetus*. Two strains of *T. foetus* (one QC strain, one field strain) were used to inoculate modified Diamonds and InPouch® (Biomed Diagnostics) media. Organism viability, motility, and morphology, and ability to detect DNA were examined at pH 4.7-6.8. Known numbers of *T. foetus* were inoculated into media with bull smegma samples that were and were not contaminated with bacteria. PCR testing was performed on aliquots from inoculated media samples at various time-points after incubation and Ct values were determined. Viability and motility of *T. foetus* decreased and pseudocysts were visible starting at pH 6.2; live organisms were rarely seen at pH 5.2 and not identified at pH 4.7. InPouches® inoculated with contaminated smegma had a pH range of 5.2-5.8, while Diamond's media inoculated with contaminated smegma had a pH range of 6.6-6.8. Media aliquots at pH 4.7 had a mean Ct value increase of 8 cycles over those with pH of 6.8. Contaminated InPouches® had a 6-9 Ct value increase over samples that were inoculated with non-contaminated smegma. Soluble DNases expressed by *T. foetus* have been shown to completely degrade a variety of DNA's in vitro and have optimal activity at pH 4.0. Bacterial contamination of InPouch® media, particularly with fecal coliforms that multiply rapidly in enrichment conditions designed to support *T. foetus*, can result in decreased pH of the media and create an environment resulting in *T. foetus* death and DNA destruction. Further investigation into optimal incubation media may help to maximize recovery and detection of *T. foetus* in clinical samples.

Poster 28

Analytical Method for the Extraction of 2-Dodecylcyclobutanone from Glycerol-Treated, Irradiated Chicken Jerky Pet Treats

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Narrative: Irradiation is an approved process useful in a broad category of food product to ensure food safety and insect control. Verification of absorbed doses can be achieved by a variety of methods with monitoring of the 2-acylcyclobutanones (2-ACB). While the correlation of 2-ACB levels in raw muscle foods has been well documented, there is a little information on the effect of processing and various additives on the observed levels. Imported irradiated chicken jerky treats have been implicated in numerous cases of illness and death in dogs, due to yet unidentified causes. It is thought that these products are irradiated at doses of about 10 kGy, though there are not any current verification tests. In addition, it is not known what, if any effect the glycerin treatment has on the final product prior to irradiation. The objectives of this study were to measure the levels of 2-dodecylcyclobutanone (2-DCB) in chicken jerky treats irradiated with different doses (0 control, 5, 10, 15, and 25 kGy) and soaked in glycerin at different times (0 control, 20 min and 24 h). 2-DCB is a common 2-ACB found in muscles of food containing palmitic acid. Samples were prepared from store bought chicken breast tenderloins, stored in Whirl-Pak bags, and then irradiated with a Cesium-137 source, at 20°C. Dosimeters were used to verify exposure time for the appropriate absorbed dose. Three replicate experiments were performed. Samples were measured for total fat by the Soxlet procedure using hexane extraction solvent. Moisture was measured by a microwave gravimetric method. For 2-DCB extraction a 5 g sample of ground chicken jerky was mixed with 50 mL acetonitrile and shaken for 20 min using a hand-shaker. The acetonitrile extract was collected and evaporated to dryness under nitrogen and injected into a gas chromatography-mass spectrometer (HP 5890 GC and 5972 detector) fitted with an HP-5MS column operated in the single ion mode monitoring ions mz 98 and 112. The concentration of 2-DCB increased linearly with the doses and had a linear correlation of R2= 0.9874. The levels of 2-DCB corresponding to the irradiation doses were 0.024 ± 0.003, 0.030± 0.007, 0.033± 0.007, and 0.041 ± 0.008 ppm for 5, 10, 15, and 25 kGy. This study showed that 2-DCB could be used to monitor the absorbed treatment dose and that soaking the chicken in glycerin at different times did not effect the detection of 2-DCB.

Poster 29

Locally Injected Autologous Platelet-Rich Plasma Improves Long Subdermal Plexus Skin Flap Survival but not Second Intention Wound Healing in Dogs

Maria Karayannopoulou[1], Panayiotis Loukopoulos[2], Dimitra Psalla[2], George Kazakos[1], Nikolaos Giannakas[1], Ioannis Savvas[1], Maria Kritsepi[3], Lysimachos G. Papazoglou[1]

