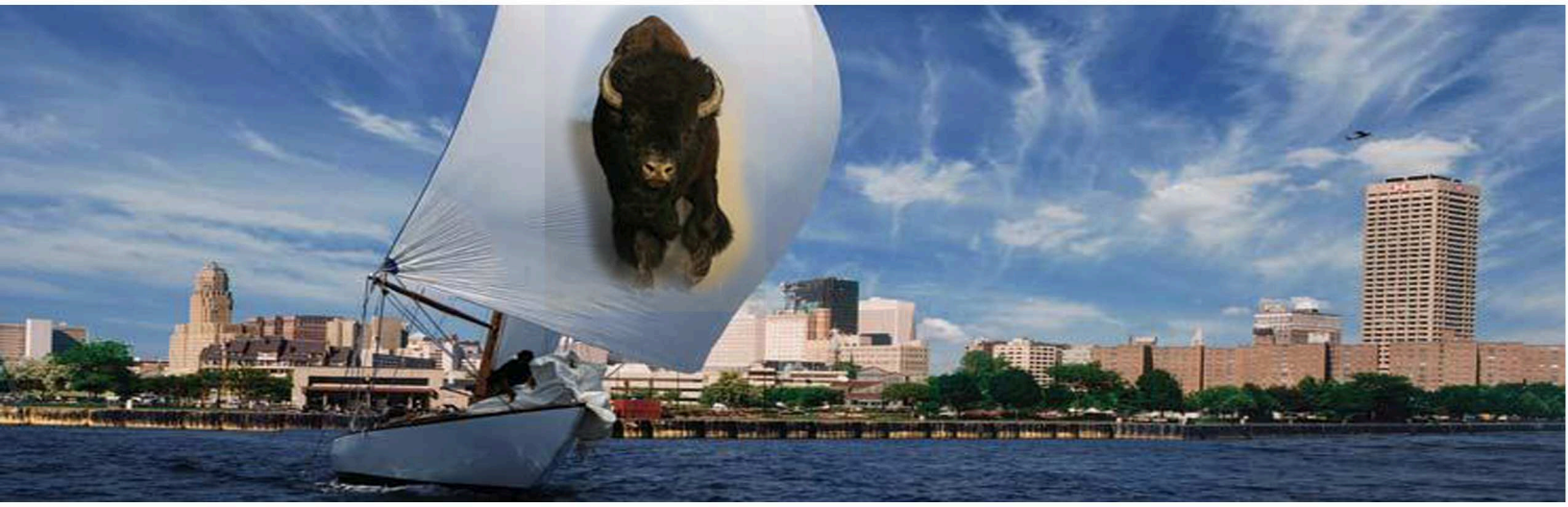
Proceedings of the

American Association of Veterinary Laboratory Diagnosticians



54th Annual Conference

Adams Mark Hotel
Buffalo, NY
September 28 - October 5, 2011

THANK YOU AAVLD 2011 Sponsors!

Diamond:

life technologies™

SYNBIOTICS®

Platinum:

PRIONICS

Test With Confidence™

IDEXX

Gold:

TREK DIAGNOSTIC SYSTEMS
MAGELLAN BIOSCIENCES

GVL®
GlobalVetLINK

GeneReach

Silver:

VLA SCIENTIFIC

VADDS

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

AAVLD Officers, 2011

President ..... Craig Carter, Lexington, KY
President-elect ..... Tim Baszler, Pullman, WA
Vice-president ..... Tom McKenna, WI
Secretary-Treasurer ..... John Adaska, Tulare, CA
Immediate Past-President ..... Gary Anderson, Manhattan, KS

AAVLD Executive Board, 2011

President ..... Craig Carter, Lexington, KY
President-elect ..... Tim Baszler, Pullman, WA
Vice-president ..... Tom McKenna, Madison, WI
Secretary-Treasurer ..... John Adaska, Tulare, CA
Northeast ..... Bruce Akey, Ithaca, NY
Southeast ..... Doris Miller, Athens, GA
North-central ..... Pat Halbour, Ames, IA
South-central ..... Bill J. Johnson, Stillwater, OK
Northwest ..... Jerry Heidel, Corvallis, OR
Southwest ..... Robert Poppenga, Davis, CA
Canada Provincial ..... Estela Cornaglia, St. Hyacinthe, QC
Canada Federal ..... Maria Perrone, Ottawa, ON
NVSL ..... Beth Lautner, Ames, IA

AAVLD Secretary-Treasurer's Office
Vanessa Garrison Fax 530-752-5680
P.O. Box 1770 Phone - 530-754-9719
Email: secretary-treasurer@aavld.org
Davis, CA 95617

Acknowledgments

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

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

****

AAVLD Program Committee, 2011

Tim Baszler, Chair
Tom McKenna, Co-Chair
John Adaska
Catherine Barr
Steven R. Bolin
Francois C. Elvinger
Scott D. Fitzgerald
Sharon K. Hietala
Lorraine J. Hoffman
Hong Li
Kristy Lynn Pabilonia
Amar Patil
Carlos Reggiardo
J. Glenn Songer
David Steffen
Patricia A. Talcott
Kyoung-Jin Yoon

****

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

New "user friendly" features added to the 2011 Annual Meeting Proceedings include author and keyword indexes. These indexes can found in the back of the book.

AAVLD Plenary Session

Bovine Tuberculosis: Here Today, Here Tomorrow??

Saturday, October 1, 2011
Grand Ballroom A/B

Moderator: Timothy Baszler

7:45 AM **Introduction by Moderator**

8:00 AM ***Mycobacterium tuberculosis*: What Makes it Such a Difficult Bug to Control?**
David Russell…… 24

8:40 AM **Advances in Immunological Ante-mortem Diagnosis: New Test Formats, Novel Antigens, Biomarkers, DIVA Diagnosis in the Face of Vaccination**
Martin Vordermeier, Sara Downs, Gareth Jones, Adam Whelan, Richard Clifton-Hadley, R. Glyn Hewinson…… 25

9:20 AM **BREAK**

9:50 AM **Progress in the Development of Vaccines Against Bovine Tuberculosis**
Bryce Buddle, Natalie Parlane, Axel Heiser, Neil Wedlock…… 26

10:30 AM **Epidemiology and Control of Bovine Tuberculosis in the United States: USDA's Perspective**
Alecia Naugle…… 27

11:10 AM **Roundtable Question and Answer Discussion**

Bacteriology Scientific Session 1

Saturday, October 1, 2011

Grand Ballroom B

Sponsor: Trek Diagnostic Systems

Moderators: Kris Clothier and Kathy Strelow

1:00 PM **Use of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Clinical Bacteriology Laboratory**
Durda Slavic…… 29

1:15 PM **Real-time PCR for Differentiation of F4 variants (F4ab, F4ac, and F4ad) of Enterotoxigenic *E. coli* Isolated From Diarrheic Piglets**
Jae-Won Byun, Byeong Yeal Jung, Ha-Young Kim, John Fairbrother, Wan-Kyu Lee……. 30

1:30 PM **Detection of Shiga Toxin Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145 by Enzyme Linked ImmunoSorbant Assay**
Chitrita DebRoy, Narasimha Hegde, Bhushan Jayarao, Vivek Kapur, Klaus Lindpaintner, Mark Muldoon…… 31

1:45 PM **An 11-gene Multiplex PCR to Detect the Seven Major Shiga Toxin-Producing *Escherichia coli* Based on Genes that Code for Major Virulence Factors and Serogroup-Specific O-Antigens**
Jianfa Bai, Zachary Paddock, Xiaorong Shi, Shubo Li, T. Nagaraja…… 32

2:00 PM **Imidocarb Dipropionate Eliminates *Theileria equi* in Experimentally Infected Horses**
Juanita Grause, Jeffrey Nelson, Donald Knowles, Massaro Ueti, Lowell Kappmeyer, Jean Laufer, Thomas Bunn…….. 33

2:15 PM **Cross-reaction of *Simplicimonas spp* Trichomonads in *Tritrichomonas foetus* Assays**
Susan Schommer, Sunny Younger, William H. Fales…… 34

2:30 PM **Comparison of Two Centrifugal Fecal Flotation Techniques for the Detection of Canine and Feline Gastrointestinal Parasites**
Juliette Carroll, Lora Rickard Ballweber…… 35

2:45 PM **Trek Diagnostic Systems Presentation**

Pathology Scientific Session 1

Saturday, October 1, 2011

Grand Ballroom A

Moderators: Melissa Behr and Dale M. Webb

AAVLD Trainee Travel Awardee (Pathology)

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Tuberculosis/Johne's Disease Special Scientific Session

Saturday, October 1, 2011

Grand Ballroom E

Moderators: Suelee Robbe-Austerman and John Adaska

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

◊ USAHA Paper

Toxicology Scientific Session

Saturday, October 1, 2011

Wright

Moderators: Patricia Talcott and Karyn Bischoff

AAVLD Trainee Travel Awardee

Virology Scientific Session 1

Saturday, October 1, 2011

Grand Ballroom D

Sponsor: GeneReach Biotechnology Corporation

Moderators: Steve Bolin and Amy Glaser

1:00 PM **Development and Bench Validation of a Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of Capripoxviruses**
Amaresh Das, Michael T. McIntosh..... 63

1:15 PM **A Novel Papillomavirus Isolated from Proliferative Skin Lesions of a Wild North American Beaver (Castor canadensis) * # +**
Artem Rogovskyy, Tim Baszler, Tom Besser, Dan Bradway, Darren. Bruning, Christine Davitt, James Evermann, Kristin Mansfield, Gary Haldorson..... 64

1:30 PM **Understanding the Role of Raccoons as Intermediate Hosts in the Evolution of Canine and Feline Parvoviruses**
Andrew Allison, Justin Brown, Mark Ruder, Kevin Keel, Carole Harbison, Israel Pagan, Karla Stucker, Jason Kaelber, Edward J. Dubovi, Edward Holmes, Colin Parrish.....65

1:45 PM **Ferret Systemic Coronavirus Infection #**
Valerie Johnson, Hana VanCampen, Sushan Han, Kathryn Holmes.....66

2:00 PM **Isolation and Molecular Characterization of Trout Infectious Pancreatic Necrosis Virus (IPNV) in Pennsylvania Aquaculture**
Huaguang Lu, Kathy Hillard..... 67

2:15 PM **Virus Isolation of Influenza A Viruses from Oral Fluid using a Check Test**
Christa Irwin, Jeff Zimmerman, Melinda Jenkins-Moore, Pravina Kitikoon, Chong Wang, Joe Anderson, Pamela Leslie-Steen, Devi P. Patnayak, Jianqiang Zhang..... 68

2:30 PM **Comparison of Virus Isolation and Real-Time RT-PCR for Detection of Avian Influenza Virus and Newcastle Disease Virus in Cloacal Swabs of Poultry and Ducks ◊**
Janice C. Pedersen, Mary Lea Killian, Nichole Hines, Barbara M. Martin, Beverly Schmitt, Monica Reising..... 69

2:45 PM **GeneReach Biotechnology Corporation Presentation:**

Detecting Influenza A Virus and Highly Virulent Chinese-Type Porcine Reproductive and Respiratory Syndrome Virus (H-PRRSV) by a Portable PCR Platform - POCKIT
Hsiao Fen Grace Chang..... 70

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

◊ USAHA Paper

Bacteriology Scientific Session 2

Sunday, October 2, 2011

Grand Ballroom B

Moderators: Kristy Pabilonia and Amar Patil

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Bovine Virus Diarrhea Special Scientific Session

Sunday, October 2, 2011

Wright

Moderators: Kathy Kurth and Beate Crossley

Epidemiology Scientific Session

Sunday, October 2, 2011

Grand Ballroom E

Moderators: Suzanne Gibbons-Burgener and Ashley Hill

8:00 AM **Bovine Neonatal Pancytopenia: Results of a 2 Year Study into this Emerging Disease in the United Kingdom**
Andrew Holliman, Kim Willoughby, Sandra F.E. Scholes, Adrian Colloff, George Caldow, Fiona Howie, Richard Smith, Sarah Lambton, Charlotte Bell…… 97

8:15 AM **IDEXX Bovine Pregnancy Test - A New Tool for Accurate and Early Pregnancy Diagnosis in Cattle**
Katherine Velek, Shona Michaud, Meghan Hart, Valerie Leathers, Christoph Egli…… 98

8:30 AM **Adaptation of a Commercial PRRS Serum Antibody ELISA to Oral Fluid Specimens ***
Apisit Kittawornrat, John Prickett, Chong Wang, Chris Olsen, Christa Irwin, Yaowalak Panyasing, Andrea Ballagi, Anna Rice, Rodger Main, Chris Rademacher, Marlin Hoogland,Jeff Zimmerman…….. 99

8:45 AM **Diagnostic Performance of a Commercial PRRS Serum Antibody ELISA Adapted to Oral Fluid Specimens: Longitudinal Response in Experimentally-inoculated Populations ***
Apisit Kittawornrat, John Prickett, Chong Wang, Chris Olsen, Christa Irwin, Yaowalak Panyasing, Andrea Ballagi, Anna Rice, Bob Rowland, Jeff Zimmerman…….. 100

9:00 AM **Diagnostic Performance of a Commercial PRRS Serum Antibody ELISA Adapted to Oral Fluid Specimens: Field Samples ***
Apisit Kittawornrat, John Prickett, Chong Wang, Chris Olsen, Christa Irwin, Yaowalak Panyasing, Andrea Ballagi, Anna Rice, Rodger Main, Chris Rademacher, Marlin Hoogland, James Lowe, Jeff Zimmerman…….. 101

9:15 AM **Epidemiology of Infection of *Mycoplasma hyorhinis* in Endemically Infected Swine Herds * #**
Maria Clavijo, Albert Rovira, Deborah Murray, Simone Oliveira…… 102

9:30 AM **BREAK**

10:00 AM **Epidemiologic Factors Associated with Genetic Variation of Serotype O Foot-and-Mouth Disease Virus in Pakistan**
Barbara Brito, Andres Perez, Luis Rodriguez…… 103

10:15 AM **Application of the Gold Standard Diagnostic Technique for Rabies to Salivary Glands as an Estimate of Viral Shedding and Potential Evolutionary Adaptation to New Species**
Cathleen Hanlon, Micheal Moore, Rolan Davis ***WITHDRAWN****…….. …… 104*

10:30 AM **Temporal and Spatial Variation in *Culicoides* Midge Abundance Determined by Different Trapping Methods in California ***
Christie Mayo, Bradley Mullens, Christopher Barker, Alec Gerry, Ian Gardner, James MacLachlan…… 105

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

Pathology Scientific Session 2

Sunday, October 2, 2011

Grand Ballroom A

Moderators: Scott Fitzgerald and Gayle C. Johnson

* Graduate Student Oral Presentation Award Applicant ◊ USAHA Paper

\+ AAVLD/ACVP Graduate Student Award Applicant

\# AAVLD Trainee Travel Awardee (Pathology)

Virology Scientific Session 2

Sunday, October 2, 2011

Grand Ballroom D

Moderators: Kyong-Jin Yoon and Alfonso Clavijo

* Graduate Student Oral Presentation Award Applicant

◊ USAHA Paper

AAVLD/USAHA Joint Plenary
Evolving Food Systems for Global Food Security:
Can Animal Production and Veterinary Infrastructure Keep Up?

Monday, October 3, 2011
Grand Ballroom A/B/C

Moderator: Terry McElwain

7:45 AM Welcome – Dave Marshall, USAHA Vice-President and Program Chair

8:00 AM **Making Safe, Affordable and Abundant Food a Global Reality**
Ted McKinney..... *137*

8:40 AM **Markets, Infrastructure and Animal Production Systems**
Tom Marsh..... *138*

9:20 AM **Role of Veterinarians in Global Food Security**
Ron DeHaven..... *139*

10:00 AM **BREAK**

10:20 AM **Veterinary Diagnostic Laboratories Role in Food Security**
Alfonso Torres..... *140*

11:00 AM **Rinderpest Control and Eradication as a Working Example of Utilizing the World's Veterinary, Laboratory, and Research Resources to Exact a Tangible, Measurable Impact on a Food Animal Disease Directly Affecting Food Security for a Significant Part of the World**
William Taylor..... *141*

11:40 AM **Roundtable Question and Answer Discussion**

AAVLD Poster Session

Friday, September 30, 2011 – Sunday, October 2, 2011

Grand Hall

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


Can't Stop Spreading? POCKIT Can!
Portable PCR Anytime, Anywhere
To learn more about POCKIT, attend our presentation at 2:45 PM on Oct 1,
Grand Ballroom D, or visit our exhibit booth
www.genereach.com

With You. For You. New to You.

ProFLOK® MG/MS Combo

Flu DETECT® BE

VISIT US AT BOOTH # 40 & 41

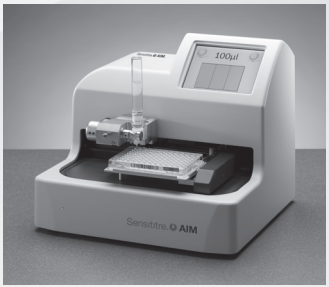
SYNBIOTICS®

Pfizer Animal Health

Come See the Sensititre® Difference...

Look for the *new* Sensititre AIM™ Automated Inoculation Delivery System, combined with numerous Sensititre Veterinary MIC Plates

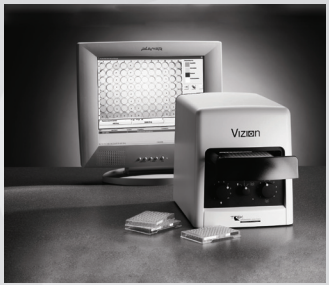
Sensititre® AIM



Precise, automated inoculation in a fast, easy-to-use bench-top device.

- Fast, accurate dosing
- Compact, modular design
- Compatible with most 96 well microtitre plates

Vizion® System



Combines automation with visual results.

- Consolidates all offline testing on to a single instrument
- Eliminate manual reading errors

Stop by booth #28 to see the new, easy-to-use Sensititre AIM System, as well as demonstrations of the Vizion System with SWIN® Software.

www.thermofisher.com www.trekds.com

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific

AAVLD Plenary Session

Bovine Tuberculosis: Here Today, Here Tomorrow??

Saturday, October 1, 2011
Grand Ballroom A/B

Moderator: Timothy Baszler

7:45 AM **Introduction by Moderator**

8:00 AM ***Mycobacterium tuberculosis*: What Makes it Such a Difficult Bug to Control?**
David Russell……. *24*

8:40 AM **Advances in Immunological Ante-mortem Diagnosis: New Test Formats, Novel Antigens, Biomarkers, DIVA Diagnosis in the Face of Vaccination**
Martin Vordermeier, Sara Downs, Gareth Jones, Adam Whelan, Richard Clifton-Hadley, R. Glyn Hewinson……. *25*

9:20 AM **BREAK**

9:50 AM **Progress in the Development of Vaccines Against Bovine Tuberculosis**
Bryce Buddle, Natalie Parlane, Axel Heiser, Neil Wedlock……. *26*

10:30 AM **Epidemiology and Control of Bovine Tuberculosis in the United States: USDA's Perspective**
Alecia Naugle……. *27*

11:10 AM **Roundtable Question and Answer Discussion**

Mycobacterium tuberculosis: What Makes it Such a Difficult Bug to Control?

David Russell

Cornell University, Ithaca, NY, USA

Narrative:

Mycobacterium tuberculosis is an extremely successful pathogen that demonstrates the capacity to modulate its host both at the cellular and tissue levels. At the cellular level the bacterium enters its host macrophage and arrests phagosome maturation, thus avoiding many of the microbicidal responses associated with this phagocyte. Nonetheless, the intracellular environment places certain demands on the pathogen, which, in response, senses the environmental shifts and up-regulates specific metabolic programs to allow access to nutrients, minimize the consequences of stress, and sustain infection. Despite its intracellular niche, *Mycobacterium tuberculosis* demonstrates a marked capacity to modulate the tissues surrounding infected cells through the release of potent, bioactive cell wall constituents. These cell wall lipids are released from the host cell by an exocytic process and induce physiological changes in neighboring phagocytes, which drives formation of a granuloma. This tissue response leads to the generation and accumulation of caseous debris and the progression of the human TB granuloma. Completion of the life cycle of tuberculosis requires damaging the host to release infectious bacteria into the airways to spread the infection. This damage reflects the pathogen's ability to subvert the host's innate and acquired immune responses to its own ends. This ability to modulate the host response is responsible, at least locally, not only for late stage damage, but also for many of the issues that face strategies to improve the immunological control of the disease. This localized control of the systemic response by Mtb is likely the underlying explanation for both the ineffectual nature of vaccines and the difficulty in identification of biomarkers that either accurately diagnose disease status or predict disease progression. This presentation discusses these practical issues in the context of the underlying biology of Mtb and speculates upon the evolutionary pressures that would have selected for such traits.

Speaker Biography:

David G. Russell, Ph.D., is Professor in the Department of Microbiology and Immunology in the College of Veterinary Medicine, Cornell University. He received his BSc. from St. Andrews University in Scotland and his Ph.D. from Imperial College, London University, in 1982. He has held positions as Group Leader at the Max-Planck-Institüt in Tübingen, Assistant Professor at NYU Medical Center and as Associate and Full Professor in the Department of Molecular Microbiology at Washington University School of Medicine. He has spent his entire career looking at host/pathogen interplay and has authored over 170 publications on the subject, including papers in Science and Nature. He was elected a Fellow of the American Association for the Advancement of Science in 2007. His research focuses exclusively on the interplay between the macrophage and the pathogen *Mycobacterium tuberculosis*. On the macrophage side of the equation the lab has been developing real-time, functional readouts for the lumenal environment within the phagosome, such as hydrolytic activity and radical production, and how these are modified by immune stimuli and infection. On the bacterial side the group is interested in how the bacterium modifies its intracellular compartment to ensure its survival, and how the bacterium responds metabolically to this changing environment. Finally, at the level of the human host, the lab is studying how human alveolar macrophages respond to infection and how the infection site evolves to either contain the infection or progress to active disease and transmission. These human studies are pursued through collaborations with the University of Cape Town, South Africa and the Wellcome Research Laboratories, Blantyre, Malawi. This work is supported by multiple grants from the National Institutes of Health and by the Bill and Melinda gates Foundation.

Advances in Immunological Ante-mortem Diagnosis: New Test Formats, Novel Antigens, Biomarkers, DIVA Diagnosis in the Face of Vaccination

Martin Vordermeier, Sara Downs, Gareth Jones, Adam Whelan, Richard Clifton-Hadley, R. Glyn Hewinson

Bacteriology, AHVLA, Addlestone, United Kingdom

Narrative:

There has been a long-term (over 25 years) increasing trend in bovine tuberculosis (bTB) incidence in cattle in Great Britain, driven by both cattle-to-cattle and badger-to-cattle transmission. The primary screening test for bTB in cattle in Great Britain is the Single Intradermal Comparative Cervical Tuberculin (SICCT) test. All cattle herds are subject to regular 'routine' testing, the frequency of which is based on the local disease incidence e.g. herds in high bTB risk areas are tested annually. The more sensitive blood-based interferon-gamma release assay (IGRA) is used in addition to the SICCT test in prescribed circumstances. Government compensation is paid to owners of cattle compulsorily slaughtered for bTB control purposes. I will briefly discuss how these tests are being used in GB. Using data from a recent meta-analysis I will discuss their performance characteristics but will also address some of their limitations. Further, I will briefly review progress in the development of serological assay formats. In the balance of my presentation, I will summarise the significant progress that has been made to develop specific antigens that allow the differentiation of BCG vaccinated and *M. bovis* infected cattle (DIVA test), and generally improving specificity compared to tuberculin alone. Prototype reagents like ESAT-6 and CFP-10 are now being routinely applied in IGRA. The search for additional antigens with similar response profiles has been greatly facilitated by the elucidation of the genome sequences of *M. tuberculosis*, *M. bovis* and BCG Pasteur and other mycobacterial species. I will describe our results applying comparative genome and transcriptome analysis to mine diagnostically useful antigens that we applied in IGRA and skin testing. Presently we are pursuing a triple-track approach to improving DIVA reagents which includes (i) a product-development objective of incremental improvement of sensitivity by defining additional specific antigens using targeted and library approaches, (ii) translational research to validate promising reagents in the field, and (iii) basic research to define additional biomarkers as read-out parameters of cellular immunity to supplement IFN-gamma and by defining disease-stage specific antigens.

Speaker Biography:

Martin studied biology in Stuttgart and Tuebingen, Germany, gaining a PhD in microbiology/immunology in 1987. Cellular immunologist with 21 years experience of mycobacterial research both in the human (MRC TB and Related Infections Unit, Hammersmith Hospital, London from 1990 - 1997) and bovine tuberculosis fields. Since 1997 he is leading a team at AHVLA investigating the immune responses in cattle to *M. bovis* infection, the development of vaccines and immuno-diagnostic reagents including the definition of molecular correlates of protection and disease, and the transmission and pathogenesis of this disease in cattle. He has also an interest in the genetics of susceptibility to TB in different cattle breeds, with particular emphasis on bovine TB in Africa. He has authored to-date to date (May 2011) more than 175 publications on this and related subjects. He is a member of the editorial boards of Clinical Vaccine Immunology, Veterinary Immunology and Immunopatrhology and other scientific journals and a past editorial board member of Infection & Immunity. He is a visiting professor at the University of Hertfordshire.

Progress in the Development of Vaccines Against Bovine Tuberculosis

Bryce Buddle, Natalie Parlane, Axel Heiser, Neil Wedlock

Hopkirk Research Institute, AgResearch, Palmerston North, New Zealand

Narrative:

Bovine tuberculosis (TB) remains a major animal health problem world-wide as well as being a zoonotic cause of human tuberculosis. Bovine TB control programs are not affordable in many countries and are less effective in countries which have wildlife reservoirs of TB. Alternative control strategies are urgently required and vaccination of domestic livestock or wildlife species which serve as reservoirs of infection has the potential to become an important TB control measure. Considerable progress has been made in the development and evaluation of vaccines to control bovine TB in cattle and wildlife based on the experimental challenge of vaccinated animals and in recent field trials. The human TB vaccine, bacille Calmette-Guérin (BCG) has been shown to induce a significant reduction in pathological lesion scores in experimental challenge studies and has reduced infection in field trials. For cattle, calves are more effectively vaccinated with BCG as neonates. The major caveats concerning the use of BCG in cattle are that protection is not complete and a proportion of vaccinated animals respond positively in the tuberculin skin test, the cornerstone of the test and slaughter control programme. New diagnostic reagents and immune assays have been developed to differentiate BCG-vaccinated and *Mycobacterium bovis*-infected animals. Research to identify effective TB vaccines for cattle has greatly benefited by leveraging from human TB vaccine studies. A range of new types of vaccines have shown particular promise in cattle including prime-boost combinations of BCG plus mycobacterial protein, DNA or virus vector expressing mycobacterial antigens as well as vaccination with attenuated strains of *M. bovis*. New TB protein vaccines have the potential to protect against TB without inducing skin test reactivity. For wildlife vaccines, a crucial development has been the formation of BCG into an oral bait delivery system which provides a practical and cost-effective means of delivering a TB vaccine to wildlife. A recent field trial in New Zealand which involved oral dosing of possums with BCG vaccine demonstrated that vaccination could assist in preventing infection rather than just modifying the severity of disease as was seen in experimental challenge trials. In conclusion, recent studies in cattle and wildlife have demonstrated the practicality and effectiveness of vaccinating animals against TB and provide much impetus for their future use.

Speaker Biography:

Bryce Buddle is the team leader of the Infectious Diseases Immunology Group at the AgResearch, Hopkirk Research Institute, Palmerston North, New Zealand and also an Adjunct Professor of Infectious Diseases at Massey University. Bryce graduated as a veterinarian from Massey University (NZ) and after spending several years in practice commenced a career in animal disease research which has spanned more than 35 years. He completed post-graduate studies at Otago University (NZ) and a PhD Virginia Tech (USA) and is a certified Diplomate of the American College of Veterinary Microbiologists. His research interests have covered control of a wide range of animal diseases through vaccination. His studies on bovine tuberculosis over the last 20 years have focussed on development and evaluation of vaccines to protect cattle and possums (wildlife reservoir of *Mycobacterium bovis* in NZ) against this disease and tests to improve the diagnosis of the disease in cattle. Bryce is co-inventor of Yersiniavax to control yersiniosis of deer, has developed commercial tests for the diagnosis of tuberculosis in cattle and shares a patent on an oral bait tuberculosis vaccine for wildlife. He has acted as a consultant to a range of international agencies on bovine tuberculosis and is currently a member of the Technical Advisory Group of the Animal Health Board (NZ) for bovine tuberculosis. He has published over 150 scientific papers in peer-reviewed journals.

Epidemiology and Control of Bovine Tuberculosis in the United States: USDA's Perspective

Alecia Naugle

USDA APHIS Veterinary Services, Riverdale, MD, USA

Narrative:

Many consider the United States Department of Agriculture's (USDA) State-Federal Cooperative Bovine Tuberculosis (TB) Eradication Program to be one of the great public and animal health achievements in the United States. This program is credited with decreasing the prevalence of *Mycobacterium bovis* infection in U.S. cattle from nearly 5 percent in 1917 to less than 0.001 percent today. However, the goal of *eradicating M. bovis* from the U.S. livestock population remains elusive. This presentation will provide an overview of the current epidemiology of bovine TB infection in the United States. Additionally, recent case examples will be used to illustrate the impact that specific factors have on current control and eradication efforts. These factors include: wildlife reservoirs; high-risk subpopulations of livestock; changes in the structure of livestock industries; increased livestock mobility; limitations of available diagnostic tests; and the use of epidemiological tools to aid in investigations and to guide policy decisions. In response to these factors, the USDA is revising the underlying regulations and policies for its TB program to reflect a comprehensive approach to disease surveillance, control, and eradication.

Speaker Biography:

Dr. Alecia Larew Naugle is a native of West Virginia where she enjoyed her childhood on her family's beef farm. She received her undergraduate degree in Animal and Veterinary Sciences from West Virginia University, followed by a DVM from The Ohio State University. After serving as an associate veterinarian in a mixed animal practice in northeast Ohio, Alecia returned to Ohio State to complete her PhD in Veterinary Preventive Medicine with a specialization in Epidemiology. Her graduate research focused on the epidemiology of Johne's disease in Ohio dairy herds, and extension activities were a significant component of her responsibilities. Upon completing her graduate work, she served as an analytical epidemiologist with the Food Safety and Inspection Service. Alecia joined Veterinary Services in March 2007 as the National Epidemiologist for the Scrapie Eradication Program, and she has also served in an interim capacity as Program Manager of the BSE Program. In September 2008, she became the Program Manager of the Bovine Tuberculosis (TB) Eradication Program. Alecia is a diplomate in the American College of Veterinary Preventive Medicine and a member of its Epidemiology Specialty.

Bacteriology Scientific Session 1
Saturday, October 1, 2011
Grand Ballroom B

Sponsor: Trek Diagnostic Systems

Moderators: Kris Clothier and Kathy Strelow

Use of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Clinical Bacteriology Laboratory

Durda Slavic

Animal Health Laboratory, University of Guelph, Guelph, ON

Narrative: Currently routine clinical diagnostic bacteriology mostly relies on the classical biochemical methods for bacterial identification. These methods usually require 12 or more hours for their completion. In contrast Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) bacterial identification can be achieved within minutes. A single bacterial colony from overnight culture, grown on different media types, is simply smeared on a MALDI-TOF MS stainless steel 96 well target and overlaid with matrix. The matrix/sample mixture is loaded into the MALDI-TOF MS instrument and protein spectra are generated. These protein spectra are then compared to the spectra in the database which are unique for each bacterial species and bacterial identification is given. Although MALDI-TOF MS is being widely used in research there is limited information available for its clinical use. As a result, the clinical validation of this instrument is underway in our laboratory. Our preliminary results suggest that this system gives a very reliable identification for Gram-negative organisms such as *Salmonella spp*., *Escherichia coli*, *Klebsiella spp*., *Serratia spp*., *Enterobacter spp., Citrobacter spp*., and *Pseudomonas aeruginosa*. For the Gram-positive bacteria tested so far, reliable results were obtained for *Clostridium perfringens*, *Clostridium difficile*, *Staphylococcus aureus*, *Streptococcus uberis*, and *Enterococcus spp*. Less reliable results were obtained for some *Staphylococcus spp.* such as *S. pseudintermedius*, *S. schleiferi subsp. schleiferi, S. schleiferi subsp. coagulans*, and *S. delfini*. More work is underway to test additional bacterial species frequently isolated in a clinical veterinary diagnostic laboratory. Based on the preliminary results, however, MALDI-TOF MS appears to be a promising new method for bacterial identification in clinical laboratories.

Real-time PCR for Differentiation of F4 variants (F4ab, F4ac, and F4ad) of Enterotoxigenic *E. coli* Isolated From Diarrheic Piglets

Jae-Won Byun[1,3], Byeong Yeal Jung[1], Ha-Young Kim[1], John Fairbrother[2], Wan-Kyu Lee[3]

[1]Animal Disease Diagnostic Center, NVRQS, Anyang; [2]Faculté de médecine vétérinaire, Université de Montréal, Montréal, QC; [3]College of Veterinary Medicine, Chungbuk National University, Cheongju

Narrative: Fimbriae play a pivotal role for attachment for enterotoxigenic *E. coli* (ETEC) to intestinal brush border in pigs. Among them, F4 fimbriae are subdivided into three variants, F4ab, F4ac and F4ad. Thus, identification of the F4 variants is important to understand the pathogenesis of colibacillosis in pigs and to develop efficient vaccines. The aims of this study were to develop a real-time PCR for the detection of the F4 variants and to investigate the prevalence of these variants in F4-positive ETEC isolated from diarrheic piglets in Korea. In 2008 and 2009, 42 F4-positive ETEC were collected from diarrheic piglets (25 from neonatal and 17 from weaned piglets) originating from 37 pig farms throughout Korea. Primers and specific probes were designed by the difference of each variant. The real-time PCR was carried out in a Rotor-gene 3000™ in a reaction volume of 20 µL, containing 2 µL of DNA solution, 2x SensiMastermix (Quantace, USA), 1 µL of primer (10 µM), and 1 µL of each probe (5 µM). The reaction conditions consisted of incubation at 95°C for 10 min followed by 50 cycles of denaturation (95°C for 20 s), and annealing/extension (61°C for 45 s). The strains that produced a threshold cycle (CT) of 45 cycles or less and an amplification plot for a particular F4 variant were considered positive for that variant. Reference strains 1019:F4ab, 7805:F4ac, 1033:F4ad were used as positive controls in each reaction. The efficacy of real-time PCR was compared to that of conventional PCR using spiked feces with or without enrichment. The sensitivity of the real-time PCR in broth dilution was 10^5, 10^4, and 10^5 CFU/mL for strains carrying F4ab, F4ac and F4ad respectively. The F4 variants were detected in feces containing 10^6 CFU/g or greater F4-positive ETEC bacteria, without enrichment. Following enrichment, the detection limit of the real-time PCR was increased by 10^2 CFU/g of F4-positive ETEC bacteria in feces, which is 10 to 100-fold higher than that of conventional PCR. All 42 field isolates were designated as the F4ac variant when examined by the real-time PCR. The purpose of this study was to develop a real-time PCR assay to differentiate the F4 variants associated with ETEC. The results showed that detection limit of F4 variants was improved 10 to 100 times higher than that of conventional PCR with enrichment samples. All 42 field isolates were designated as the F4ac variant when examined by the real-time PCR. Hence, the present real-time PCR assay can be useful to differentiate the F4 variants of pathogenic *E. coli* both directly in feces and following enrichment.

Detection of Shiga Toxin Producing *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145 by Enzyme Linked ImmunoSorbant Assay

Chitrita DebRoy[1], Narasimha Hegde[1], Bhushan Jayarao[1], Vivek Kapur[1], Klaus Lindpaintner[2], Mark Muldoon[2]

[1]Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA;
[2]Microbiology, SDIX, Newark, DE

Narrative: Shiga toxin producing *Escherichia coli* (STEC) serogroups O26, O45, O103, O111, O121, O145 have been identified as the "top six'' non-O157 STEC by the U.S. Centers for Disease Control and Prevention as causative agents of diseases with high morbidity and mortality. While there are methods available for identification of O157, no analogous detection procedures are yet available for rapid detection of these serogroups other than by conventional serotyping and PCR assays. The objective was to develop an Enzyme Linked ImmunoSorbent Assay (ELISA) to detect the six STEC O groups, O26, O45, O103, O111, O121, O145 using polyclonal antibodies specific for each O group. Rapid and easy detection and identification methods are crucial for determining the prevalence of the STEC O groups in food samples for controlling the pathogens to improve food safety and public health. Polyclonal antibodies for each of these O groups were kindly provided by SDIX. Sandwich ELISAs were developed for each O group by coating 96- well plates with monoclonal anti-lipid A antibodies. The antigen, heat killed *E. coli* strains at different concentrations, were allowed to bind to lipid A antibodies for 1 h at 37C. The bound complexes were washed with PBS. Antibodies against each of these O groups were added and the reaction was continued for another 1 h at 37C. Anti-rabbit IgG linked to horse radish peroxidase was added to bind to lipid-antigen-antibody complex. The color reaction was developed using TMB (3,3',5,5'-tetramethyl benzidine) solution and the plates were read at 450 nm. The ELISAs developed for all six O groups could detect the reference strains and test clinical samples belonging to the STEC O group. By spiking ground beef samples with different concentrations of known STEC strains belonging to one of the six serogroups, the assays could detect 20 CFU of STEC following enrichment for 16 h at 37C in tryptic soy broth. The ELISAs for each O group were highly specific and did not react positively with most of the 173 different known *E coli* O groups or other Enterobacteriaceae. Current methods, for the detection and identification of STEC O groups, that have been adopted by CDC and FSIS are time consuming and require several days to complete. The ELISAs described here are rapid, sensitive, specific and easy to use that ideally lend themselves to application by the food industry for rapid detection of STEC O groups.