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Narrative: Peripheral flap necrosis remains a serious complication of subdermal plexus skin flaps. Platelet-rich plasma (PRP) is rich in growth factors and cytokines implicated in tissue repair; thus, topical PRP supplementation has the potential to improve wound healing. To evaluate histologically the effect of locally injected autologous PRP on second intention wound healing of acute full-thickness skin defects ("wounds"), and on the survival of long (length-to-width ratio 5:1) subdermal plexus skin flaps ("flaps") in dogs, three 2x2 cm full-thickness skin defects and one dorsally based 2x10 cm subdermal plexus flap were created in six purpose-bred beagle dogs on opposite sides of the dorsal midline. Randomly, one side received autologous PRP injections and the other side was left untreated (control). Autologous PRP (2.5 ml), prepared using the Magellan-Medtronic platelet separator system, was immediately equally distributed beneath the flap, through sutures. Skin flap survival was evaluated macroscopically, by Laser Doppler Flowmetry measurements of tissue perfusion, and histologically. Samples taken on days 4, 10 and 20 post-surgery were examined histologically; edema, collagen production and neovascularization were scored. The survival rate was 96.3% in PRP-treated flaps and 79.7% in controls. Tissue perfusion was significantly higher in PRP-treated flaps. Wounds showed higher collagen production and better structure on day 20 in the PRP group. In flaps, necrosis was observed at the distal part of most of the controls; less edema and greater collagen production were found in the PRP group compared to the controls ($P = 0.01$ and 0.011, respectively), on day 10. In both wounds and flaps, no significant differences in neovascularization were found between groups. Locally injected autologous PRP improves the survival of long subdermal plexus skin flaps, but not second intention wound healing of acute wounds, in dogs.

Poster 30

Uroperitoneum and Bilateral Renal Rupture in a Buck following Urethral and Urinary Bladder Obstruction due to Carbonate Apatite/Struvite Urolithiasis

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Narrative: Urolithiasis is encountered less frequently in goats compared to other ruminants. When followed by lower urinary tract obstruction, it may, if not diagnosed and treated early, lead to urethral or bladder rupture, azotemia and death. We describe a case of obstructive carbonate apatite/struvite urolithiasis in a buck, causing complete urethral and bladder blockage followed by bilateral renal rupture and uroperitoneum. A buck from a herd from Asvestohori, Thessalonica, Greece, with a history of dysuria that had progressed to anuria and death was submitted for necropsy in May 2011. The other buck of the herd showed similar signs albeit to a milder degree. The animals were fed straw, alfalfa hay, and commercial concentrates, and were allowed to graze for a few hours daily. The two months prior to presentation, the water supplied to the animals came from a well within the farm compounds. On necropsy, perineal erythema, severe abdominal distention due to severe uroperitoneum and bilateral renal rupture were noted. A large number of spherical smooth olive-green stones 1mm to1.5cm in diameter was present in the bladder and urethra, causing their complete obstruction, severe distention of the bladder, and hemorrhagic content throughout the urethral lumen. Bacterial culture of urethral samples in aerobic conditions was negative. Chemical analysis showed that the uroliths were composed of struvite (40%) and carbonate apatite (60%). Urolithiasis in the buck may be due to carbonate apatite-struvite uroliths. Bilateral renal rupture and uroperitoneum due to obstructive urolithiasis is rarely reported in the goat.

Poster 31

Prevalence and Spectrum of Johne's Disease Lesions in Cattle Slaughtered at Two Abattoirs in Kampala, Uganda

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Narrative: This study was conducted to determine the prevalence and characteristics of Johne's disease (JD) lesions in Ugandan cattle slaughtered at two of the main abattoirs in Kampala. JD, or paratuberculosis, is a chronic wasting disease that affects a wide range of domestic and wild animals and is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), an acid-fast bacterium. It has serious negative impact on the productivity of the affected animals. The control and eradication of the disease is constrained by the lack of early diagnosis tools. Identification of the affected animals can be based on clinical signs, demonstration of the organism in feces and tissues of affected animals using various bacteriological and molecular methods; serological testing in live animals, and gross and microscopic lesions. In countries where JD has never been reported or has very low prevalence, facilities for culture and molecular diagnosis of the disease might not be in existence. Under such circumstances, pathological diagnosis is very important. Although gross lesions can lead to a correct diagnosis of the disease in advanced stages, in some cases, even animals with advanced disease may lack such lesions. Moreover, in early stages of the disease, the lesions may be so subtle as to escape recognition. Ileocecal junction and the associated lymph nodes of 1,022 cattle were examined for gross and microscopic lesions, followed by Ziehl Neelsen staining of the tissues bearing lesions. Confirmation of MAP infection was done in some of the tissues using culture and IS900 PCR. The lesions were then described, characterised and tabulated. Characteristic JD granulomas were found in 4.7% of the samples examined, derived from Zebu, Ankole longhorn, Friesian breeds of cattle and their crosses. Lesions were found both in the lymph nodes and ileocecal junction mucosa. The lesions tended to be more severe in the lymph node than in the mucosa. There were also some unique and atypical lesions found in association with JD granulomas. The diagnostic value of various gross lesions and criteria of lesion classifications and diagnosis are revisited and discussed based on the findings of this study. The prevalence of JD lesions among slaughtered cattle in Kampala's two abattoirs indicates that the disease is well established in the cattle population in the country. The diverse manifestations in lesions of JD, particularly early, focal or mild lesions, need to be considered when making histological diagnosis in tissues where the disease is suspected.

Poster 32

Non-Metastatic Pancreatic Adenocarcinoma with Persistent Hyperglycemia in a Horse

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Narrative: Pancreatic cancer is rarely diagnosed in horses, unlike in humans. Here, we report the only, to our knowledge, case of pancreatic adenocarcinoma that was shown to be associated with endocrine disorders. Only nine other cases of exocrine pancreatic adenocarcinoma and one case of a pancreatic islet cell tumor have been reported to date. A 20 year old crossbred gelding was diagnosed with persistent hyperglycemia of unknown origin, without clinical signs, in October 2012. In early January, he developed inappetance and fever; hematologic and serum biochemistry aberrations included elevated triglycerides, hyperglycemia, elevated fibrinogen, AST and GGT, and increased monocytes. A right abdominal mass was diagnosed by ultrasonography. Following 5 weeks of treatment, euthanasia was elected due to the persistent fever and blood profile abnormalities. On necropsy, a ~30 cm in diameter mass had replaced the pancreas, occupied the adjacent space, extending to the liver, gastric, duodenal, and colonic serosal surfaces from which it was separated by thick fibrous tissue, grossly sparing the above organs, but invading the liver capsule. The mass was partitioned by multiple fibrous bands, and was multifocally necrotic and/or cystic. Histologically, the normal pancreatic and peripancreatic architecture had been effaced by a poorly cellular, moderately well demarcated, infiltrative and unencapsulated tumor. Neoplastic cells were arranged in variably complete, variably sized and shaped, often tortuous tubules and acini, supported by an extensive, moderately dense fibrous stroma or by granulation tissue. Cells showed moderate anisokaryosis and anisocytosis, and low mitotic index. The tumor was separated from the liver and the duodenal, colonic and gastric serosa by a thick layer of fibrotic and granulation tissue. Multifocal, focally extensive mineralization of the vascular walls and the neuropil was noted in the cerebellum and cerebrum. Immunohistochemically, tumor cells were positive for cytokeratin, indicating epithelial origin, and negative for vimentin, synaptophysin, S-100, glucagon, chromogranin A, insulin, and Neuron Specific Enolase (NSE). Remnants of compressed islet cell islands, that were positive for synaptophysin, S-100, glucagon, chromogranin A, insulin, and NSE, were surrounded by tumor acini. The persistent hyperglycemia and other serum biochemical abnormalities observed likely resulted from beta cell depletion due to compression of the islet cells by the tumor. To our knowledge, clinical signs relating to endocrine aberrations were not noted in previous cases of exocrine pancreatic neoplasia. Unlike most previously reported pancreatic cancer cases, no metastases were observed in this case, despite extensive expansion in peri-pancreatic organs.

AAVLD Trainee Travel Awardee (Pathology)

Poster 33

Development of Multiplex Real Time PCR Assays for the Detection and Quantification of the Six Major Non-O157 *Escherichia coli* Serogroups in Cattle Feces # *

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Narrative: Shiga toxin-producing *E. coli* (STEC) are major food-borne pathogens associated with various outbreaks throughout the world. *E. coli* O157:H7 is the major STEC, however, non-O157 STEC are gaining more attention in recent years as they are responsible for more than 60% of human STEC infections. Non-O157 *E. coli* are under recognized and under estimated since the detection methods, including molecular methods, for non-O157 *E. coli* have not yet been fully developed and validated. Hence the objective of this study was to develop multiplex real time PCR assays to detect and quantify the six major non-O157 STEC: O26, O103, O111, O45, O121 and O145 in cattle feces. Primers were designed targeting the O-antigen genes, *wzx* for serogroups O26, O103, O111, O45 and O145, *wbqE* and *wbqF* for O121. Two serogroup specific assays, O26, O103, O111 in assay 1, and O45, O121, and O145 in assay 2, were developed. Specificity of the assays was assessed by testing 148 strains of top six non-O157 *E. coli* and 100 strains representing 42 other *E. coli* serogroups. Analytical sensitivity of the assays was determined with 10-fold serial dilutions of pooled pure cultures of non-O157 STEC strains. Also, fecal samples determined to be PCR-negative for non-O157 were spiked with 10-fold serial dilutions of pooled cultures of 1) O26, O103, and O111; 2) O45, O121 and O145; and 3) O26, O103, O111, O45, O121 and O145. Spiked fecal samples were enriched in *Escherichia coli* broth at 40°C for 6 hours. Fecal DNA was extracted before and after the enrichment and subjected to real time PCR. The assays were specific for all the target genes and no cross-reactions with non-targeted serogroups were observed. Correlation coefficients of the assays were >0.99 for pure culture, and >0.95 and >0.98 for fecal samples before and after the enrichment, respectively. PCR amplification efficiencies ranged from 94-102% for pure culture, 88-95% for fecal samples before enrichment and 90-101% after enrichment. The detection limits of the assays were 10^3 CFU/ml, 10^4 CFU/g, and 10^2 CFU/g for pooled pure cultures, before and after enrichment of spiked fecal samples, respectively. The two sets of multiplex real time PCR assays are being evaluated for their applicability in the detection of six non-O157 STEC serogroups in cattle feces and other matrices.