An 11-gene Multiplex PCR to Detect the Seven Major Shiga Toxin-Producing *Escherichia coli* Based on Genes that Code for Major Virulence Factors and Serogroup-Specific O-Antigens

Jianfa Bai[1], Zachary Paddock[2], Xiaorong Shi[2], Shubo Li[1], T. Nagaraja[2]

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

Narrative: Cattle harbor Shiga toxin-producing *E. coli* (STEC) in the gastrointestinal tract, particularly in the hindgut, and serve as a major reservoir. The STEC is shed in the feces, which serves as the major source of food contamination and human infections. Among STEC, the O157 serogroup has long been recognized as a major foodborne pathogen. Recently, non-O157 serogroups, belonging to six O groups, O26, O111, O103, O121, O45, and O145, have been recognized as a growing public health concern. According to CDC, all STEC account for approximately 175,000 illnesses annually, and of those O157 and non-O157 are responsible for 36 and 64% of total STEC infections, respectively. The traditional cultural method to identify *E. coli* O157 relies on the use of immunomagnetic separation beads, plating on selective medium, and agglutination with serogroup-specific antisera for confirmation. Such methods for isolation of non-O157 STEC have not been developed. Moreover, the beads are not available for all non-O157 STEC. Regardless, culture-based methods are laborious and are not adaptable for high-throughput settings. We have previously developed a 6-gene multiplex PCR to detect four major virulence genes, *stx* 1, *stx* 2, *eae* and *hly* A, and the serotype-specific O157 (*rfb* E) and H7 (*fli* C) antigens. Monday and colleagues have developed a PCR assay to detect O157 and five of the six major non-O157 serogroups from culture. In this study, we developed an 11-gene multiplex PCR assay to detect the four virulence genes as well as the O157 and the six major non-O157 serogroups. Molecular target for the four virulence genes and the O157 antigen were the same in the 6-gene PCR. The *wzx* and *wbq* E-F genes were used for six non-O157 serogroup designs. The amplicon sizes of the 11-gene multiplex PCR are 890, 740, 655, 587, 523, 477, 417, 375, 296, 230, and 199 bp for O45, O103, *stx* 1, O121, O145, *stx* 2, O26, *eae*, O157, O111, and *hly* A respectively. The bands were separated well on a 1.2 % agrose gel and on a Qiagen QIAxcel automated electrophoresis system. The test was validated with 138 *E. coli* strains consisting of 18 strains of O26, 3 of O45, 23 of O103, 28 of O111, 9 of O121, 13 of O145, and 44 of O157. Sensitivity test for pure culture was 10^4 CFU/ml, and for spiked cattle feces was 10^5 CFU/ml. The test was also validated with a number of field cattle fecal samples.

Imidocarb Dipropionate Eliminates *Theileria equi* in Experimentally Infected Horses

Juanita Grause[1], Jeffrey Nelson[1], Donald Knowles[2], Massaro Ueti[3], Lowell Kappmeyer[2], Jean Laufer[4], Thomas Bunn[1]

[1]Diagnostic Bacteriology Lab, National Veterinary Services Laboratory, Ames, IA; [2]Animal Disease Research Unit, Agricultural Research Service, Pullman, WA; [3]Program in Vector-Borne Diseases, Washington State University, Pullman, WA; [4]National Animal Disease Center, Agricultural Research Service, Ames, IA

Narrative: *Theileria equi*, one of the causative agents of equine piroplasmosis, is endemic in many regions of the world, and is considered a foreign animal disease in the United States of America (US). Stringent practices including serological screening of imported horses have maintained a low incidence of *T. equi* infection in the US. The discovery of *T. equi* infection in US horses that had been previously imported using a less sensitive complement fixation test was an impetus for this study. Additionally, recent *T. equi* outbreaks have led to finding additional animals through increased testing for movement within the US. Current options available in cases of infected domestic horses include euthanasia and permanent quarantine. Chemotherapeutics that eliminate infection and therefore transmission risk are a critical need for management of infected horses. This study sought to determine whether imidocarb dipropionate would be efficacious in the elimination of *T. equi* infection. Previous studies testing the efficacy of imidocarb dipropionate yielded conflicting results. Here, ten horses were experimentally inoculated with *T. equi*, and six of these were treated. Parasite elimination was demonstrated in all but one horse by the following tests of clearance: negative nested polymerase chain reaction (nPCR) of peripheral blood in three successive bleeds, failure to transmit infection to a naïve horse by intravenous blood transfusion, survival following splenectomy, and negative nPCR of bone marrow and spleen samples. In addition to elimination of *T. equi*, reversion to seronegativity was a result of successful chemotherapy. These data show imidocarb dipropionate is was capable of eliminating infection in 83% of the horses experimentally infected with the *T. equi* strain utilized in this study.

Cross-reaction of *Simplicimonas spp* Trichomonads in *Tritrichomonas foetus* Assays

Susan Schommer, Sunny Younger, William H. Fales

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

Narrative: The demand for *Tritrichomonas foetus* (TF) testing continues to increase due to the rapid expansion of regulations on bull movement and awareness of the disease. Most states accept a single PCR or 3 cultures for regulatory purposes, making the classification of a single TF PCR sample critical. Although many labs utilize the same primers and probe for real-time PCR detection of TF, there is not a standardized procedure for the processing of samples. Previous AAVLD presentations have established that proper handling of the sample is critical for accurate test results. Death of the TF organism is believed to release DNases that can decrease the sensitivity of TF PCR, leading to high Cts in a truly positive animal. This can happen as a result of prolonged incubation in the InPouchTM TF (BioMed Diagnostics) or incubation at suboptimal temperatures. Our laboratory has been performing TF PCR based on the McMillen assay (McMillen, L. and Lew, AE, 2006, Vet. Parasitology 141:204-215) for approximately two years. In 2010, our laboratory recognized that some samples with high Cts on the TF PCR were the result of a cross -reaction with a recently described tritrichimonad, *Simplicimonas moskowitzi* (Cepicka, I., et. al. 2010, Protist 161:400-433). Our laboratory is now performing a restrospective study on samples that were previously run on our TF assay with Cts of 35 or higher. These samples are being amplified for sequencing to determine the genetic variation of *Simplicimonas spp* detected in Missouri cattle, establish if any animals were incorrectly identified as *Tritrichomonas foetus* positive, and then create a panel of samples to be used for improving the TF assay. To date, three sequence variants of the *Simplicimonas spp* have been identified in Missouri cattle with a 98% nucleotide homology. The McMillen primers have 5 nucleotide mismatches in the forward primer and 3 mismatches in the reverse making it appear on paper that there should not be any cross-reaction. However, the probe has 100% homology and we have identified *Simplicimonas* in assays with a Ct as low as 38. We have not found any cattle that were incorrectly identified as positive on this assay. A panel of these cross-reacting samples is being established so that modifications to our current assay, as well as the Life Technologies assay, can be tested. At this time, we have evidence that the McMillen assay, typically used for TF PCR can cross-react with a recently described related organism *Simplicimonas moskowitzi*. Setting strict Ct guidelines may falsely classify animals, a diagnosis that could be costly and detrimental to the herd. It is for these reasons, samples yielding high Ct's should be regarded with caution before classification as positive or negative and modifications of the currently accepted assays should be evaluated.

Comparison of Two Centrifugal Fecal Flotation Techniques for the Detection of Canine and Feline Gastrointestinal Parasites

Juliette Carroll, Lora Rickard Ballweber

Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, CO

Narrative: Fecal flotations are still the mainstay of gastrointestinal parasite detection in small animal practice. Simple flotation techniques are popular over centrifugation due to their simplicity and perceived cost benefit. However, studies have shown that detection of parasite eggs/cysts/oocysts is enhanced with centrifugation. Recently, a new single use, disposable device for centrifugal flotion was introduced. Using a coring tool to obtain the sample, it is designed to minimize mess while maintaining the benefits of centrifugation. Fifty-five feline and 70 canine fecal samples were used to evaluate this new technique in the detection of gastrointestinal parasites in animals from an area of relatively low parasite prevalence. All samples were mixed, split and processed using both the new technique (OT) and a modified double centrifugation technique (MDCF) using Sheather's sugar (sp.g.=1.27). Samples were scored as positive if any parasite egg/cyst was detected. Eggs/gram were determined for all helminth eggs while a qualitative system was used to score the relative intensity of *Giardia* cysts for all samples. Overall, 25/70 (35.7%) canine and 16/50 (32%) feline fecal samples had one or more parasites present. When samples were simply scored as parasites detected or not detected, substantial agreement occurred between techniques; however, when compared with the MDCF, OT detected fewer positive samples (35 vs 40) and fewer parasites (45 vs 54). This discrepancy centered primarily around *Toxocara canis* (OT=8; MDCF = 11) and *Toxocara cati* (OT=8; MDCF=10). These samples tended to have low egg counts which may explain the differences. Discrepancies were also noted in the detection of *Giardia* cysts (OT=11; MDCF=15). *Taenia* eggs were detected by OT but not by MDCF on one sample. Increasing the recommended flotation time for OT when using sugar, which is more viscous than salt solutions, may solve these differences in detection.

Pathology Scientific Session 1

Saturday, October 1, 2011

Grand Ballroom A

Moderators: Melissa Behr and Dale M. Webb

AAVLD Trainee Travel Awardee (Pathology)

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Post-mortem Findings in 54 Cases of Anesthetic Associated Death in Cats from Two Spay-neuter Programs +

Jodie Gerdin[1], Margret Slater[3], Kathleen Makolinski[3], Andrea Looney[2], Leslie Appel[4], Nicole Martin[3], Sean P. McDonough[1]

[1]Anatomic Pathology, Biomedical Sciences, Cornell College of Veterinary Medicine, Ithaca, NY; [2]Clinical Sciences, Cornell College of Veterinary Medicine, Ithaca, NY; [3]American Society for the Prevention of Creulty to Animals, New York City, NY; [4]Shelter Outreach Services, Ithaca, NY

Narrative: Anesthetic associated death (AAD) in cats is infrequent, but occurs far more frequently than in people. Post-mortem investigations of AAD in cats are uncommon, and their results only sporadically published. Here we report the findings in 54 cases of AAD in cats. Significant gross and/or microscopic disease, including pulmonary, cardiac, and systemic disease, was detected in 33% of cases. Pulmonary disease was most frequently diagnosed (n=13), and included 5 cases of Aelurostrongylus abstrusus. Heart disease, including 2 cases of hypertrophic cardiomyopathy, was less frequent (n=6). Surgical complications were infrequent (4% of cases; n=2). No significant gross or microscopic disease was detected in 63% of cases. Additional studies are needed to determine if the findings in these cases are similar to those of cats with regular access to veterinary care. This study demonstrates that post-mortem investigation of AADs is an important and worthwhile endeavor.

+ AAVLD/ACVP Graduate Student Award Applicant

Trends in Medicolegal Pathology Cases Submitted to the Animal Health Laboratory 1998-2010

Beverly McEwen

Animal Health Laboratory, University of Guelph, Guelph, ON

Narrative: Pathologists at veterinary diagnostic laboratories receive medicolegal cases from a variety of animal species for post-mortem examination. A search of computerized records of the Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario, Canada from 1998 to 2010 identified 1706 medicolegal cases. Almost 25% of all horses, 15% of cats and 10% of dogs submitted for post-mortem examination to the AHL were medicolegal cases. These were categorized according to the history as criminal investigations, anesthetic-related deaths, insurance, litigation, malpractice and regulatory cases. The latter are racehorses that have died within a prescribed interval following racing or training. Statistically significant linear trends in the proportion of medicolegal cases for all animals and criminal cases for companion animals were identified. Companion animals had significantly greater odds of being a medicolegal case in all categories except for insurance and regulatory cases, compared to non-companion animals. Pathology reports for the criminal cases identified that 43.1% were consistent with neglect, 29.2% were compatible with non-accidental injury, 4.80% were poisonings, 10.7% were consistent with natural disease and 11.43% were inconclusive. The results of this study indicate that forensic pathology cases have increased significantly at the AHL, particularly cases of animal abuse and neglect. It is unknown if these data are representative of the pathology caseload in other laboratories, however, educating veterinary students, veterinarians and pathologists in the scientific method as it applies to forensic science is essential to ensure that veterinary forensic science withstands the scientific and legal scrutiny applied to its medical counterpart.

Immunophenotypic Characteristics of Equine Blood Monocytes and Alveolar Macrophages

Solomon Odemuyiwa, Dorothee Bienzle

Pathobiology, University of Guelph, Guelph, ON

Narrative: Equine recurrent airway obstruction (RAO) is a chronic non-septic inflammatory condition with similarity to human allergic asthma. Th2 polarization is the hallmark of human allergic asthma but T-helper lymphocyte responses in RAO are incompletely characterized. T-helper cell responses are orchestrated by monocytes and macrophages, which are also functionally divided into M1 (pro-inflammatory) and M2 (anti-inflammatory) types on the basis of surface markers, metabolic pathways, and secretion of specific cytokines and chemokines. It is currently unknown whether equine monocytes and macrophages differentiate into M1 or M2, but based on the observed inflammatory response in RAO, we hypothesized that there is a preponderance of M1 but not M2 cells. Blood and bronchoalveolar lavage fluid (BALF) monocytes and macrophages, respectively, from normal and affected horses were subjected to flow cytometric analysis with antibodies to CD14, CD90, CD163, MHC class II, and CD206. Results showed that 13-85% of blood monocytes expressed CD163, and 1-26% co-expressed CD14 with CD163. Further, CD90 labeled equine neutrophils but not monocytes. There was significantly higher expression of MHC-II in CD163+ than CD163- monocytes. Expression of CD163 was upregulated in horses with systemic inflammation, but downregulated on blood monocytes of horses with experimentally induced RAO. Airway macrophages expressed only low levels of CD14, but more than 85% of cells positive for the mannose receptor CD206 also expressed CD163. These results suggest that either CD163 is not a marker of M2 cells in horses, or that RAO is not characterized by M2-type of inflammatory responses. We further characterized M1-M2 polarization using real-time reverse transcriptase polymerase chain reaction (RT-PCR) to quantify cytokine and chemokine genes in alveolar and monocyte-derived macrophages. Following activation with lipopolysaccharide (LPS), macrophages were probed for expression of TNF and IL-12 (M1) and IL-10, CCL17 and TIE-2 (M2) mRNA. Preliminary results show significantly higher expression (3-6 fold) of IL-10 and TIE-2 in CD163+ compared to CD163- macrophages. The results of cytokine and chemokine expression in alveolar macrophages of RAO horses suggest there may be distinct functional profiles in alveolar macrophages.

An Outbreak of Suspected Nutritional Chondrodysplasia in Calves

Ada Cino Ozuna[1], Gregg Hanzlicek[1], Matt Miesner[2], Brad M. DeBey[1]

[1]Diagnostic Medicine/Pathobiology, Kansas State University - College of Veterinary Medicine, Manhattan, KS; [2]Department of Veterinary Clinical Sciences, Kansas State University - College of Veterinary Medicine, Mahattan, KS

Narrative: Nine calves in a herd of approximately 90 first-calf heifers were born with similar skeletal defects. The calves had varying degrees of a dwarflike appearance, with short limbs and joint laxity. There was no previous history of similar abnormalities in the herd. A one-day-old calf was submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) for necropsy. On physical examination the calf had a blocky posture with shortened long bones of the limbs and abnormal joint articulations. Gross and histologic sections of bones of the calf were compared with a normal similar-age calf. The most significant lesions were in the long bones of all four limbs. There was moderate limb-trunk disproportion, with short extremities and hyper-extensibility of the joints. The shape of the humerus, radius, metacarpus, femur, tibia, and metatarsus was distorted with irregularly enlarged epiphyses, and curved and short diaphyses when compared to the control. On longitudinal sections, there were irregularities in the growth plates. The growth plates were thin, sometimes jagged (scalloped). The marrow cavity contained increased spongiosa as compared to the control. No significant gross changes were seen in the other organs. Microscopically, growth plates were irregularly shaped, diffusely thin, and multifocally discontinuous. The hypertrophic zone was reduced in width. The columns of chondrocytes were short with reduced numbers of chondrocytes that were not arranged in orderly columns. The reserve and the proliferative zones often contained degenerated target chondrocytes. The primary spongiosa in the metaphyses was thicker than that of the control and in areas formed horizontal lattices that were parallel to the physis. The gross and microscopic lesions indicate a chondrodysplastic syndrome. Known causes of chondrodysplasia in calves include genetic, infectious, and nutritional (toxic). In this case, a genetic cause was excluded by epidemiologic investigation. Serum from the calf was negative for antibodies to BVD and bluetongue viruses, indicating lack of in-utero immune response to these viruses. The gross and microscopic findings are very similar to previously reported chondrodysplasia associated with manganese deficiency.

Glioblastoma Multiforme (High Grade Astrocytoma) in Two Free-ranging Raccoons (Procyon lotor) # +

Federico Giannitti[1], Leslie Woods[1], Asli Mete[1], Deana Clifford[2], Melanie Piazza[3], Diane Naydan[4], Robert Higgins[4]

[1]California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California, Davis, CA; [2]Wildlife Investigations Laboratory, California Department of Fish and Game, Rancho Cordova, CA; [3]WildCare, San Rafael, CA; [4]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA

Narrative: Intracranial neoplasms in wildlife are rarely reported and only two primary central nervous system neoplasms have been documented in raccoons, both classified as astrocytomas. Here we describe the pathological and immunocytochemical aspects of glioblastoma multiforme (GBM, World Health Organization grade IV astrocytoma) in two free-ranging, adult female raccoons that exhibited blindness, lethargy, ataxia and other neurologic signs at a wildlife rescue facility in Marin County, California. The animals were euthanized due to poor prognosis and submitted to the California Animal Health and Food Safety Laboratory System, Davis for postmortem examination. Grossly, there were well-demarcated, ellipsoid, 2-cm-diameter, space-occupying, soft, cavitated masses in the cerebral hemispheres (right olfactory bulb) in both cases. Microscopically, both masses were well-circumscribed, infiltrative and unencapsulated. Two distinct characteristic cell patterns were easily recognized on subgross examination. The predominant portion of the examined sections (>60%) was comprised of densely cellular areas while the rest was represented by multiple coalescing, bridging acellular irregular and interconnected pale eosinophilic areas of necrosis (serpentine necrosis). Neoplastic cells were largely spindle to fusiform and were arranged in densely-to sparsely-packed interlacing bundles often forming streaming patterns; had indistinct borders, moderate to scant amounts of fibrillar eosinophilic cytoplasm, ovoid to elongate nuclei with finely stippled chromatin, frequently containing a single round central to paracentral light magenta nucleolus. Multifocally, fusiform neoplastic cells oriented perpendicularly to necrotic foci at the borders of the necrotic areas (pseudopalisading). Scattered throughout the neoplasm were multiple blood vessel proliferations often forming prominent glomeruloid structures lined by hypertrophied endothelial cells (microvascular proliferation). There was moderate cellular pleomorphism, anisocytosis and anisokaryosis, single-cell necrosis and occasional karyomegalic and multinucleated cells. There were average 10 (case 1) and 19 (case 2) mitoses per 400x power field (n=10 fields), including both normal and bizarre mitotic figures. Multifocally throughout the tumors there were frequent lymphoplasmacytic and eosinophilic infiltrates. Neoplastic cells were immunohistochemically positive for glial fibrillary acidic protein (GFAP) and vimentin, and negative for pancytokeratin (Lu-5) and oligodendrocyte transcription factor 2 (OLIG2). Vascular proliferations were positive for human von Willebrand factor VIII and α-smooth muscle actin (α-SMA). To the best of our knowledge this is the first written communication of GBM in wildlife species.

AAVLD Trainee Travel Awardee (Pathology)

+ AAVLD/ACVP Graduate Student Award Applicant

Multiple Testicular Neoplasms in a Canine * +

Anwar Sarah, Tanya Graham, David Henry Zeman

ADRDL-SDSU, Brookings, SD

Narrative: An 11-year-old, male dog was presented to a local practitioner with a testicular tumor. The right testicle was approximately 5 times the size of the left testicle, which appeared atrophied. The testicles were surgically removed. Two testicles in formalin were submitted to the SDSU-ADRDL for histologic examination. Within the parenchyma of the right testicle was a slightly oval 35mm by 30mm mass. The mass elevated the capsule and occupied the seminiferous tissue. In addition to this mass, there was another half circle mass in the right testicle measuring 20mm by 12mm. The left testicle was diffusely white and firm and was interpreted to be severely atrophic. It had two small tumors. The first was a crescent shaped mass measuring 15mm by 8mm. The second mass within the left testicle was a small tumor located adjacent to the crescent shaped tumor, measuring 9mm by 7mm. The histologic examination showed that the large right testicular tumor was a malignant seminoma, the medium-sized testicular tumors in the right and left testicles were a benign interstitial cell tumor (Leydig Cell Tumor), and the small left testicular tumor was a benign sertoli cell tumor. This case is quite interesting having three types of testicular neoplasms (seminoma, interstitial cell tumor and sertoli cell tumor) simultaneously. Most neoplasms cause enlargement of the testis, but each neoplasm presents different gross and histologic lesions. According to the case history, the right testicle was enlarged and about five times the size of left testicle. This is considered a consequence of hormone production by the sertoli cell tumor as indicated by atrophic seminiferous tubules. About a third of sertoli cell tumors produce estrogen (hyperestrogenism) which may also have other feminizing effects, such as gynecomastia. Other etiologies reported for testicular atrophy by direct or indirect (hormonal) pathways include heat stress, brucellosis, phytoestrogens, mycotoxins, vitamin and mineral deficiencies, high dose of heavy metals, organochlorine contamination, and genetic factors.

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Hypovitaminosis D in a Swine Herd * +

Elisa Salas[1], Steve M. Ensley[2]

[1]Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, NE; [2]Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA

Narrative: Di-hydrocholecalciferol (1, 25 Vitamin D3) is important for enteric calcium absorption and calcium homeostasis. A dietary lack of this nutrient and insufficient exposure to sunlight has been linked to metabolic bone disease (rickets and fibrous osteodystrophy) secondary to hypocalcemia. Eleven pigs were presented to the University of Nebraska-Lincoln Veterinary Diagnostic Center and ten pigs were presented to the Iowa State University Veterinary Diagnostic Laboratory from the same herd for investigation. One hundred and fifty pigs from a pen of 220 were dead by the conclusion of the investigation. The history and clinical signs consisted of tremors, short-strided gaits, joint pain, weakness, cyanosis, recumbency, normal mentation, and one femoral fracture. Eighteen of twenty-one pigs were presented alive and were euthanized. Significant gross lesions were lumbosacral fractures and mid-lumbar vertebral fractures. Histologic lesions included bone marrow emboli in the pulmonary vasculature of three pigs and myocardial necrosis and fibrosis in six pigs. Bone lesions in two pigs consisted of atrophy and discontinuity of the cortices, multifocal cartilagenous necrosis and infarction, rare fractures of cancellous bone, metaphyseal hemorrhage and decreased osteoblasts in cancellous bone. Twelve serum samples were submitted for 25 dihydrocholecalciferol assays and testing revealed decreased amounts in 8 pigs; <2 ng/mL for six pigs, 8.3 ng/mL in one pig and 11.0 ng/mL in one pig. Four pigs were within normal limits (30-35ng/mL). Bone ash analysis, the burning and evaluation of boney calcium composition, performed on the 2nd rib of seven pigs showed low bone ash in all, ranging from 8%-35%, well below the normal rib bone ash level of 58-62%. Blood calcium was low in all pigs tested (4/4 pigs), ranging from 4.0-5.8 mg/dL. Unfortunately, no feed analysis was pursued in this case, but, often, the inciting feed is no longer present on the farm. The data from the investigation indicated that these pigs suffered from hypocalcemia as a consequence of hypovitaminosis D. Multiple cases were presented to the Iowa State University Veterinary Diagnostic Laboratory, likely making hypovitaminosis D an under reported phenomenom in swine herds and one that warrants a high index of suspicion and investigation.

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Multiple Endocrine Neoplasia in a Dog

Leah Kuhnt

Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, AL

Narrative: An 11-year-old Basset Hound-mix dog was presented to the Thompson Bishop Sparks State Diagnostic Laboratory in Auburn, AL for necropsy. The dog had been previously diagnosed with an oral malignant melanoma and was undergoing treatment at another institution. The dog had previous cytologically confirmed lymph node metastasis of melanoma, and radiographically evident bilateral lung masses which were presumed to be metastatic melanoma lesions as well. At the time of necropsy, multiple masses were found in a variety of organs. There were enlarged submandibular, cervical and mediastinal lymph nodes, and variably-sized masses within the mediastinum and thoracic inlet, within the lungs, kidney, liver, spleen, adrenal, thyroid, skin, urinary bladder, and small intestine. Immunohistochemistry was performed on many of the masses, and included stains directed against Melan-A, thyroglobulin, calcitonin, chromogranin-A, S-100 protein, a-inhibin, synaptophysin, NSE, smooth muscle actin, c-KIT, cytokeratin, vimentin, CD3, and CD79a. The masses were determined to include dermal melanoma and metastatic melanoma within several lymph nodes, but other masses throughout the body represented a wide variety of other endocrine and non-endocrine related lesions. These included bilateral thyroid adenoma, ectopic thyroid tissue, thyroglossal duct mass, primary bilateral pulmonary bronchioloalveolar carcinoma, adrenal cortical carcinoma with renal metastasis, and leiomyoma of both the urinary bladder and small intestine. The presence of multiple endocrine, as well as many non-endocrine, neoplastic masses and lesions within this dog suggests the likelihood of a multiple endocrine neoplasia (MEN) syndrome. Although there are few published reports of MEN in dogs, the types of lesions noted often do not fall directly or neatly into the classification scheme of human MEN. In people, cases range from Type 1, Type 2a and 2b, and are associated with genetic mutations in genes such as MEN or RET. This case is unusual in that it represents a previously unreported grouping of neoplastic and non-neoplastic lesions, and may help provide further insight into how MEN in dogs may be diagnosed and classified.

Tuberculosis/Johne's Disease Special Scientific Session

Saturday, October 1, 2011

Grand Ballroom E

Moderators: Suelee Robbe-Austerman and John Adaska

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

◊ USAHA Paper

Meta-Analysis on 15 Field Studies Comparing the Performance of the Skin test with the Gamma-Interferon Test (Bovigam®) for the Detection of Bovine Tuberculosis in Cattle

Alex Raeber, Bjoern Schroeder

R&D, Prionics AG, Schlieren

Narrative: BOVIGAM® (the Gamma Interferon test) received approval by the USDA in June of 2003 as an official supplemental test for diagnosis of bovine tuberculosis (bTB) in cattle. However, BOVIGAM® currently has a very limited role in the US bTB eradication campaign in its role as a supplementary test to the Caudal Fold Test (CFT). Approval as a primary diagnostic test requires equivalency with the CFT as the currently approved primary diagnostic test. Here we report on a meta-analysis to review field trials comparing the diagnostic performance of BOVIGAM® and the tuberculin skin test. A total of 15 field studies with 16,664 animals sourced from more than 175 herds in 8 different countries and published between 1991 and 2006 were analysed. Field studies were separately assessed based on the type of skin test (CFT: Caudal Fold Test; SCT: Single Cervical Test; CCT: Comparative Cervical Test) used. As reference test for all sensitivity studies and for some specificity studies, tested animals were slaughtered and subjected to examination for lesions and culture of *M.bovis* from tissue samples. Specificity studies were conducted in herds with no history of bTB. The reported performance estimates for the two tests were analysed for statistical significance by the Fisher's Exact Test. A test was considered significantly different if a p-value < 0.05 was obtained. In 6 of 15 studies involving 8704 animals, BOVIGAM® was significantly better than the skin test. Four of these studies assessed sensitivity (1 CFT, 1 SCT, 2 CCT), and 2 studies specificity (2 CFT). In a further 7 studies with 1266 animals, BOVIGAM® was equivalent to the skin test. In 4 studies the sensitivity was determined (2 CFT, 1 SCT, 1 CCT) and 3 studies were aimed to estimate specificity (1 SCT, 2 CCT). Finally, in 2 of 15 studies with 2543 animals, the skin test performed better than BOVIGAM®. Both of these studies investigated specificity between the CCT and BOVIGAM®. The CFT/SCT was determined to have a median sensitivity value of 75.3% and a median specificity value of 96.97%. The CCT was found to be slightly more specific with a median specificity value of 99.95% and a median sensitivity value of 74.3%. The corresponding median values for BOVIGAM® were calculated as 84.6% for sensitivity and 97.4% for specificity. Analysis of data from 15 field studies that were conducted to compare the diagnostic sensitivity and specificity of the tuberculin skin tests and the BOVIGAM® assay show that BOVIGAM® performed equal or better than the single intradermal skin tests CFT and SCT with regard to both sensitivity and specificity while the CCT had a higher specificity than BOVIGAM® but lower sensitivity. Based on these data, BOVIGAM® should be considered for approval as official primary diagnostic test in the US bTB program. This would allow use of BOVIGAM® in new and more cost effective testing strategies such as for Test and Slaughter, Import Testing, Pre-movement Testing and Herd Screening.

Association between Caudal Fold Tuberculin Reactions and *Mycobacterium avium* subsp. *paratuberculosis* ELISA and Fecal Culture Test Results in Dairy Herds #

Barbara Brito[1], Randy Anderson[2], Sharif Aly[3], Ian Gardner[1]

[1]Medicine and Epidemiology, School of Veterinary Medicine, UC Davis, Davis, CA; [2]Animal Health Branch, California Department of Food and Agriculture, Sacramento, CA; [3]Department of Population Health and Reproduction, School of Veterinary Medicine, UC Davis, Davis, CA

Narrative: The caudal fold tuberculin CFT is the most commonly used USDA-approved screening test for TB. On average, the CFT is estimated to be about 97% specific with values ranging between 76 and 99%. Mycobacteria from the avium complex have been implicated as a cause of CFT cross-reactivity, including *Mycobacterium avium* subspecies *paratuberculosis* (MAP) which causes paratuberculosis, a chronic gastrointestinal disease that is present in more than two-thirds of US dairy herds and causes substantial financial loses to cattle producers. The objective of the present study was to evaluate the association between CFT reactors and MAP ELISA and fecal culture (FC) test results. Caudal fold tuberculin and MAP test results records from one dairy herd located in California (n=4924), and three dairy herds in Colorado (n=1869, 777 and 2054, respectively) were analyzed. The herds were free of tuberculosis and had ELISA seroprevalences for MAP ranging from 1.9 to 4.0%. All herds were participants in the Johne's Disease Demonstration Herd Project. The California herd CFT testing took place in 2004 and 2008, and the Colorado herds in 2005 and 2008-2009. MAP ELISA and FC results obtained within 1 year before or after the CFT test were used in the analysis. Two binary logistic regression models were constructed to measure the association between the CFT and MAP (ELISA or FC) test results, where the dependent outcome was the CFT status of the animal (negative or positive reactor) and explanatory variables were the MAP status (ELISA or FC), lactation number and herd. MAP status as defined by ELISA and FC results was strongly associated with CFT positive reactions. The odds for a CFT reactor compared with a non-reactor being positive to MAP ELISA and FC were 4.7 (95%CI=3.0-7.2) and 5.6 (95%CI=3.5-9.0), respectively. In conclusion, MAP status needs to be considered when interpreting CFT test results in a dairy herd. In addition, inoculation of bovine purified protein-derivative tuberculin may increase the probability of having false-positive serum ELISA results for MAP.

AAVLD Trainee Travel Awardee

Factors Associated with the Detection of Bovine Interferon-γ Response in Blood Collected During Exsanguination of Cattle Sensitized to *Mycobacterium bovis* * #

Chika Okafor[1,2], Daniel Grooms[1,2], Steven R. Bolin[3], Tara Gravelyn[1], John Kaneene[1,2]

[1]College of Veterinary Medicine, Michigan State University, East Lansing, MI; [2]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI; [3]Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI

Narrative: The interferon-gamma (IFN-γ) assay measures bovine IFN-γ which is released by T-lymphocytes as part of cell-mediated immunity to *Mycobacterium bovis*. Integrating this assay with the currently used visual inspection of carcasses at slaughter could help detect more bovine tuberculosis (BTB) exposed herds and aid in a more timely eradication of BTB. However, it is unknown if a bovine IFN-γ response, sufficient enough to classify cattle exposed to *M. bovis* as being positive for BTB, can be obtained using blood collected at exsanguination. We hypothesized that the IFN-γ responses/interpretations between blood collected pre-slaughter and at exsanguination would not change. Two separate trials were conducted using cattle experimentally sensitized to killed *M. bovis* (n=16), creating a similar immune response found in BTB affected cattle, and unsensitized control cattle (n=4). In trial 1, the IFN-γ assay was performed on blood collected immediately before stunning and at exsanguination from 15 cattle (12 sensitized and 3 control); from a subset of these cattle (4 sensitized and 1 control) additional blood was collected 5 minutes following exsanguination. Trial 2 consisted of 1 control and 4 sensitized cattle. From this group, total white blood cells (WBC) and lymphocyte counts were performed alongside the IFN-γ response on the blood collected at the farm, at exsanguination, and at one minute intervals for 3 minutes. The probability that cattle sensitized to *M. bovis* remained IFN-γ positive on blood collected at exsanguination was 0.75 (95% CI 0.54, 0.88). Using paired t-test analysis, there was a significant decrease in the mean ELISA adjusted optical density (OD) readings from pre-slaughter to exsanguination ($p = 0.03$). Although IFN-γ ELISA OD readings of individual cattle dropped at exsanguination, a change in the IFN-γ ELISA interpretation only occurred in cattle that were borderline positive pre-slaughter. The decline in bovine IFN-γ responses following exsanguination were in parallel with a corresponding decline in total WBC and lymphocyte counts. At 5 minutes following exsanguination, sensitized cattle were found IFN-γ negative for BTB. This study demonstrates that most cattle with a positive IFN-γ response pre-slaughter will remain positive at exsanguination. The decline in total WBC and lymphocyte numbers following exsanguination could be responsible for the corresponding decline in bovine IFN-γ responses. Furthermore, timing of blood collection at slaughter is critical in reducing false negative results. These findings support further development and use of the IFN-γ assay on blood collected during exsanguination as part of a BTB surveillance program.

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

Interferon-γ Assay on Blood Collected During Exsanguination of Cattle: A Surveillance Tool for Bovine Tuberculosis *

Chika Okafor[1,2], Daniel Grooms[1,2], Steven R. Bolin[3], James Averill[4], John Kaneene[1,2]

[1]College of Veterinary Medicine, Michigan State University, East Lansing, MI; [2]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI; [3]Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI; [4]Animal Industry Division, Michigan Department of Agriculture and Rural Development, Lansing, MI

Narrative: Improving bovine tuberculosis (BTB) surveillance is imperative for continued progress in global BTB eradication in a timely and cost effective manner. Interferon-gamma (IFN-γ) assay performed on blood collected during exsanguination of cattle experimentally exposed to killed *Mycobacterium bovis* has been reported as a potential tool for targeted surveillance of BTB. However, this assay has not been validated in cattle naturally exposed to *M. bovis.* The aim of this study was to determine: (1) if the use of the bovine IFN-γ assay on blood collected during exsanguination of cattle at slaughter could identify any cattle as IFN-γ positive for BTB; and (2) if IFN-γ positive cattle are more likely to originate from herds with a history of BTB exposure. Cattle from three risk groups (herds) were used in this study: (1) herds from which BTB infected animal(s) was/were isolated; (2) a herd that purchased cattle from a BTB affected herd; and (3) herds with no known history of BTB infection or exposure from the BTB modified accredited advanced zone in Michigan. To evaluate the first aim, bovine IFN-γ assay was performed on blood collected during exsanguination of cattle at slaughter. BTB history of the herds, identification of BTB-like gross lesions via routine slaughter inspection and isolation of *Mycobacterium bovis* by culture from lymph nodes were used as the criteria for evaluating the second aim of this study. Cattle, both with and without BTB-like gross lesions, were identified as IFN-γ assay positive for BTB using blood collected at exsanguination. All IFN-γ positive cattle originated from BTB affected herds and a BTB exposed herd. No IFN-γ positive cattle originated from herds with low risks/no history of BTB exposure. This study demonstrates that the use of the IFN-γ assay on blood collected during exsanguination as part of a BTB surveillance program will help identify BTB exposed herds. The addition of this assay to the currently used slaughter surveillance for BTB could be advantageous in the control and eradication of BTB.

* Graduate Student Oral Presentation Award Applicant

Effect of Positive Test Results for *Mycobacterium avium* subsp. *paratuberculosis* on Weaning Weights in Beef Cow-calf Herds *

Bikash Bhattarai[1], Jason Osterstock[3,4], Charles Fossler[7], Seong Park[6], Allen Roussel[5], Geoffrey Fosgate[2]

[1]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX; [2]Department of Production Animal Studies, University of Pretoria, Onderstepoort; [3]Texas AgriLife Research, Amarillo, TX; [4]Pfizer Animal Genetics, Kalamazoo, MI; [5]Department of Veterinary Large Animal Clinical Sciences, Texas A&M University, College Station, TX; [6]Texas AgriLife Research and Extension Center, Vernon, TX; [7]NAHMS, USDA-APHIS-VS-CEAH, Fort Collins, CO

Narrative: Johne's Disease Demonstration Herd Project data was obtained to evaluate losses associated with Johne's disease in beef cow-calf herds based on serological and microbiological tests for *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The primary economic outcome for the study was calf weaning weight. Data for 205 day adjusted weaning weights (AWW), fecal culture results (n=2,103 cow-calf pairs) and ELISA results (n=3,482) were analyzed. To account for clustering of observations, multilevel mixed models were developed including random effects to account for repeated tests within cow, and cow nested within herd. Potential confounding of the associations between test status and AWW associated with herd and animal-level covariates was evaluated on the basis of change in regression coefficient after inclusion of the covariate in the model. Univariate analyses identified a significant reduction of 57.9 lbs (95% CI: 24.49 to 91.23) in AWW when the dam was fecal culture positive and a non-significant reduction of 7.6 lbs (95% CI:-2.71 to 17.96) when the dam was ELISA positive. Multivariable models estimated 69.2 lbs (P<0.001; 95% CI: 36.94 to 101.46) lower AWW in calves from fecal culture positive cows adjusted for cow age, lactation number and years since the inception of a control program in the herd. Similarly, ELISA positive dams were associated with an AWW reduction of 11.4 lbs (P=0.029; 95% CI: 1.18 to 21.66) adjusted for the effects of cow age and years since the inception of a control program in the herd. Results from this study indicate that there is a significantly lower AWW in the offsprings of cows positive for MAP by fecal culture or ELISA. These findings support the contention that Johne's disease in beef herds is associated with significant economic losses attributed to decreased AWW within infected herds. These findings will be important for producers and other stakeholders in the industry to better understand the importance of control of Johne's disease in beef herds.