AAVLD Trainee Travel Awardee (Bacteriology/Mycology)
* Graduate Student Poster Presentation Award Applicant

Poster 34

Simple and Rapid Method for the Detection of *Salmonella* in Environmental Samples in the Presence of High Background Flora by Real-Time PCR

Quoc Hoang, Robert Tebbs, Michael Pettit, Rick Conrad

Life Technologies, Austin, TX

Narrative: *Salmonella* is a ubiquitous problem in the poultry industry. *Salmonella* can survive and persist almost anywhere in the process from hen house to poultry processing plant and can therefore be classified as an environmental pathogen. This makes environmental testing for Salmonella an important step in reducing *Salmonella* contamination. Traditional culture methods require an additional 3 to 5 days to confirm a result after an initial overnight sample enrichment. Some environmental samples may contain low levels of *Salmonella* in a background containing very high concentrations of other micro-flora that would compete for nutrients often causing false negative results. Here, we present a real-time PCR-based workflow, the RapidFinder™ Direct Lysis system, that provides a simple and rapid protocol for the detection of *Salmonella* in about an hour post-enrichment. The RapidFinder™ Direct Lysis protocol uses a lysis solution that allows the lysate to be used directly with the MicroSEQ® *Salmonella* spp. Detection Kit. Environmental sponge samples were prepared either artificially or were provided from a food manufacturing facility and evaluated for detection of *Salmonella* that was spiked onto the sponges at 2-5 CFU. The samples were stored at 4°C overnight prior to analysis to mimic standard practice for shipping environmental samples by overnight express at 4°C. To enrich, the samples were combined with 100 mL of BPW and incubated overnight at 37°C ±1°C. Aliquots from the enriched samples were removed and prepared for real-time PCR using a previously AOAC validated magnetic bead-based nucleic acid extraction kit or with the RapidFinder™ Direct Lysis protocol. A culture-based confirmation method was also used to confirm real-time PCR results. To mimic background flora normally found in environmental samples, the artificially-prepared environmental sponges were spiked with a mixture of *Klebsiella pneumonia*, *Citrobacter freundii*, and *Enterobacter cloacae* at increasing concentrations up to 108 CFU. The real-time PCR method detected 4 CFU of *Salmonella Poona* even in the presence of 108 CFU of background flora. The results from real-time PCR and the culture reference method matched for 36 out of 36 samples tested after 20 hours of enrichment. The RapidFinder™ Direct Lysis protocol offered the simplest solution with minimal hands-on time and results in about an hour from enriched sample.

Poster 35

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

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

Poster 36

Rapid Isothermal Detection of *Bovine Viral Diarrhea Virus* (BVDV) RNA

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Narrative: *Bovine viral diarrhea virus* (BVDV) is an increasingly important threat to both dairy and beef cattle, resulting in increased operational costs and lost productivity. Testing of newborn calves for BVDV promises to help prevent the spread of the disease by persistently infected calves, one of the most challenging sources of infection for this disease. This project is intended to address the need for a sensitive, specific, affordable, and fast solution for penside detection of BVDV. As a first step in providing a penside test for BVDV, we have designed an isothermal molecular detection chemistry for both type 1 and type 2 BVDV RNA. This test is based on reverse transcription, loop mediated isothermal amplification (RT LAMP) using OmniAmp DNA polymerase. This enzyme is uniquely suitable for RT LAMP due to its innate reverse transcriptase and strand displacement activities. Its high thermostability allows high temperature melting of target RNA structures, which proved critical to the successful detection of BVDV targets. Detection is based on signal generation by a fluorescent intercalating dye that binds to the double stranded RT-LAMP DNA product. This chemistry is intended to eventually be used with a low cost, easily operable instrument being developed concurrently that should facilitate penside detection of BVDV and a range of other agricultural pathogens. The OmniAmp Polymerase-based RT-LAMP chemistry allowed detection of purified viral RNA from both type 1 and type 2 BVDV reference strains that were independently confirmed as positive by real-time PCR. The time to result was under 20 minutes. Preliminary studies suggest that the limit of detection was comparable to RT PCR for both the type 1 and type 2 samples tested with no false positives in the negative control samples. The combination of performance, time to result, ease of operation and interpretation, low cost and compatibility with less complex instrumentation point to the potential of this test platform as an alternative to antibody-mediated point of care tests.

Poster 37

An Immunofluorescence Antibody Test: A Novel Diagnostic Tool for Detection of *Mycoplasma synoviae* in Chickens