* Graduate Student Oral Presentation Award Applicant

Evaluation of Real-time PCR for the Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Alpacas and the Prevalence of Johne's Disease in Alpacas Presented to Four Veterinary Teaching Hospitals in the United States

Marie-Eve Fecteau[1], Robert Whitlock[1], Susan McAdams[1], Terry Fyock[1], Daniela Bedenice[2], Christopher Cebra[3], Toby Pinn[4], Raymond Sweeney[1]

[1]Clinical Studies-NBC, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA; [2]Clincial Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA; [3]Clinical Sciences, Oregon State University College of Veterinary Medicine, Corvallis, OR; [4]Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

Narrative: Johne's disease (JD) is a chronic intestinal infection of domestic and wild ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The prevalence of JD in alpacas in the United States is unknown. Real-time polymerase chain reaction (RT-PCR) has been used in cattle to quickly and accurately identify MAP in fecal specimens. However, the limits of detection in alpaca feces by use of PCR have not been determined. The objectives of this study were: (1) to validate the PCR test for use in alpaca feces, and define the limits of MAP detection in these samples; and (2) to estimate the prevalence of MAP shedding in feces of alpacas presented to 4 veterinary teaching hospitals in the United States.

Objective 1- Ten 5-fold dilutions of a MAP strain (3.8×10^7 colony forming units [CFU]/mL) obtained from an alpaca with clinical signs of JD were added to known-negative alpaca fecal samples. All 10 fecal dilutions were processed for MAP detection using a commercially available RT-PCR assay, and for MAP culture on Herrold's Egg Yolk Medium (HEYM) and the MGIT liquid broth method. For each dilution, the PCR's number of cycles to threshold (Ct), the number of CFU/g of feces on HEYM, and the time to detection (TTD) in MGIT were recorded and the lowest limit of detection for each method was determined.

Objective 2- Fecal samples from alpacas presenting to 4 United States veterinary teaching hospitals from November 2009 through February 2011 were collected and processed for MAP via PCR and HEYM culture. The prevalence of MAP fecal shedding was determined as the fraction of PCR-positive samples.

The lowest MAP dilution detectable via PCR was 243 MAP CFU/g of feces. At that concentration, MAP growth was also detectable on HEYM and MGIT culture medium. Ten (6%) of the 180 fecal samples collected from hospitalized alpacas were positive on PCR. The 95% confidence interval was determined to be between 3% and 9% of the population tested. These results indicate that: 1) PCR can provide an accurate and rapid detection of MAP fecal shedding in alpacas, and that it may be a useful tool in the detection of JD in clinical and subclinical alpacas; and 2) the prevalence of MAP fecal shedding in hospitalized alpacas in US veterinary teaching hospitals is low.

Toxicology Scientific Session

Saturday, October 1, 2011

Wright

Moderators: Patricia Talcott and Karyn Bischoff

Rapid Screening for Toxicants Using DART and UHPLC-High Resolution Mass Spectrometry

Michael Filigenzi, Robert Poppenga

Toxicology, California Animal Health and Food Safety Laboratory, Davis, CA

Narrative: Incidents involving poisoning of animals by unknown agents are challenging from a diagnostic standpoint. Screening analysis for a wide range of analytes is often used as an initial step in determining the toxicant in question. Speed is essential for such screening methods, as time is often a critical element in the investigation of such incidents. This presentation will describe a set of procedures which together provide for the screening of a wide array of chemicals in various matrices in as little as several minutes for preliminary results to several hours for more refined results at low detection limits. The first procedure involves analysis of the sample using a Direct Analysis in Real Time (DART) ion source coupled with a high resolution mass spectrometer. A second procedure involves the preparation of a dilute extract of the sample using the QuECHERS (for Quick, Easy, Cheap, Effective, Reliable, Safe) method and analysis of this extract using ultra high pressure liquid chromatography coupled with the same high resolution mass spectrometer (UHPLC-HRMS). The third procedure involves concentration of the QuECHERS extract and analysis by UHPLC-HRMS. Results of all three procedures are available within three hours of receipt of the sample in the laboratory. Analytes tested through this method include plant toxins, a variety of pesticides, and some veterinary drugs. Detectible levels ranged from 1 ppb to 1 ppm. Several cases will be presented in which unexpected toxicants were detected using this method.

Identification of Protoxins, Mechanism of Action and Possible Microbial Basis for Red Maple (Acer rubrum) Toxicosis in Equines #

Karan Agrawal[1], Joseph Ebel[1], Karyn Bischoff[1,2], Craig Altier[1,2]

[1]Animal Health Diagnostic Center, Cornell University, Ithaca, NY; [2]Population Medicine & Diagnostic Sciences, Cornell University, Ithaca, NY

Narrative: *Acer rubrum* (Red Maple) is a deciduous tree native to northeastern North America and commonly found in pastures. The leaves of *A. rubrum*, especially when wilted in the fall, cause severe oxidative damage to equine erythrocytes, leading to potentially fatal methemoglobinemia. Gallic acid and tannins from *A.rubrum* leaves have been implicated as the toxic compounds responsible for red maple toxicosis, but this study found that pyrogallol is a more potent inducer of methemoglobinemia. Given that the conversion of gallic acid to pyrogallol involves a decarboxylation reaction, and the literature suggests a microbial basis for the metabolism of tannins to gallic acid and subsequent pyrogallol production, it is hypothesized that the hydrolysable tannins and free gallic acid in in *A. rubrum* leaves are protoxic and undergo metabolism to the oxidative agent pyrogallol as outlined above. The objective of this study is to confirm the proposed pathway, identify sites in equines where this pathway occurs, and identify microbes in these sites capable of carrying out this pathway. *A. rubrum* leaves do not contain free pyrogallol, therefore any pyrogallol formation observed is due to the decarboxylation of gallic acid. Incubation of equine gastrointestinal contents and liver with ground, dried *A. rubrum* leaves demonstrated maximum pyrogallol formation in the Ileum, trace pyrogallol formation in the jejunum and liver, and no pyrogallol formation in the stomach, colon or cecum, therefore this the pathway occurs primarily in the ileum. Pyrogallol persisted in the ileum for up to 96 hours after initial formation in vitro, and is therefore stable enough to allow absorption into the bloodstream in vivo. Bacteria isolated from the ileum are pending identification and characterization, but ileum contents filtered through a 0.22µm filter (to exclude bacteria) and incubated with dried and ground *A. rubrum* leaves did not demonstrate gallic acid or pyrogallol formation. This study is important because, despite cases of red maple toxicosis being documented in the literature as far back as 1981, the mechanism of action for the syndrome is as yet unknown. The current recommended treatment for red maple toxicosis is methylene blue injection. More information on the mechanism of action will make it possible to expand the treatment options to include other antioxidants and possibly antimicrobials or probiotics to disrupt bacterial production of pyrogallol.

AAVLD Trainee Travel Awardee

Quantitation and Confirmation of the Insecticide Chlorfenapyr in Liver using GC/ECD and GC/MS

Christina Wilson[1,2], Kimberly Meyerholtz[2], Adam Stern[3,4], Stephen B. Hooser[2]

[1]Comparative Pathobiology, Purdue University, W. Lafayette, IN; [2]Indiana Animal Disease Diagnostic Laboratory, Purdue University, W. Lafayette, IN; [3]Veterinary Diagnostic Laboratory, University of Illinois, Urbana, IL; [4]Pathobiology, University of Illinois, Urbana, IL

Narrative: Introduction: Chlorfenapyr is a halogenated, pyrrole insecticide used to control pests or insects resistant to pyrethroids and other common insecticides. The mechanism of toxicity involves bioactivation to a toxic metabolite that uncouples oxidative phosphorylation, ultimately disrupting production of ATP leading to cell death. Concerns regarding toxicity in non-target species, in addition to its ability to persist in the environment and bioaccumulate, warrant the need to develop methods to detect chlorfenapyr in biological samples in suspect cases of exposure.

In this study, a method has been developed to quantitate and confirm chlorfenapyr in liver using GC/ECD and GC/MS. Briefly, this method involves homogenizing approximately 5 grams of liver in acetonitrile and NaCl (10:1, v/w). Analyte isolation and removal of matrix interferences from the acetonitrile extract was achieved using ENVI-Carb™-II/PSA solid-phase extraction cartridges. The resultant eluate was evaporated to dryness under nitrogen and reconstituted with 0.5 mL toluene for GC/ECD and GC/MS analysis. The GC/ECD conditions were: injector port temperature of 215 degrees C, column flow set to 1.0 mL/min, an initial column temperature of 100 degrees C with a 20 degrees C/min ramp to 300 degrees C, and the ECD temperature was set at 300 degrees C. The GC/MS parameters were: injector port temperature 220 degrees C, column flow 1.0 mL/min, column temperature program was initially set at 90 degrees C (held 0.10 min) with a 7.1 degrees C/min ramp to 300 degrees C. The MS transferline heater was 220 degrees C, the ion trap temperature was 150 degrees C, and a manifold temperature of 40 degrees C. EI-MS scan from 40 to 650 m/z was used to monitor ions. For both GC/ECD and GC/MS analyses, 1 µL of standards and samples were injected onto a VF-5ms column (30m x 0.32mm, ID; DF = 0.25).

GC/ECD analysis of positive control liver samples, fortified at 40 ppb chlorfenapyr, resulted in ≥ 80% recovery and a retention time of approximately 16 minutes. GC/MS analysis of chlorfenapyr standards and positive control liver samples (fortified at 40 ppm chlorfenapyr) resulted in retention times of approximately 21 minutes and detection of ions characteristic of chlorfenapyr: m/z 59 and m/z 247.

This study details development of a method for isolating and detecting chlorfenapyr in liver samples. In cases of suspect toxicosis, this method can be used to quantitate and confirm exposure to chlorfenapyr in non-target species.

Ocular Fluid Nitrate and Nitrite Concentrations in Aborted, Stillborn and Newborn Equines

Cynthia Gaskill, Lori Smith

Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY

Narrative: Equine abortion due to nitrate intoxication is rarely reported in the literature. In cattle, fetal and neonatal ocular fluid nitrate concentrations > 25-30 ppm can be associated with nitrate-induced abortions while ocular fluid nitrate concentrations < 20 ppm are generally considered normal. However, no published reports of normal reference ranges for fetal and neonatal equine ocular fluid nitrate concentrations could be found in the literature. The purpose of this study was to establish a normal reference range for ocular fluid nitrate and nitrite concentrations in aborted, stillborn, and neonatal foals that died in central Kentucky. A secondary goal was to compare two methods of nitrate/nitrite concentration determinations in equine ocular fluid: ion chromatography (IC) and the EM Quant strip rapid, semi-quantitative colorimetric method.

Aqueous humor was collected from 67 aborted or neonatal foals presented to the University of Kentucky Veterinary Diagnostic Laboratory for post-mortem examination from March 1 to May 1, 2011. Nitrate/nitrite concentrations were determined by IC and by EM Quant strip analysis. Data collected included age of foal, sex, breed, gestation time, month of abortion, and clinical history. Cause of death diagnoses were obtained from case reports after post-mortem examinations and related testing were completed. Statistical analyses included comparison of analysis methods and associations between nitrate concentrations and age, sex, breed, month of abortion, and diagnosis.

Ocular fluid nitrate concentrations determined by IC ranged from below the quantitation limit of 5 ppm (6 samples) to a high of 12.8 ppm, with a median of 7.4 ppm and a mean of 7.8 ppm (+/-2.07) for the 61 samples with nitrate concentrations > 5 ppm. Nine samples had nitrate concentrations between 10 and 12.8 ppm. Nitrite was not detected in any sample (minimum level of quantitation: 1 ppm). Thoroughbreds were over-represented (48 foals). Causes of death included nocardioform placentitis (14), other placentitis causes (13), dystocia/asphyxia/aspiration (20), flexure deformities/scoliosis/other deformities (7), miscellaneous causes (7) such as equine herpesvirus type 1, premature placental separation, uterine torsion, pneumonia; and no diagnosis (4). There were no significant associations between ocular fluid nitrate concentration and age, breed, gestation length, sex, month of abortion, or diagnosis. Test strip color changes correlated well with IC results but large jumps between pad color cut-offs (0, 10, and 25 ppm) made interpretation somewhat subjective.

Ocular fluid concentrations in fetal, stillborn, or neonatal foals dying from causes other than nitrate poisoning ranged from < 5 ppm to 12.8 ppm. EM Quant test strips appear to be an appropriate rapid semi-quantitative screening analysis. Additional investigations are underway.

The Detection and Interpretation of Liver Anticoagulant Rodenticide Concentrations in Diverse Avian and Mammalian Wildlife Species

Robert Poppenga[1], Mike Filigenzi[1], Seth Riley[2], Terra Kelly[3], Mourad Gabriel[4], Pam Swift[5], Laurel Klein[6], Chris Kreuder[3], Deana Clifford[5], Walter Boyce[3], Winston Vickers[3], Jessie Quinn[8], Leslie Woods[1], Erin Boydston[7]

[1]California Animal Health and Food Safety Laboratory, Davis, CA; [2]National Park Service, Santa Monica Moutains National Recreation Area, Thousand Oaks, CA; [3]Wildlife Health Center, UCD School of Veterinary Medicine, Davis, CA; [4]UCD School of Veterinary Medicine, Davis, CA; [5]Wildlife Investigations Laboratory, California Department of Fish and Game, Davis, CA; [6]Department of Ecology and Evolution, UCLA, Los Angeles, CA; [7]Western Ecological Research Center, USGS, Thousand Oaks, CA; [8]Department of Fish, Wildlife and Conservation Biology, UCD, Davis, CA

Narrative: Anticoagulant rodenticides (AR) are readily available and widely used for the control of small mammalian pests such as rats and mice. Currently available AR are classified as either first generation (warfarin, diphacinone, chlorophacinone and coumachlor) or second generation (brodifacoum, bromodiolone, difethialone) AR. Second generation AR are more toxic and have long half-lives once absorbed. The potential for secondary intoxication of non-target species is well known and has led the USEPA to impose restrictions on their use. We have had the opportunity to test a number of avian and mammalian wildlife species including mountain lions, bobcats, badgers, pacific fishers, coyotes, red foxes, turkey vultures and golden eagles for the presence of AR over the last several years. Samples were obtained opportunistically from animals found in a variety of urban, peri-urban and rural environments. While there were some species differences in terms of the frequency of detection, AR were detected in all species tested. In many individuals, multiple AR were found. The toxicity of AR for non-target species is largely unknown, although there do appear to be substantial species differences. The ingestion of AR-exposed or poisoned prey is the most likely source for non-target species exposure, in contrast to exposure via direct consumption of AR-containing bait. However, the identification of exposure pathways has been difficult since prey items are infrequently available for analysis. Antemortem assessment of target and non-target species exposure has been challenging due to low concentrations of AR in available antemortem samples such as serum and, in some species such as field mice or wood rats, limited sample volumes. From assessment of data collected to date, there does not appear to be liver AR concentration ranges that are consistently associated with acute adverse effects. It is likely that such ranges, if they can be established, will differ by species. At the present time, the only way to confirm AR morbidity or mortality in wildlife species is to detect the presence of one or more AR in appropriate antemortem or postmortem samples and confirm the concurrent presence of a coagulopathy. The subclinical and population level effects of AR exposure on non-target species have not been determined. However, it is hoped that steps to restrict AR use in the future will help lesson non-target species exposure and intoxication.

Detection of Toluene-2,4-diisocyanate in Nesting-material Associated with Mortality in Pigeon Chicks

Motoko Mukai[1], Samuel Stump[2], Jeanne Smith[3], Francisco Uzal[4], Robert Poppenga[2], Leslie Woods[2], Birgit Puschner[2]

[1]Animal Health Diagnostic Center, Cornell University, Ithaca, NY; [2]California Animal Health and Food Safety Laboratory System, University of California, Davis, CA; [3]Avian Health Services, , Placerville, CA; [4]California Animal Health and Food Safety Laboratory System, University of California, San Bernardino, CA

Narrative: Toluene diisocyanate (TDI) is a highly reactive compound commonly used in the production of polyurethane foams for furniture, bedding, and car upholstery. It is considered one of the most hazardous inhalant toxicants for humans and animals. Here we report two cases of mortality in pigeon chicks that were associated with nesting material contaminated with 2,4-TDI. Nesting pads were sold as “Black European Felt Nesting Pads” made from a recycled textile material manufactured in Belgium and purchased from retail stores in the United States. The first case was submitted by a racing pigeon breeder in January 2010, who had lost 100% (total of 129) of the hatchlings immediately after changing the nesting material to this particular product . Hatchability did not significantly change with the use of the nesting pad (approx. 90-95%) and adult birds appeared healthy. All of the hatched chicks died at 2 days of age, except for one that lived to 14 days. Necropsy of 3 of the chicks at day 2 showed edematous lungs and focal hepatic necrosis was found in the liver of one bird. The second case was submitted in April 2010 by another pigeon breeder with a history of low hatchability. All hatchlings died within 2 days (N=100). No gross lesions were found in necropsied chicks or embryos. The nesting pads were screened for toxic compounds using gas chromatography-mass spectrometry (GC/MS) and 2,4-TDI was detected in both cases. Both breeders noted significant recoveries in hatchling-survival with the change of nesting pads to a different material. There is limited information on the toxicity of 2,4-TDI to birds. According to one report, the oral LD50 in red-winged blackbirds is 100 mg/kg, which is much lower than that for other species (>3,000 mg/kg). Information on inhalational and developmental toxicity or transfer across egg shells is not available in any avian species. In several animal species, inhalant exposure to 2,4-TDI leads to pulmonary edema and death at low concentrations, which was consistent with necropsy findings in one case. Although it is difficult to definitively determine whether the presence of 2,4-TDI in the nesting pads caused hatchling death, its presence in the nesting materials is concerning, requires awareness, and needs to be further investigated.

Iron Intoxication in a Dog Consequent to the Ingestion of Oxygen Absorber Sachets in Pet Treat Packaging

Ahna Brutlag[1], Charlotte Flint[1], Birgit Puschner[2,3]

[1]SafetyCall International and Pet Poison Helpline, Minneapolis, MN; [2]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, Davis, CA; [3]California Animal Health and Food Safety Laboratory System, University of California, Davis, Davis, CA

Narrative: Oxygen absorbers are commonly added to packages of dried or dehydrated foods (e.g., beef jerky, dried fruit, processed meat) in order to prolong the shelf life and protect food from discoloration and decomposition caused by oxidation or aerobic microorganisms. They typically contain reduced iron as the active ingredient although this is rarely stated on the external packaging. Additionally, information on the iron salt formulation and amount of elemental iron can be difficult to obtain. Although reduced iron has minimal oral bioavailability, such products are potential sources of iron poisoning in companion animals and, possibly, children. We present a case of a canine ingestion of an oxygen absorber from a bag of rawhide dog treats that resulted in iron toxicosis necessitating chelation therapy.

A 7 month old, female Jack Russell terrier presented for evaluation of vomiting and melena 8-12 hours after ingesting the contents of 1-2 oxygen absorber sachets from a package of rawhide dog treats. Abdominal radiographs revealed granular, metallic radiodense material in the distal colon. The serum iron concentration and ALT 8-12 hours post-ingestion were elevated at 436 ug/dL (reference range = 46-214 ug/dL) and 175 U/L (reference range = 10-100 U/L), respectively. The dog was treated with intravenous (IV) deferoxamine, IV fluids, gastrointestinal protectants, maropitant, and metronidazole. Clinical signs resolved within 12 hours of initiating treatment, but the ALT remained elevated (217 U/L) at the 3-month post-exposure examination.

The ingestion of reduced iron in humans has been reported to cause mild elevation of serum iron concentrations with minimal clinical effects. However, this case demonstrates that iron intoxication can occur following the ingestion of oxygen absorbers. To our knowledge, no cases of intoxication subsequent to the ingestion of oxygen absorbers have been reported in humans or animals. Due to the risk of poisoning, the lack of ingredient information on the packaging and the difficulty obtaining specific ingredient information, the authors performed chemical analysis on a selection of oxygen absorbers. Results indicate that oxygen absorbers contain 50-70% elemental iron.

The reduced iron in oxygen absorbers, when ingested, can result in iron intoxication. Human and veterinary medical personnel need to be aware of this effect and monitor serum iron concentrations as chelation may be necessary.

Screening and Confirmation of Veterinary Drugs in Milk and Milk Products by LC-MS/MS

Elizabeth Tor, Linda Aston, Robert Poppenga

Toxicology Laboratory, CAHFS, Davis, CA

Narrative: A simultaneous screening and confirmation method for the detection of thirty veterinary drug residues in milk and milk products using an LC-MS/MS technique was developed. This method was applied to milk samples prepared according to FDA method 4443 (Turnipseed et al., 2008). A high performance liquid chromatograph (Microm BioResources Inc., Auburn, CA) coupled with a hybrid triple quadrupole/linear ion trap mass spectrometer, AB/SCIEX 4000 QTRAP, was used in all analyses. The analytical column was a 100 x 2 mm x 3 µm YMC-AQ (Waters Corp.). The mobile phase consisted of: (A) 0.1% formic acid in water; and (B) acetonitrile at a flow rate of 250 µL/min. The injection volume was 20 µL. Mass spectral data were acquired in positive ion electrospray ionization (ESI) mode, using the multiple reaction monitoring (MRM) scan function. The precursor ion and the optimal fragmentation conditions for each of the compounds on the screen were determined by infusion of pure analytical standards into the mass spectrometer using a syringe pump. The MRM method was constructed with two transition ions for each compound. This assured meeting the 5-point EU criteria for confirmation of positives. Each set of samples contained a reagent blank, control and five point extracted standard curve. Samples were typically run it duplicates. The method was used to rapidly determine if veterinary drug residues were present at levels above the FDA tolerance or safe limits. Our results demonstrate that liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a reliable technique for unambiguous determination of veterinary drug residues in milk and milk products.

Vet-LRN - Center For Veterinary Medicine's Laboratory Response Network Focusing on Animal Feeds and Drugs

Renate Reimschuessel[1], Sarah Yachetti[1], Andriy Tkachenko[1], April Hodges[2]

[1]Center For Veterinary Medicine, FDA, Laurel, MD; [2]Center For Veterinary Medicine, FDA, Rockville, MD

Narrative: Vet-LRN is the Center for Veterinary Medicine's new program which will coordinate facilities, equipment, and professional expertise of government and veterinary diagnostic laboratories across the country and Canada to respond to high priority chemical and microbial feed/drug contamination events. This network will provide the means for rapid response to reports of animal injury and will establish protocols to facilitate veterinary diagnostic reporting to FDA. Vet-LRN will work with the veterinary diagnostic laboratories to document, investigate and diagnose animal feed or drug related illnesses. These efforts can contribute to overall food safety as animal feed events could signal potential issues in the human food system. During 2011, several FERN laboratories are participating in one chemical and one microbial Vet-LRN cooperative agreement projects. These will be described and preliminary findings presented.

Virology Scientific Session 1

Saturday, October 1, 2011

Grand Ballroom D

Sponsor: GeneReach Biotechnology Corporation

Moderators: Steve Bolin and Amy Glaser

1:00 PM **Development and Bench Validation of a Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of Capripoxviruses**
Amaresh Das, Michael T. McIntosh…… 63

1:15 PM **A Novel Papillomavirus Isolated from Proliferative Skin Lesions of a Wild North American Beaver (Castor canadensis) * # +**
Artem Rogovskyy, Tim Baszler, Tom Besser, Dan Bradway, Darren. Bruning, Christine Davitt, James Evermann, Kristin Mansfield, Gary Haldorson…….. 64

1:30 PM **Understanding the Role of Raccoons as Intermediate Hosts in the Evolution of Canine and Feline Parvoviruses**
Andrew Allison, Justin Brown, Mark Ruder, Kevin Keel, Carole Harbison, Israel Pagan, Karla Stucker, Jason Kaelber, Edward J. Dubovi, Edward Holmes, Colin Parrish…….. 65

1:45 PM **Ferret Systemic Coronavirus Infection #**
Valerie Johnson, Hana VanCampen, Sushan Han, Kathryn Holmes…… 66

2:00 PM **Isolation and Molecular Characterization of Trout Infectious Pancreatic Necrosis Virus (IPNV) in Pennsylvania Aquaculture**
Huaguang Lu, Kathy Hillard…… 67

2:15 PM **Virus Isolation of Influenza A Viruses from Oral Fluid using a Check Test**
Christa Irwin, Jeff Zimmerman, Melinda Jenkins-Moore, Pravina Kitikoon, Chong Wang, Joe Anderson, Pamela Leslie-Steen, Devi P. Patnayak, Jianqiang Zhang…… 68

2:30 PM **Comparison of Virus Isolation and Real-Time RT-PCR for Detection of Avian Influenza Virus and Newcastle Disease Virus in Cloacal Swabs of Poultry and Ducks ◊**
Janice C. Pedersen, Mary Lea Killian, Nichole Hines, Barbara M. Martin, Beverly Schmitt, Monica Reising…… 69

2:45 PM **GeneReach Biotechnology Corporation Presentation:**

Detecting Influenza A Virus and Highly Virulent Chinese-Type Porcine Reproductive and Respiratory Syndrome Virus (H-PRRSV) by a Portable PCR Platform - POCKIT
Hsiao Fen Grace Chang…… 70

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

◊ USAHA Paper

Development and Bench Validation of a Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of Capripoxviruses

Amaresh Das, Michael T. McIntosh

Proficiency and Validation Services and Foreign Animal Disease Laboratory, APHIS, USDA, Plum Island Animal Disease Center, Greenport, NY

Narrative: Capripoxviruses (CaPVs) including Sheeppox virus (SPPV), Goatpox virus (GTPV) and Lumpy skin disease virus (LSDV) are spread by aerosol or biting insects and are responsible for economically significant diseases in sheep, goats and cattle, respectively, in Africa, parts of Asia and the Middle East. In this study we reported the bench validation of a sensitive and cost effective loop-mediated isothermal amplification (LAMP) assay for rapid detection of CaPVs. The LAMP primers were designed to target Orf068 of CaPV genome, which encodes the highly conserved poly-A polymerase small subunit gene. Optimum LAMP was achieved at 60C for 1 hr, and the reactions were monitored by addition of hydroxyl naphthol blue (HNB) prior to amplification. A color change from violet to sky blue indicated positive reactions that could also be confirmed by gel electrophoresis. The LAMP assay was specific for CaPVs with no cross-reactivity to other known poxviruses or other viruses that are clinically indistinguishable from capripox. The LAMP assay had a similar analytical sensitivity as real time PCR (rPCR) with a limit of detection of 8 copies. In comparison to rPCR, the diagnostic sensitivity and diagnostic specificity were 96% and 100%, respectively, as assessed on 299 positive and 26 negative clinical samples from experimentally infected sheep, goats and cattle and included oral, nasal or conjunctival swabs, blood and tissues. Using swabs, the earliest detection of the virus was determined to be 2 days post inoculation (dpi) in sheep, 4 dpi in goats and 6 dpi in cattle. From blood, the earliest detection was 6 dpi in sheep and goats and 9 dpi in cattle. From tissues (skin nodules and skin lesions), the earliest detection was 6 dpi in sheep, goat and cattle by LAMP and rPCR. The CaPV LAMP assay described in this study provides a robust, sensitive and cost effective solution to the diagnosis of CaPVs in endemic countries and for surveillance in free countries.

A Novel Papillomavirus Isolated from Proliferative Skin Lesions of a Wild North American Beaver (Castor canadensis) * # +

Artem Rogovskyy, Tim Baszler, Tom Besser, Dan Bradway, Darren. Bruning, Christine Davitt, James Evermann, Kristin Mansfield, Gary Haldorson

Washington Animal Disease Diagnostic Laboratory and Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA

Narrative: Cutaneous papillomatosis was diagnosed in an adult North American beaver (Castor canadensis). Gross lesions included numerous exophytic roughly circular, lightly pigmented lesions on hairless areas of fore and hind feet and the nose. The most significant histopathologic findings were multifocal spongiosis within the stratum granulosum of the epidermis, and multifocal ballooning degeneration of keratinocytes, often with large, darkly basophilic intranuclear inclusion bodies. A virus with properties consistent with papillomavirus (PV) was recovered by virus isolation of skin lesions, utilizing RK-13 and CrFK cell lines. The presence of the virus was confirmed by papillomavirus-specific polymerase chain reaction. Partial sequences of E1/L1 of beaver papillomavirus (BePV) were concatenated head-to-tail to form a super-gene alignment with concatenated full E1/L1 gene sequences of 50 other PV species. A neighbor-joining phylogenetic tree placed BePV together with 5 other PVs, Bovine papillomavirus 1 (BPV 1), Capreolus capreolus PV 1 (CCPV 1), Camelus dromedus PV 1 (CDPV 1), Deer PV 1 (DPV 1), and European elk PV (EEPV) in a monophyletic branch that encompasses the Delta papillomavirus genus. This report confirms the papillomaviral etiology of cutaneous exophytic lesions in the beaver and molecularly characterizes the novel virus through phylogenetic analysis.

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Understanding the Role of Raccoons as Intermediate Hosts in the Evolution of Canine and Feline Parvoviruses

Andrew Allison[1,2], Justin Brown[1], Mark Ruder[1], Kevin Keel[1], Carole Harbison[2], Israel Pagan[3], Karla Stucker[2], Jason Kaelber[2], Edward J. Dubovi[4], Edward Holmes[3], Colin Parrish[2]

[1]Population Health, Southeastern Cooperative Wildlife Disease Study, Athens, GA; [2]Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY; [3]Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park, PA; [4]Department of Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY

Narrative: Canine parvovirus (CPV) is a host range variant of feline panleukopenia virus (FPV) which emerged as a new pathogen in 1978 (designated as CPV-2) and subsequently spread worldwide, infecting >80% of the world's dog population. In 1979, a variant of CPV-2 (designated as CPV-2a) also spread globally and by 1980 had supplanted CPV-2. Although the molecular changes between CPV-2 and CPV-2a are well known, the evolutionary pathways by which they arose remain obscure. Herein we genetically and functionally characterize FPV- and CPV-derived viruses from raccoons and show that raccoons served as the intermediate host in the evolution of CPV-2a from CPV-2, and possibly in the emergence of CPV-2 from FPV. All raccoon viruses derived from FPV or CPV had key substitutions in the VP2 capsid protein that altered their interactions with the cellular transferrin receptors (TfR) of different host species. The mutations also altered the antigenic structure of the capsids, as determined by virus-specific monoclonal antibody binding. Cloning and expression of the raccoon TfR showed it was genetically intermediate between the feline and canine TfRs and that it was bound by both FPV and CPV-2, supporting the role of raccoons in the host adaptation of these parvoviruses in dogs and cats.

Ferret Systemic Coronavirus Infection #

Valerie Johnson[1], Hana VanCampen[1], Sushan Han[1], Kathryn Holmes[2]

[1]Department of Microbiology, Immunology and pathology, Colorado State University, Fort Collins, CO; [2]Department of Microbiology, University of Colorado, School of Medicine, Aurora, CO

Narrative: A highly fatal condition has recently been recognized in juvenile domestic ferrets with clinical and pathological manifestations similar to the non-effusive form of feline infectious peritonitis (FIP) in cats. This disease has been termed ferret systemic coronavirus infection (FSCV) following demonstration of a group 1 coronavirus in tissues of affected animals. Enteric coronavirus infections in ferrets have been previously described in association with epizootic catarrhal enteritis, a diarrheal disease of ferrets. Partial characterization of the structural and nonstructural proteins of FSCV and ferret enteric corona virus (FECV) demonstrate a significant phylogenetic relationship between the two viruses. This raises the possibility that this disease may arise from a mutation of FECV to a macrophage trophic form capable of inducing systemic infection as has been a hypothesized pathogenesis in FIP in cats. This report documents a case of systemic coronavirus infection in a 1 year old ferret with a 2 month history of vague gastrointestinal signs. Gastrointestinal biopsies were performed after initial onset of clinical signs revealing lyumphoplasmacytic inflammation of the jejunum and pyogranulomatous inflammation in the pancreatic lymph node. The clinical disease was progressive despite supportive treatment and the ferret was euthanized. At necropsy, marked perivascular and necrotizing pyogranulomatous lesions in the intestine and mesentery were observed consistent with previously described pathologic lesions associated with FSCV. Immunohistochemical analyses were performed using anti-feline corona virus (FCoV) monoclonal antibody FIPV3-70 on paraffin embedded mesentery, intestines and abdominal lymph nodes. Positively-stained macrophages were found in pyogranulomatous perivascular foci within the mesentery and lymph nodes consistent with intra-cytoplasmic FSCV. Virus isolation is currently being performed using Crandell feline kidney (CrFK), Madin-Darby canine kidney (MDCK) and Vero cells to provide virus for further studies to investigate differences between FSCV and FECV with respect to host receptor specificity. Characterization of cellular and tissue trophism of FSCV and FECV and investigation of the pathogenesis of FSCV could have important implications regarding systemic coronavirus infection in other species. Currently antemortem diagnosis of non-effusive FIP presents a challenge in the feline population and could potentially be more problematic in domestic ferrets as the condition has only recently been described and cases are few. Further research into this area could have widespread benefits applicable to multiple species.

AAVLD Trainee Travel Awardee

Isolation and Molecular Characterization of Trout Infectious Pancreatic Necrosis Virus (IPNV) in Pennsylvania Aquaculture

Huaguang Lu, Kathy Hillard

Animal Diagnostic Laboratory, Pennsylvania State University, University Park, PA

Narrative: Infectious pancreatic necrosis (IPN) is an infectious viral disease that affects young fish of Salmonid species held under intensive rearing conditions. The disease most characteristically occurs in rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*) and several Pacific salmon species (*Oncorhynchus spp*.). In Pennsylvania, an aquaculture surveillance program has been started in late 1990s and conducted more extensively in recent years for surveillance and diagnostic tests of trout IPN virus (IPNV) at the Pennsylvania State Animal Diagnostic Laboratory (ADL). From May 2008 to the present time, a total number of 1555 samples were submitted for testing IPNV (also other fish viruses) at ADL. IPNV was identified in 122 samples tested by virus isolation in fish cell cultures using the chinook salmon embryo (CHSE) cell line and/or the fathead minnow (FHM) cell line. Viral cytopathic effects (CPE) usually occurred at the first or second cell passages if a test fish was IPNV infected. Immunoblot assay using monoclonal antibodies specific to IPNV was used to confirm IPNV from the CPE positive cell culture materials. The 122 IPNV isolates were mostly identified from rainbow trout and also a variety of other trout species. About 15% of these IPNV isolates, which represent different trout species, seasonal patterns and geographic locations, are currently selected for further molecular characterization of the IPNV field strains. Detail results of IPNV surveillance tests during the last three year period and molecular characterization studies on selected IPNV isolates will be presented and discussed.

Virus Isolation of Influenza A Viruses from Oral Fluid using a Check Test

Christa Irwin[1], Jeff Zimmerman[1], Melinda Jenkins-Moore[4], Pravina Kitikoon[5], Chong Wang[1,2], Joe Anderson[3], Pamela Leslie-Steen[6], Devi P. Patnayak[7], Jianqiang Zhang[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Statistics, Iowa State University, Ames, IA; [3]Kansas State University Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; [4]National Veterinary Services Laboratories, USDA- APHIS, Ames, IA; [5]National Animal Disease Center, USDA- ARS, Ames, IA; [6]Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, SD; [7]University of Minnesota Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN

Narrative: Oral fluid (OF) has been shown to be an excellent surveillance tool for several swine respiratory viruses (Hoffman et al. 2008; Prickett et al., 2008; Prickett et al., 2008). Preliminary data suggested that IAV detection in the field might be improved through the use of OF samples. The objective of this study was to determine whether diagnostic laboratories could recover IAV from OF by virus isolation. For this study, OF was inoculated ("spiked") with either contemporary H1N1 or H3N2 influenza virus and submitted to diagnostic laboratories in a "ring test" format. To prepare samples, 5.4 liters of OF were collected from 4 PRRSV-, IAV-, M. hyopneumoniae-, and ADV-negative sows, centrifuged, and pooled. To create ring test samples, 8 10-fold serial dilutions were prepared from Ohio '07 H1N1 and Illinois '09 H3N2 stock solutions. The initial concentrations of the two isolates were 1 x 10^1 TCID50 different, but this was not determined until the inocula were back-titrated. Samples were randomized, grouped into sets of 180 samples (10 samples from each of the 8 dilutions of each virus plus 20 negative OF samples), frozen at -80C and submitted to 6 U.S. diagnostic laboratories using overnight delivery. Laboratories were contacted to confirm samples arrived frozen. Each laboratory performed virus isolation according to their established protocol. Results showed significant differences between laboratories in the ability to detect influenza in oral fluid as virus concentration decreased. Detection differences between influenza subtypes appeared to be related to the difference in starting concentrations rather than virus type. One negative control sample among 20 provided was reported positive in one laboratory. Some of the differences between laboratory methods were: volume of sample used, tissue culture surface area, filtering or further processing. Preliminary analysis confirms influenza virus can be successfully isolated from oral fluid, but the ability to isolate virus at lower concentrations is dependent upon laboratory protocol and technique. Field samples must be assessed to confirm the applicability of these observations.

Acknowledgements This work was supported by USDA/APHIS (NCAA: 10-9100-1314-CA).

References:

Hoffmann P et al., March 8-11, 2008. 38th Annual Meeting of the AASV, 301-302.

Prickett et al., 2008. J Vet Diagn Invest 20,156-163.

Prickett et al., 2008. J Swine Health Prod 16(2), 86-91.