Vijay Durairaj, Victoria Laibinis, Ruth Wooten, Naola Ferguson-Noel

Poultry Diagnostic and Research Center, The University of Georgia, Athens, GA

Narrative: *Mycoplasma synoviae* is an infectious pathogen affecting chickens and turkeys worldwide. It causes devastating economic losses in the poultry industry. *M. synoviae* causes synovitis in chickens and may cause clinical or subclinical upper respiratory tract infections. *M. synoviae* outbreaks occurring in poultry operations may be associated with horizontal or vertical transmission. Aerosol exposure is one of the major routes for disease transmission. Exposure to an aerosol antigen causes mucosal insult in the upper respiratory system and stimulates the mucosal immune system. As a consequence of this antigenic stimulation, secretory IgA antibodies are produced in the upper respiratory tract. The objective of this study was to evaluate the detection of these specific IgA antibodies as a predictor for *M. synoviae* infection in chickens. The experimental trial was conducted with three-week-old SPF chickens. Sixty chickens were reared in ten Horsefall-Bauer-type isolation units (6 chickens/unit) and provided with feed and water *ad libitum*. Chickens in five isolation units (n = 30) were infected with a *M. synoviae* field isolate by the intratracheal, intraocular and intranasal routes. Chickens in the remaining isolation units were uninfected and maintained as negative controls. Blood samples, choanal cleft swabs and tracheal swabs (in PBS tubes) were collected on alternate days from 3 days post infection (DPI) up to 11 DPI. Blood samples were centrifuged and serum separation was performed and used in serological testing using established methods. Choanal cleft samples were analyzed by real time PCR for detection of the infectious agent. Tracheal swabs in PBS were centrifuged and the supernatant was used for the immunofluorescence test to detect specific IgA antibodies. *M. synoviae* cultures grown on modified Frey's agar in a 48- well plate was used for this study. The supernatant collected from tracheal swabs were used as primary antibody followed by anti-chicken IgA antibody conjugated with FITC. Positive and negative results were based on the presence and absence of immunofluorescence respectively. By applying this method, positive reactions were observed as early as 3 days after infection, and the intensity of the positive reactions was significantly high in samples collected around 11 DPI. Several field samples were analyzed by using this method and a scoring system was established for evaluation of field samples.

AAVLD Trainee Travel Awardee (Bacteriology/Mycology)

Poster 38

Development of Serum Neutralization Assay for Detecting Neutralizing Antibodies against *Mycoplasma synoviae* *

Vijay Durairaj, Naola Ferguson-Noel

Poultry Diagnostic and Research Center, The University of Georgia, Athens, GA

Narrative: *Mycoplasma synoviae* is one of the pathogenic avian mycoplasma organisms affecting chickens and turkeys. *M. synoviae* adversely affects the health status of chickens by causing synovitis and/or airsacculitis. *M. synoviae* outbreaks cause significant economic losses in the poultry industry by affecting egg production in layers and condemnation losses in broilers. So, the assays used for early detection of *M. synoviae* outbreaks would play a major role in *M. synoviae* surveillance programs. Currently, there are various diagnostic assays employed in screening poultry for *M. synoviae* infections. In *M. synoviae* surveillance and monitoring programs, serological tests are frequently used as first-line diagnostic tests due to their amenability to high throughput and low cost. The early and specific detection of infection is critical to the control of *M. synoviae* infections. The main objective of this research was to develop a serum neutralization assay for detecting neutralizing antibodies against *M. synoviae*. This assay was performed by adding a standard concentration of *M. synoviae* culture to diluted concentrations of serum. The results of the test were read after ten days based on color changing units. Growth of a culture (color change) indicates that there was no antibody to prevent the growth of *M. synoviae* culture. Absence of growth of the culture (no color change) indicates that the antibodies inhibited the growth of *M. synoviae* culture. Based on this assay, we have tested several field samples and compared the results with other serological assays. One of the advantages of this assay is that the results are both qualitative and quantitative. Since the titer values of this assay are based on the amount of neutralizing antibodies it helps in understanding the protective antibodies against *M. synoviae*. This assay can be used as an additional test to detect *M. synoviae* outbreaks in chicken flocks and can be used in *M. synoviae* monitoring and surveillance programs.

* Graduate Student Poster Presentation Award Applicant

Poster 39

A Rapid Molecular Diagnostic Test for Detection of Swine Pathogens

Yogesh Chander, David Mead, Thomas W. Schoenfeld, Michael J. Moser

Lucigen Corporation, Middleton, WI

Narrative: Pork production is an economically important component of US agriculture, requiring efforts to keep pigs free of diseases and pathogens. Biosecurity, customer safety, and the well-being of the herd makes it critical to control and prevent swine diseases. The best preventative measure to defend against local and foreign diseases is the ability to rapidly detect and identify disease pathogens so that appropriate control strategies could be implemented to prevent disease outbreak. The objective of this project is to address the need for a sensitive, specific, affordable, and rapid solution for penside detection of various swine pathogens. As a first step, we have designed an isothermal molecular detection system for two important swine pathogens: *Clostridium difficile* and *Swine influenza virus*. This test is based on loop mediated isothermal amplification (LAMP) chemistry using OmniAmp DNA polymerase and can be completed in 30 min when coupled with a rapid, easy to use sample preparation method. OmniAmp polymerase is the only known thermostable enzyme that combines both adequate strand displacement activity for isothermal LAMP and strong reverse transcriptase (RT) activity to amplify RNA, thus making it suitable for amplification of both DNA and RNA based targets under uniform reaction conditions. This chemistry is intended to be used with a low cost, easily operable instrument that should facilitate penside detection of a wide range of swine pathogens. Primers targeting the highly conserved region of cytotoxin gene B (for *C. difficile*) and matrix gene (for SIV) were designed and reaction conditions were optimized. Initially, serial 10-fold dilutions of target (DNA or RNA) were used as template. SYBR-Green I dye in the reaction mixture allowed real-time monitoring of amplification products and post reaction thermal melt analysis was used to confirm the correct amplification product. Both the assays were found to be 100% specific for target pathogen, the limit of detection was <500 copies of DNA/RNA for both the targets, which is comparable to that obtained with PCR based methods. For SIV, amplification was achieved for all currently circulating subtypes including the H3N2v. These results indicate that this new LAMP assay method is a rapid and effective method for detection of *C. difficile* and SIV. This method can be used for development of rapid molecular diagnostic tests for detection of other swine pathogens.