Comparison of Virus Isolation and Real-Time RT-PCR for Detection of Avian Influenza Virus and Newcastle Disease Virus in Cloacal Swabs of Poultry and Ducks

Janice C. Pedersen[1], Mary Lea Killian[1], Nichole Hines[1], Barbara M. Martin[1], Beverly Schmitt[1], Monica Reising[1,2]

[1]U. S. Department of Agriculture, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Ames, IA; [2]United States Department of Agriculture, Center for Veterinary Biologics, Ames, IA

Narrative: Cloacal (CL) swabs from poultry and ducks are known to contain substances that are inhibitory to polymerase chain reaction (PCR) testing. Fecal and dietary inhibitors can reduce the efficiency of amplication or completely prevent amplification of the target leading to weak positive or false negative PCR results. A NAHLN Methods Comparison Study was conducted to compare the limit of detection (LOD) for virus isolation (VI) and real-time RT-PCR (rRT-PCR) for RNA extracted with magnetic bead technology for CL swabs from poultry and domestic ducks (DD) to determine if CL swabs could be an approved sample matrix for rRT-PCR testing. Ten-fold serial dilutions of A/turkey/Ontario/18-2/2000 H7N1 and APMV-1 B1 viruses were prepared in chicken (CK) and DD CL and CK tracheal (TR) supernate and tested by VI and rRT-PCR. Five replications of each dilution series were conducted each day, and the testing was performed on three consecutive days. The LOD, amount of analyte in which there is a 95% probability of classifying the sample as positive, was determined for each species, sample type and virus and was expressed as an estimated log10 EID50. The EID50 for CK TR swabs with AI for VI was determined to be 1.9, and the EID50 for rRT-PCR was determined to be 1.0. For TR swabs with APMV-1 the EID50 for VI was determined to be 0.8-2.0, and the estimate for rRT-PCR was 1.5. For CL swabs the EID50 for CK swabs with AI for VI was 1.7, and the estimate for rRT-PCR was 1.8. For APMV-1, the EID50 for CK CL swabs with VI was 3.2, and the estimate for rRT-PCR was 0.8-2.0. For DD CL swabs spiked with AI the EID50 for VI was 1.5, and the estimate for rRT-PCR was 1.2-2.2. Using TR swabs spiked with AI and APMV-1 the rRT-PCR tests were estimated to be more sensitive by approximately 0.9 to 0.5 EID50, respectively. Using CK CL swabs with AI, VI tests were estimated to be more sensitive by 0.1 EID50 while PCR was estimated to be more sensitive by 2.4 to 1.2 EID50 for CK CL swabs with APMV-1. Using DD CL swabs, VI was estimated to be more sensitive than PCR by 0.7 EID50. The estimated difference is <1 0 EID50 for both CK and DD CL swabs samples with AI and APMV-1, which is considered to be comparable to VI. Cloacal specimens will be an approved sample matrix for rRT-PCR when RNA is extracted with approved magnetic bead RNA extraction procedures.

Detecting Influenza A Virus and Highly Virulent Chinese-Type Porcine Reproductive and Respiratory Syndrome Virus (H-PRRSV) by a Portable PCR Platform - POCKIT

Hsiao Fen Grace Chang

GeneReach Biotechnology Corporation, Taichung City

Narrative: A highly sensitive field diagnostic platform is an essential tool for farmers to effectively control infectious diseases. It allows quick decision making to avoid significant economic impact. POCKIT is a qualitative PCR detection system based on insulated isothermal PCR (iiPCR). The PCR reaction is driven by the Rayleigh-Bénard convection principle. A natural thermal convection phenomenon is induced inside a capillary tube with a single heating source applied at the bottom, which results in a temperature gradient between 60°C to 95°C inside the tube. The convection will repeatedly circulate the reagents through different temperature zones for the three steps of PCR -denaturing, annealing, and extension respectively. Field tests were conducted to detect influenza A virus and highly virulent Chinese-type PRRSV (H-PRRSV) by POCKIT. For influenza A virus, a set of PCR primers and a FAM labeled TaqMan probe were designed to specifically amplify and detect the matrix gene, whereas for H-PRRSV, the primers and FAM labeled TaqMan probe were designed to detect H-PRRSV but not normal strains including Asian origin, NA origin, and EU origin. The RNA was prepared by a 15-minute spin-column protocol. The results showed that the POCKIT system was capable of detecting the field samples effectively. When verified and quantified with the real-time PCR, the titers of the field samples were in the range of 103 to 105 viral particles/µl of extracted samples. In addition, the POCKIT influenza A virus system was able to detect H3N2, H5N1, H6N2, H7N2, and H9N2 subtypes. For the POCKIT influenza A virus assay system, Newcastle disease virus was introduced as a specificity control and the result was negative accordingly. For the H-PRRSV system, POCKIT did not detect the normal PRRSV BSL vaccine strain. The POCKIT system is specifically aiming for field diagnosis though it could be used in reference labs as a screening test. The system comes as a carry-on hard-shell suitcase package including an iiPCR instrument, a mini-centrifuge and two micro pipettes. The reagents are lyophilized for room temperature shipping. The system can take up to eight samples per run. The total run time from sample to result is less than 1.5 hours. With its high sensitivity and specificity, ease-of-use and short-turn-around-time, POCKIT can provide farmers and field consultants a powerful tool for veterinary disease management.

Bacteriology Scientific Session 2

Sunday, October 2, 2011

Grand Ballroom B

Moderators: Kristy Pabilonia and Amar Patil

11:00 AM **Evaluation of Polymerase Chain Reaction (PCR) and Culture as Diagnostic Methods for Identifying *Brachyspira* Species in Swine**
Leslie Bower, Joann M. Kinyon, Kent Schwartz, Kristin Clothier, Janet Hill, John Harding, Erin Strait…….. 83

11:15 AM **Comparison of Serological Assays for *Actinobacillus pleuropneumoniae* (serotypes 1-9) on Serum from Pigs Experimentally Infected with APP or Vaccinated with APP Bacterins**
Michelle Hemann, Sheila Heinen, John Johnson, Patrick G. Halbur, Tanja Opriessnig…….. 84

11:30 AM **Development of a Novel Universal and Species-specific *Haemophilus parasuis* ELISA Test * #**
Nubia Macedo, Albert Rovira, Simone Oliveira…….. 85

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

+ AAVLD/ACVP Graduate Student Award Applicant

Molecular Epidemiology of *Brucella abortus* in Livestock in the US: 2011 Update

James Higgins[1], Tod Stuber[1], Angela Berte[1], Christine Quance[1], Hank Edwards[2]

[1]Mycobacteria and Brucella Section, NVSL/APHIS/USDA, Ames, IA; [2]Wyoming Game and Fish Department, , Laramie, WY

Narrative: Since 2008 the Mycobacteria and Brucella Section of the NVSL has been genotyping field isolates of *Brucella abortus* using a variable number tandem repeat (VNTR) assay. The VNTR assay queries each isolate for the presence of tandem repeats at 10 loci ('Hoofprints' 1, 3, 4, and 8, and VNTR 2, 5A, 5B, 16, 17, and 21). As of early May, 2011, n = 370 isolates, recovered from cattle, elk, and bison, have been genotyped. Allelic diversity (h) indices for these 10 loci range from 0.01 to 0.8; four loci displayed a high discriminatory capability (i.e., h ≥ 0.75). Considerable variability in VNTR profile was observed, even for sets of isolates generated from the same animal. Minimum spanning tree analysis of all isolates resulted in the formation of two large clusters, one primarily composed of bison isolates, the other, of cattle; elk isolates are partitioned among both of these clusters. UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) analysis was used to demarcate isolates associated with selected outbreaks of brucellosis in cattle. Isolates recovered from cattle in the Park County, WY area in 2010- 2011 had as 'nearest neighbors' isolates recovered from elk. Isolates associated with an infected cow in Navarro County, Texas, in the Fall of 2010 showed genetic similarity to *B. abortus* Strain 19, while isolates recovered in early 2011 from infected cattle in Starr County, Texas, displayed limited similarity to entries in the NVSL genotyping database. While the VNTR assay is not as powerful as similar assays in use for other genera of bacteria, it continues to be a useful tool for supporting Veterinary Services' efforts to investigate and prevent outbreaks of brucellosis among US cattle.

Streptococcus suis Associated Disease in Calves

Patricia Blanchard, John Adaska, Robert B. "Moeller, Jr."

California Animal Health and Food Safety Laboratory, University of California, Tulare, CA

Narrative: *Streptococcus suis*, a well-documented pathogen of swine, has been sporadically reported in association with septicemia and pneumonia in cattle. This organism has become a frequent isolate from calves submitted to the California Animal Health and Food Safety laboratory (CAHFS) at Tulare, CA. A 3-year review of cases (May 2008 to May 2011) at CAHFS-Tulare identified 130 animals from which *S. suis* was isolated. Most cases are found in calves at calf ranches (110/130= 84.6%) with only 1 case from a feedlot animal and 14.6% (n=19) from dairies of which 3 were in adult cows. The most common disease found was pneumonia (97/130 = 74.6%) followed by septicemia and/or meningoencephalitis (27/130 = 20.8%). The organism was isolated from the tympanic bullae in 6 cases of otitis media of which 5 also had pneumonia and in 3 cases of arthritis of which 2 also had pneumonia. Among the pneumonia cases the ages ranged from 5 days to 7 months, mean 34.6 days, median 21 days, excluding the 3 adult cows. *S. suis* was rarely the sole respiratory pathogen and was most often accompanied by indole positive *Mannheimia sp.* and/or *Bibersteinia trehalosi*. However, all other respiratory pathogens have also been isolated in conjunction with this organism. In animals over 3 weeks of age, *Mycoplasma sp* (most often *M. bovis*) was also commonly found. Cases of septicemia or meningoencephalitis alone due to *S. suis* have increased over the 3 year period. The ages of affected animals range from 5 - 23 days, mean 9.8 days. The most common lesions are meningitis or meningoencephalitis and nephritis. Less consistent findings are myocarditis, polyarthritis and interstitial pneumonia. Species identification of *Streptococcus* is initiated when an alpha-hemolytic, gram positive, catalase negative cocci is found in pure culture or is the predominant organism or when found only with other respiratory pathogens from lung or ear cultures. Initial screening includes salicin fermentation broth, bile esculin slant and 6.5% sodium chloride broth. If the latter two are negative, an API 20 Strep (bioMerieux SA) strip is set up. The isolates typically key out as *Streptococcus suis* II with some variation in biochemical reactivity but are consistently negative for Voges-Proskauer and hippurate hydrolysis and are esculin positive.

Genotypic Characterization of Selected Resistant *Mannheimia haemolytica* and *Pasteurella multocida* Associated with Bovine Respiratory Disease from the Pfizer Animal Health Susceptibility Surveillance Program 1999-2007

Robert Murray[1], Ellen Portis[1], Susan Kotarski[1], Lacie Johansen[1], Kristina Kadlec[2], Geovanna Michael[2], Jeffrey Watts[1], Stefan Schwarz[2]

[1]Pfizer Animal Health, Kalamazoo, MI; [2]Friedrich-Loeffler-Institute, Neustadt-Mariensee

Narrative: The Pfizer Animal Health Susceptibility Surveillance Program (PAHSSP) for bovine respiratory disease (BRD) consists of bacterial isolates from veterinary diagnostic laboratories since 1998 to the present originating from 44 states and 7 provinces in the United States and Canada. The purpose of this study was to examine the clonal diversity of macrolide-resistant strains of *Mannheimia haemolytica* and *Pasteurella multocida* found in the PAHSSP.

A subset of BRD-associated *M. haemolytica* and *P. multocida* PAHSSP isolates from 1999 to 2007 (N=230) were examined by pulsed-field gel electrophoresis. For each organism, a matched set of tilmicosin^{R}tulathromcyin^{R}tetracycline^{R} (TIL^{R}/TUL^{R}/TET^{R}), TIL^{R}/TUL^{S}/TET^{R} and TIL^{S}/TUL^{S}/TET^{S} isolates (if available) from the same year and state/province of the program was examined. Decreased tetracycline susceptibility or tetracycline resistance was consistently observed with macrolide resistance in the PAHSSP collection. The TIL^{S}/TUL^{R}/TET^{R} phenotype was not observed.

M. haemolytica (N=79) clustered at 91% similarity into 5 clonal groups of 4-7 members each. *P. multocida* (N=151) clustered at 91% similarity into a major clonal group of 70 members and a minor, 15 member clonal group. *M. haemolytica* and *P. multocida* clonal groups consisted of TIL^{R}/TUL^{R}/TET^{R}, TIL^{R}/TUL^{S}/TET^{R} and TIL^{S}/TUL^{S}/TET^{S} isolates and occurred in multiple states/provinces over multiple years. The *erm*(42) gene occurred in TIL^{R}/TUL^{S}/TET^{R} isolates; *erm*(42) and *msr*(E)-*mph*(E) genes occurred in TIL^{R}/TUL^{R}/TET^{R} isolates.

BRD-associated, macrolide-tetracycline resistant *Mannhemia haemolytica* and *Pastuerella multocida* from the PAHSSP representing various regions in North America over nine years were distributed among multiple clonal groups with varied macrolide susceptibility. In the isolates examined from North America, the existence of two major *Pastuerella multocida* clonal groups across time and region suggest broad distribution of a limited number of macrolide resistant clonal strains.

Real-time Multiplex PCR Assay for Rapid Detection of *Clostridium difficile* Toxin Genes in Feces and Ground Meat

Beth Houser, Arthur L. Hattel, Bhushan Jayarao

Veterinary and Biomedical Sciences, Penn State Animal Diagnostic Lab, University Park, PA

Narrative: *Clostridium difficile* is considered an important, well established pathogen capable of causing disease in humans and animal species. In our study, we developed and evaluated a real-time multiplex PCR assay for the rapid detection of *C. difficile* genes encoding toxin A (tcdA), toxin B (tcdB), and binary toxin (cdtA and cdtB). The detection limit of the standardized real-time multiplex PCR assay for toxin genes of *C. difficle* was 103cells/g and 101cells/g for non-enriched and enriched fecal and ground meat samples, respectively. The assay was used to screen for genetic elements of toxigenic *C. difficile* in fecal samples from 71 pre-weaned calves and 53 retail ground meat samples. All samples were also examined for *C. difficile* using traditional culture techniques to validate the PCR assay. A total of 24 fecal samples (33.80%) were positive for toxigenic *C. difficile* using either multiplex real-time PCR or culture. *Clostridium difficile* toxin genes were detected in 23 enriched fecal samples using the multiplex real-time PCR assay and only 15 samples using culture techniques. Sensitivity, specificity, positive predictive value, and negative predictive value for the multiplex real-time PCR assay were 0.93, 0.84, 0.61, and 0.98 for enriched fecal samples and 0.67, 0.86, 0.55, and 0.91 for non-enriched fecal samples. *C. difficile* was not detected in ground meat by traditional culture or real time PCR assay. Eleven fecal samples were positive for all 4 toxin genes suggesting pre-weaned calves may be a likely source for toxigenic *C. difficile*. Based on the findings of our study it can be concluded that real-time multiplex PCR carried out on samples enriched for *C. difficile* is a reliable, sensitive, and specific diagnostic tool for rapid screening and identification of samples contaminated with *C. difficile*.

Identification of *Brucella canis* in Canine Blood by a Duplex Real-time PCR Assay

Jianfa Bai[1], William Fortney1[1], Tanya Purvis[1], Brian Lubbers[1], T. Nagaraja[2], Gary A. Anderson[1]

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

Narrative: *Brucella canis* is the primary causal agent of canine brucellosis, a contagious disease with venereal and oral modes of transmission that produces late gestation abortions in females and epididymitis and prostatitis in males. The transmissibility of the canine brucellosis is related to enhanced environmental persistence of *B. canis* compared to other *Brucella* species. Although mortality is relatively low, the infection in males often leads to sterility. Other *Brucella* species, *abortus, melitensis* and *suis* can occasionally cause brucellosis in dogs. We have developed a duplex real-time PCR assay that can specifically detect *B. canis* or any other *Brucella* species. The assay was designed to target the spacer region between the 16S and 23S rRNA genes that is common to all *Brucella* species, and a nearly 1 kb deletion that occurs only in *B. canis* strains. Primers for *B. canis*-specific target were designed flanking the nearly 1 kb deletion, and the probe was designed on the deletion junction so that it should generate signal specific for *B. canis* strains. The performance of the assay was tested with 676 dog blood samples and compared with the traditional cultural method. One milliliter of the whole blood was added to 5 ml of BHI broth, and was frozen at -20C for overnight. The mixture was subsequently incubated at 37C for 24 h and used for DNA extraction for PCR assay and for streaking on a blood agar plate for isolation. Culture method identified 26 blood samples positive for *B. canis*. In addition to the 26 culture positives, the duplex PCR assay identified 13 more samples positive for *B. canis*. The amplicons of selected positives were confirmed by DNA sequencing. The analytical sensitivity of the assay was determined using blood samples inoculated with serially diluted (ten-fold dilutions) pure culture of *B. canis*. Blood samples were mixed with serially diluted *B. canis* culture and allowed to set for 15 min, then added to 5 ml BHI broth. The tubes were incubated at 37C for 24 and 48 h after freezing overnight at -20C. Selected dilutions were plated on blood agar plates to determine bacterial cell concentrations. The experiment was repeated three times using three different *B. canis* strains. After 24 h enrichment in BHI, the minimum detection limit was 14 CFU/ml. At 48 h post enrichment, we were able to detect 1.4 cells/ml. Culture method was only evaluated at 48 h post enrichment. The PCR assay had the same analytical sensitivity as culture method in one replication, and was more sensitive than the culture method in two other replications. The duplex real time assay may provide a reliable and rapid method to diagnose *B. canis* infections in dogs.

Respiratory Disease in Ferrets (*Mustela putorius*) Associated with an Unknown *Mycoplasma* # +

Jennifer Lamoureux[1], Danielle Desjardins[1], Cathy Johnson-Delaney[2], Ailam Lim[1], Carole Bolin[1], Steven R. Bolin[1], Michael Gardner[3], Matti Kiupel[1]

[1]Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI; [2]Eastside Avian and Exotic Animal Medical Center, Kirkland, WA; [3]Northwest ZooPath, Monroe, WA

Narrative: An outbreak of respiratory disease characterized by a non-productive cough was observed in 6- to 8-week-old domestic ferrets. The coughing was paroxysmal, honking, and violent often with the ferret moving backwards during the process. Over a four year period approximately 8,000 ferrets were affected from a commercial breeding facility in Canada. Morbidity reached 95% with almost no mortality. Treatments with broad spectrum antibiotics, bronchodilators, expectorants, nonsteroidal anti-inflammatories, nebulization, and low dose prednisolone temporarily decreased the clinical symptoms, but failed to alleviate recurrences. Clinical diagnostic testing included bronchiolar lavage and cytology, bacterial cultures, canine distemper PCR/serology, influenza serology, radiographs, heartworm antigen screening, and ultrasonography of the heart and thorax. However, the cause of the clinical signs was not determined. Complete blood counts and chemistries were within normal ranges. Bronchiolar lavage and cytology yielded some inflammatory cells, but routine cultures yielded only various non-pathogenic bacteria, and ultrasonography showed no abnormalities in the thorax. Radiographs showed mild bronchiolar patterns with possible thickening of bronchi. On necropsy of affected ferrets, the only gross lesions were observed in the lungs and consisted of multifocal, tan to gray semi-firm nodules surrounding airways with markedly narrowed lumina. Microscopically, affected lungs exhibited severe bronchiole associated lymphoid tissue (BALT) hyperplasia, bronchointerstitial pneumonia and diffuse pulmonary edema. An infection with a *Mycoplasma sp.* was suspected based on the clinical presentation and the described lesions. Immunohistochemistry using an anti-*Mycoplasma bovis* antibody exhibited strong labeling along the bronchiolar epithelial cells. Transmission and scanning electron microscopy of the lungs from affected ferrets further supported an infection with *Mycoplasma sp.* Bronchoalveolar lavage samples from 12 affected ferrets yielded a fast growing, glucose-fermenting *Mycoplasma*. PCR for the 16s ribosomal RNA and rpoB gene of *Mycoplasma* yielded amplicons that supported the diagnosis of a novel *Mycoplasma sp*. with highest sequence identity with *Mycoplasma molare* and *Mycoplasma lagogenitalium*. In order to determine the incidence of *Mycoplasma* infections in ferrets, numerous healthy animals from a commercial breeder were euthanized, but all lung samples cultured negative for *Mycoplasma*. Our findings support a causal relationship between a novel *Mycoplasma* species and pulmonary disease in ferrets. Experimental reproduction of the respiratory disease to better elucidate the pathogenesis of this novel ferret respiratory pathogen is currently in progress. Based on our results, it may be necessary to redefine the cause of a commonly reported "allergic" cough in ferrets and to consider mycoplasmosis as a differential for pulmonary disease in ferrets.

AAVLD Trainee Travel Awardee

+ AAVLD/ACVP Graduate Student Award Applicant

Salmonella enteritidis Surveillance in Iowa Following FDA's Egg Safety Rule

Timothy Frana, Darrell Trampel

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

Narrative: On July 9, 2010, the Food and Drug Administration (FDA) Egg Safety Rule became mandatory for egg producers with 50,000 or more laying hens. Environmental culturing of chicken houses for *Salmonella enteritidis* (SE) must be done when pullets are 14 to 16 weeks of age, when laying hens are 40 to 45 weeks old, and during the period from 4 to 6 weeks after hens are molted. If environmental cultures are positive, eggs from positive houses must be diverted to breaking plants for pasteurization or egg testing must begin. Each egg test consists of 1,000 fresh eggs from a day's production and 4 tests must be completed at 2-week intervals. If even one egg tests positive, eggs must be diverted to pasteurization until 4 consecutive tests at 2-week intervals yield negative test results. In July, 2010 the Centers for Disease Control (CDC) identified an increase in the number of human SE infections with a specific pulse-field gel electrophoresis (PFGE) pattern. Traceback investigations found the sources of human exposure were linked with eggs from producers in Iowa. These investigations led to the largest egg recall in U. S. history. To support FDA's testing requirements for these events, more than 3,000 chickens have been euthanized and necropsied, and over 27,000 tissues have been collected using aseptic procedures at the Iowa State University Veterinary Diagnostic Laboratory.. In addition to bacteriological examination of tissue samples, more than 8,200 environmental samples and more than 191,000 eggs have been cultured through April 2011. Bacteriological procedures to isolate and identify *Salmonella enteritidis* have progressively evolved to increase efficiency while concurrently reducing costs and time required for obtaining culture results.

Detection of *Salmonella enteritidis* in Pooled Poultry Environmental Samples Using a SE-specific RT PCR Assay

Timothy Frana, Derek Adams, Wendy Stensland, Karen Harmon, Erin Strait

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

Narrative: Real-time (RT) PCR assays have been developed in recent years as a rapid testing methodology for the detection of *Salmonella enteritidis* (SE) in a variety of matrices. Numerous studies have been conducted to examine the efficacy of pooling environmental samples for testing by culture. However, very little research has been done with RT PCR to examine whether the same level of accuracy can be found in pooled samples as opposed to individual samples. The purpose of this study was to evaluate an SE-specific RT PCR in samples inoculated with strains of SE and environmental samples from poultry facilities.

Serial dilutions of three strains of SE were prepared (10^{-1} to 10^{8} CFU/ml). One militer of each dilution was used to inoculate environmental drag swabs. Each sample was cultured according to National Poultry Improvement Plan (NPIP) guidelines using 100ml Tetrathionate (Tet) for enrichment and then incubating for 24 hours. Serial dilutions, pre-incubation, and post-incubation samples were collected for RT PCR. Two hundred and eight environmental drag swab samples were collected from several poultry facilities previously found to be positive for SE and cultured by NPIP guidelines. Post-incubation Tet samples were collected and tested by RT PCR individually and in pools of two, three and four. Positive cut-off (Ct) values were evaluated based on results from inoculated samples and agreement with culture.

The range of Ct values in the serially inoculated samples prior to incubation was 17.2 to 37.3 with the average level of detection at 2.7×10^{3} CFU/ml. After incubation in Tet, all samples had Ct values between 15.1 and 16.5. SE was isolated in 7 of 208 environmental samples (3.4%). Culture agreement with RT PCR at Ct < 36 and < 30 was 99.0% and 100%, respectively. For pooled samples with at least one culture positive sample at Ct < 36, agreement was 98.1%, 97.1%, and 98.1% for pools of two, three, and four, respectively. Likewise at Ct < 30, agreement was 99.0%, 100%, and 100%.

The results indicate that pooling of environmental samples after enrichment does not impact sensitivity when compared to testing individual samples by culture or RT PCR. Given the time and cost savings that sample pooling represents to the poultry industry, this maybe a future option when testing poultry facilities for SE.

Regulation of *Streptococcus equi* subspecies *equi* by the Control of Virulence Sensor, covS

Sheila Patterson[1], Luke Borst[2], Carol Maddox[1]

[1]Pathobiology, University of Illinois, Urbana, IL; [2]Population Health and Pathobiology, North Carolina State University, Raleigh, NC

Narrative: *Streptococcus equi* subspecies *equi* (*S. equi*), the highly contagious causative agent of strangles, a pharyngeal lymphadenitis of horses, is controlled in the U.S. largely through vaccination with an intranasal modified live product. The Pinnacle® IN vaccine was created by means of nitrosoguanine mutagenesis and numerous single nucleotide polymorphisms (SNPs) contribute to the attenuation of the vaccine strain. Deletion of a guanidine residue 189 bp upstream of the M-like protein gene (szp) appears to be a highly conserved mutation. Another SNP is a G-A substitution at bp 769 of the virulence sensor (covS) gene. The resultant leucine to phenylalanine substitution at a.a. 257 in the HAMP domain of covS may interfere with ligand-binding or signaling kinase domains. This conformational change in the α-helix may limit if not eliminate covS function. Numerous isolates recovered from abscesses have been characterized biochemically and genotypically, to determine if they were of wild type or vaccine origin. Many isolates were initially of interest, as they presented as mucoid forms of the normally dry vaccine strain morphology, some with restored hemolysis as well. To study the role of covS in capsule and hemolysin production, a deletion was created in covS, introduced through use of a suicide vector pJRS233. Complementation of the covS mutant strain (#34) was accomplished using a 2100 bp product that encompasses the entire covS gene cloned into a plasmid. Deletion of covS resulted in an 11.4-fold decreased expression of hasA (capsule), 6.8-fold reduction in expression of hemolysin (sagD), and ~4-fold reductions in expression of super antigens (speM, seeI, and seeH) as determined by qRT-PCR in a Syber-green assay. Expression of these virulence genes in a covS deletion mutant (strain 34) was comparable to the expression levels of the Pinnacle IN vaccine strain and both could be complemented by introduction of the plasmid containing the covS gene (pSKP34). Mucoid revertants of Pinnacle have been shown to contain compensatory SNPs in covS with hasA expression restored to wild type levels. Compensatory covS SNPs have also been shown to affect expression of sagD and the super antigens. The expression of the szp gene, encoding the M-like outer membrane protein of *S. equi*, was shown to be independent of covS. While szp serves well as a genotypic marker for Pinnacle, expression and sequencing of the covS gene may be more informative with regard to virulence of abscess isolates.

Antimicrobial Susceptibility Patterns of Nocardioform Bacteria Causing Placentitis in Horses

Erdal Erol, Laura Kennedy, Stephen Sells, Stephen Locke, Jacqueline Smith, Neil Williams, Craig N. Carter

Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY

Narrative: Nocardioform actinomycetes have been reported as a significant cause of bacterial placentitis and abortion in horses in Kentucky. Historically, they have been referred to as nocardioform actinomycetes or gram positive branching bacilli, which include several species. Sequence analysis of 16S rRNA genes of isolated bacteria from placentitis cases shown that the most common nocardioform actinomycetes are *Crossiella equi sp. nov., Amycolatopsis spp.* and *Streptomyces spp*..The nocardioform related placentitis causes foal losses from late abortions, still-births, premature foaling, or early neonatal deaths. The foals are not infected, but may be small or emaciated. Portals of entry for infection and transmission are as yet unknown. Detection is based on culture of placenta and PCR. The bacteria have never been isolated from other fetal tissues. During pregnancy, the ultrasonaghrophic examination of placenta (thickness of placenta) can help diagnose nocardioform infections. In this study, we evaluated antimicrobial susceptibility patterns of nocardioform actinomycetes that cause placentitis and abortion in horses, which have never been reported. In the 2010-2011 reproductive season, the pathologists and a diagnostic surveillance tool monitored by the epidemiology section of the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) noticed a significant increase of nocardioform placentitis in horses. Nocardioform actinomycetes were isolated and identified by routine bacteriologic methods and PCR. Thirty four *Amycolatopsis spp.*, 20 *Cr. equi* and 10 unidentified species of nocardioform actinomycetes including reference strains of *Amycolatopsis spp.* and *Cr. equi* were included in broth micro-dilution antimicrobial susceptibility test following Clinical and Laboratory Standards Institute (CLSI) guidelines. RapMyco plates (Trek Diagnostics), which were designed for rapidly-growing mycobacteria, nocardia and other aerobic actinomycetes, were used in this study. Overall, linezolid, trimethoprim/sulfamethoxazole, ceftriaxone, amoxicillin/clavulanic acid and doxycycline showed 93.75%, 86.93%, 84.37%, 82.81% and 82.81% of susceptibility, respectively. The other antimicrobials including amikacin, cefepime, cefoxitin, ciprofloxacin, claritromycin, imipenem and tobramycin showed lesser degree of susceptibility. Highly susceptible antimicrobial drugs can be used if the mare is suspected to have nocardioform related placentitis.

Evaluation of Polymerase Chain Reaction (PCR) and Culture as Diagnostic Methods for Identifying *Brachyspira* Species in Swine

Leslie Bower[1], Joann M. Kinyon[1], Kent Schwartz[1], Kristin Clothier[2], Janet Hill[3], John Harding[3], Erin Strait[1]

[1]Veterinary Diagnostic Laboratory, Iowa State University College of Veterinary Medicine, Ames, IA; [2]California Animal Health and Food Safety Lab System, University of California Davis, Davis, CA; [3]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK

Narrative: The genus *Brachyspira* includes several species of anaerobic bacteria that have been shown to cause enteric diseases in swine. The most virulent species, *Brachyspira hyodysenteriae*, causes swine dysentery, resulting in significant losses to swine producers worldwide. While other species are considered less virulent, they still have a negative impact on swine health and production economics. It has recently been suggested that new virulent species may also exist. Diagnosis of *Brachyspira* infection is difficult because traditional anaerobic culture methods are not reliably discriminatory to the species level, and cultures containing multiple species can be problematic to identify. Polymerase chain reaction (PCR) may be a more specific method for *Brachyspira* identification, but assays designed to detect multiple species are neither widely-used nor well-validated. In this study, several PCR assays targeting the *nox* gene were evaluated to compare the specificity of each test to identify the following *Brachyspira* species: *hyodysenteriae*, *pilosicoli*, *intermedia*, *murdochii*, *innocens*, and in collaboration with the University of Saskatchewan, Canada, a potentially new species, *Brachyspira sp. Sask30446*. Seventy-five field isolates and 13 clinical specimens, all previously identified as *Brachyspira*-positive, were obtained from the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). DNA was extracted for use in the PCR assays in order to establish species identity. Isolates were cultured on Trypticase Soy Blood agar with 5% bovine blood, incubated anaerobically at 42°C for 2-6 days, and then examined for culture phenotype. The combined PCR assays were able to type all of the samples, 18 of which were positive for the proposed novel species. Culture phenotypes between species showed distinct variation. In addition, an unexpectedly high amount of phenotypic variation also existed among samples within each species. The sample origin from which DNA was extracted for use in PCR was also compared for the clinical cases. The PCR results from culture samples did not always agree with those directly from clinical samples. In addition, PCR results varied among multiple samples originating from the same culture plate, indicating the presence of mixed species. The implementation of the PCR assays evaluated in this study significantly broadens diagnostic capabilities, particularly for cases that were previously undiagnosed. The results of this study will lead to updated and improved diagnostic capabilities in the ISU-VDL and suggest the need for further studies related to the potential emergence of a new pathogenic *Brachyspira* species.

Comparison of Serological Assays for *Actinobacillus pleuropneumoniae* (serotypes 1-9) on Serum from Pigs Experimentally Infected with APP or Vaccinated with APP Bacterins

Michelle Hemann, Sheila Heinen, John Johnson, Patrick G. Halbur, Tanja Opriessnig

Iowa State University, Ames, IA

Narrative: *Actinobacillus pleuropneumoniae* (APP) is found worldwide and is a major cause of bacterial pneumonia in swine. APP can act as a primary pathogen and is frequently associated with high death loss when introduced in naïve populations. APP is a gram-negative, small, coccoid bacteria. There are 12 known serotypes ranging in pathogenicity from mild (serotype 3) to highly pathogenic (serotypes 1, 5, and 7). Several serological assays are currently available for the detection of APP, but some debate exists as to which test to use in different diagnostic situations and which is the “gold standard”. Recently, three new serogroup specific ELISAs (1-2-9-11; 4-5-7, and 3-6-8-15) were introduced and are now available through many veterinary diagnostic laboratories. The objective of this study was to determine the diagnostic performance of the complement fixation (CF) assay compared to the three new ELISAs. Serum samples tested included samples from pigs vaccinated intramuscularly with inactivated APP or inoculated intranasally with live APP. This study was conducted in two parts: In part A, eight 8-week-old pigs were randomly divided into four groups of two pigs each and each group was vaccinated with inactivated APP of serotype 1, 3, 5 or 7, respectively. In part B, eighteen 8-week-old pigs were randomly divided into nine groups of two pigs each and each group was inoculated with live APP serotype 1 through 9, respectively. In both parts, blood was collected weekly and serum tested by CF and ELISA for APP-specific antibodies. Vaccinated animals became positive for APP-specific antibodies by ELISA at DPI 14, but only 3/8 animals were positive by the end of the study. When the CF assay was used, only 2/8 animals were positive at DPI 21 but all pigs were negative on all other days tested. This indicates that vaccination using inactivated bacterins did not result in a detectable IgG response in 62.5% (5/8 of the vaccinated pigs). Results on animals challenged with live bacteria indicate that seroconversion was detected as early as 7 days after APP inoculation by both the ELISA and the CF assay. Differences in ability of assays to detect antibodies were likely due to differences in Ig types (IgM versus IgG) and overall magnitude of the immune reaction against live APP challenge versus vaccination. In conclusion, the new ELISAs appear to be slightly more sensitive than the CF assay.

Development of a Novel Universal and Species-specific *Haemophilus parasuis* ELISA Test * #

Nubia Macedo, Albert Rovira, Simone Oliveira

University of Minnesota, Saint Paul, MN

Narrative: Control of *Haemophilus parasuis* mortality in the nursery is an important concern for swine veterinarians and producers. There is no reliable serological test available to characterize the development of antibodies in sows and piglets, which is critical to understand herd immunity and to identify the best timing for vaccination. In this study, we have utilized a highly immunogenic and species-specific antigen in *H. parasuis* to develop and validate a *H. parasuis*-specific ELISA test. We used convalescent serum samples obtained from 10 pigs that survived a *H. parasuis* outbreak to screen 6 outbreak strains using whole cell protein profiles and western blot analysis for the presence of immunodominant proteins. An immunodominant protein present in all strains was sequenced and identified as the *H. parasuis* oligopeptide permease A (OppA) protein. This species-specific protein was present in 14 *H. parasuis* reference strains from different serovars, but absent in all non-*H. parasuis* swine bacterial pathogens tested.

The OppA gene of *H. parasuis* was cloned and expressed in *E. coli* and the recombinant protein was used as the coating antigen for ELISA. A checkerboard titration was performed to establish the optimal dilutions. Once the conditions of the ELISA were defined, serum samples obtained from convalescent pigs were used as positive control. Eleven SPF and 80 healthy pigs naturally colonized with *H. parasuis* were used as negative controls, in order to standardize the test. Field samples used to validate the ELISA test included sera collected from 60 non-vaccinated 3 week-old pigs, 60 non-vaccinated 8 week-old pigs, and 60 pigs vaccinated at 3 and 6 weeks of age. Evaluation of anti OppA ELISA titers obtained for control and field samples suggests that pigs with S/P ratios above 0.2 have significant titers against *H. parasuis*. By using a cut-off of 0.2, all outbreak survivors, 8 week-old and vaccinated pigs were positive for anti-*H. parasuis* antibodies. Only 10 of the 80 healthy 3-week old pigs were positive for anti-OppA antibodies using the 0.2 cut off. Nine of them decreased their titers in 3 weeks, indicating that they were positive due to maternal immunity. These pigs were colonized by *H. parasuis* and this data suggests that colonization is not enough to generate anti-OppA antibodies. In conclusion, we have successfully developed an OppA-based ELISA test to detect anti-*H. parasuis* antibodies. This data suggests that this ELISA can be used to detect active immune response against *H. parasuis*, define best timing for vaccination, and track seroconversion post vaccination.

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

Bovine Virus Diarrhea Special Scientific Session

Sunday, October 2, 2011

Wright

Moderators: Kathy Kurth and Beate Crossley

BVD Milk ELISA Compared with Ear Notch Testing for Detection of PI Cattle, and Effects of Cow Characteristics and 4 Milk Sample Handling Methods on ELISA Results

David J. Wilson[1], Kerry Rood[1], Greg Goodell[2]

[1]Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT; [2]The Dairy Authority, Greeley, CO

Narrative: Results of a milk ELISA test for antibody (Ab) against p80/125 non-structural protein (p80) of Bovine Viral Diarrhea (BVD) virus were compared with standard ear notch testing for cows persistently infected with BVD-PI. Association of results with cow characteristics and with 4 different milk sample handling methods was also evaluated. Dairy Herd Improvement Association (DHIA) milk meter-collected milk samples from a dairy herd with past diagnoses of abortions caused by BVD and presence of PI cows were used. BVD MLV vaccine was administered to calves at 3 and 4 mo of age, to all cows at dry off and 15-21 days in milk (DIM) after calving. One month (mo.) apart, 247 and 258 cows were tested using a competitive ELISA for milk Ab binding to p80 BVD protein. Results are reported as % binding by a second test kit anti-BVD Ab after each milk sample was placed into p80 BVD protein coated test wells and then washed. Increased second Ab binding means the milk had less anti-BVD p80 Ab. Interpretation of % binding by the second Ab is as follows: 90-100%, little anti-BVD Ab - PI or vaccine failure if consistent; 60-89%, moderately low anti-BVD Ab; 30-59%, moderate anti-BVD Ab; 10-29%, high anti-BVD Ab; 0-9%, very high anti-BVD Ab. In accordance with various DHIA milk handling practices, 4 samples from each cow were handled differently: fresh milk, fresh with preservative pill, frozen 7 days, room temp 7 days with preservative. Ear notches were collected concurrently from all cows for BVD antigen (Ag) testing. Some descriptive means for all cows were: 173 DIM; 305ME (Mature Equivalent) milk production 13,450 kg; and daily milk 40 kg. No PI cows were found from ear notch Ag tests of 345 cows; this included all cows tested at least once using the milk test. Milk handling method was significantly associated with differences in milk BVD results; fresh milk mean 49% second Ab binding was higher than other methods, 7 days preserved was especially lower at 42% ($P < 0.01$, ANOVA, Tukey's). (Nevertheless, there was little practical significance in classifying cows for BVD among the 4 milk handling methods.) All further results here are from fresh milk. Binding ranged from 3%-98%, quartiles 29%, 47%, 62% 1st mo., and 35%, 56%, 71% 2nd mo.. 15 cows had 90-98% binding on one test, but 14 were milking each mo. and were below 90% on the other test; mean binding the other mo. was 60%. Therefore no PI or vaccine failures (consistently >90%) were found by milk ELISA. For cows > 90% binding, DIM that mo. was 41-188, 305ME mean 12,935 kg, and daily milk mean 44 kg. DIM significantly affected Ab binding: 1-9 DIM, 16%*; 10-30 DIM, 34%*; 31-60 DIM, 46%; 61-150 DIM, 60%*; 151-300 DIM, 47%; 301-360 DIM, 40%; and >360 DIM, 46% (* = $P < 0.025$, ANOVA, Tukey's). Lactation number did not affect binding. The milk ELISA agreed with ear notch testing in finding no PI cows. Anti-BVD Ab was high in early lactation and then decreased. Further study of the ELISA is indicated.

Establishment of a Diagnostic Cutoff for BVDV Persistently Infected Cattle

Quoc Hoang[1], Jeff Zinza[1], Angela Burrell[1], Catherine O'Connell[1], Kelly Foster[2], Richard Hesse[2], Richard Oberst[2], Daniel Thomson[2], Gary A. Anderson[2], Lalitha Peddireddi[2]

[1]Animal Health Group, Life Technologies, Austin, TX; [2]College of Veterinary Medicine, Kansas State University, Manhattan, KS

Narrative: Bovine Viral Diarrhea Virus (BVDV) causes infection in cattle leading to major economic losses in the beef and dairy industries. In utero BVDV infection can induce immunotolerance, causing animals to be persistently infected (PI). PI animals continuously shed the virus and are the major source of BVDV infection in herds. Rapid detection of PI cattle is essential for BVDV control. Antigen capture ELISA (ACE), virus isolation (VI), immunohistochemistry (IHC), and RT-PCR are the most common methods of BVDV detection. PI animals are routinely diagnosed by retesting samples 2-3 weeks after an initial positive result. Animals that are acutely or transiently infected will pass the disease and will be BVDV negative upon re-testing. The VetMAX™- Gold BVDV Detection Kit was evaluated to determine whether a suitable diagnostic cut-off could be established for ear punch samples obtained from a mixed population of cattle including uninfected, persistently infected (PI) and acutely/transiently infected animals characterized by various methods. After confirming that an assay cut-off could be established, statistical analysis of study data was performed to determine a suitable one-sided upper Ct cut-off for detection of PI cattle. In this study, we show a method for PI determination that does not require retesting of samples after an initial positive test. Ear punch samples were used since ear punches will show high viral titers if an animal is PI, but not if the animal is acutely infected. In contrast, serum or blood BVDV testing will show high viral titers from both persistently and acutely infected animals and cannot be used to distinguish PI from acutely-infected animals. To determine PI status, ear notch samples need to be standardized to one size; in this study, 3 mm ear punches were used. Purified RNAs tested with the Bovine Virus Diarrhea RNA Test Kit were obtained from 63 BVDV PI positive cattle infected with BVDV of various subtypes, and 53 healthy BVDV-negative cattle co-mingled with PI animals to create transiently-infected (TI) cattle. RNA from the ear punches was isolated using MagMAX Viral Isolation followed by real-time RT-PCR with the VetMAX™- Gold BVDV Detection Kit on the Applied Biosystems 7500 Fast Real-Time PCR System. Testing the VetMAX™- Gold BVDV Detection Kit on diagnostic ear punch samples of known BVDV status produced acceptable results with respect to distinguishing PI from acutely infected animals. The results of this field study support the establishment of a diagnostic cut-off for PI cattle (Ct ≤ 30.07) using the VetMAX™ - Gold BVDV Detection Kit and 3 mm ear punch samples. Data from this study was filed with USDA/APHIS-Center of Veterinary Biologics and approval of the labeling claim is pending review.

Detection of BVDV Antigens in Hair Samples from Alpaca, Deer and Cattle

Andrew Read[1], Jing Zhang[1], Daniel Givens[3], Clayton Kelling[2], Daniela Bedenice[4], Lalitha Peddireddi[5], Peter D. Kirkland[1]

[1]Virology Laboratory, EMAI, Camden, NSW; [2]Nebraska Center for Virology, University of Nebraska, Lincoln, NE; [3]College of Veterinary Medicine, Auburn University, Auburn, AL; [4]Cummings School of Veterinary Medicine, Tufts University, North Grafton, MD; [5]Veterinary Pathology, Kansas State University, Manhattan, KS

Narrative: Antigen-capture ELISA technology has been used for many years for the diagnosis of bovine virus diarrhea virus (BVDV) infection by the detection of viral antigens in blood and tissues. More recently, the assay has been modified to detect antigen in serum and skin. The pestivirus antigen capture ELISA (PACE) is the most commonly used assay to detect cattle persistently infected (PI) with BVDV. The testing of skin samples has also facilitated the detection of PI animals and is widely used in control and eradication programs. This study involves the evaluation of hair samples from cattle, alpaca and deer as alternative specimens for BVDV detection. Initially beef or dairy cattle that had previously been identified as being PI using PACE on blood or skin samples were included in this study. New samples (blood, hair and skin) were collected from all animals and tested for BVDV antigens by PACE and for BVDV antibodies. A total of 173 PI cattle and 394 non-PI infected animals were examined. At the outset, antigens were eluted from the hair samples in conventional soaking buffers and extracts tested in a PACE. Sample processing was optimised by the inclusion of different amounts of sample, different soaking times and by the evaluation of different soaking solutions. The effect of different storage conditions for both original samples and also extracts was examined. Finally, testing was also varied to include use of a pan-pestivirus real time PCR (qRT-PCR) and the capacity to test pooled samples investigated. The optimised methods were applied to the examination of archived samples from alpaca and deer and compared with results on blood, skin or tissue samples. The results of tests conducted on hair samples showed very high sensitivity (>99%) and specificity (>99%) The results for hair samples are not affected by maternally derived antibodies. The results also showed that testing of hair was more specific for the detection of PI animals than tests using skin samples. With the selection of an appropriate sample soaking buffer, the same extract could be tested by both qRT-PCR and PACE. This supports a strategy for cost-effective testing with pooling of samples which are screened by qRT-PCR with individual positive animals identified by PACE. This approach has been applied to test more than 20,000 cattle and has been shown to be equally well suited for the detection of BVDV in alpaca and deer The primary purpose of testing for the detection of BVDV infected animals is to identify PI animals since these act as reservoirs of the virus in a population. Ease of sample collection and use of a non-invasive method are important considerations to encourage testing. The method described in this study is faster and easier that the collection of either blood or skin samples and does not require specialised skills. The samples may be obtained quickly and easily by untrained personnel, are not invasive or disfiguring to the animal and may be easily shipped by courier or postal service.

Experimental Evaluation of BVDV Transient Infections in Cattle Exposed to PI Animals

Lalitha Peddireddi[1], Kelly Foster[2], Richard Oberst[1], Richard Hesse[1], Jianfa Bai[1], Joe Anderson[1], Kayla Hoskins[1], Daniel Thomson[2], Gary A. Anderson[1]

[1]Kansas State Veterinary Diagnostic Laboratory, Deapartment of Diagnostic Medcine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS; [2]Department of Clinical Sceinces, College of Veterinary Medicine, Kansas State University, Manhattan, KS

Narrative: Bovine viral diarrhea virus (BVDV) has a significant negative impact on the cattle industry worldwide. A source of BVDV infection is exposure to persistently infected animals (PIs), which are lifelong carriers of the virus. Acute or transient infections (TI) are a result of cattle exposure to BVDV, but the infections do not persist. Some reports suggest PIs are the predominant source of BVDV persistence in a herd, capable of creating new PIs, but little information is available on TI animals and their potential to spread BVDV. The objectives of this study were to: 1) identify PI cattle representing different genotypes, 2) determine the PIs ability to generate TI in non-PI cattle after co-mingling; 3) determine the onset and length of TI; and 4) to compare which sample type is preferred to identify PI or TI infections by real-time RT-PCR (rRT-PCR). To generate TIs, ten PIs, representing various genotypes and strains, were selected from the PI pool of 63 cattle and co-mingled with 53 cattle of non-PI status in a single pen for a 27-day period. All animals were monitored for their health and performance. Sera, buffy coats, and nasal swabs were collected two days prior to, and daily for 8 days, followed by every three days after co-mingling. Ear notches were collected on day 8, 13, and 20 after co-mingling. Approximately 50% of the cattle showed typical bovine respiratory disease (BRD) clinical signs such as cough, runny nose, lethargic, and elevated temperature (104-106°F). rRT-PCR was performed on sera, buffy coats and ear samples (nasal swabs samples are currently being evaluated), while VI was performed on nasal swabs and buffy coats. rRT-PCR results on sera and sera identified ~96% (51/53) of the non-PIs were transiently infected during the course of the co-mingle. A majority of TIs were positive by rRT-PCR by ~6 days (range: 1-8) after co-mingling. Duration of TI lasted ~9 days (range: 5-14, serum rRT-PCR data). VI confirmed positive BVDV status on all 10 PIs throughout the study, whereas only 26.4% (14/53) of TIs were VI positive. All the sample types tested by rRT-PCR were highly sensitive for PI detection (100%). To detect TIs, serum appear to be more sensitive (51/53) than ear punches (41/53). Low real-time RT-PCR Ct values (suggestive of high virus load) on buffy coat and serum samples from TIs indicate that these animals have the potential to infect other cattle and perpetuate an infectious cycle within a group of animals.

Detecting and Evaluating Gentotype/Strain Transmission of Bovine Viral Diarrhea Virus from Persistently Infected Cattle to Non-PI Cattle When Co-mingled

Lalitha Peddireddi[1], Elizabeth Poulsen[1], Kelly Foster[2], Taghreed Mahmood[1], Jianfa Bai[1], Daniel Thomson[2], Richard Hesse[1], Richard Oberst[1], Gary A. Anderson[1]

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

Narrative: In a co-mingle experiment (reported at this conference entitled, "Experimental evaluation of BVDV transient infections in cattle exposed to PI animals" by L. Peddireddi et al.) we were able to demonstrate transmission of bovine viral diarrhea viruses (BVDV) from persistently infected (PI) cattle to non-PI individuals. Buffy coats and sera that demonstrated rRT-PCR (Bovine Virus Diarrhea RNA Test Kit, Life Technologies) Ct values of <36 were subjected to additional 5'UTR sequencing to define BVDV genotype and strain designation. Sequencing data from day-8 indicated that 4/53 (7.5%) cattle were PCR negative (Ct > 36); 7/53 (13%) were infected with a genotype 1A strain; 12/53 (23%) had 1B infections (with one indicating mixed sequences); 27/53 (51%) had 2A infections (with four individuals indicating mixed sequences); and 3 (6%) had mixed infections with genotypes as yet undetermined. Day 13 samplings indicated that 10/53 (19%) were PCR negative; 5/53 (9%) had 1A infections (with one indicating mixed sequences); 7/53 (13%) had 1B infections; and 31/53 (58%) had 2A infections (with four individuals indicating mixed sequences and not resolved). Day 20 samplings indicated that 19/53 (36%) were PCR negative; 4/53 (8%) had 1A infections (with one indicating mixed sequences and not yet resolved); 7/53 (13%) had 1B infections (with one indicating mixed sequences and not yet resolved); 22/53 (42%) having 2A infections (with 5 indicating mixed sequences and not yet resolved); and 1 sample had genotype as yet undetermined. Certain strain preferences could be recognized in non-PI cattle as one 2A strain was identified in 63/80 (78.8%) of all 2A transient infections and ~52% (63/122) of all strains currently detected. Only one strain of 1A was identified in 16 samples from 9 cattle; 4 strains of genotype 1B were identified with one strain infecting 3 animals on 4 occasions, one 1B strain infecting 2 animals on 2 occasions, one strain infecting 5 animals on 5 occasions, and three PI strains were not identified at this time. This data strongly suggests the existence of BVDV strain predominance in an infectious cycle within a group of cattle, and can serve as a basis for future studies that investigate BVDV strain dominance in infection and transmission. The results of this study confirm the extreme sensitivity of this molecular diagnostic approach to identify, characterize, and evaluate the transmission of BVDV in cattle. It also further confirms the value of the co-mingle model for future studies evaluating BVDV diagnostics, biosurveillance, strain dominance, and disease prevention.

A Non-invasive, Novel Testing Method to Detect the Presence of Cattle Persistently Infected with Bovine Viral Diarrhea Virus

M. Shonda Marley, Kay Riddell, Patricia Galik, YiJing Zhang, Maurice Daniel Givens

College of Veterinary Medicine, Auburn University, Auburn, AL

Narrative: This project evaluated the impact of multiple factors on the ability of consumption surface swabbing to detect bovine viral diarrhea virus (BVDV) within a group of cattle consuming a mixture of soy hull and corn gluten pellets. A group of 10 seronegative, uninfected steers were commingled (Day 0) with two persistently infected (PI) calves for three days and separated thereafter. After commingling, the 10 exposed calves and the two PI calves were separated in two different pens to determine the impact of acute versus persistent infection on the results of testing. On Days 6, 7, 8, 9 and 10, consumption surface samples were collected from both the PI and exposed calf groups. Additionally, virus isolation was performed on blood and nasal samples to confirm acute infections. The commingled cattle shared 86-cm-wide polyethylene feed troughs with a total length of 6 meters. Three Dacron-tipped swabs and three synthetic fiber scour pads were used for each sampling at 20 min, 2 h, 6 h and 23.5 h post-feeding. The samples were handled by refrigerating (4°C) for 1 to 3 d, refrigerating 7 to 10 d, or freezing (-23°C) 7 to14 d before reverse transcription-nested PCR (RT-nPCR). All 96 refrigerated samples from feed troughs obtained within 6 h of feeding of commingled or PI calves were positive for BVDV compared to only 42 of 48 frozen samples regardless of moderate ranges in environmental temperature, precipitation, and humidity. Synthetic fiber scour pads tended to facilitate viral detection better than Dacron-tipped swabs if samples were frozen or if obtained at 23.5 hours post-feeding. While exposed steers exhibited acute infections, viral RNA was not detected from the feed troughs used only by acutely infected steers. Ranges in environmental temperature, humidity and precipitation had no effect on the detection of BVDV. Consumption surface swabbing within 6 h of feed consumption is a sensitive and non-invasive method to determine if PI animals are present within a group of cattle. Samples should be refrigerated 10 d or less prior to performing RT-nPCR.

A Rare Case of Persistent Testicular Infection with Bovine Viral Diarrhea Virus Causes Consistent Shedding of Infectious Virus in Semen

Maurice Daniel Givens[1], Kathy L. Kurth[2], Yan Zhang[3], M. Shonda Marley[1]

[1]College of Veterinary Medicine, Auburn University, Auburn, AL; [2]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin-Madison, Madison, WI; [3]Animal Disease Diagnostic Laboratory, Ohio Department of Agriculture, Reynoldsburg, OH

Narrative: One previous case of persistent testicular infection with bovine viral diarrhea virus (BVDV) was diagnosed in a Holstein-Friesian bull, "Cumulus," in New Zealand in 1995 (Voges et al., 1998. Veterinary Microbiology 61:165-175). Recently, in the United States, a dairy bull was diagnosed as the second confirmed case of persistent testicular infection. Similar to the prior case, characteristics of the exposure to BVDV in the most recent case are unknown. Between 6 and 24 months of age, this U.S. bull lacked BVDV in seven sequential serum samples and two peripheral white blood cell samples as determined by virus isolation, antigen capture ELISA, and PCR assays. The bull was seropositive to type 1, BVDV strains with serologic endpoints of 256, 2048, and 4096 at 8, 19, and 22 months of age, respectively. At 24 months of age, the bull exhibited a serum neutralizing antibody titer of 4096 to the strain isolated from his semen. The bull produced 25 collections of semen from 14 to 22 months of age that consistently contained BVDV as determined by PCR and virus isolation when semen was shipped to the laboratory in a liquid nitrogen dry shipper. The concentration of infectious virus in semen ranged from < 250 to 6250 $CCID_{50}$/mL with an average of 1773 $CCID_{50}$/mL and a median of 1250 $CCID_{50}$/mL. The detected concentration of infectious BVDV is clearly greater than previously detected concentrations in semen from acute infections, less than previously detected concentrations in semen from classical persistent infections, and consistent with the sole report of a prior case of persistent testicular infection. Using centrifugal separation and PCR, virus was not detected free in seminal plasma but was readily detected in association with spermatozoa. Thus, this persistent testicular infection resulted in association of spermatozoa with BVDV beginning with the initial semen collection at 14 months of age through the most recent collection at 22 months of age. Sequencing of 248 nucleotides from the 5′ nontranslated region of the viral genome revealed that the persisting virus was a 1a subgenotype of BVDV. Epidemiologic investigation within a bull stud housing this unique case provided evidence that BVDV was not transmitted to directly contacted bulls and steers. In conclusion, this bull validates that exposure to a 1a strain of BVDV can result in a unique persistent testicular infection causing contamination of semen with readily detectable infectious virus for a duration of at least eight months.

Comparison of Transport and Detection Methods for Bovine Viral Diarrhea Virus in Semen

Kathy L. Kurth[1], Daniel Givens[2], Yan Zhang[3], Robert Stolen[1], Craig Radi[1], Dave Krueger[1], Jared VanThiel[1], Francine Cigel[1], Rhiannan Schneider[3], Kerri Lawrence[3], YiJing Zhang[2], M. Shonda Marley[2]

[1]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin- Madison, Madison, WI; [2]College of Veterinary Medicine, Auburn University, Auburn, AL; [3]Animal Disease Diagnostic Laboratory, Department of Agriculture, Reynoldsburg, OH

Narrative: Testing of semen for bovine viral diarrhea virus (BVDV) and other bovine pathogens is mandated by most countries prior to export from the USA. Laboratories approved for export testing follow virus isolation and nucleic acid detection methods described in the Office of International Epizootics (OIE) Terrestrial Manual. Transport of semen has been done in several ways including nitrogen vapour tanks, dry ice and cold packs (wet ice). Recently, a dairy bull was identified as the second confirmed case of persistent testicular infection. A set of 20 extended semen samples collected at different time points over the course of 6 months was collected from this bull. These samples were tested in 3 laboratories all of whose methods were in compliance with guidelines in OIE. However, identification of BVDV positive samples was inconsistent. In order to determine the cause of the false negative samples an inter-laboratory comparison of methods was undertaken. Transport issues were addressed first. A comparison between transport on ice packs (approximately 0 degrees Celsius) and transport in a liquid nitrogen vapour tank (approximately -130 Celsius) demonstrated that shipment on ice packs significantly reduced the ability to detect viable BVDV using virus isolation (VI). Transport conditions did not account for all the differences observed with virus isolation. Additional experiments are in progress to investigate differences due to cell line receptivity, rinsing steps, confirmation methods and length of incubation. In addition to VI, real-time reverse transcription PCR (rRT-PCR) assays were performed using in-house assays or commercial kits for detection of BVDV RNA. All were performed as relative rPCR assays rather than absolute quantitative assays. Detection of BVDV RNA was usually possible even when virus could not be isolated. Equivalent CT values were observed in samples transported by liquid nitrogen as compared to those transported on ice packs. One striking observation is the considerable variability in relative CT levels among the rRT-PCR procedures. Differences in CT levels spanned up to 16 CTs (~4.8 logs) for the same sample presumably due to the robustness of the enzyme in the presence of residual inhibitors. Identification of this positive bull provided a unique opportunity to compare and achieve better uniformity in semen testing procedures.

Epidemiology Scientific Session

Sunday, October 2, 2011

Grand Ballroom E

Moderators: Suzanne Gibbons-Burgener and Ashley Hill

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

Bovine Neonatal Pancytopenia: Results of a 2 Year Study into this Emerging Disease in the United Kingdom

Andrew Holliman[1], Kim Willoughby[7], Sandra F.E. Scholes[6], Adrian Colloff[5], George Caldow[3], Fiona Howie[2], Richard Smith[4], Sarah Lambton[4], Charlotte Bell[7]

[1]Regional Laboratory, Animal Health and Veterinary Laboratories Agency, Penrith; [2]Disease Surveillance Centre, Scottish Agricultural Colleges, Edinburgh; [3]Disease Surveillance Centre, Scottish Agricultural Colleges, St. Boswells; [4]Centre for Epidemiology and Risk Analysis, Animal Health and Veterinary Laboratories Agency, Weybridge; [5]Regional Laboratory, Animal Health and Veterinary Laboratories Agency, Truro; [6]Pathology, Animal Health and Veterinary Laboratories Agency, Lasswade, Edinburgh; [7]Virology, Moredun Research Institute, Edinburgh

Narrative: Bovine neonatal pancytopenia (BNP) was first diagnosed in the UK in 2009 but had been reported in Europe since 2007. Since March 2009, up until the end of March 2011, 181 cases had been confirmed on 124 farms in England and Wales, and 166 cases had been confirmed on 139 farms in Scotland. Clinical signs were seen typically at around 12 days of age, with a maximum of 28 days recorded. Most affected calves died within a few days of onset. Haematological examination revealed a characteristic loss of three blood cell types, hence pancytopenia. Limited haematological studies found evidence of haematological parameters being normal at birth and subsequently deteriorating in accordance with the lifespan of peripheral blood cells. Virological examination using a range of techniques including cell culture, RT-PCR, electron microscopy and microarray analysis found no evidence of viral agents. Deep sequencing of bone marrow looking for foreign nucleic acid found no evidence of an infectious agent. Histological examination of 333 calves falling within the case definition revealed a characteristic reduction of all three main blood cell types in the bone marrow (tri-lineage hypoplasia). The possibility of a genetic predisposition was investigated but no striking differences were found between the frequencies of the most common alleles in a case versus control comparison. A case-series study in 2009 identified a number of factors to investigate further in a case-control study (CCS) in 2010. These included bovine virus diarrhea virus (BVDv) vaccination of the dam. Risk factors identified in the CCS included the use of one specific BVDv vaccine, (PregSure). Bone marrow was analysed by FACS to determine whether antibody in the serum from dams of affected calves might bind to neonatal bone marrow but we were unable to demonstrate this effect. However this still remains the most plausible aetiological hypothesis and future work should continue to investigate the likelihood of this mechanism. Although our study has provided some information on the pathogenesis of BNP, the underlying mechanism initiating the disease process is still unclear. The exact role of the PregSure vaccine is uncertain and it must be borne in mind that, although observational epidemiological studies can reveal associations between certain exposures and a disease, they do not confirm causation.

IDEXX Bovine Pregnancy Test - A New Tool for Accurate and Early Pregnancy Diagnosis in Cattle

Katherine Velek, Shona Michaud, Meghan Hart, Valerie Leathers, Christoph Egli

IDEXX, Westbrook, ME

Narrative: Introduction: Accurate and timely detection of pregnancy in dairy cows is an essential component of today's reproductive management programs. Veterinarians and farmers use early detection of non-pregnant (open) cows to enable faster rebreeding and shorten the calving interval, thereby maximizing milk production and revenue for the farm. IDEXX Laboratories, Inc. has developed an ELISA for the accurate detection of pregnancy as early as 28 days post breeding, providing veterinarians and dairy farmers with another tool for the early identification of open cows.

The IDEXX Bovine Pregnancy Test detects the presence of early pregnancy-associated glycoproteins (PAGs) in bovine serum or EDTA plasma as a marker for pregnancy in cows. This study was conducted to evaluate the sensitivity and specificity of the IDEXX Bovine Pregnancy Test in dairy and beef cows and heifers, starting at 28 days after insemination and/or 60 days after calving. Serum and EDTA plasma samples were obtained from multiple sites in the US as well as Beijing, China. Trans-rectal ultrasound was also performed at day 28 or later to confirm the pregnancy status of bred cows. A total of 1181 serum samples and 1214 EDTA plasma samples were tested on the IDEXX Bovine Pregnancy Test following the package insert protocol.

In this evaluation, the sensitivity of the IDEXX Bovine Pregnancy Test was greater than 99% when testing either serum or plasma taken from pregnant animals at least 28 days after insemination. Specificity was 93.8% for serum and 95.1% for plasma samples taken from heifers or from cows that were confirmed open by ultrasound after artificial insemination. Additional analysis of the data shows that after calving, the IDEXX ELISA detects a rapid decline in PAGs , and by 50 days after calving the assay values returned to baseline. Specificity was 100% for serum (n=227) or plasma (n=205) samples taken 50-200 days post-calving.

This evaluation of the IDEXX Bovine Pregnancy Test indicates that the test can be a useful adjunct to existing reproductive management programs. It offers a reliable method to distinguish between pregnant and open animals at 28 days after breeding, and throughout the course of pregnancy. As with any diagnostic test, the IDEXX Bovine Pregnancy Test should be used under the guidance of a veterinarian as part of the farm's overall health and reproductive management program.

Adaptation of a Commercial PRRS Serum Antibody ELISA to Oral Fluid Specimens *

Apisit Kittawornrat[1], John Prickett[1], Chong Wang[1,2], Chris Olsen[1], Christa Irwin[1], Yaowalak Panyasing[1], Andrea Ballagi[3], Anna Rice[3], Rodger Main[1], Chris Rademacher[4], Marlin Hoogland[4], Jeff Zimmerman[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Deparment of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA; [3]IDEXX Laboratories, InC., WestBrook, ME; [4]Murphy-Brown LLC, Ames, IA

Narrative: Oral fluid samples are increasingly used for the surveillance of porcine reproductive and respiratory syndrome virus (PRRSV) infection in commercial swine operations using PCR-based assays (Chittick et al., 2011; Kittawornrat et al., 2010). While PCR-based assays are useful for detecting the circulation of PRRSV, antibody-based assays are informative regarding herd immunity and history of prior infection. The feasibility of detecting antibody in oral fluids has already been addressed: antibody-based assays using oral fluid specimens are already widely available in human diagnostic medicine for a variety of pathogens (Prickett et al., 2010). The purpose of the present study was to optimize a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) to the oral fluid matrix. ELISA parameters assessed in the optimization process included: sample volume, sample dilution, incubation time, secondary antibody isotype (IgM, IgA, IgGH&L, IgGFc), and secondary antibody dilution. To reduce oral fluid sample-to-sample response variation during this process, 11 oral fluids ("Reference Standards") were used in the optimization process to measure the effects of changes in parameters. Reference standards were collected from one commercial wean-to-finish barn (1,100 pigs) prior to the day of PRRS vaccination (Ingelvac® PRRS MLV) and on DPV 0, 10, 15, 20, 28, 35, 41, 49, 56, 75, and 91. (Reference standards available upon request.) Results showed that the ELISA was readily adapted to detect IgM, IgA, and IgG in oral fluid specimens. The protocol that we have developed for detection of IgG is readily amenable to the routine performance of the assay in a diagnostic laboratory.

* Graduate Student Oral Presentation Award Applicant

Diagnostic Performance of a Commercial PRRS Serum Antibody ELISA Adapted to Oral Fluid Specimens: Longitudinal Response in Experimentally-inoculated Populations *

Apisit Kittawornrat[1], John Prickett[1], Chong Wang[1,2], Chris Olsen[1], Christa Irwin[1], Yaowalak Panyasing[1], Andrea Ballagi[3], Anna Rice[3], Bob Rowland[4], Jeff Zimmerman[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA; [3]IDEXX Laboratories, Inc., Westbrook, ME; [4]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS

Narrative: Previous work (AAVLD abstract "Adaptation of a commercial PRRS serum antibody ELISA to oral fluid specimens") showed that a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) could be adapted to detect antigen-specific IgM, IgA, and IgG antibodies to porcine reproductive and respiratory syndrome virus (PRRSV) in oral fluid specimens. Further, the protocol for the IgG ELISA for oral fluid samples was readily amenable to the routine performance of the assay in high throughput diagnostic laboratories. This suggests the possibility of a cost-effective method to routinely monitor commercial swine populations for maternal antibody, vaccination compliance, and herd immune parameters using oral fluid sampling. The purpose of the present study was to evaluate the ability of the PRRS oral fluid IgG ELISA to detect anti-PRRSV IgG antibody in pen-based oral fluid samples from experimentally inoculated pigs over time. In nine trials, ~200 pigs per trials were intramuscularly (IM) inoculated with PRRSV isolate NVSL 97-7895. Oral fluid samples were collected on 0, 5, 7, 9, 11, 14, 17, and 21 days post inoculation (DPI). All oral fluid samples were randomized and tested for anti-PRRSV antibodies using the PRRS ELISA protocol for oral fluids: 1:2 oral fluid sample dilution, 16 hour incubation at 4°C, reaction detected using anti-swine IgGFC. Anti-PRRSV IgG antibodies were detected as early as 7 DPI and all samples were positive by DPI 9. These results indicated that the ontogeny of anti-PRRSV antibodies in oral fluid is amenable to rapid detection of infection. Testing based on oral fluids could provide an efficient, cost-effective approach to PRRSV monitoring in commercial herds and surveillance in elimination programs.

* Graduate Student Oral Presentation Award Applicant

Diagnostic Performance of a Commercial PRRS Serum Antibody ELISA Adapted to Oral Fluid Specimens: Field Samples *

Apisit Kittawornrat[1], John Prickett[1], Chong Wang[1,2], Chris Olsen[1], Christa Irwin[1], Yaowalak Panyasing[1], Andrea Ballagi[3], Anna Rice[3], Rodger Main[1], Chris Rademacher[4], Marlin Hoogland[4], James Lowe[5], Jeff Zimmerman[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames, IA; [3]IDEXX Laboratories, Inc., Westbrook, ME; [4]Murphy-Brown LLC, Ames, IA; [5]Carthage Veterinary Service, Ltd., Carthage, IL

Narrative: Oral fluid samples are of interest because of their ease of collection and documented use in surveillance of porcine reproductive and respiratory syndrome virus (PRRSV) and other pathogens (Kittawornrat et al., 2010; Prickett et al., 2011). Previous work showed that a commercial PRRS ELISA (HerdChek® PRRS X3 ELISA) could be adapted to detect anti-PRRSV IgG in oral fluid specimens (IgG ELISA). The objective of the current study was to evaluate the ability of the assay to detect anti-PRRSV IgG antibody in pen-based oral fluid field samples. Positive samples were derived from a longitudinal field study in 10 wean-to-finish barns (Ramirez et al., 2011). At each site, oral fluid samples were collected from the same 6 pens at 2-week intervals (total of 10 sampling points per barn). Positive oral fluid samples were defined as all samples collected from a pen after the first PRRSV PCR positive oral fluid sample from that pen (n = 250). Negative oral fluid (n = 284) field samples were diagnostic samples submitted to the ISU VLD for PRRSV qRT-PCR testing from expected-negative herds. Of 284 expected-negative field samples, all were negative on the IgG ELISA (S/P <0.40). 223 of 250 expected positive samples were positive. The 27 negative results on expected positive samples were from pens that initially tested positive and became negative over time. The results indicated that anti-PRRSV antibody from natural PRRSV infection can be effectively detected in oral fluids using the IgG ELISA.

* Graduate Student Oral Presentation Award Applicant

Epidemiology of Infection of *Mycoplasma hyorhinis* in Endemically Infected Swine Herds * #

Maria Clavijo[1], Albert Rovira[2], Deborah Murray[3], Simone Oliveira[2]

[1]Veterinary Population Medicine, University of Minnesota, Saint Paul, MN; [2]Veterinary Diagnostic Laboratory, University of Minnesota, Saint Paul, MN; [3]New Fashion Pork Inc, , Jackson, MN

Narrative: *Mycoplasma hyorhinis* has recently emerged as an important cause of mortality in nursery pigs. Approximately 50% of the cases with polyserositis received at the Minnesota VDL in 2010 had the involvement of this pathogen based on isolation or PCR. The objective of this study was to characterize the pattern of *M. hyorhinis* colonization in endemically infected herds. Three 6000 sow farrow-to-wean herds and their nurseries located in Minnesota and South Dakota were selected. These herds had a diagnostic history of recurrent mortality associated with *M. hyorhinis* isolation from systemic sites. Nasal swabs were collected from 60 sows, 60 piglets in each group of 1, 7, 14 and 21 days of age as well as 30 pigs in each group of 28, 35, 42, 49, 56, 63, 70 and 77 days of age. Oral fluids were also collected from the same post weaning pigs. Tissue samples were collected from ten clinically affected and ten clinically healthy pigs necropsied at the age of the peak of mortality. All samples were tested by a real-time PCR developed in our laboratory. *M. hyorhinis* was detected in the nasal cavity of 5/60 sows in herd one, 3/60 in herd two and none in herd three. In herd one and two, that had clinical cases suggestive of *M. hyorhinis*, the colonization prevalence in suckling piglets was low (avg=8%) and high in post-weaning pigs (avg=98%). In contrast, in herd three where *M. hyorhinis* clinical signs were absent, colonization in pigs was very low until the last week in the nursery. A total of 7/8 oral fluids tested *M. hyorhinis* positive in herd one and two, while 1/8 tested positive in herd three. Polyserositis was not observed in any of the healthy necropsied pigs from all three herds, nor in the diseased pigs from herd three. However, in herds one and two polyserositis was observed in 9/10 and 4/10 diseased pigs respectively. Isolation of *M. hyorhinis* from the pericardium was achieved only in herds one and two. *M. hyorhinis* was detected by PCR in the pericardium of 9/10 diseased pigs in herd one and 3/10 in herd two. In the healthy pigs only one sample tested PCR positive. In herd three *M. hyorhinis* was not detected in any of the necropsied pigs. In summary, *M. hyorhinis* can be detected by PCR in nasal swabs, tonsil swabs and oral fluids. The pathogen can colonize pigs at day one of age; however, most of the pigs become colonized sometime in the nursery. High prevalence of *M. hyorhinis* nasal colonization in weaned pigs appears to be correlated to the presence of *M. hyorhinis* associated disease and the detection of the agent in polyserositis cases in nursery pigs.

AAVLD Trainee Travel Awardee

* Graduate Student Oral Presentation Award Applicant

Epidemiologic Factors Associated with Genetic Variation of Serotype O Foot-and-Mouth Disease Virus in Pakistan

Barbara Brito[1], Andres Perez[1], Luis Rodriguez[2]

[1]Medicine and Epidemiology, Center For Animal Disease Modeling and Surveillance, UC Davis, Davis, CA; [2]United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Davis, CA

Narrative: Foot-and-mouth disease (FMD) is one of the most devastating and difficult to control animal diseases because of its broad host range, high morbidity, rapid replication rate, multiple transmission routes, ability to cause subclinical disease and persistent infection in some animals, genetic diversity, and lack of cross-protective immunity. In the Indo-Pakistan subcontinent, serotype O foot-and-mouth disease virus (FMDV) is highly prevalent and, along with the also prevalent serotypes Asia 1 and A, it causes substantial economic losses to farmers in one of the regions with the largest rural milk production of the world. Vaccination has shown to be a key measure to control FMD. However, vaccine effectiveness depends on a number of factors, including selection of protective vaccine strains. Selection of FMD vaccine strains is challenging in endemic countries such as Pakistan because the FMDV has high mutation rates and evolves into new lineages that replace previous ones quite frequently. Factors associated with such genetic variation in Pakistan and other endemic regions of the world are yet to be elucidated. The association between the pair-wise genetic distances of serotype O FMDV isolates and epidemiological factors, such as time, space, and species was assessed. Data from the VP1-coding region of 67 serotype O FMDV collected between 2005 and 2009 were analyzed. A mixed Bayesian binary logistic regression model was formulated, in which the probability of nucleotides differing between each pair of sequences followed a binomial distribution, and explanatory variables were geographic distance (in km), time between sample collection (in days), and species (whether samples were taken from the same or different species). Time and distance were significantly ($P<0.01$) associated with genetic variation of serotype O FMDV in Pakistan. However, genetic diversity of VP1-coding region was not influenced by variation in the species in which samples were collected. This initial assessment of the genetic variation of FMDV in Pakistan will contribute to the evaluation of vaccine effectiveness and formulation of risk-driven control programs in one of the most important regions of the world in terms of subsistence of the FMDV infection.

Application of the Gold Standard Diagnostic Technique for Rabies to Salivary Glands as an Estimate of Viral Shedding and Potential Evolutionary Adaptation to New Species

Cathleen Hanlon, Micheal Moore, Rolan Davis

Rabies Laboratory, Kansas State University, Manhattan, KS

Narrative: The direct fluorescent antibody (dFA) technique performed on fresh brain material is the Gold Standard for rabies diagnosis. Through this technique, there are between 6,000 and 10,000 animal rabies cases diagnosed every year, mostly in wildlife, and consisting of a number of virus variants sustained through transmission primarily within a single reservoir species. Although exposure may result in a case of rabies among individual animals of other species, it is rare to detect serial transmission of a variant outside of its primary reservoir. With the high genetic diversity of RNA viruses, there exists an innate capability of current variants to rapidly adapt to new host populations. For example, a variant previously associated with Big Brown bats in Arizona is now being maintained in striped skunks. Our investigative hypothesis is that the presence and quantity of rabies virus in the salivary glands of the primary reservoir host will be greater in incidence and amount than in other naturally infected non-reservoir species. If a rabies virus variant is reliably detected and in high amounts within the salivary glands of a particular spillover species, then this could signal that this is a permissive species for this variant and/or that the variant is capable of emerging in this species through viral adaptation. We applied the dFA test to touch impressions of salivary glands from rabid animals including 423 skunks, 4 red fox, 2 each of coyote, raccoon and bobcat, 67 cats, 16 dogs, 12 cows, and 2 each of ferrets and horses infected with South-Central Skunk variant; 20 skunks, 5 cats, and 2 raccoons infected with the North-Central Skunk variant; and 34 raccoons, 8 cats, and 2 bobcats infected with raccoon variant. Among salivary glands, the dFA test was positive for virus antigen in the majority of skunks infected with the South-Central variant (n=399 positive (99%) of 423) and all of those infected with the North-Central variant (n=20; 100%) and 97% of raccoons (n=34) positive for the raccoon variant. Among domestic species (cats, dogs, cows, ferrets and horses), none to 75% were positive by dFA and where present, antigen distribution was sparse. Conversely, salivary glands from wild carnivores such as foxes, coyote, and bobcat had abundant antigen. The results are intriguing for the extrapolation of the differential risk of viral adaptation and emergence among spillover species. Continued investigation will include quantification of live virus, and evaluation of viral genomic material. The application of these tools to rabid animals will illuminate the potential of viral evolution, adaptation, and emergence, and also provide information directly applicable to the estimation of public health risk of transmission among these various species, according to unique variants.