Poster 40

HoBi-like Pestiviruses Persistently Infected Calves Transmit the Virus to Calves, Sheep, Goats and Pigs

Fernando Bauermann[1], Shollie Falkenberg[1], Nicola Decaro[2], Aaron Harmon[3], Julia F. Ridpath[1]

[1]Ruminant Immunology Group, USDA-ARS-National Animal Disease Center, Ames, IA; [2]Veterinary Medicine, University of Bari, Valenzano, Italy; [3]Novartis Animal Health, Larchwood, IA

Narrative: Similar to *Bovine viral diarrhea virus* (BVDV), HoBi-like viruses can establish persistently infections in cattle. The purpose of this study was to determine if HoBi persistently infected (PI) calves could transmit the virus to cattle and other species. Ten calves, eight sheep, seven goats and ten pigs free of pestivirus infection and antibodies against pestiviruses were used. Animals from each species were divided into two groups. One group (PICG) was exposed to a HoBi PI and the other group (ING) was inoculated with $2.5x10^5$ TCID (tissue culture infecting dose) of each of the two HoBi-like virus strains (HoBi-like HoBi_D32/00 and Italy-1/10-1 strains). The PICG, which were composed of five calves, four sheep, four goat and five pigs housed by species, were exposed to two HoBi PI calves (one PI harboring the strain HoBi_D32/00 and one the strain Italy-1/10-1) for 30 minutes, twice a day, for seven days. HoBi PI calves status was determined based on virus isolation from buffy coat from samples collected two weeks apart. Animals were monitored for 24 days, nasal swab and blood samples were collected at days 3, 6, 9, 11, 13 and 18 post-infection (ING) or following the first day of contact with the PI calves (PICG). RT-PCR was performed using primers specific for HoBi-like viruses. Pyrexia and viremia were observed in all calves on at least one time point. The average number of days with pyrexia was 2.5 for ING and 3.6 for PICG. One calf died at day 17 post-infection and virus was detected in the buffy coat. Nasal discharge was observed in the four sheep in the ING and one at the PICG (one day average). One sheep in the ING presented pyrexia at day 5 post-infection, and diarrhea and ocular discharge for four days. Virus was detected in four and three sheep in the ING and PICG, respectively. Two goats were viremic and three goats developed pyrexia in both the ING and PICG. The average number of days in pyrexia was 5.3 and 3.7 for ING and PICG. Virus was detected in two pigs in the ING and one in the PICG. In addition, two of the ING pigs and all of the PICG pigs developed pyrexia. While the most efficient transmission was observed with cattle, transmission was observed with all species tested indicating that this virus may infect multiple domestic species and thus may prove a threat to multiple industries.

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American Association of
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Exhibit Directory

San Diego, CA
October 17-23, 2013

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www.qualtrax.com

SafePath Laboratories, LLC

Booth **32**

5909 Sea Lion Place, Suite D
Carlsbad, CA 92010
www.safepath.com
Contact: Steve Ness
949-436-8854
steve@safepath.com

SafePath Laboratories is a USDA licensed veterinary diagnostics manufacturing facility. SafePath is located in Carlsbad, CA, and has experience in manufacturing assays on a variety of platforms to include 96-well ELISA and rapid lateral flow. SafePath consists of a team of professionals with over twenty-five years experience and a proven record of innovation, in the development and manufacture of high performance immunoassays.

SAGE

Booth **29**

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

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

Tetracore, Inc.

Booth **7**

9901 Belward Campus Drive, #300
Rockville, MD 20850
www.tetracore.com
Contact: Pete Pillay
240.268.5400
ppillay@tetracore.com

Company Representatives: Dr. William Nelson, Rolf Rauh, John Kelly, and Pete Pillay

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

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

Thermo Scientific

Booth **24**

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

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

AAVLD/USAHA
Upcoming Meetings

2014: October 16-22
Kansas City, Missouri

VMRD, Inc.

Booth **6**

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

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

ZOETIS

Booth **17-18**

100 Campus Drive
Florham Park, NJ 07932
www.zoetisus.com
Contact: Constantina Poga
973.443.2738
constantina.poga@zoetis.com

Zoetis (pronounced zō-EH-tis), formerly Pfizer Animal Health, is a leading developer, manufacturer and marketer of immunodiagnostic test platforms for reference laboratories worldwide. We are building in more than 60 years of experience to deliver quality medicines, vaccines, diagnostic products, genetics tests and a range of other services to the animal health industry and its stakeholders, from veterinarians to the livestock farmers and pet owners they assist. We are committed to ensuring the long-term health and sustainability of veterinary medicine by addressing the challenges facing the profession. Our goal is to support veterinarians along the continuum of care for disease management and advance the profession through our research and development and continuing education and training.