Temporal and Spatial Variation in *Culicoides* Midge Abundance Determined by Different Trapping Methods in California *

Christie Mayo[1], Bradley Mullens[2], Christopher Barker[1], Alec Gerry[2], Ian Gardner[1], James MacLachlan[1]

[1]VM: PMI, UC Davis, Davis, CA; [2]Entomology, UC Riverside, Riverside, CA

Narrative: Bluetongue virus (BTV) is the causative agent of bluetongue (BT), an OIE reportable and re-emerging arboviral disease of ruminants that is transmitted by various species of *Culicoides* midges (gnats). Twenty-four (likely 26) serotypes of BTV are recognized globally, four (serotypes 10, 11, 13, 17) of which are endemic in much of the western United States (US). The global distribution of BTV infection coincides with that of competent *Culicoides* vectors, historically ranging between 40-50°N and 35-40°S. However, since 1998, ten previously exotic serotypes have been isolated in the southeastern US and eight novel serotypes of BTV invaded and spread throughout extensive portions of Europe and the Mediterranean Basin precipitating an economically devastating epidemic. One especially disconcerting aspect of this expansion of BTV into Europe included the emergence of several apparently new Palearctic vector species. There is no doubt that the global distribution and nature of BTV infection of livestock has changed, and climate change has been implicated as the cause of this dramatic global event because of its potential impact on the vectorial capacity of populations of *Culicoides* midges. To better characterize the role of *Culicoides* midges in the epidemiology of BTV infection of California dairy cattle, objectives of our current studies are to establish the abundance of *Culicoides* insects on individual farms, estimate attack rates by performing animal baited aspirations, and determine the seasonality *Culicoides* midge abundance. Four dairy farms were enrolled where trapping of *Culicoides* was performed weekly for 1 year using downdraft suction traps with or without a 4W blacklight (UV) bulb, and mechanical aspiration directly from cows using a modified handheld vacuum. Five seronegative adult sentinel cattle were used for mechanical aspiration of insects. *Culicoides* midges were sorted independently based on collection method and recorded as to their relative abundance. Blood was collected from sentinel cattle for detection of viral RNA by BTV-specific quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). Preliminary analysis of the data indicates that there are marked differences in *Culicoides* abundance as determined by different trapping methods. Abundance levels peak during August as determined by both light and CO2 trapping methods, whereas numbers of insects collected by animal baited aspiration do not peak until September. This suggests that measuring abundance (including that of specific midge species) with only conventional trapping methods, estimates typically used in current BTV models, might be misleading and inappropriate for determining true attack rates (biting rates) or vector to host ratios. Data from this study have identified transmission parameters (attack rates) and quantified variables that influence the spatial heterogeneity of BTV infection in ecologically diverse regions of California.

* Graduate Student Oral Presentation Award Applicant

Evaluation of Viral Hemmorhagic Septicemia Virus Real-time RT-PCR Assays

Geoffrey Groocock[1], Rodman Getchell[1], Nicholas Phelps[2], Kathy L. Kurth[3], Janet Warg[4], Andrew Goodwin[5], Cem Giray[6], Elizabeth Brown[7], Robert Kim[8], Yan Zhang[9], Gael Kurath[10]

[1]Aquatic Animal Health Program, Cornell University, Ithaca, NY; [2]Minnesota Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN; [3]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI; [4]National Veterinary Service Laboratories, USDA-APHIS, Ames, IA; [5]Aquaculture/Fisheries Center, University of Arkansas at Pine Bluff, Pine Bluff, AR; [6]Micro Technologies Inc., Richmond, MA; [7]Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD; [8]College of Veterinary Medicine, Michigan State University, East Lansing, MI; [9]Animal Disease Diagnostic Laboratory, Ohio Dept. of Agriculture, Reynoldsburg, OH; [10]Western Fisheries Research Center, USGS, Seattle, WA

Narrative: The emergence of a new genotype of viral hemmorhagic septicemia virus in the Great Lakes basin has highlighted the need for an accepted method of PCR assay detection. A collaboration of laboratories was formed under the guidance of USDA-APHIS. Participating laboratories were asked to evaluate currently available real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays for the purpose of surveillance and screening fish for movement. The specific study objectives include evaluating up to four rRT-PCR assays to determine the analytical sensitivity and specificity to all VHSV genotypes, and based on these results further evaluating up to two assays for diagnostic sensitivity and specificity. The first objective has been completed. The initial four assays were chosen based on previous validation data, available primer/probe sequences and potential for widespread acceptance. Three laboratories with previous VHSV rRT-PCR experience were chosen. These laboratories used identical platforms and followed standard protocols to minimize variability. The results show that limits of detection for the North American genotypes (VHSV types IVa & IVb) were comparable for all assays. Three assays were sensitive for all genotypes of VHSV, while one assay did not detect the VHSV type I genotype. Two one-step assays were chosen for the second objective based on these results. This objective is currently ongoing with participation by the remaining collaborating laboratories. The potential for sample type inhibition was also evaluated. The two assays chosen for the second objective were tested on a range of sample types including whole viscera, ovarian fluid, kidney/spleen and a no-tissue control. Of the samples tested thus far, preliminary data shows a 10-fold limit of detection reduction between the kidney/spleen and entire viscera samples.

Geospatial Analysis of Canine Leptospirosis Risk Factors *

Ram Raghavan[1], Kenneth Harkin[2], Gary A. Anderson[1]

[1]Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; [2]Department of Clinical Sciences, Kansas State University, Manhattan, KS

Narrative: Canine leptospirosis is a widely prevalent zoonotic disease and recent research shows a steady increase of cases received at hospitals. Of the many factors contributing to this disease among dogs, environmental (land-use/land-cover), climate, and socio-economic conditions of pet owners are suspected to play a prominent role. Identifying specific risk factors for this disease could help form effective preventive strategies such as vaccination and behavior modification. Using GIS (Geographic-Information-Systems) we analyzed risk factors for canine leptospirosis based upon cases received at Kansas State Veterinary Diagnostic Laboratory (KSVDL) between 2002 and 2009. Nintey-four cases from the KSVDL database were selected predominantly based upon positive polymerase-chain reaction (PCR) results for known pathogenic leptospires in the urine. Addresses of selected dogs' residences were geocoded using GIS software. Different thematic layers of geospatial information including the land-use/land-cover, pet owners' socio-economic and demographic information, and hydrologic/soil-hydrologic information were analyzed using geospatial methods and multivariate logistic regressions in a case-control study to derive risk factors. Geospatial analysis revealed that urban areas in general (OR = 2.013, 95% C.I = 1.355, 2.991) and specifically medium-intensity urban areas (OR = 1.866, 95% C.I = 1.443, 2.412), lack of plumbing facilities in households (OR = 2.880, 95% C.I = 1.867, 4.442), poverty status (OR = 2.079, 95% C.I = 1.763, 2.451), proximity to water features (OR = 0.828, 95% C.I = 0.795, 0.863), hydrologic density (OR = 2.809, 95% C.I = 1.588, 4.969), and frequently flooded areas (OR = 4.051, 95% C.I = 2.172, 7.555) were significant risk for dogs. With the identification of these risk factors, geospatial analysis has clarified uncertainties in previous works along with the identification of newer risks to dogs. Risk factors identified using GIS in this study can be used for strategizing prevention/spread of canine leptospirosis. Furthermore, the geospatial methods developed are applicable for studying other infectious diseases. The inclusion of geospatial technology (GIS and remote-sensing) to veterinary diagnostic laboratories has the potential to add a powerful new dimension to how we collect animal/public health information, conduct research and provide real-time, value-added services to clientele. While the technology appears to be relevant and useful to veterinary researchers and practitioners, there is a general lack of expertise or service providers that could facilitate its application. Diagnostic laboratories can fill this gap in coming years while at the same time evaluating the usefulness of geospatial technology in improving animal/public health.

* Graduate Student Oral Presentation Award Applicant

Pathology Scientific Session 2

Sunday, October 2, 2011

Grand Ballroom A

Moderators: Scott Fitzgerald and Gayle C. Johnson

8:00 AM **Recurring Outbreaks of Hemorrhagic Pneumonia Due to Pseudomonas on a Mink Ranch**
Scott D. Fitzgerald, Joy Gary, Karla Fenton, Matti Kiupel……. *109*

8:15 AM **Necrotic Enteritis in Chickens Associated with *Clostridium sordellii* + #**
Guillermo Rimoldi, Richard Chin, Francisco Uzal, Muhammad Ilyas, Moeller Robert, H. L. Shivaprasad…… *110*

8:30 AM **Gross and Histopathologic Characterization of Caprine Melioidosis after Aerosol Challenge with *Burkholderia pseudomallei* ◊**
Carl Soffler, Tawfik Aboellail, Angela Marolf, Angela Bosco-Lauth, Richard Bowen…… *111*

8:45 AM **Severe Hoof Deformities in Free-Ranging Elk in Western Washington State**
Sushan Han, Kristin Mansfield…… *112*

9:00 AM **Bovine Herpesvirus-2 Ear and Facial Skin Infection in Holstein Calves**
Patricia Blanchard, James Reynolds, Naomi S. Taus, Hong Li……. *113*

9:15 AM **Limb Cellulitis Caused by *Mycoplasma bovis* in Dairy Cows**
Alfonso De la Mora, Janet Moore, Farshid M. Shahriar, Francisco Uzal…… *114*

9:30 AM **Fatal Bovine Respiratory Disease with Syncytial Cell Formation: Histologic Findings with Lack of Etiologic Agent Identification**
Kelli M. Almes, Richard Hesse, Joe Anderson, Mike Hays, Thomas Waltzek, Gregory Gray…… *115*

9:45 AM BREAK

10:15 AM **Analysis of Lesion Patterns in Post-Natal Bovine Herpesvirus 1-Associated Encephalitis**
Sandra Scholes…… *116*

10:30 AM **Nocardioform Placentitis in Central Kentucky**
Laura Kennedy, Neil Williams, Craig N. Carter, Erdal Erol, Jacqueline Smith, Stephen Sells…… *117*

10:45 AM **Gram-Positive Cocci Causing Equine Enterocolitis**
Jane Kelly, Tom Baldwin, Kimberly Cavender, Ramona Skirpstunas…… *118*

11:00 AM **Diaphragmatic Paralysis Due to Phrenic Nerve Degeneration in an Alpaca**
Francisco Uzal, Motoko Mukai, Leslie Woods, Robert Poppenga, Jana Smith…… *119*

11:15 AM **Garden Hose Scalding Syndrome ***
Erin Quist, Mika Tanabe, Joanne Mansell, Jeffrey Edwards…… *120*

* Graduate Student Oral Presentation Award Applicant
\# AAVLD Trainee Travel Awardee (Pathology)
\+ AAVLD/ACVP Graduate Student Award Applicant
◊ USAHA Paper

Recurring Outbreaks of Hemorrhagic Pneumonia Due to Pseudomonas on a Mink Ranch

Scott D. Fitzgerald, Joy Gary, Karla Fenton, Matti Kiupel

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

Narrative: An 80,000 animal mink ranch experienced repeated episodes of high mortality in 3 separate outbreaks in June, August and September, 2010. Representative juvenile mink were submitted to the Diagnostic Center for Population and Animal Health for necropsy during each outbreak. The final submission included feed samples as the owner attempted to identify the source of the infection. Clinical signs included poor appetites, sudden death, and a few animals exhibited bloody nasal discharge. Gross findings included mottled red to dark brown lungs, with the pneumonia being either diffuse or lobar in distribution, and the pleural cavities were filled with bloody pleural effusion. The gastro-intestinal tracts were generally empty or had scant contents. Histopathology of the lobar pneumonia was characterized by filling of alveoli and airways by edema, fibrin, hemorrhage, neutrophils and macrophages. Interlobular septa were expanded by lymphocytes and plasma cells. There was widespread pulmonary parenchymal necrosis, and heavy colonization of necrotic areas by small bacterial bacilli. The pleura were thickened by accumulations of fibrin and neutrophils. Bacterial culture of pooled lung samples revealed numerous *Pseudomonas aeruginosa*, admixed with moderate to numerous beta-hemolytic Streptococcus, *Klebsiella pneumoniae*, or *Enterococcus spp*. *Pseudomonas aeruginosa* was isolated in high numbers from all 3 submissions, and was the only organism isolated from the second submission. The Pseudomonas isolates also exhibited an extremely resistant antibacterial susceptibility profile. We ruled out underlying canine distemper virus by PCR testing and immunohistochemical staining. Feed samples on the final submission revealed few to no Pseudomonas organisms on culture, leading us to believe that water or subclinical carrier adult mink may have been the more likely sources of the infectious agent. *Pseudomonas pneumonia* in ranch-raised mink has been a recognized cause of hemorrhagic pneumonia for over 40 years. It is classically characterized by high mortality, especially in the late summer and early fall associated with stressors such as hot, humid weather and fur molt. Mortality reportedly varies from 1-50% in affected ranches, with rapid spread by aerogenous infection. Killed bacterins have been shown to be efficacious in the face of outbreaks. We recommended rapid removal of moribund or dead mink, treatment of the entire ranch by antibiotics in the water, and immediate vaccination using a commercial bacterin to all unvaccinated juvenile animals. While treatment was successful in limiting mortality during each of the 3 outbreaks, the ultimate source of the infectious agent was not determined.

Necrotic Enteritis in Chickens Associated with *Clostridium sordellii* + #

Guillermo Rimoldi[1], Richard Chin[1], Francisco Uzal[2], Muhammad Ilyas[1], Moeller Robert[1], H. L. Shivaprasad[1]

[1]Pathology, CAHFS UCDAVIS, Tulare, CA; [2]Pathology, CAHFS UCDAVIS, San Bernardino, CA

Narrative: Necrotic enteritis (NE) is a common clostridial disease affecting poultry. It usually affects young chickens and is characterized by sudden onset and high mortality. NE is caused by *Clostridum perfringens* type A and occasionally type C. It is characterized by intestinal necrosis that may affect all layers of the intestine; duodenum and mid jejunum are mostly affected. It is very common to find concomitant coccidiosis together with NE. In February 2011, five live and seven dead 18-day-old male broiler chickens were submitted for necropsy with a history of increased mortality in the flock. Grossly, segmental dilation of duodenum and jejunum with watery content, gas and mucoid exudate were seen in all birds. The enteric mucosa had a roughened uneven surface and was covered with fibrin strands in five of the birds. Coccidia oocysts were readily detected on wet mount preparations of intestinal scrapings. Histological examination of the small intestine revealed severe mucosal necrosis with myriad intralesional gram-positive bacilli and the presence of coccidian parasites in different developmental stages. *Clostridium sordellii* was isolated in large numbers from pooled intestinal swabs cultured anaerobically followed by identification by RAP ID ANA II Systems by Remel, and fluorescent antibody test. No *Clostridium perfringens* was isolated. *C. sordellii* is responsible for gas gangrene in humans and for gas gangrene and enteric disease in several species including, but not restricted to, cattle, sheep and horses. Recently, this microorganism has been associated with fatal endometrial infection in women. In birds, *C. sordellii* has been reported to produce gangrenous dermatitis in chickens and turkeys and enteritis and hepatitis in ostriches. *C. sordellii* produces necrotizing and hemolytic toxins, some of which share characteristics with *C. perfringens* type A alpha toxin (e.g., lecythinase activity). In poultry NE is usually diagnosed on gross findings and histological examination. A fast and accurate diagnosis allows producers to set measures to decrease or stop mortality rates in affected flocks. Cultures are not always done. As this report suggests, classical NE lesions might not be produced exclusively by *C. perfringens*, and *C. sordellii* might be also responsible for NE in chickens. In this case, previous intestinal lesions produced by coccidiosis might have acted as a predisposing factor for *C. sordellii* infection. Coccidiosis is also considered the main predisposing factor for *C. perfringens* NE.

+ AAVLD/ACVP Graduate Student Award Applicant

AAVLD Trainee Travel Awardee (Pathology)

Gross and Histopathologic Characterization of Caprine Melioidosis after Aerosol Challenge with *Burkholderia pseudomallei*

Carl Soffler[1], Tawfik Aboellail[1], Angela Marolf[2], Angela Bosco-Lauth[3], Richard Bowen[3]

[1]1 Department of Microbiology, Immunology, and Pathology, Colorado State University, 300 W. Lake St.,, Fort Collins, Colorado, 80523, USA, CO; [2]2 Department of Environmental and Radiological Health Sciences, Colorado State Univeristy, Fort Collins, CO; [3]3 Department of Biomedical Sciences, Colorado State University, Fort Collins, CO

Narrative*:* *Burkholderia pseudomallei* is the causative agent of meliodosis, which is endemic to Southeast Asia and northern Australia. While the disease was first described almost 100 years ago (1), it still remains a serious emerging infectious disease. Melioidosis is currently the third leading infectious cause of death in northeast Thailand (2). *B. pseudomallei* has also been designated a Category B Select Agent by the United States Centers for Disease Control and Prevention because of its potential use in bioterrorism. The research on the pathogenesis of melioidosis has primarily used mouse models. However, mice are not naturally infected with *B. pseudomallei*, nor do murine models readily allow for serial assessment of vital parameters, clinical pathology, and immunologic events because of their small size and limited blood volume, limiting the evaluation of disease progression on a human-relevant scale. Twelve goats were infected intratracheally by an aerosol of 105 CFU *B. pseudomallei*. Goats were sacrificed on days 2, 7, 14, and 21 post-infection (PI). Bronchointerstitial pneumonia was grossly apparent by day 7 PI and systemic dissemination was evident in multiple organs namely spleen, kidneys, adrenal glands, and testicles as early as day 14-16 PI. Histopathology revealed that the early lesions of mucopurulent bronchopneumonia soon progressed to a more severe fibrinopurulent and histiocytic to proliferative bronchointerstitial pneumonia with eventual formation of characteristic meliodosis pyogranulomas. Lesions spread within the lungs along interlobular septa and subpleural stroma where leukocytoclastic vasculitis appears to be the central step in hematogenously disseminating the organism to extra-pulmonary tissues. 1. Whitmore A. An Account of a Glanders-Like Disease Occurring in Rangoon. The Journal of Hygiene1913;13(1):1-34. 2. Limmathurotsakul D, Wongratanacheewin S, Teerawattanasook N, Wongsuvan G, Chaisuksant S, Chetchotisakd P, Chaowagul W, Day NP, Peacock SJ. Increasing incidence of human melioidosis in Northeast Thailand. Am J Trop Med Hyg2010 Jun;82(6):1113-7.

Severe Hoof Deformities in Free-Ranging Elk in Western Washington State

Sushan Han[1,2], Kristin Mansfield[3]

[1]Dept of Microbiology Immunology Pathology and Diagnostic Medicine Center, Colorado State University, Fort Collins, CO; [2]Washington Animal Disease and Diagnostic Laboratory, Washington State University College of Veterinary Medicine, Pullman, WA; [3]Department of Wildlife Health, Washington Department of Fish and Wildlife, Spokane Valley, WA

Narrative: Free-ranging Roosevelt elk (*Cervus elaphus roosevelti*) and Roosevelt-Rocky Mountain (*Cervus elaphus nelsoni*) hybrid elk in South Western Washington State were recently reported with severely overgrown and deformed claws. Reports of elk with deformed hooves have occurred sporadically for over a decade, but the number and distribution of these reports increased significantly in 2008. In the spring of 2009 we determined the geographical distribution of affected herds of elk, characterized hoof lesions, and examined possible etiologies of the claw deformities, with the goal of establishing a baseline of health within a defined study area. Herds were visually evaluated, and landowners and hunters were surveyed to estimate the prevalence and distribution of affected elk within 10 counties and 11 game management units in the Cowlitz River Basin, and approximately 55% of all herds in the study area had 30 to 90% of individuals affected by deformities of one or more claws, with marked variation in severity. Affected animals represented all age and sex classes. Eight elk with abnormal or normal hooves representing three herds were collected. Elk were necropsied, and tissue and biological specimens and distal limbs were collected. Limbs and all viscera were examined by radiology, gross dissection, histopathology, viral and bacterial culture and polymerase chain reaction (PCR), serology, and hepatic trace mineral concentration analysis, and feces were examined for parasite burden. Results of laboratory tests were unremarkable indicating no primary underlying disease condition(s). Grossly, deformed claws varied from intact, but abnormally long and curved, to sloughed or broken, with common severe sole abscesses. Histology of affected and normal claws identified acute and chronic laminitis in most cases, with negative culture of hooves for common hoof rot pathogens. Hepatic selenium and copper levels in all elk were severely deficient. Chronic laminitis likely has an important role in the pathogenesis of lameness and aberrant growth of hooves, and may be indicative of a nutritional etiology. Additionally, marked copper deficiency may affect hoof keratin integrity predisposing elk to sole abscesses and hoof deformities.

Bovine Herpesvirus-2 Ear and f
Facial Skin Infection in Holstein Calves

Patricia Blanchard[1], James Reynolds[2], Naomi S. Taus[3], Hong Li[3]

[1]California Animal Health and Food Safety Laboratory- Tulare, University of California- Davis, Tulare, CA; [2]Large Animal Food Production, Western University, Pomona, CA; [3]Animal Disease Research Unit USDA ARS, Washington State University, Pullman, WA

Narrative: A ~5000 head calf ranch experienced an outbreak of alopecia, scab formation and peeling skin on the ears and alopecia and scab formation above the eyes of 95-100% of two to three-week old calves in August. Ear lesions were most common on the dorsal portion. Calves were bright and alert, afebrile, thin but eating and drinking normally. The ranch manager reported the lesions resolved within two to four weeks of onset. Microscopic examination of ear scabs from nine calves revealed intranuclear inclusion bodies within the commonly seen multinucleate syncytial cells. Electron microscopy of scabs from two calves confirmed the presence of herpesvirus particles. Immunohistochemistry testing for Bovine Herpes I virus was negative on all 9 animals. Bovine viral diarrhea virus antigen ELISA was negative on ear tissue from 3 calves tested. Bovine herpesvirus -2 (BHV-2) was identified using consensus polymerase chain reaction on ear scab samples from two calves. Three of 4 clones from each calf were BHV-2 and there was one unidentified herpesvirus clone from each animal. The analysis was performed by Dr. Naomi Taus in the laboratory of Dr. Hong Li at USDA Animal Disease Research Unit, Washington State University. This was an unusual presentation for Bovine herpesvirus-2 which is most commonly associated with vesicles, scabs and ulcers on one or more teats of lactating cows and rarely in the mouth and on the muzzle of nursing calves. A second syndrome associated with BHV-2 is pseudo-lumpy skin disease with skin lesions over the whole body and fever and depression. The histopathology of this case matches that described for bovine herpes dermatopathic disease but calves in this case did not show evidence of fever or depression at the time of, nor prior to, onset of the skin lesions. The location of lesions and high transmission rate on this calf ranch may reflect the presence of face flies which were common at the time of the outbreak.

Limb Cellulitis Caused by *Mycoplasma bovis* in Dairy Cows

Alfonso De la Mora, Janet Moore, Farshid M. Shahriar, Francisco Uzal

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

Narrative: *Mycoplasma bovis* is an important cause of pneumonia, mastitis, arthritis and otitis media in cattle. We present here a cluster of cases of limb cellulitis in cows caused by *M. bovis*. Four cows with a history of lameness and one or more swollen legs were submitted for necropsy and diagnostic work up. Gross examination showed one or two swollen front legs with diffuse subcutaneous edema, hemorrhage and multifocal pockets of necrosis and pus affecting mostly the dorsal aspects of the carpal, metacarpal and phalangeal areas. The pre-scapular and axial lymph nodes were markedly enlarged in all cases. Three out of the four cows had mild, non-perforating (1-3 cm in diameter) skin lacerations on the dorsal carpal or dorsal phalangeal areas. Microscopically, chronic-active, necro-suppurative and lymphoplasmacytic cellulitis and tendinitis with proliferation of granulation tissue were observed in the affected areas. Gram and PAS stains did not reveal the presence of bacteria or fungal elements, and aerobic, anaerobic and salmonella cultures were negative. *M. bovis* was isolated from, and/or detected by immunohistochemistry in the subcutaneous tissue of all affected limbs, and was also isolated from the lungs of one cow. Cellulitis has been reported in cattle before, caused by *Arcanobacterium pyogenes*, *Bacteroides spp*., *Clostridium septicum* and *Staphylococcus spp*., amongst others. In this case, we found a unique association of *M. bovis* with limb cellulitis. In view of the presence of skin lacerations on the fore limbs of three of the cows, and the ability of *M. bovis* to penetrate broken skin, wound contamination is one possible route of infection. Alternatively, *M. bovis* bacteremia occurring secondary to respiratory tract or mammary gland infection, may have been the origin of the infection. Pre-existing trauma of the distodorsal aspect of the forelimbs (a common finding in dairy cows), may have provided a favorable environment for localized *M. bovis* growth. A source for *M. bovis* infection may have been the hospital milk from cows with clinical or subclinical mastitis. Attempts to isolate *Mycoplasma spp*. from the milk of some of these cows, and environment water were unsuccessful. Further work is needed to elucidate the pathogenesis of this unusual manifestation of *M. bovis* infection in cattle.

Fatal Bovine Respiratory Disease with Syncytial Cell Formation: Histologic Findings with Lack of Etiologic Agent Identification

Kelli M. Almes[1], Richard Hesse[1], Joe Anderson[1], Mike Hays[1], Thomas Waltzek[2], Gregory Gray[2]

[1]Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; [2]Global Pathogens Laboratory, College of Public Health and Health Professions, University of Florida, Gainesville, FL

Narrative: From July to October 2008 the Kansas State Veterinary Diagnostic Laboratory received 17 cases of fatal respiratory disease in 13 cattle, 3 sheep, and 1 goat. These cases were suspected to be caused by Bovine Respiratory Syncytial Virus (BRSV) because each case yielded at least one of the following criteria for BRSV: positive by fluorescent antibody testing (10/10 cases tested), positive by ELISA testing (14/14 cases tested), or syncytial cell formation on routine histopathology of lung tissue (7/14 cases tested). All lung samples were negative for virus isolation on embryonic bovine lung cells. Polymerase chain reaction (PCR) was also negative for nucleic acid coding for the fusion and G proteins of BRSV and the F protein of Parainfluenza-3 virus (PI-3). Histopathologic changes observed, in addition to syncytial cell formation, included bronchopneumonia, interstitial pneumonia, or a combination of the two. Thin section electron microscopy was conducted on formalin fixed, paraffin embedded bovine lung tissue from one case exhibiting syncytia. Virus-like particles were observed in the cytoplasm of multinucleated cells that appeared to be of macrophage origin. Due to the lack of conclusive BRSV and PI-3 confirmatory testing, these cases are considered open for viral agent identification. Emergence of a new viral agent associated with bovine respiratory disease complex (BRDC) should be considered. Future pathogen testing including, in-situ hybridization, microarray and pyrosequencing is planned for a select set of lung samples from these cases in an attempt to identify the presence of an apparent unknown agent associated with fatal bovine respiratory disease in feedlot cattle.

Analysis of Lesion Patterns in Post-Natal Bovine Herpesvirus 1-Associated Encephalitis

Sandra Scholes

AHVLA-Lasswade, AHVLA, Midlothian

Narrative: Encephalitides associated with viral infections of the CNS frequently are characterised by a non-suppurative response, although the expression is influenced by many factors including virus characteristics and route of infection. Histological analysis of patterns of CNS viral diseases, based on the location and nature of the neuroparenchymal lesions, has been used to classify human encephalitides to facilitate differential diagnosis. This approach was applied to Bovine herpesvirus (BoHV) 1 encephalitis. Eleven standardised sites representing the major neuroanatomical regions of the brain were examined histologically in 6 cattle, with varying ages and clinical presentations, in which BoHV1 was confirmed by DNA sequencing. The anatomical distribution and histological characteristics were compared with published data on BoHV1/5 encephalitis. Rabbit polyclonal (recognising BoHV1/5) and BoHV1-specific monoclonal antibodies were used for immunohistochemistry. In all cattle, the gray matter and white matter were involved (pan-encephalitis). BoHV1 antigen was detected in association with neuroparenchymal inflammation, in neurones / neuronal processes and astrocytes. In 4 calves (10 days - 3 weeks) with upper respiratory / alimentary BoHV1 lesions, the encephalitis was limited to caudal brainstem and included the fasciculus and nucleus solitarius; intralesional BoHV1 antigen was detected in 3, consistent with centripetal spread of BoHV1 to the CNS via visceral afferent fibres originating in the alimentary and respiratory tract lesions. In one 2 weeks old calf that died following brief neurological signs, loose glial foci were widespread in gray matter, including cerebral cortex associated with neuronal necrosis, and in white matter, together with consistent presence of BoHV1 antigen. The distribution of the lesions was compatible with hematogenous localisation; simultaneous detection of BoHV1 DNA in the liver of this calf supported the possibility of viremia. In a yearling steer with nervous disease, extensive destructive lesions with marked glial reaction and abundant intense labelling for BoHV1 were present predominantly in cerebral cortex gray and white matter, particularly rostrally. The lesion distribution is closely similar to that observed following experimental BoHV infection via the nasal route or recrudescence of latent BoHV acquired via the nasal route (cribriform plate). Although 3 distinct neuroanatomical distributions were recorded, in all cattle both gray and white matter lesions (pan-encephalitis) were present, according with the classification of human encephalitides; herpesviral encephalitis is a differential diagnosis for panencephalitis in man. The lesion distribution correlated with the likely route of neuroinvasion by BoHV1. These findings suggest that pattern diagnosis may be applicable to the diagnostic investigation of the etiology and route of neuroinvasion in non-suppurative encephalitides in animals.

Nocardioform Placentitis in Central Kentucky

Laura Kennedy, Neil Williams, Craig N. Carter, Erdal Erol, Jacqueline Smith, Stephen Sells

Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY

Narrative: In November and December of 2010, there was a marked increase in the number of nocardioform placentitis cases submitted to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL). This increase was detected through the observations of pathologists and a diagnostic surveillance tool monitored by the epidemiology section. The trend of increasing incidence of nocardioform placentitis continued into 2011, causing growing concern among practitioners, farm owners and managers, the UKVDL and the horse industry media. Following several meetings with stakeholders in the Kentucky equine industry, an ad hoc task force was assembled to further investigate the unusual number of cases. Full participation by the equine industry, including subsidies for placental examinations by local organizations, led to the largest number of nocardioform placentitis submissions on record, greater than 300 confirmed cases. Nocardioform placentitis was first identified in Central Kentucky in the mid-1980s. The term “nocardioform” was adopted due to similarities of the causative agents to the nocardioform actinomycetes. Through sequence analysis of 16S rRNA genes the most common organisms have been identified as *Crossiella equi sp. nov*, *Amycolatopsis spp*, and *Streptomyces spp*. The pathogenesis of nocardioform placentitis is poorly understood. The clinical ramifications of nocardioform placentitis range from late gestation abortions and stillbirths to small, weak foals that may or may not survive. Due to the fact that even mild cases of nocardioform placentitis were submitted in 2011 a number of foals were healthy and vigorous at birth. Gross and histologic lesions of nocardioform placentitis are distinctive. The cervical star region is spared and lesions most commonly occur in the body of the placenta at the bifurcation of the horns. Lesions may be single or multiple. The affected chorion is covered by a thick, light brown, tenacious exudate overlying a rough, tan chorion with marked villous loss. Expansion of the allantoic stroma by nodular masses (adenomatous hyperplasia) is frequently observed. The characteristic histologic lesions include necrosis of trophoblasts, marked villous loss, squamous metaplasia of the chorionic epithelium, chronic suppurative inflammation, adenomatous hyperplasia, and intra-lesional gram positive branching bacilli. The bacteria do not reach the fetus and fetal lesions are limited to those of placental insufficiency. It is hoped that through the tremendous number of cases received and collaboration between practitioners, owners, the UKVDL and the University of Kentucky Gluck Equine Research Center that significant inroads can be made in the understanding of this unusual disease process.

Gram-Positive Cocci Causing Equine Enterocolitis

Jane Kelly, Tom Baldwin, Kimberly Cavender, Ramona Skirpstunas

Springville, UT

Narrative: In the past 2 years, 7 cases of equine enteric disease have been attributed to infection by Gram-positive cocci at the Utah Veterinary Diagnostic Laboratory. Most of the cases were in foals, but cases occurred in adult horses also. In spring 2010, two foals, 3 days and 7 days of age, from the same ranch were submitted for necropsy with a history of watery diarrhes with blood flecks for 24 hours before death. On necropsy, severe necrotizing, fibrinosuppurative enterocolitis with surface colonization by numerous small coccoid bacteria was diagnosed in each animal. *Enterococcus faecalis* was isolated from the intestine of the older foal and *Enterococcus faecium* from the younger. In spring 2011, a 6 week-old foal had profuse diarrhea, a painful abdomen, and refused to nurse over a 3-day period. Severe ulcerative, necrosuppurative and fibrinous enterocolitis, with Gram-positive cocci lining ulcerativesegments of intestine was diagnosed. *Enterococcus faecium* was isolated from the small intestine. In the spring of 2011, three stallions and a colt from the same facility died without observed clinical signs. In all four horses there was severe necrotizing, fibrinosuppurative, hemorrhagic enterocolitis. *Streptococcus bovis* was isolated from the intestinal content in all 4 horses. Although reported infrequently in the literature, findings suggest that Gram-positive infections of the equine intestinal tract are an important cause of enterocolitis.

Diaphragmatic Paralysis Due to Phrenic Nerve Degeneration in an Alpaca

Francisco Uzal[1], Motoko Mukai[2], Leslie Woods[2], Robert Poppenga[2], Jana Smith[3]

[1]CAHFS San Bernardino, UCDavis, San Bernardino, CA; [2]CAHFS Davis, UCDavis, San bernardino, CA; [3]Private Practitioner, NA, Somas, CA

Narrative: A 6-month-old 12 kg female alpaca cria, with a history of 24 hr respiratory distress before death, was presented for post-mortem examination. No significant gross abnormalities were observed at necropsy. Histologically, the diaphragm revealed multifocal areas of angular atrophy of a small number of contiguous myofibers, and fiber size variation, with internal nuclei. Scattered necrotic fiber segments with loss of cytoplasm and striations, cytoplasmic vacuolation and pyknotic or karyorrhectic nuclei were also seen. Both phrenic nerves exhibited multifocal and marked degenerative changes consisting of dilated myelin sheaths, swollen axons and occasionally foamy macrophages within dilated myelin sheaths (digestion chambers). No other significant histological abnormalities were observed in any of the other tissues examined. No significant aerobic or anaerobic bacterial pathogens were isolated from liver, lung or small intestine. Heavy metals and vitamin E concentrations were within expected ranges in liver, blood and serum. No organophosphorus insecticides or tin were detected in liver and no unusual compounds were detected in the liver by GC-MS screen. The lesions in the diaphragm were characteristic of denervation atrophy, which suggests that the degenerative lesions in both phrenic nerves were the primary event that led to diaphragmatic degeneration and paralysis. Diaphragmatic paralysis associated with neuropathy of phrenic nerves was previously described in a llama and several alpacas although the etiology was not determined. The etiology of the neuropathy of the phrenic nerve in the present study also remains undetermined. The postmortem diagnosis of diaphragmatic paralysis associated with phrenic nerve neuropathy can be challenging and it will be missed unless samples of phrenic nerves and diaphragm are examined histologically. This report stresses the need to examine diaphragm and phrenic nerves in cases of respiratory difficulty without evident gross changes in camelids.

Garden Hose Scalding Syndrome *

Erin Quist[1], Mika Tanabe[2], Joanne Mansell[1], Jeffrey Edwards[2]

[1]Veterinary Pathobiology, Texas A & M University, College Station, TX; [2]Antech Diagnostics, Irvine, CA

Narrative: In this report, we present a series of cases of thermal burns (scalds) in dogs resulting from exposure to hot water from a garden hose that has been lying in the sun. These dogs typically inhabited the southern and western regions of the United States where the recorded high temperatures exceed 32C (90F) during the warm summer months. Dogs with thermal scald injury in these cases typically presented with a variety of macroscopic lesions dependant upon the degree of burn exposure, ranging from local erythema to ulcerated, necrotic and sloughing skin. Chronic, healed wounds were often alopecic, with markedly thickened skin and characteristically smooth and glassy scar tissue formation. Histologically, the lesions of thermal scald injury in these dogs were indistinguishable from any other 2nd or 3rd degree burn, and consisted of full-thickness dermal and epidermal necrosis with occasional fibrinoid necrosis of the vessel wall, vasculitis and intravascular thrombosis. Here, we closely examine 10 cases of dogs with thermal burns collected from Texas, Arizona, California, Utah, Nevada, Indiana, Michigan and North Carolina and propose the term “Garden Hose Scalding Syndrome (GHS)” to describe this unusual type of scald injury.

* Graduate Student Oral Presentation Award Applicant

Virology Scientific Session 2
Sunday, October 2, 2011
Grand Ballroom D

Moderators: Kyong-Jin Yoon and Alfonso Clavijo

* Graduate Student Oral Presentation Award Applicant

◊ USAHA Paper

Observations from Serological Monitoring of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) "Negative" Herds: Getting to the Final Answer *

Kevin O'Neill, John Johnson, Tanja Opriessnig

Iowa State University, Ames, IA

Narrative: Diagnosticians are consistently challenged by porcine reproductive and respiratory syndrome virus (PRRSV). PRRSV continually alters its genome and occasionally this results in genetic and antigenic changes that affect the sensitivity of diagnostic assays. Working with their veterinarians, boar stud and breeding herd owners have come up with a variety of testing protocols to reduce the risk of introducing PRRSV into naïve or stable herds. This usually involves the use of some combination of PCR and serology. While PCR can be a very accurate method, it is fairly expensive and constant attention must be focused on maintaining current primer-probe combinations to avoid false negative results. Serology on the other hand, is a much less expensive diagnostic tool. One of the primary disadvantages of PRRSV serology is the time and expense invested in dealing with the false negative or false positive samples. For the purposes of this presentation, false positive reactors are identified as being an individual animal from a group that is otherwise negative. Explanations for false positive results could be inconsistent plate coating, recent vaccination, unsuitable cut-off selection, cross-reactivity of the antibodies with other pathogens present, and many more. The current recommended process of testing is ELISA first, then if there is a false positive reactor; verify the results with indirect fluorescence assay (IFA) and PCR. The objective of this presentation was to summarize observations and outcomes from serological monitoring of PRRSV negative herds. Periodically testing of a subset of animals from three PRRSV negative flows resulted in (1) detection of type 1 PRRSV RNA without evidence of PRRSV seroconversion by an ELISA (IDEXX PRRS X3 Ab test) and an IFA test, (2) detection of seroconversion by one (IDEXX PRRS 2XR Ab test) of two ELISAs but not by IFA and no PRRSV RNA was detected, and (3) detection of seroconversion by two ELISAs (IDEXX PRRS X3 or 2XR Ab tests) and no PRRSV RNA was detected. Two cases of interest encompass the first and second outcomes. Case A involved a negative herd, which was serologically confirmed via ELISA and IFA, but later proven type 1 PRRSV positive by PCR. Case B involved one farm where five positive animals were identified from a group of 30. Both the farm and diagnostic laboratory serology confirmed PRRSV, but PCR and IFA were negative. The results indicate that current methods for detection of PRRSV infection differ substantially making timely, definitive decisions difficult.

* Graduate Student Oral Presentation Award Applicant

"Catch Me if You Can!" - The Ongoing PRRSV PCR Challenge

Karen Harmon, Sarah Jones, Amy Chriswell, Erin Strait

VDPAM, Iowa State University, Ames, IA

Narrative: Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus causing reproductive failure in sows and respiratory problems in piglets and growing pigs. The high economic impact of this agent dictates the need for rapid, accurate diagnosis. RNA viruses such as PRRSV have high mutation rates resulting in rapid evolution and genetic variability. The TaqMan® North American (NA) and European (EU) PRRSV PCR reagents (Applied Biosystems™)(AB) and VetAlert™ (Tetracore®) NA and EU PRRSV PCR reagents contain multiple primers and probes for detecting both NA and EU subtypes, providing enhanced ability to detect field strains compared to traditional real-time PCR assays with one primer/probe set for each target. Even with improved detection capabilities, instances occur where isolates fail to be detected, or are only weakly detected. When alerted to this possibility, these samples are retested using alternate PCR assays. The confirmed positive sample and/or appropriate sequence information is forwarded to the manufacturer for further analysis. Recently, AB's bioinformatics group verified mismatches between their NA PRRSV PCR primers and probes and the corresponding target region in some contemporary field isolates. An additional primer/probe set was developed to supplement the existing PRRSV PCR reagents, allowing for broader detection of NA viruses without diminishing the assay's sensitivity. However, additional testing revealed a consistent unexpected weak false positive signal with the AB updated assay. An alternative supplemental primer/probe set was designed to add to the core reagents, and the weak false positive signal was eliminated. Subsequently a comparison of AB's updated reagents versus the Tetracore® reagents was performed on 888 samples, representing 440 clinical cases, submitted to the ISU VDL. Reagents from the two manufacturers performed similarly in a large proportion of the field isolates represented. One case was positive with the Tetracore® reagents but negative with the AB reagents and 11 cases were positive by the AB reagents but negative with the Tetracore® reagents. Seven of the discrepant samples were confirmed positive by conventional PCR and/or sequencing. Confirmation of PRRSV status was not achieved for the remaining discrepant samples. These results emphasize the importance of maintaining current primers and probes for the detection of circulating field isolates, especially against agents known to possess a high mutation rate such as PRRSV. Traditionally, good communication between the client and diagnostician(s) has been the primary mechanism to alert labs to potentially false-negative results. In the future, a proactive effort to compare field isolates on an ongoing basis is planned in order to maintain the diagnostic sensitivity of PRRSV PCR.

In vitro Interaction of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV2)

Xue Lin[1], Michelle Hemann[1], Avanti Sinha[1], Huigang Shen[1], Nathan Beach[2], Xiang-Jin Meng[2], Chong Wang[1], Patrick G. Halbur[1], Tanja Opriessnig[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia Polytechnic Institute and State University, Blacksburg, VA

Narrative: In diagnostic submissions where PCV2 and PRRSV coinfection is confirmed, it is not unusual for producers to report severe clinical respiratory disease and wasting with substantial mortality in the group. Based on field data, PCV2 subtype b (PCV2b) is believed to be more pathogenic than PCV2 subtype a (PCV2a). Co-infections may increase the disease severity by enhancing viral replication and by manipulating the host innate immune response. The objectives of this study were: 1) to establish an *in vitro* PCV2/ PRRSV co-infection model and 2) to further investigate the significance of the PCV2 signature motif located in open reading frame (ORF) 2 by utilizing chimeric infectious clones of PCV2a and PCV2b. Pulmonary alveolar macrophages (PAMs) were infected singularly or with combinations of PCV2b/b (ORF1 and ORF2 from PCV2b), PCV2a/a (ORF1 and ORF2 from PCV2a), chimeric PCV2a/b (ORF1 from PCV2a; ORF2 from PCV2b) and chimeric PCV2b/a (ORF1 from PCV2b; ORF2 from PCV2a), and one type 1 PRRSV isolate (SD 01-08) or one of four type 2 PRRSV isolates (SDSU-73, NC-16845, VR-2332, MN-184, JA-142). Culture supernatant was collected at 24, 48, 72 and 96 hours post inoculation (hpi). Real-time PCR assays were used to evaluate presence and amount of PRRSV RNA, PCV2 DNA and PCV2 RNA viral loads. Commercially available quantitative ELISAs were used to measure IFNγ and IL-10 levels. PCV2 replication was confirmed by presence of PCV2 RNA in all groups at 24 hpi. By 48 hpi, PCV2 replication was limited to groups inoculated with ORF1 of PCV2a. As expected, PRRSV RNA PCR indicated that the type 1 PRRSV isolate replicated slower compared to all type 2 PRRSV isolates. There were no significant differences in presence and amount of PCV2 DNA, IFNγ, and IL-10 production among the groups. In summary, in vitro differences in PCV2 replication, PRRSV replication, IFNγ production, and IL-10 production in PAMs inoculated with different combinations of PCV2 and PRRSV were minimal and independent of ORF1 or ORF2 origin.

Development and Validation of VetMAX™-Gold SIV Detection Kit

Angela Burrell, Quoc Hoang, Rohan Shah, Ivan Leyva-Baca, Catherine O'Connell

Life Technologies, Austin, TX

Narrative: Swine Influenza Virus (SIV) is a negative-sense RNA virus of the Influenzavirus A genus, family Orthomyxoviridae. SIV infection results in poor performance of market and breeding animals causing major economic losses to swine producers. Influenza classification is based on the antigenic characteristics of the nucleoprotein and matrix genes. The surface glycoproteins hemagglutinin and neuraminidase are used to further subtype SIV, with H1N1, H3N2, and H1N2 being the most prevalent subtypes. We have validated an SIV detection workflow consisting of high throughput nucleic acid purification and SIV detection from porcine nasal swab samples. The VetMAX™-Gold SIV Detection Kit was evaluated with 121 SIV-positive and 105 SIV-negative nasal swab field samples and virus stocks originating from samples across the US. The SIV status of each sample was confirmed with Virus Isolation (VI) and/or sequencing prior to the start of the study. Of the 121 characterized positive samples processed in this study, 62.8% were of the H1N1 genotype, 14.0% were of the H3N2 genotype, 21.5% were H1N2, and 1.7% was H2N3. Collaborator laboratories purified the viral nucleic acid using the MagMAX™-96 Viral RNA Isolation Kit (AM1836) and MagMAX™-Express instruments. 50 uL of porcine nasal swab virus or supernatant was used for SIV nucleic acid purification. 20,000 copies/rxn Xeno™RNA was spiked into each reaction to serve as an internal positive control for PCR inhibition and nucleic acid recovery. 8 uL of purified RNA was used for each 25 uL PCR reaction amplified with the SIV RNA Test Kit on the AB 7500-Fast Real-Time PCR system using standard ramp mode. RNA isolated from diagnostic nasal swab samples of known SIV status (n=226) were used to determine diagnostic sensitivity and specificity of VetMAX™-Gold SIV Detection Kit. The assay produced 98.4% sensitivity, 99.1% specificity, 99.2% predictive value of a positive test, and 98% predictive value of a negative test for nasal swab samples. Upon further investigation, it was determined that the discrepant positive samples were very low titer samples amplifying near the assay's limit of detection, which resulted in inconsistent detection upon re-testing. The single discrepant negative sample was confirmed to be negative upon re-isolation and repeated qRT-PCR. The original positive test was likely due to cross-contamination during isolation or qRT-PCR set-up. In conclusion testing the Swine Influenza Virus RNA Test Kit on RNA isolated from diagnostic nasal swab samples of known SIV status provides an economical and rapid solution for SIV identification.

Clinical and Pathological Effects of Experimental Bungowannah Virus Infections in Weaner Pigs and Pregnant Pows

Deborah Finlaison[1], Katherine King[1], Melinda Gabor[2], Peter D. Kirkland[1]

[1]Virology Laboratory, Elizabeth Macarthur Agricultural Institute, Camden, NSW; [2]State Veterinary Diagnostic Laboratory, Elizabeth Macarthur Agricultural Institute, Camden, NSW

Narrative: In 2005 the novel pestivirus Bungowannah was identified in pigs affected with the porcine myocarditis syndrome (PMC). The clinical effects of Bungowannah virus following post-natal and *in utero* infection were assessed in two studies. In the first study, 30 weaner pigs were infected with one of six doses of Bungowannah virus to determine if post-natal infection results in disease and/or haematological changes. No disease was observed, although mild temperature rises and decreases in total leukocyte counts were recorded. In the second study, 24 pregnant gilts were challenged with Bungowannah virus at approximately 35, 55, 75 or 92 days gestation and allowed to farrow. Infection of pregnant pigs with Bungowannah virus resulted in reproduction of PMC. Maternal infection at around 35 days gestation had the most severe effects including a combined stillbirth and foetal mummification rate of 42% and a preweaning mortality rate of 70%. Live born pigs were often very weak and showed a limited ability to suckle. At birth, lesions observed included subcutaneous oedema and purpura. Of the 11 pigs that survived to weaning, 9 were persistently infected with this pestivirus. These pigs became severely stunted soon after weaning and died or were euthanased by 11 weeks of age. Infection at 55 or 75 days gestation had the least effects. Progeny with “chronic infections” were identified from sows that were infected at 55 days gestation. These pigs became stunted after weaning, but remained healthy despite prolonged viraemia and virus excretion and seroconverted at about six months of age. Infection at 92 days gestation resulted in an increased preweaning mortality (29%) with some pigs exhibiting signs of cardiorespiratory compromise prior to death. In these pigs infected in late gestation, a multifocal to coalescing necrotising myocarditis was observed in some of the stillborn pigs, and in many of the animals that died suddenly or exhibited cardiorespiratory signs. In conclusion, Bungowannah virus is a significant foetal pathogen in the pig with some clinical outcomes and pathology similar to *in utero* infection with low virulence strains of classical swine fever virus. Clinical and pathological effects following post-natal infection are limited.

Virology and Serology Studies of Experimental Bungowannah Virus Infections in Weaner Pigs and Pregnant Sows

Deborah Finlaison, Katherine King, Peter D. Kirkland

Virology Laboratory, Elizabeth Macarthur Agricultural Institute, Camden, NSW

Narrative: In 2005 the novel pestivirus Bungowannah was identified in pigs affected with the porcine myocarditis syndrome (PMC). During studies of post-natal and *in utero* infection with Bungowannah virus the host-pathogen interaction was measured. Virus loads and virus excretion were monitored by real-time RT-PCR and serological responses by peroxidase linked assay and virus neutralisation test. In the first study, 30 weaner pigs were challenged with one of six doses of Bungowannah virus and the infectious dose determined. Viraemia and viral excretion were detected from 3 days post-inoculation and seroconversion from 10 days post-inoculation. Viral shedding was greatest and most frequently detected in oropharyngeal and nasal secretions, and generally detected in lower amounts and less frequently in conjunctival secretions and faeces. Seroconversion was associated with a marked reduction in viraemia and viral excretion and chronic infections like those seen in some cases of classical swine fever were not observed. In the second study 24 pregnant pigs were challenged with Bungowannah virus at approximately 35, 55, 75 or 92 days gestation. The foetuses from 20/23 of the challenged dams became infected. With the exception of one piglet, all foetuses within the litters of infected sows were also infected and all foetuses were still viraemic at birth regardless of the stage of gestation at which infection had occurred or whether the foetus had mounted a humoral immune response to the virus. Few foetuses developed antibodies after infection at 35 days of gestation whereas all foetuses infected at 75 days were seropositive at birth. Post-natally, Bungowannah virus was cleared most rapidly from the progeny of dams infected at approximately 92 days gestation. Persistently and 'chronically' infected pigs were identified following infection of the dam at 35 and 55 days gestation respectively. The 'chronically' infected pigs seroconverted at a variable but lengthy time after birth and ceased shedding virus. These studies show that the course of infection following post-natal infection with Bungowannah virus is typical of other pestiviruses with viraemia and viral excretion resolving after seroconversion. Following *in utero* infection prolonged infections are observed despite a serological response by the foetus. In addition, persistent and 'chronic' infections also occur following infection in early gestation.

Evaluation of DNA Purification and Quantitative PCR Methods for the Rapid Detection of Bovine Herpesvirus 1

Megan Schroeder, Mangkey Bounpheng, Alfonso Clavijo

Molecular Diagnostics, Texas Veterinary Medical Diagnostic Laboratory, College Station, TX

Narrative: Bovine herpesvirus 1 (BoHV-1) is an economically important pathogen that causes infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, infectious balanoposthitis, and abortions in cattle. BoHV-1 is transmitted through respiratory and genital secretions, and by artificial insemination (AI) with BoHV-1 contaminated semen. Following initial infection, the virus enters neural cells and establishes a life-long latent infection in the sensory ganglia that can later be re-activated. Infected bulls are life-long carriers and may shed virus in their semen intermittently, resulting in potential virus transmission through AI. Due to the wide use of AI in the cattle breeding industry, BoHV-1 detection is critical to the international bovine semen trade and maintenance of BoHV-1-free status in disease-free countries. Several testing methods for BoHV-1 exist, including VI, ELISA, and VN, however, the most rapid and sensitive is the OIE prescribed quantitative PCR (qPCR) which utilizes Chelex 100, Proteinase K, and DTT method for DNA purification and TaqMan PCR for BoHV-1 detection. To streamline our semen diagnostic testing, we compared the OIE method with our method consisting of the automated magnetic bead based DNA purification technology (MagMAX TM reagents and Kingfisher Magnetic Particle Processor) and Path-ID PCR Master Mix; identical primers and probe sequences were used for both methods. Dilutions (10-fold, spanning 5 logs) of reference strains (n=3) were spiked in negative semen to compare analytical sensitivity and negative semen was used to compare specificity. Both methods produced equivalent specificity, however the OIE method was 1 log (~3 Ct) less sensitive and less consistent/reproducible (higher standard deviations among replicates). Based on these comparisons we selected the MagMAX and Path-ID method for our routine semen diagnostic testing. We have tested 824 bovine semen samples using our method with the following results interpretation guidelines. Samples with Ct ≤ 35.0 were considered positive; Ct 35.1-39.9 were retested in triplicates (samples permitting) and if amplification was observed in all replicates, samples were considered positive. Of 824 samples tested, 13 samples were Ct ≤ 35.0 (Ct 24.5-35) , 27 samples were Ct 35.1-39.9 and were retested; of the 27 retests, 2 samples (Ct 35-36) were repeatable; 25 samples (Ct >36) were non repeatable. Based on these results, we have established the Ct cutoff value for BoHV-1 positive samples to be 36.0 and have identified 15 positive samples (~1.8% positive rate). In conclusion, we have developed a streamlined nucleic acid and qPCR method for easy and fast (~2 hrs) BoHV-1 detection in semen.

Bovine Herpesvirus 1 Isolated from Multiple Abortions in Recently Vaccinated Herds

Binu Velayudhan[1], James Trybus[1], Alfonso Clavijo[2], Robert Sprowls[1]

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

Narrative: Bovine herpesvirus 1 (BoHV-1) causes an economically significant respiratory/reproductive disease in cattle called infectious bovine rhinotracheitis (IBR) or infectious pustular vulvovaginitis (IPV). Though vaccination has reduced the occurrence of clinical disease to a great extent in cattle, sporadic incidences are not uncommon. In February-March, 2010, our laboratory received three cases of multiple abortions from two breeding herds in the Texas Panhandle. The heifers were vaccinated with a modified live virus vaccine (MLV) containing IBRV and bovine viral diarrhea virus (BVDV) annually and at pregnancy check during the second trimester. One submission consisted of aborted fetus with moderate post-mortem autolysis. Fetal tissues were inoculated into various cell culture systems for virus isolation. Bovine turbinate cells inoculated with brain tissue sample showed cytopathic effect in the form of rounding and clumping (grape-like clusters) of cells. The cells were fixed and stained with BoHV-1 specific antibodies conjugated with fluorescein isothiocynate. The cells showed positive nuclear staining specific for BoHV-1. Histopathology revealed multifocal necrotizing hepatitis. Immunohistochemistry (IHC) of liver showed cytoplasmic staining for BoHV-1 antigen. The second submission included serum samples and fresh and fixed placenta. This ranch had 130 bred heifers. Heifers had received three MLVs containing IBRV and BVDV before breeding and then one MLV at pregnancy check. Abortions started in the third trimester. Serology showed positive titers for IBRV and BVDV. BoHV-1 was isolated from placenta and was confirmed by fluorescent antibody staining. The heifer from which IBRV was isolated had a BoHV-1 antibody titer of >256. Histopathology of placenta demonstrated suppurative inflammation. Subsequent IHC staining showed the presence of BoHV-1 antigen. The third submission consisted of sera and fresh and fixed placenta. The placenta showed moderate histologic suppurative inflammation. IHC showed staining for BoHV-1 antigen. Serum was positive for IBRV antibodies. BoHV-1 was isolated from placenta via cell culture and confirmed by fluorescent antibody staining. Analysis of gD gene segment of BoHV-1 showed high homology between the vaccine strain and the clinical isolates. Though more detailed analyses are required for a definitive conclusion, these results suggest that the virus we isolated could have originated from the vaccine strain. The use of MLV in pregnant heifers/cows is a topic that needs further investigation. A more thorough study on these and similar cases may reveal why these vaccine-induced abortions occur and also support development of more effective and appropriate intervention strategies with less risk of undesired outcomes

Survey of Bovine Respiratory Disease Complex (BRDC) Pathogens from Clinical Cases Submitted to Kansas State Veterinary Diagnostic Laboratory (KSVDL) Using a Real-Time PCR Panel

Richard Hesse, Joe Anderson, Barbara Breazeale, Alex Fuller, Jianfa Bai, Elizabeth Poulsen, Gary A. Anderson, Mike Hays, Richard Oberst

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

Narrative: The KSVDL initiated the use of a BRDC real-time PCR panel for diagnostic use in the summer of 2010. Samples routinely tested included clinical submissions from fatal BRDC cases as well as nasal swabs from live animals exhibiting respiratory disease. As of April 2011, a total of 530 samples have been submitted for BRDC real-time PCR testing; 322 were from tissues obtained at necropsy and 208 were swab samples usually received in the mail. Positive PCR results for one or more of the pathogens were observed in 54% of the tissue samples and 43% of the nasal swabs. Individual pathogen results from tissue samples were: *Mycoplasma bovis* (*M. bovis*) 41%, bovine viral diarrhea virus (BVDV) 38%, infectious bovine rhinotracheitis (IBR) 12%, bovine coronavirus (BoCV) 17%, bovine respiratory syncytial virus (BRSV) 5% and bovine parainfluenza virus (PI3) 4%. Concurrent infections from the tissue samples demonstrated that *M. bovis* and BVD were most frequent and infections where *M. bovis* was excluded demonstrated that BVDV and BoCV were the most frequent followed by BVDV and IBR. Individual pathogen results from swab samples were: *M. bovis* 18%, BVDV 5%, IBR 13%, BoCV 30%, BRSV 1% and PI3 4%. Concurrent infections from swab samples demonstrated that BoCV and *M. bovis* were the most frequent followed by BoCV and IBR. BVDV genotyping and isolation were conducted on a subset of the submissions; genotypes 1A, 1B and 2A were observed in approximately equal frequency and a number of the viruses were cytopathic in cell culture. Frequency and sequence alignments of the genotypes detected will be discussed. The preliminary data obtained in this survey demonstrate the utility of the BRDC real-time PCR assay as a diagnostic tool for pathogen detection and subsequent analysis of those agents.

A New Fluorescent Microsphere Immunoassay Platform and Comparison with the Existing Platform

Jessica Green[1], Michaela Hoffmeyer[2], Tammy Koopman[1], Richard Hesse[1], Bob Rowland[1]

[1]Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS; [2]Life Science Research and Food Safety, Luminex Corporation, Austin, TX

Narrative: Fluorescent microsphere immunoassay (FMIA) is a relatively new technique for serologic diagnosis of infectious disease. Advantages to the use of FMIA over traditional serologic techniques such as ELISA include the detection of multiple targets in a shorter time period with a single, small volume of sample; greater sensitivity and specificity; and decreased cost. This technology can also be adapted for use on non-serum samples such as oral fluid. The current technology (e.g. Bio-Plex 200) uses fluorescently labeled polystyrene beads coated with antigen combined with flow cytometric instrumentation to simultaneously categorize bead types and detect bound antibody. A newer modification of FMIA technology is the MAGPIX from Luminex which relies on the use of magnetic microspheres. This simplifies and reduces the time and cost of washing steps in both coupling and assay procedures. The magnetic property of the microspheres also allows their adherence in a monolayer and detection by LED optics and a digital imaging system. This results in decreased cost, ease of use, and a smaller lab bench footprint for the MAGPIX. Magnetic beads can be used in the Bio-Plex 200 instrument, but the MAGPIX instrument will not accept non-magnetic beads. The purpose of this project was to compare the analytical performance of the MAGPIX to the Bio-Plex 200. Recombinant porcine viral antigens including PCV2 capsid protein and PRRS type I and II nucleocapsid proteins were expressed, column purified and bound to magnetized polystyrene beads. Antigen coated beads were then tested with a panel of 36 positive and negative serum samples previously characterized by “gold standard” tests, IFA for PCV2 and a commercial IDEXX ELISA for PRRSV. The same 96-well plate was run on both instruments and instruments were calibrated using manufacturer recommended calibration kits. The results indicate MFI values correlated well between the two instruments and correlated well with gold standard results. In some cases the MAGPIX exhibited a greater dynamic range. The results show that the MAGPIX instrument is comparable to the existing Bio-Plex 200 platform. The retention of good analytical performance combined with reduced costs for the MAGPIX instrument and associated sample preparation creates the opportunity for the use of FMIA by smaller diagnostic laboratories or as an additional instrument run in parallel in laboratories which require high throughput capabilities.

Equine Herpesvirus 1 Outbreak at a Racetrack in Ohio

Yan Zhang, Jing Cui, Alex Hamberg, David Newman, Jeff Hayes, Tony Forshey, Beverly Byrum

ADDL, Ohio Dept of Agriculture, Reynoldsburg, OH

Narrative: An outbreak of neurologic disease occurred at a horse racetrack between December 20, 2010 and January 8, 2011. Two horses were euthanized on site due to neurologic disease. Nonsupporative meningoencephalomyelitis was observed in both horses, suggesting a viral infection. Nasal swab and paired serum samples were collected from three additional febrile horses from the same barn as the dead horses and were submitted to Ohio Animal Disease Diagnostic Laboratory. PCR and virus isolation were positive for equine herpesvirus 1 from all swab samples from those horses. Viral neutralization test performed on the paired serum samples from the febrile horses showed four-fold or greater increase of antibody titer against equine herpesvirus 1 virus. A quarantine was placed on the entire racetrack and a strict biosecurity policy was implemented to prevent the spread of the disease. Fever watch followed by PCR testing was used for surveillance of the disease for the entire facility. Due to the efficient measures and control procedures, the outbreak was controlled and limited to only one barn.

Isolation of Equine Rhinitis A Virus from Stallion Semen

Donna Johnson, Eileen Ostlund, Beverly Schmitt

National Veterinary Services Laboratories, Ames, IA

Narrative: Semen from a healthy, four year old quarter horse was submitted to the National Veterinary Services Laboratories (NVSL) for virus isolation testing for equine arteritis virus (EAV). The testing was required to qualify the EAV-seropositive stallion for export. Upon arrival at the NVSL, the semen was visually inspected for suitability for testing and microscopically examined to confirm presence of sperm, as per EAV testing requirements. Ample spermatozoa were observed, however it was noted that the semen sample was an unusual yellow color. The sample was inoculated onto rabbit kidney cell cultures. Five days post inoculation, cytopathic effects were observed in the inoculated cell culture. Electron microscopic examination of the isolate revealed particles with the morphologic appearance of picornaviruses. The cell culture isolate was ultimately identified as equine rhinitis A virus (ERAV) by RT-PCR and sequencing. Subsequent RT-PCR testing of the semen confirmed the presence of ERAV in the original sample submitted for testing. This is the first report of an isolation of ERAV from equine semen. However, isolation of ERAV from the urine of infected horses is well documented. It is suspected that the semen submitted was contaminated with ERAV-positive urine during collection. Laboratories should be aware of the possibility of ERAV contamination when testing equine semen.

Pathological Lesions and Patterns of Luciferase Luminescence in CD-1 Mice Exposed to Aerosol and Subcutaneous Infection with a Recombinant Neurovirulent Western Equine Encephalitis Virus

Aaron Phillips, Tawfik Aboellail, Kenneth Olson

Colorado State University, Fort Collins, CO

Narrative: Two groups (10 mice each) of 5-6 weeks old, outbred CD-1 mice were infected either intranasally or subcutaneously dorsal to the cervical spine with a recombinant western equine encephalitis virus (WEEV). The virus was constructed based on the infectious clone of the severely neurovirulent McMillan strain of WEEV. The recombinant virus possesses a duplicate subgenomic promoter that drives the expression of firefly luceiferase. *In vivo* imaging was conducted on an IVIS 200 instrument to compare degree and pattern of luminescence in infected mice versus control mice (3 uninfected animals) 10 minutes after injection of 150 mg/kg luciferin. For the *Ex-vivo* images, mice were injected with 150 mg/kg luciferin five minutes prior to euthanization. After humane and expeditious euthanasia with inhalant gas (Iso-Flo), the heads of sacrificed mice were sagittaly split along the midline and immediately imaged at 24, 36, 48, 72, 84, 96 and 108 hours respectively. The severity of clinical symptoms and mortality rate was significantly higher in the intranasally infected mice than in the subcutaneous group. The degree and pattern of luciferase luminescence suggests that the virus spreads via olfactory nerve to the olfactory bulb in the intranasal route of infection. Pathologic lesions in the olfactory bulb are more concentrated in the glomeural layer, external plexiform layer and granular layer being more severe in the external plexiform layer. Multifocal neuronal and glial cell necrosis with status spongiosis of the neuropil and infiltration of affected areas by neutrophils are evident in the olfactory bulb as early as 48-72 hours postinfection (PI). In the subcutaneously infected mice, Luciferase luminescence was evident in the more caudal quadrants of cerebral hemispheres and tends to spare the cerebellum. Histologically those mice show more randomly scattered areas of perivascular necrosis and inflammation that are not restricted to the olfactory bulb.

AAVLD/USAHA Joint Plenary
Evolving Food Systems for Global Food Security:
Can Animal Production and Veterinary Infrastructure Keep Up?
Monday, October 3, 2011
Grand Ballroom A/B/C

Moderators: Terry McElwain

7:45 AM **Welcome – Dave Marshall, USAHA Vice-President and Program Chair**

8:00 AM **Making Safe, Affordable and Abundant Food a Global Reality**
Ted McKinney…….. *137*

8:40 AM **Markets, Infrastructure and Animal Production Systems**
Tom Marsh……. *138*

9:20 AM **Role of Veterinarians in Global Food Security**
Ron DeHaven……. *139*

10:00 AM **BREAK**

10:20 AM **Veterinary Diagnostic Laboratories Role in Food Security**
Alfonso Torres…… *140*

11:00 AM **Rinderpest Control and Eradication as a Working Example of Utilizing the World's Veterinary, Laboratory, and Research Resources to Exact a Tangible, Measurable Impact on a Food Animal Disease Directly Affecting Food Security for a Significant Part of the World**
William Taylor…….. *141*

11:40 AM **Roundtable Question and Answer Discussion**

Making Safe, Affordable and Abundant Food a Global Reality

Ted McKinney

Elanco, Greenfield, IN

Narrative:

By 2050, we'll need 100% more food, and according to the U.N. FAO, 70% of it must come from efficiency-enhancing technologies. We must call a truce to the debate about the role of technology in the sustainable production of safe, affordable and abundant food if we are to protect the Three Rights: 1) Ensuring the human right of all people around the world to have access to affordable food 2) Protecting all consumers' rights to spend their food budget on the widest variety of food choices 3) Creating a sustainable global food production system, which is environmentally right. Key Point: The challenge of world hunger is complex and multifaceted. Allowing the entire food chain access to safe, efficiency-enhancing technologies is an essential component of a comprehensive solution to the challenge-both locally and globally. In addition, protecting the right to choose these technologies can make the dream of safe, affordable and abundant good a reality worldwide.

Speaker Biography:

Ted McKinney is Director, Global Corporate Affairs, for Elanco Animal Health, a division of Eli Lilly and Company. Prior to Elanco, McKinney spent many years in a variety of Corporate Affairs positions at Dow AgroSciences, the last being as lead for global Food Chain & State Government Affairs. During his Dow AgroSciences tenure, he took leave in 2000 to serve as co-founder and Interim Executive Director for the Council for Biotechnology Information, a public information program sponsored by a consortium of companies involved with the development of crop biotechnology. He also led the company's biotechnology public affairs efforts. He began his career in 1981 with Elanco Products Company, a division of Eli Lilly and Company, and spent time in several sales and marketing assignments. In 1986, he moved to Lilly, the parent company, with responsibility for media relations for the agriculture business and for environmental issues. In 1990, he joined DowElanco, a joint venture company formed by The Dow Chemical Company and Lilly. There, he established the Community Affairs and Industry Associations programs and later, added responsibilities for State Government and Public Affairs for the Midwestern States. He is co-chair of the 2006-12 National FFA Conventions Local Organizing Committee and a member of the Indiana State Fair Commission. He serves on the board of directors for the Washington, D.C.-based International Food Information Council (IFIC) and serves as Food Technology & Sustainability Committee Chair. He is also on the Lead Team for the International Federation of Animal Health (IFAH), and liaison to a number of food chain related associations. He also recently completed a year as Chair of the Indiana Agriculture Regulatory Structure Task Force, commissioned by the Lt. Governor of the State of Indiana. McKinney is a 1990-91 graduate of the Indiana Agricultural Leadership Program and a 1991-92 graduate of the Stanley K. Lacey Indianapolis Executive Leadership Series. He was honored in 2004 by the National FFA Organization with an honorary American FFA Degree. In 1981, McKinney received a B.S. Degree in Agricultural Economics from Purdue University. There, he received the G.A. Ross Award as the outstanding senior male graduate. In 2002 he was named a Purdue Agriculture Distinguished Alumnus.

Markets, Infrastructure and Animal Production Systems

Tom Marsh

Washington State University, Pullman, WA

Narrative:

World population has more than doubled since 1960, with continued growth projected in the future. Consequently, increased population and income growth have fueled a rising demand for protein in developed and developing countries. This includes protein from livestock. Global demand for livestock protein is here to stay in the foreseeable future. Among the developed countries, the U.S. is one of the largest consumers of livestock and meat products. Simply put, we like our meat. In contrast, in less developed countries, culture and economic circumstances can dictate demand for livestock protein. For instance, in Eastern Africa, smallholder pastoralists are dependent upon milk from cattle. A preponderance of empirical evidence on the demand for food suggest that it would take major structural changes in economies and in cultures/traditions across the world to permanently shift preferences away from consumption of livestock products or to alter strongly formed consumption habits. A safe and secure livestock supply chain is critical to meet demand, now and in the future. An efficient, productive, and sustainable livestock sector is simply not feasible without adequate veterinarian infrastructure. Investment in infrastructure in the U.S. has enabled producers of livestock to successfully combat diseases, and as a result obtain loans, grow assets, retain value, and accumulate wealth. Except for isolated events (e.g., BSE) the U.S. has had access to international markets for exports. This is not so in other parts of the world. In East Africa, there are limits on resources for veterinarian infrastructure/services, surveillance, control of transboundary diseases, and response. As a result, smallholders can lose a majority of their calf crop to livestock diseases and they have limited if any access to international markets. Because livestock are their primary assets and the main source of wealth for the smallholder, these circumstance help perpetuate a poverty trap. In the current economic circumstances, it is understandably important to scrutinize federal or state budgets and to prioritize resource allocations. However, short term political decisions should not outweigh critical investment into livestock and human health infrastructure and services. These investments are necessary to combat livestock diseases, facilitate sustainable production systems, provide safer and more secure food, and maintain or improve household well-being.

Speaker Biography:

Dr. Thomas L. Marsh is a Professor in the School of Economic Sciences at Washington State University, and holds affiliated appointments in the Paul Allen School for Global Animal Health and Department of Statistics at WSU. Dr. Marsh's major areas of study are marketing and international trade, quantitative methods, and natural resource economics. Specific areas of research include estimating the impact of public food safety information on consumer demand, as well as measuring welfare and trade impacts of livestock diseases (including BSE, FMD, and E. Coli) and policy responses. Dr. Marsh was raised on a cattle ranch near the Canadian border in Northeastern Montana, which continues to be operated in the family as a cow-calf and backgrounding operation. Dr. Marsh is a member of the Washington State Academy of Sciences, and also serves as Director of the IMPACT Center at Washington State University. He teaches Ph.D. courses in econometrics and an undergraduate course in management economics. He is an associate editor of the top economic field journal in agricultural, the American Journal of Agricultural Economics.

Role of Veterinarians in Global Food Security

Ron DeHaven

American Veterinary Medical Association (AVMA), Schaumburg, IL

Narrative:

National and State veterinary services in the U.S. and elsewhere have a proud and distinguished history of animal disease exclusion, detection, control, and eradication. Animal production in the U.S. has advanced to its current global leadership role due, in no small part, to the partnership between federal, state, and industry animal health officials. By working collaboratively to promote and improve the health of our livestock and poultry populations, these groups are able to produce the safest, most abundant food supply in the world. Global demand for animal protein has been increasing exponentially for several years and that demand is expected to accelerate as the world population continues to grow and as the economies of many developing countries continue to improve. Terrestrial and aquatic production systems are becoming larger and more integrated, making the potential impact of disease introduction much greater. The everyday movement of animals and animal products in a global marketplace has exaggerated the potential for disease spread, with the incubation period of many emerging and re-emerging disease shorter than the time it takes to move people and animals from one corner of the globe to another. Ultimately we have a growing dependence on animal protein at the same time that the risks to systems that produce that protein are at an all-time high. The systems designed to protect that animal production infrastructure reside largely in national veterinary services. At a time when national and state (public) veterinary services need to be stronger and more effective than ever, many countries are actually experiencing an alarming decline in the investment in national veterinary services. Similarly, private sector veterinary practice in developed countries has been impacted by the consolidation of animal operations, resulting in fewer veterinarians overseeing larger populations of animals, leaving many large rural areas totally without the benefit of veterinary professionals. This has not only impacted the delivery of necessary veterinary medical care to small producer operations, but also reduced the trained workforce necessary for early detection and rapid, effective response to a disease outbreak. Driven in large part by the global economic downturn, politicians and government officials are looking increasingly at cutting funding to animal health infrastructure within their national and state veterinary services as a source of savings. The irony is that the relatively small savings that can be realized by such funding cuts puts at risk huge production system as well as economically important domestic and international markets.

Speaker Biography:

Dr. Ron DeHaven is the Chief Executive Officer and Executive Vice President of the American Veterinary Medical Association (AVMA), where he serves over 81,500 members of the AVMA as they work to meet the challenges of improving both human and animal health in the 21st century. Dr. DeHaven has more than two decades of experience with the United States Department of Agriculture's (USDA) Animal Plant Health Inspection Service (APHIS) and gained national prominence in 2003 and 2004 when bovine spongiform encephalopathy and avian influenza were making headlines. Dr. DeHaven received the President's Rank Awards (Meritorious and Distinguished) for his leadership. He also received the Secretary's Honor Award twice. The AVMA honored Dr. DeHaven's contributions to the veterinary profession with the Meritorious Service Award in 2004. He also received the Roswell Award from the Scientists Center for Animal Welfare and an honorary Doctor of Science degree from Purdue University in 2005. Dr. DeHaven has been the CEO of AVMA since August 2007. As APHIS administrator from 2004 to 2007, Dr. DeHaven was ultimately responsible for the protection of U.S. agriculture and natural resources from exotic pests and diseases, administering the Animal Welfare Act, and carrying out wildlife damage management activities. Prior to starting work at APHIS, Dr. DeHaven was commissioned into the U.S. Army Veterinary Corps and served in the U.S. Army Reserves and National Guard. Dr. DeHaven obtained his doctor of veterinary medicine degree from Purdue University in 1975 and a master's degree in business administration from Millsaps College in 1989.

Veterinary Diagnostic Laboratories Role in Food Security

Alfonso Torres

College of Veterinary Medicine, Cornell University, Ithaca, NY

Narrative:

It is well recognized that animal disease outbreaks, particularly those that are highly contagious or of foreign or emerging nature, often cause serious local to global negative socioeconomic consequences. A key element in the prevention and control of such disease outbreaks is the ability to provide a prompt and accurate diagnosis of the event. Veterinary diagnostic laboratories, whether federal, state, or private, provide a critical front line defense for the prevention and control of these potentially catastrophic animal or zoonotic disease events. We should be proud of the fact that the discipline of veterinary diagnostics has reached a high scientific level, comparable to (and in cases better than) that used for the diagnosis of transmissible diseases in humans. The same can be said for the state of veterinary diagnostics in areas of anatomical and clinical pathology for non-infectious conditions. The development of veterinary and public health diagnostic laboratory networks in the United States has been a great accomplishment serving as a model for the rest of the world, particularly for the development of regional laboratories in developing nations. The recent outbreaks of highly pathogenic avian influenza with their potential role in generating a human influenza pandemic eventually demonstrated to the world the need to invest in developing a robust network of influenza diagnostic laboratories all over the globe. Unfortunately, the sustainability of those laboratories is now in jeopardy as the "influenza bubble" has passed, and unfortunately we continuing to face a national and global crisis in the provision of vital veterinary diagnostic services for many diseases at a time when the demands for veterinary diagnostic services is increasing due to: (a) increasing demands on productivity of our farms to feed an ever increasing global population that requires more and more protein of animal origin; (b) increased risk of introduction of transboundary animal diseases due to greater than before travel, trade and geopolitical instabilities; (c) continuous threat of bioterrorism; and (d) the continuing possibility of the emergence of new or altered infectious diseases. To compound those challenges, the affordable delivery of quality veterinary medical and diagnostic services is being seriously challenged due to the current national and global economic crisis.