2015: October 22-28
Providence, Rhode Island

Exhibits and Poster Presentations

Sponsor Presentations Saturday – October 19, 2013

IDEXX	6:00-6:15 pm	Pacific Salon 2	The Role of Oral Fluids in Swine Herd Management
PRIONICS	6:15-6:30 pm	Pacific Salon 3	PrioCHECK® Salmonella Antibody Tests: New Tools to Screen Bovine Serum, Plasma and Milk Samples
ZOETIS	6:30-6:45 pm	Pacific Salon 3	An empirical approach to confidence intervals of Positive and Negative Predictive Values Extrapolated from case control studies.
QIAGEN	6:30-7:00 pm	Pacific Salon 2	What's new at QIAGEN?

Exhibit Hall Times

Saturday, October 19
9:00 am - 10:00 am
11:30 am - 1:00 pm
3:00 pm - 6:00 pm

Sunday, October 20
9:00am -10:30 am

Refreshments available during exhibit times

Poster Session

9:00 am-6:00 pm Saturday, Oct 19
7:00 am-10:30 am Sunday, Oct 20

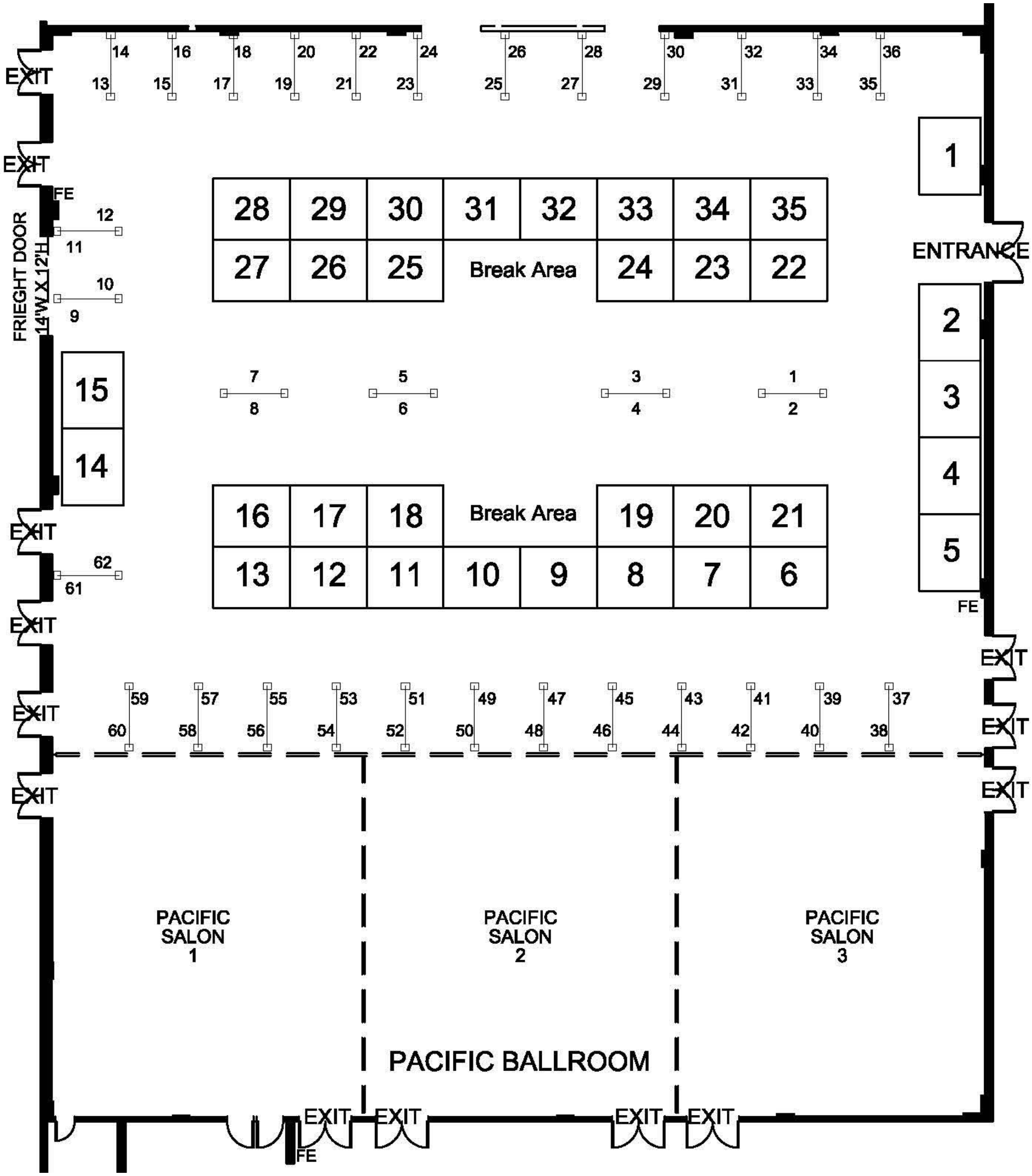
Authors present 3:00-4:00 pm on Saturday, Oct 19, 2013

Booth #	Company	Booth #	Company
25	Advanced Technology Corp. VADDS	22-23	Life Technologies
28	Anaerobe Systems	16	LIMS Pro, Inc.
26	Biocare Medical	31	Mitsubishi Gas Chemical America, Inc.
27	Biolog, Inc.	4	Nat'l Instit. Animal Agriculture (NIAA)
14	BioMerieux	11	Omega Bio-Tek
10	Bio-Rad AbD Serotec	8	PRI Bio (Progressive Recovery, Inc.)
34	Biovet Inc.	5	Prionics
12	Bruker Daltonics	2-3	QIAGEN, Inc.
35	Centaur, Inc.	30	Qualtrax
33	Computer Aid, Inc.	32	SafePath Laboratories, LLC
1	ECL2/Q-Pulse	29	SAGE
9	GeneReach Biotechnology Corporation	7	Tetracore, Inc.
21	GlobalVetLINK	24	Thermo Scientific
13	Hardy Diagnostics	6	VMRD, Inc.
19-20	IDEXX Laboratories	17-18	Zoetis (was Pfizer Animal Health)

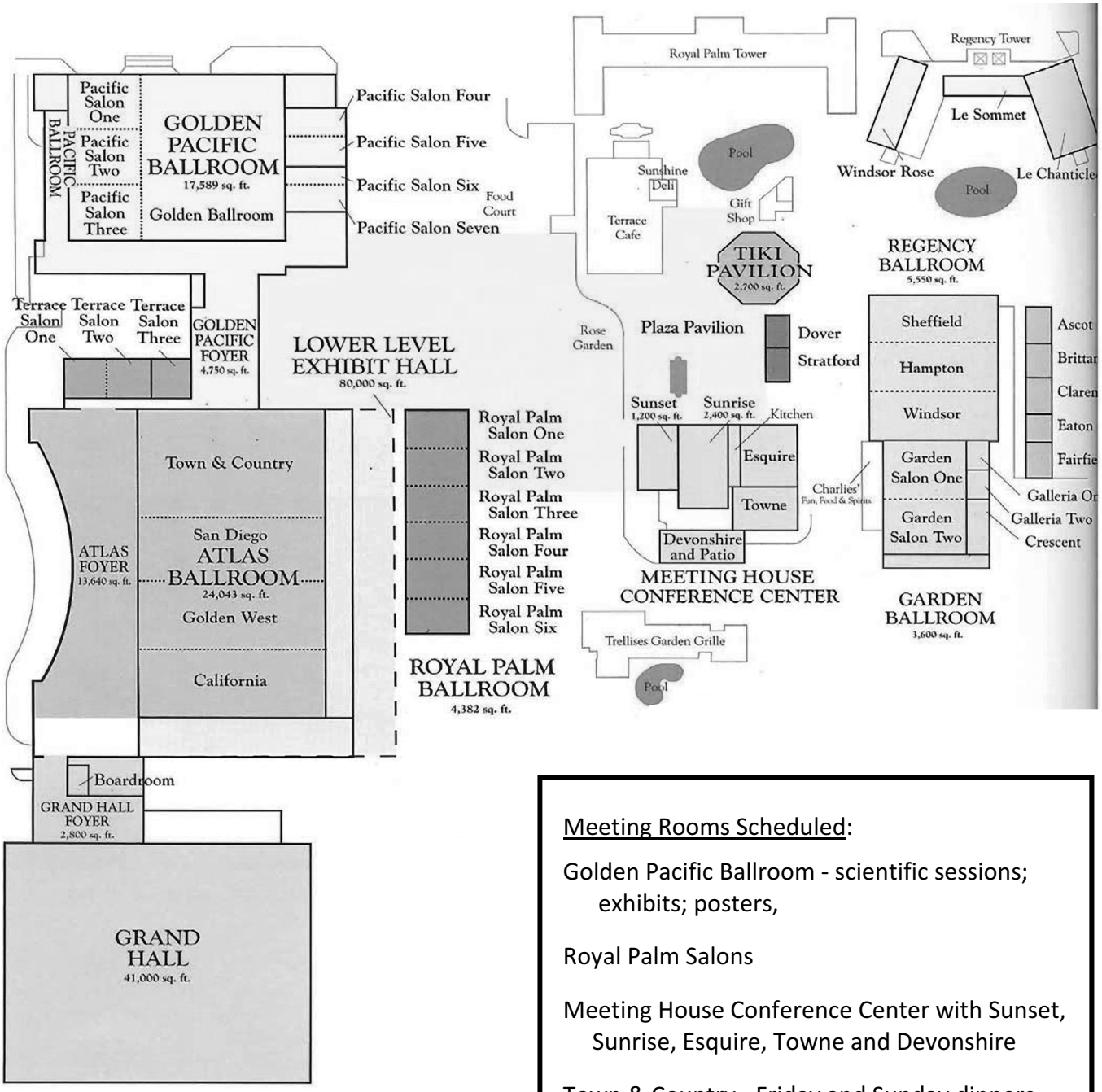
Exhibits and Posters

October 19-20, 2013

Golden Ballroom



Town and Country Resort & Convention Center



Meeting Rooms Scheduled:

Golden Pacific Ballroom - scientific sessions; exhibits; posters,

Royal Palm Salons

Meeting House Conference Center with Sunset, Sunrise, Esquire, Towne and Devonshire

Town & Country - Friday and Sunday dinners