Speaker Biography:

A native of Bogotá, Colombia, Dr. Torres holds a doctor of veterinary medicine degree from the National University of Colombia. He also has earned a Master of Science degree in veterinary pathology from the University of Nebraska, and a doctorate in medical microbiology, specializing in virology, from the University of Nebraska Medical Center. Dr. Alfonso Torres served as Deputy Administrator of USDA-APHIS-Veterinary Services from 1999 to 2002. In that capacity he was also the United States' Chief Veterinary Officer and the US delegate to the World Organization for Animal Health - OIE. From 1996 to 1999, Dr. Torres was the Director of the Plum Island Animal Disease Center (PIADC) on Plum Island, NY. While at PIADC, Dr. Torres also served as chief of USDA's Foreign Animal Disease Diagnostic Laboratory (FADDL) from1994 to1996. Prior to serving at the USDA, he worked for SmithKline Beecham Animal Health from 1987 until 1991. Dr. Torres served as an Associate Professor of virology at Cornell University's College of Veterinary Medicine, from 1983 until 1987. He held a similar position at the University of Nebraska from 1978 through 1983. Dr. Torres returned to Cornell in 2002 as professor and Associate Dean for Public Policy. He coordinates all government relations and international programs for the College of Veterinary Medicine and supervises the College biosafety program. He teaches the Foreign and Emerging Animal Disease Course and coordinates the USDA-funded "Smith-Kilborne Foreign Animal Disease Program" that brings every year one veterinary student from all the US Colleges to Cornell and to PIADC, Plum Island. He also coordinates the USDA-funded International Course on Transboundary Animal Diseases delivered at PIADC, Plum Island, for veterinarians from many parts of the world. Dr. Torres interests are in the area of transboundary and emerging animal diseases, global animal health, bioterrorism, biosecurity and animal health public policy. Contact information: Alfonso Torres, DVM, MS, PhD. Professor & Associate Dean for Public Policy College of Veterinary Medicine Cornell University S2-005C Schurman Hall Ithaca, NY 14853-6401 Tel.: (607) 253-3480 Fax: (607) 253-3701 e-mail: at97@cornell.edu.

Rinderpest Control and Eradication as a Working Example of Utilizing the World's Veterinary, Laboratory, and Research Resources to Exact a Tangible, Measurable Impact on a Food Animal Disease Directly Affecting Food Security for a Significant Part of the World

William Taylor

Independent Consultant, Littlehampton

Narrative:

In the 17th and 18th centuries rinderpest spread unchecked across Europe causing the death of millions of cattle and thereby earned its fearsome reputation. Eventually, legislative measures, the establishment of veterinary schools and state veterinary services brought the disease under control and by the end of the 19th century rinderpest had been eradicated from Europe. Further progress in control awaited the end of the Second World War when China rapidly eradicated rinderpest using an intensified strategy that embraced newly available, large scale vaccination. Around 1960 an international appreciation of the need to eradicate rinderpest as a food security issue began to form. Starting in south Asia, FAO became involved in introducing vaccine technology in Cambodia and Thailand and eradication campaigns in these, and neighbouring countries, were quickly successful. The international community was also looking at Africa in the realisation that considerable external financial assistance would be required to achieve a similar objective. This came, firstly through USAID, later through FAO and finally from the EU. After three decades of mass vaccination the incidence of rinderpest in Africa finally fell to zero in 2001. In the 20th century the control of rinderpest became increasingly reliant on improvements in vaccine production technology which ultimately yielded egg, goat, rabbit and cell culture attenuated strains and finally, a thermostable variant of the latter. Applied research demonstrated the absence of reservoir hosts in wild life and showed the presence of three lineages of the virus with variable R0 values. Rinderpest diagnostics moved on from the simple agar gel immunodiffusion test and virus isolation procedures to the RT-PCR for virus confirmation and the immunocapture ELISA for large scale serum testing. In 1954 India embarked on her national eradication campaign relying on mass vaccination but required 40 years and a major strategy review to complete the task. Nevertheless India saw her last case of rinderpest in 1995 and from then to the last case globally took a mere six years. This demonstrated the need for management practices to keep pace with strategy concepts, which were proceeding apace, with the OIE developing a pathway approach dependent on a zero incidence of disease, an end to vaccination and a subsequent evidence-based clinical and serological assessment. Throughout, FAO provided the necessary elements of international co-ordination and cohesiveness. Globally rinderpest was eradicated in 2001 and over the next decade all recently infected countries provided evidence of this achievement at a technical level.

Speaker Biography:

Dr Taylor graduated BVM&S from Edinburgh University in 1962 and BSc in1963. In 1972 he was awarded his PhD from the Australian National University, Canberra. From 1963 to 1968 he worked at the East African Veterinary Research Organisation at Muguga, Kenya where he studied the pathogenesis of rinderpest virus in cattle, the epidemiology of the virus in game animals and the isolation of field strains in cell culture: he also initiated the manufacture of Plowright's famous attenuated cell culture rinderpest vaccine in East Africa. From 1973 to 1978 he worked at the National Veterinary Research Institute at Vom, in northern Nigeria where he established field evidence that Peste des Petits Ruminants was a new morbillivirus and not, as hitherto proposed, a rinderpest mutant. He also worked extensively on Bluetongue and African Horse Sickness showing each was an endemic but sub-clinical condition of local cattle and horses respectively but with the potential to cause severe disease in imported stock. From 1979 to 1985 Dr Taylor worked at the IAH, Pirbright, UK on bluetongue in the Middle East and on rinderpest in the Gulf States. From 1986 to 1988 he worked in Nairobi with the Pan African Rinderpest Campaign, initially as FAO epidemiologist, later as an EU Technical Advisor. In 1989 he moved to New Delhi, India as the EU Technical Advisor to the Government of India's National Project for Rinderpest Eradication where he designed a strategy that took India from a rinderpest infected to a rinderpest-free country. In 1997 he assisted FAO in halting a rinderpest epidemic in Tanzania and for the next seven years, regularly visited Pakistan as an FAO consultant, assisting that country to qualify as rinderpest-free. In 2010 he chaired a Joint FAO-OIE Committee to evaluate the soundness of a global declaration of rinderpest eradication.

AAVLD Poster Session

Friday, September 30, 2011 – Sunday, October 2, 2011

Grand Hall

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

The National Science Advisory Board for Biosecurity (NSABB)-Will Their Work Impact Yours?

Tanya Graham

Veterinary & Biomedical Sciences Department, South Dakota State University, Brookings, SD

Narrative: The NSABB is a federal advisory committee that was established in 2005 as a result of concern over dual use research and the potential implications for bioterrorism. The board is chartered to "[p]rovide advice, guidance, and leadership regarding biosecurity oversight of dual use research." [2], According to the NSABB charter, the NSABB board may have no more than 25 voting members. Voting members are considered special government employees with secret security clearances and are considered subject matter experts. These experts provide expertise in molecular biology, microbiology, clinical infectious diseases, laboratory biosafety and biosecurity, public health/epidemiology, health physics, pharmaceutical production, veterinary medicine, plant health, food production, bioethics, academia, national security, biodefense, intelligence, national security, and law and law enforcement. Voting members also provide perspectives in medical and scientific journal publishing, industry, public awareness, institutional biosafety committees, recombinant DNA, and export control. The NSABB does not authorize or ban specific experiments and only provides guidance on individual experiments at the request of the Secretary of HHS. The NSABB does, however, advise the Secretary of Health & Human Services (HHS), the Director of the National Institutes of Health (NIH), and the heads of federal agencies that conduct or support life sciences-related research by "...recommend[ing] specific strategies for the efficient and effective oversight of federally conducted or supported dual use biological research, taking into consideration national security concerns and the needs of the research community."[1,2] The current poster will discuss the development of the NSABB and their Proposed Framework for the Oversight of Dual Use Life Science Research: Strategies for Minimizing the Potential Misuse of Research Information. This document is intended to form a framework for the federal government to develop a system for the "Responsible identification, review, conduct, and communication of dual use research."[3]

References:

1. Atlas R. Biosecurity concerns: Changing the face of academic research. Chemical Health & Safety 2005;12(3):15-23. Available at: http://biossegurancaemfoco.com/wp-content/uploads/2009/10/Biosecurity-Concerns.pdf. Accessed Feb. 18, 2011.

2. National Science Advisory Board for Biosecurity (NSABB). Available at: http://oba.od.nih.gov/biosecurity/about_nsabb.html. Accessed Feb. 18, 2011.

3. National Science Advisory Board for Biosecurity (NSABB). Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information. 2007.

Available at: http://oba.od.nih.gov/biosecurity/pdf/Framework%20for%20transmittal%200807_Sept07.pdf.

USDA/NAHLN Quality Management System Training

Barbara M. Martin[1], Terry McElwain[2], Patricia Lukens[2], Kelly Burkhart[1], David S. Korcal[3], Laura Torchin[4], Sharon Hietala[4], Joseph Kellum[5], Tina Buffington[6], Shawna Middleton[6], Eleanor Britten[6], Kathryn Moser[6], Ruth Smith[6], Katherine Burch[7]

[1]National Animal Health Laboratory Network, United States Department of Agriculture, Ames, IA; [2]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA; [3]Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI; [4]California Animal Health & Food Safety Laboratory System, University of California, Davis, CA; [5]Mississippi Veterinary Research & Diagnostic Laboratory, Mississippi State University, Starkville, MS; [6]National Veterinary Service Laboratories, United States Department of Agriculture, Ames, IA; [7]Center for Plant Health Science and Technology, United States Department of Agriculture, Raleigh, NC

Narrative: Laboratories in the United States and around the world are increasingly relied upon to produce critical information important to animal disease surveillance, identification, and eradication. To establish confidence in their diagnostic test results, laboratories must have well-implemented and well-managed quality systems. Additional national and global sources for training in quality system principles and practices are needed to assist laboratory staff in understanding, creating and using quality systems. To achieve this, the USDA/NAHLN has developed a standardized, comprehensive training program, Quality System Management, to provide training in the principles, implementation and management of a quality system. Topics covered in the Quality Management System training have been gleaned from common areas of deficiency observed during AAVLD and NAHLN audits. The course covers some of the most challenging elements of a quality system including: Overview of Quality Management System Requirements, Implementation of a Quality System, Document Control, Records, Corrective Action/Root Cause, Equipment, Continuous Improvement/Control Trend Tracking, Training, Complaints, Internal Audits, and Management Reviews. It is intended for laboratory staff new to quality system principles. The instructors, from USDA/APHIS/NALHN and State animal disease diagnostic laboratories, have included examples of documents, practical tips and best practices for implementation and management of quality processes. In addition, workshops on Corrective Action/Root Cause and Management Review allow trainees to practice the principles learned during the presentations. Interactive games are used to review concepts. The final day is a “wet lab”, providing an opportunity to audit a mock laboratory and use the skills necessary to evaluate laboratory policies, documents and processes to assess compliance with the standard. Three QMS training sessions have been held to date; the last included trainees from the Eastern Europe and Africa. Improvements and additions are continuously made from trainee evaluation and feedback. On line training modules for access by laboratorians worldwide are under development, with modules on corrective action/root cause investigation and internal auditing currently being assessed. These modules consist of interactive exercises and challenges supported by animated audio/visual mini-lectures explaining the topic and providing experienced advice. Real laboratory examples are used; with explanations of acceptable vs. unacceptable documents and procedures included. Development of this training is an important step forward in the global implementation of standard quality system principles.

Vet-LRN - Center for Veterinary Medicine's Laboratory Response Network Focusing on Animal Feeds and Drugs

Renate Reimschuessel[1], Sarah Yachetti[1], Andriy Tkachenko[1], April Hodges[2]

[1]Center for Veterinary Medicine, FDA, Laurel, MD; [2]Center for Veterinary Medicine, FDA, Rockville, MD

Narrative: Vet-LRN is the Center for Veterinary Medicine's new laboratory response network which will coordinate facilities, equipment, and professional expertise of government and veterinary diagnostic laboratories across the country and Canada to respond to high priority chemical and microbial feed/drug contamination events. Vet-LRN will provide the means for rapid response to reports of animal injury and will establish protocols to facilitate veterinary diagnostic reporting to FDA. Vet-LRN will work with the veterinary diagnostic laboratories to document, investigate and diagnose animal feed or drug related illnesses. The network will collaboratate with existing networks including NAHLN, AAVLD and FERN. These efforts can contribute to overall food safety as animal feed events could signal potential issues in the human food system

Standardized Sample Preparation for Multiple Sample Matrices

Rohan Shah, Adam Toguchi, Quoc Hoang, Angela Burrell, Ivan Leyva-Baca, Catherine O'Connell

Life Technologies, Austin, TX

Narrative: Sample preparation is an integral component of the pathogenic nucleic acid amplification workflow. Coordinated development of workflows with consideration of the sample matrix, nucleic acid of interest, as well as format of downstream analysis can have a profound impact on the successful amplification of target nucleic acids from animal sourced matrices. Effective sample preparation from a wide variety of sample matrices is critical to accurate testing, and the development of sample preparation methodologies in the context of the entire testing workflow is key to ensure optimal performance. There are many different methods to process different sample matrices. This can lead to confusion and frustration for researchers working with multiple sample types. This abstract describes the MagMAX™ Pathogen RNA/DNA kit which is designed to achieve a more standardized solution so labs can order just one kit for a variety of sample matrices as well as different input volumes for each sample. This will allow labs to work with many different sample matrices using the same chemistry and instrumentation. Sample matrices that can be processed using this kit include blood, serum, swabs, semen, feces, cell supernatant and others, from a sample volume of 50 µL up to 400 µL.

Thiosulfate Determination in Urine a Possible Diagnostic Tool for Supporting Polioencephalomalacia Diagnosis for Bovine on High Dietary Sulfur Diet.

Paula Imerman[1], Steve M. Ensley[1], Mary Drewnoski[2], Stephanie Hansen[2]

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

Narrative: Dried distiller's grain + soluble (DDGS) can have a high sulfur content resulting from its production. With the increased feeding of DDGS to bovine an increase in polioencephalomalacia (PEM) cases has been seen at our Diagnostic Lab. PEM is characterized by a laminar necrosis of the cortical gray matter in the brain. High sulfur content in feed and water has been associated with a predilection for PEM. Dietary sulfur is reduced to sulfide by microflora in the rumen and this flora then can produce hydrogen sulfide gas. Sulfide is a potent toxicant that affects the CNS in mammals. Sulfide is thought to cause damage to proteins and depress the respiratory centers in the brain. Many cases of suspected PEM lack the characteristic brain lesion. Our study was to investigate other diagnostic tests to correlate with exposure to high dietary sulfur. The two groups consisted of 8 control steers and 8 steers fed a low roughage diet with DDGS and sodium sulfate. Steers were cannulated for gas cap measurement of hydrogen sulfide. Rumen fluid was also collected along with urine and serum samples. The rumen fluid was measured for sulfide and the urine samples for thiosulfate. Animals on the high sulfur diet showed high hydrogen sulfide in the gas cap vs. the control animals. Hydrogen sulfide gas cap levels reached as high as 16,000 ppm in post mortem readings. The mean for urine thiosulfate in control animals ranged from 1.5 to 7.5 ppm where as the mean for the high sulfur diet animals ranged from 137 to 536 ppm. Urine thiosulfate analysis looks promising as an ante-mortem diagnostic tool to indicate exposure to a high sulfur diet. Rumen fluid sulfide analysis was seen as a weaker indicator for high sulfur exposure than urine thiosulfate. Serum analysis for thiosulfate and sulfide is still pending.

Pooling of *Tritrichomonas foetus* Cultured Samples Followed by MagMAX™ Sample Preparation System and Amplification with Applied Biosystems qPCR Reagents

Ivan Leyva Baca[1], Marilyn Simunich[2], Lee J. Effinger[3], Catherine O'Connell[1]

[1]Aninal Health, Life Technologies, Austin, TX; [2]Animal Health Laboratory, Division of Animal Industries Idaho State Department of Agriculture, Boise, ID; [3]Animal Health & ID Division, Department of Agriculture, Salem, OR

Narrative: Bovine trichomoniasis is a sexually transmitted infection caused by *Tritrichomonas foetus* resulting in significant monetary losses to the cattle industry worldwide. *T. foetus* is a flagellated protozoan found in bovines that colonizes the uterine, vaginal and preputial epithelium, resulting in early embryonic death, abortion, and infertility. Although bulls are the main carriers of *T. foetus*, they remain asymptomatic for their entire life. Increased interest in pooling *T. foetus* culture media samples has being identified in private and government diagnostic labs to save time, labor, DNA purification, and qPCR reagents costs during mandatory government eradication programs or monitoring status in clean herds. The feasibility of pooling these samples for nucleic acid isolation and quantitative real-time PCR has not been well researched, and the optimal number of pooled samples and volumes has not being determined. Robust studies need to be conducted to determine the number of samples and volumes to be pooled that can accurately lead to positive and negative *T. foetus* calls. The aim of this study is to assess the feasibility of pooling cultured samples followed by MagMAX™ Sample Preparation Systems and amplification with Experimental *T. foetus* DNA Test Kits. Sample identification will be conducted with the collaboration of 7 feeding diagnostic state labs (CA, CO, KS, NV, OK, and two TX), which will receive samples from different regions of United States. Samples from 100 positive and 400 negative bulls will be collected and each sample will be individually processed for DNA extraction with MagMAX™ Total Nucleic Acid Purification Kit using cultured media and tested with Experimental *T. foetus* DNA Test Kits. Once the positive and negative samples are identified and confirmed, each cultured sample (4 negatives and 1 positive) will be pooled for DNA purification and *T. foetus* real-time PCR amplification. Results will be discussed. A U.S. Veterinary Biological Product Application has been filed with the USDA Center for Veterinary Biologics for the *T. Foetus* DNA Test Kit. Data from this study will be filed with USDA to support licensing evaluation.

The Detection of Antibodies to Different Subtypes of Influenza A Virus in Swine Using a Multispecies Blocking ELISA

Christa Irwin[1], Chong Wang[1,2], John Johnson[1], Jeff Zimmerman[1], Apisit Kittawornrat[1], John Prickett[1]

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

Narrative: Hemagglutination inhibition (HI) assays are widely used to detect anti-influenza A virus (IAV) serum antibodies. However, HI antibody titers are strongly affected by the challenge virus in the assay. Commercial indirect swine influenza ELISA assays are also available, but have poor sensitivity for the detection of antibodies against contemporary IAVs (Barbé et al., 2009; Yoon et al. 2004). The objective of this study was to evaluate the diagnostic performance of an influenza blocking ELISA using serum samples of known status. The ELISA is based on the detection of serum antibody against IAV nucleoprotein (NP). Eighty-two, 3-week-old weaned piglets from a known PRRSV-, IAV-, and *M. hyopneumoniae*-negative source were isolated for 30 days and confirmed negative to these pathogens by repeated testing. A subset (n=28 pigs) was vaccinated against IAV using a commercial multivalent vaccine (Flusure XP®, Pfizer Animal Health). Following isolation, pigs were transported to a research facility and randomly assigned but balanced by vaccination status, to one of 3 treatment groups: (1) inoculation with Ohio '07 H1N1, (2) inoculation with Illinois '09 H3N2, or (3) negative control. Serum samples were collected weekly DPI -7 to +42, randomized, and assayed using AI MultiS-Screen Ab ELISA (IDEXX® Laboratories). Receiver operator characteristic analysis (MedCalc® v9.5.2.0) was used to calculate the optimized cut-off and associated diagnostic sensitivity (Se) and specificity (Sp) estimates. Based on results from 279 negative and 288 positive samples, the optimized cutoff was S/N ≤ 0.60 (AUC = 99.3). This cutoff resulted in an overall estimated Se of 95.5% (95% CI: 92.4%, 97.6%) and Sp of 99.6% (95% CI: 98.0%, 99.9%). Differences in the duration of ELISA positivity were observed in unvaccinated+challenged vs. vaccinated+challenged groups by DPI 21. The cut-off of S/N ≤ 0.60 provided excellent diagnostic specificity and sensitivity for detection of antibodies against IAV. These results support previous work in swine and other species (Ciacci-Zanella et al. 2010; Brown et al, 2009), the NP ELISA appears to have potential as a universal influenza A antibody detection assay.

Acknowledgements: This work was supported in part by the National Pork Board (#09-193), Pfizer® Animal Health and IDEXX® Laboratories, Inc. A special thanks to the Serology department of the Iowa State University Veterinary Diagnostic Laboratory. Contemporary virus isolates were generously provided by Drs. Amy Vincent (National Animal Disease Center) and Marie Gramer (University of Minnesota).

References: Barbé et al. (2009). J Vet Diagn Invest 21, 88-96. Yoon et al. (2004). J Vet Diagn Invest 16,197-201. Ciacci-Zanella et al. (2010). J Vet Diagn Invest 22, 3-9. Brown et al. (2009). Clin Vaccine Immunol. 16(6), 824-829.

Real-Time rt-PCR Detection of Influenza Virus A in Oral Fluid Using a Check Test

Christa Irwin[1], Jeff Zimmerman[1], Chong Wang[1,2], Richard Hesse[3], Karen Harmon[1], Jane Christopher-Hennings[6], Tracy Otterson[7], Jodi McGill[4], Amy Vincent[5], Rohan Shah[8], Rolf Rauh[9]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Statistics, Iowa State University, Ames, IA; [3]Kansas State University Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; [4]National Veterinary Services Laboratories, USDA-APHIS, Ames, IA; [5]National Animal Disease Center, USDA-ARS, Ames, IA; [6]Veterinary and Biomedical Sciences Department, South Dakota State University, Brookings, SD; [7]University of Minnesota Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN; [8]Life Technologies ™, Houston, TX; [9]Tetracore™, Rockville, MD

Narrative: Oral fluid (OF) has been shown to be an excellent surveillance tool for several swine respiratory viruses (Hoffman et al. 2008; Prickett et al., 2008; Prickett et al., 2008). Preliminary data suggested that influenza A virus (IAV) detection in the field might be improved through the use of OF samples. The objective of this study was to determine whether diagnostic laboratories could repeatedly detect IAV from an OF ring test by rt-PCR. In this study, OF was inoculated ("spiked") with either contemporary H1N1 or H3N2 influenza virus for submission to diagnostic laboratories in a "ring test" format. To prepare samples, 5.4 liters of OF were collected from 4 PRRSV-, IAV-, *M. hyopneumoniae*-, and ADV-negative sows, centrifuged, and pooled. To create ring test samples, 8 10-fold serial dilutions were prepared from Ohio '07 H1N1 and Illinois '09 H3N2 stock solutions. The initial concentrations of the two isolates were 1 x 101 TCID50 different, but this was not determined until the inocula were back-titrated. Samples were randomized, grouped into sets of 180 samples (10 samples from each of the 8 dilutions of each virus plus 20 negative OF samples), frozen at -80°C and submitted to 8 U.S. laboratories using overnight delivery. Laboratories were contacted to confirm samples arrived frozen. Each laboratory performed rt-PCR using the method(s) of their choice. Rt-PCR results demonstrated significant differences in detection of influenza virus as virus concentration decreased between laboratories. To quantitate virus detection, cycle threshold values were reported and these were also different between laboratories, however within laboratory, results demonstrated strong linearity as virus titer declined. Seven of the 8 laboratories reported as positive at least one of the 20 negative control samples. Analyses and comparisons of both extraction and PCR methods are in progress. Preliminary analysis suggests that successful rt-PCR detection is dependent upon laboratory protocol. Field samples must be assessed to confirm the applicability of these observations.
Acknowledgments: This work was supported by USDA/APHIS (NCAA: 10-9100-1314-CA).
References: Hoffmann P et al., March 8-11, 2008. 38th Annual Meeting of the AASV, 301-302. Prickett et al., 2008. J Vet Diagn Invest 20,156-163. Prickett et al., 2008. J Swine Health Prod 16(2), 86-91.

Antimicrobial Susceptibility Patterns and Sensitivity to Tulathromycin in Goat Respiratory Bacterial Isolates

Kris Clothier[2,1], Joann M. Kinyon[2], Ronald Griffith[1]

[1]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA;
[2]Veterinary Department of Production Animal Medicine, Iowa State University, Ames, IA

Narrative: Bacterial pneumonia is a common and often life-threatening respiratory problem in goats. Antimicrobials approved for use in this species are severely limited, requiring extra-label drug administration to treat these infections. Data on antibiotic susceptibility patterns in goat respiratory isolates can provide valuable information to prescribing veterinarians making treatment decisions. Tulathromycin, a triamilide macrolide antimicrobial drug shown to be effective against swine and cattle respiratory bacterial agents, has been identified as a potentially useful drug in caprines. The present study was conducted to determine the susceptibility patterns of recognized bacterial respiratory pathogens to commonly prescribed antimicrobials, with a particular emphasis on the efficacy of tulathromycin against these agents. A collection of 45 *Mannheimia haemolytica*, 11 *Pasteurella multocida*, and 11 *Bibersteinia trehalosi* isolates recovered from the lungs of goats with clinical pneumonia were identified for this study. Minimum inhibitory concentrations (MIC) were determined using microbroth dilution against a panel of antimicrobials at the listed dilutions, including ampicillin, ceftiofur, chlortetracycline, danofloxacin, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, sulfadimethoxine, trimethoprim-sulfamethoxazole, tulathromycin, and tylosin. To further characterize efficacy of tulathromycin against these pathogens, minimum bactericidal concentration (MBC) testing and kinetic killing assays were conducted. Significantly ($P > 0.05$) greater MIC values were seen in *B. trehalosi* to ampicillin, chlortetracycline, danofloxacin, florfenicol, neomycin, oxytetracycline, and penicillin. *M. haemolytica* demonstrated significantly higher MIC values against florfenicol, gentamicin, penicillin, and tulathromycin. While no Clinical and Laboratory Standards Institute (CLSI) interpretive criteria have been established for goats, use of criteria established for these same agents in cattle can yield valuable information. Most isolates were susceptible to the antimicrobials tested; however, increased resistance was seen in all species to penicillin and tylosin; in *P. multocida* to sulfadimethoxine, and in *B. trehalosi* to the tetracyclines. All isolates were susceptible to tulathromycin, which demonstrated a high killing efficiency in both bactericidal assays. Results of this study indicate that most goat pneumonic bacterial pathogens remain susceptible to commonly-prescribed antibiotics, although some evidence of resistance was seen to certain drugs; and that tulathromycin is highly effective against goat respiratory pathogens which could make it a valuable medication in this species.

Gout Due to Suspected Nephrotoxicity Associated with Prolonged Antibiotic Therapy in an Alligator (*Alligator mississippiensis*)

Marcia Regina Da Silva Ilha, Sreekumari Rajeev

Veterinary Diagnostic and Investigational Laboratory, University of Georgia, Tifton, GA

Narrative: A 4-year-old American alligator (*Alligator mississippiensis*) was presented for necropsy. This alligator was part of a group of 10 alligators from a zoological facility. Two animals developed chronic crusting lesions on the ventral aspect of their feet. The lesions were initially attributed to the concrete floor of the cage and secondary bacterial infection. The lesions persisted for 4 months despite of topical treatment and systemic antibiotic therapy with enrofloxacin and amikacin. This alligator developed subcase swelling of all four legs and reluctance to walk. It died 2 weeks after the swelling of the legs was first observed. On necropsy, there were a few crusted skin lesions in the upper jaw and both front feet had 1 to 2cm in diameter, brown and crusting lesions on the foot pads. All four legs had moderate subcutaneous edema. Several joints of all four legs had periarticular and articular deposits of a white dry chalky material. These deposits were also observed on subcutis, skeletal muscle, apex of heart, spleen, and both kidneys. Histologically, the renal lesions were characterized by tubular loss and intratubular and interstitial deposits of eosinophilic amorphous to basophilic needle-like material consistent with tophi (gout deposits) surrounded by multinucleated giant cells and epithelioid macrophages. Similar histological lesions were seen in the periarticular tissues, mucosa of the stomach, small intestine, lung, and spleen. The skin from the foot was ulcerated and covered by a thick layer of cellular debris and superficial bacterial colonies. HE and GMS stains showed fungal hyphae embedded within the skin crust. *Fusarium sp.* was cultured from the foot skin lesions. Many predisposing causes have been associated with gout in crocodilians. Overfeeding can cause visceral gout in young alligators. Secondary renal failure due to streptomycin was suspected as the cause of visceral gout in a caiman. Hypovitominosis A and bacterial pyelonephritis has been associated with gout in crocodiles. This alligator was the only animal affected in this group. All alligators were kept in similar environment and received the same diet. Two out of 10 alligators from this facility received prolonged antibiotic therapy; however only this animal developed gout. The prolonged antibiotic therapy with amikacin, a potentially nephrotoxic drug, was considered a possible predisposing factor in this case for renal failure and secondary gout.

References: Jacobson ER. J Zoo An Med. 15: 38-45, 1984. Buenviaje GN, Ladds PW, Melville L, Manolis SC. Aust Vet J. 71: 165-173, 1994.

Comparison of the Rapid Detection Methods to Reference Bacterial Culture Methods for the Detection of *Salmonella enteritidis* in Eggs and Environmental Drag Swabs

Ellen King, Beth Houser, Tammy Matthews, Traci Pierre, Valerie Linter, Bhushan Jayarao, Subhashinie Kariyawasam

Veterinary and Biomedical Sciences, The pennsylvania State University, University Park, PA

Narrative: Consumption of eggs contaminated with *Salmonella enteritidis* (SE) has been recognized as one of the important causes of human foodborne salmonellosis. As a result, several Egg Quality Assurance Programs (EQAPs) are in place to reduce SE contamination of shell eggs. While some EQAPs adopted by National Poultry Improvement Plan (NPIP) and individual states (e.g. Pennsylvania Egg Quality Assurance Program and California Egg Quality Assurance Program) are purely voluntary, the recently introduced egg rule by the Food and Drug Administration is a mandatory program. Until recently, all these programs used culture methods for detecting SE in eggs and environmental drag swabs obtained from commercial layer complexes. The turnaround time for SE culture and identification can be up to 4 to 10 days depending on the sample type and the culture method, and additional time is required to serotype group D Salmonella isolates. To improve the turnaround time of SE detection, many different rapid diagnostic methods have been developed and validated. The objective of this study was to compare 5 rapid SE detection assays to FDA Bacteriological Analytical Manual (BAM) procedure and NPIP bacterial culture method. The rapid methods included 3 polymerase chain reactions (SE-specific end-time PCR, group D- specific Salmonella real-time PCR and SE-specific PCR from Life Technologies) and 2 lateral flow tests (SDIX's RapidChek® SELECT" SE and Neogen's rapid SE test kit). One hundred egg pools and 100 environmental samples were spiked with SE at four different levels: no-spike (0 CFU/1 ml), low-spike (2 CFU/1ml), moderate-spike (4 CFU/1ml), and high-spike (5 CFU/1ml). The samples were coded to create a blind set of 200 samples. The diagnostic sensitivity, diagnostic specificity and test agreement of the testing methods were compared statistically. The results of the study indicated that the five candidate methods (rapid detection methods) and two reference methods (FDA BAM and NPIP culture methods) were comparable.

An Unusual Gram-Staining of an *Arcanobacterium pyogenes* Isolated from a Milk Sample: A Case Report

Tammy Matthews, Valerie Linter, Traci Pierre, Bhushan Jayarao, Subhashinie Kariyawasam

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

Narrative: An unusual *Arcanobacterium pyogenes* isolate was recovered from a bulk tank milk sample submitted to Animal Diagnostic Laboratory at the Pennsylvania State University. This organism produced small grey colonies with small zones of beta-hemolysis on blood agar after 24 hours of incubation at 37°C. The organism was coagulase-negative as determined by direct tube coagulase test. Gram staining of a pure culture of the organism yielded a Gram-positive organism with abnormal microscopic cell morphology with a filamentous structure. An automated biochemical testing (Sensititre®, Trek Diagnostic Systems, Inc., Cleveland, Ohio, USA) identified the organism as *A. pyogenes*. Partial nucleic acid sequencing of bacterial 16S rRNA gene confirmed the organism as *A. pyogenes* with 100% homology at the nucleotide level. Repeated subculturing of the organism on blood agar yielded a cell morphology which is typical of *A. pyogenes* on Gram-stained smears. However, when the organism grew in the presence of penicillin in the laboratory the cell morphology was restored back to its initial filamentous morphology. This suggests that this unusual gram-stain finding of *A. pyogenes* is perhaps due to defective cell division subsequent to antibiotic therapy.

Obstructive Hepatopathy Secondary to Choledochophytolithiasis in a Racehorse

Federico Giannitti[1], Mark Anderson[1], Jerry Parker[2], Marcia Booth[1]

[1]California Animal Health and Food Safety (CAHFS) Laboratory System, School of Veterinary Medicine, University of California, Davis, CA; [2]Equine Sports Medicine & Surgery, Richmond, CA

Narrative: A 9-year-old Thoroughbred bay gelding presented with a 2½-month history of intermittent fever and anorexia since late December 2010. Biochemical analyses of serum, and complete and differential blood cell counts performed in early and late January revealed increased activity/values of γ-glutamyl transferase (676 and 613 IU/L, reference: 2-30), alkaline phosphatase (624 and 635 IU/L, reference: 50-300), total bilirubin (3.9 and 2.7 mg/dL, reference: 0.1-2.5) and direct bilirrubin (0.9 and 0.7 mg/dL, reference: 0.0-0.5), as well as neutrophilia (8585 -85%- and 12864 -96%- cells/uL, reference: 2700-6700) and lymphopenia (1313 -13%- and 268 -2%- cells/uL, reference: 1500-5500). The horse was euthanized due to poor prognosis on March 11, 2011 and submitted to the California Animal Health and Food Safety Laboratory, Davis for postmortem examination. Grossly, the liver was globally and markedly enlarged, had thick margins and rounded edges (hepatomegaly), weighed 18.2 kg (3.6% of body weight -505 kg-, reference: 1.5%) and had a firm consistency. A diffuse enhanced reticular pattern characterized by a delicate reticular gray meshwork encircling a green-tinted hepatic parenchyma was evident on external examination of the capsular surface and on cut sections. There was a 6.5 x 4 cm ellipsoid, orange-brown to tan-black concretion (choledocholith) lodged in the lumen of the most distal aspect of the common (extrahepatic) bile duct just proximal to the choledochoduodenal junction (major duodenal papilla). The choledocholith was firm but could be cut with a knife, had a laminar/faceted appearance with a central core within which there were seedheads of *Avena fatua* (wild oat). Microscopically in sections of liver there was diffuse severe portal bridging, centrilobular perivenous and sinusoidal fibrosis, often disrupting the hepatic cord architecture and surrounding small nests of hepatocytes, with marked bile duct/oval cell hyperplasia and diffuse moderate hepatocellular atrophy. There was abundant bile pigment within the cytoplasm of Kupffer cells that were markedly distended and were often bi/multinucleated, forming scattered microgranulomas engulfing bile throughout the sinusoids. There was also multifocal mild to moderate lymphohistiocytic portal hepatitis with fewer neutrophils and plasma cells and multifocal mild sinusoidal hemorrhage. Chemical analysis of the choledocholith revealed it was composed 100% of bilirubin (infrared spectroscopy). We speculate that the wild oat seedhead reached the common bile duct through the major duodenal papilla during normal intestinal peristalsis, serving as a nidus for bilirubin deposition and consequent choledocholith formation, leading to clinical obstructive hepatopathy in this horse.

Prevalence of Virulence Genes in *Escherichia coli* Strains Recently Isolated from Piglets with Diarrhea Submitted to Iowa State University Veterinary Diagnostic Laboratory: 2006 to 2008

Subhashinie Kariyawasam[1], Curt Thompson[2], Chitrita DebRoy[1], Thomas Denagamage[1]

[1]Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA;
[2]Vterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

Narrative: Enterotoxigenic *Escherichia coli* (ETEC)-associated post-weaning diarrhea is an economically important disease for the swine industry. Most veterinary diagnostic laboratories in the US perform virulence genotyping of porcine ETEC by means of polymerase reaction (PCR) assays. These PCR assays often detect the following 9 virulence genes of *E. coli*: faeG (K88), fedA (F18), F41 (F7), fasA (987P/F6) and fanA (K99) fimbrial genes; and elt (LT), estA (STa), estB (STb) and stx2e (Shiga toxin-2e variant) toxin genes. This study was carried out to determine whether it is necessary to expand the gene coverage in current virulence gene panels to include recently described virulence genes such as astA (*E. coli* heat-stable enterotoxin 1 or EAST1) and sepA toxin genes, and aidA (adhesin involved in diffuse adherence, or AIDA-I) and paa (porcine attaching and effacing-associated factor) adhesin genes in porcine strains of ETEC. A total of 1119 E. coli isolates obtained from diarrheic piglets submitted to the Iowa State University Veterinary Diagnostic Laboratory from January 2006 to July 2008 were screened for a total of14 genes: 5 fimbrial genes (faeG, fedA, F41, fasA and fanA); 6 toxin genes elt, estA, estB, stx2e, astA and sepA); 3 adhesin genes (aidA, paa and eae (attaching and effacing factor) by PCR. Of 1119 E. coli isolates tested, 227 strains (20.28%) possessed fedA, 176 strains (15.72%) possessed faeG, 9 strains (0.8%) possessed F41, and 1 strain (0.08%) each possessed fanA and fasA. Among toxin genes, astA, estB, elt, estA, sepA and stx2e, were present in 429 (38.33%), 308 (27.52%), 262 (23.41%), 104 (9.29%), 104 (9.29%) and 45 (4.02%) isolates, respectively. The adhesin gene eae was present only in 10 (0.89%) isolates whereas paa and aidA were present in 282 (25.2%) and 137 (12.24%) isolates, respectively. This study demonstrated that recently described virulence genes are common in porcine ETEC and it may be useful to incorporate them into current virulence genotyping panels.

Antimicrobial Susceptibility of Bacteria Isolated from Milk Samples Submitted to The Pennsylvania State Animal Diagnostic Laboratory

Valerie Linter, Tammy Matthews, Traci Pierre, Bhushan Jayarao, Subhashinie Kariyawasam

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

Narrative: Antimicrobial resistance has become an increasingly important global public health issue. This study was conducted to determine whether antimicrobial susceptibility patterns of major mastitis pathogens isolated from milk samples of dairy cows have changed over time. Samples included 1136 bacterial isolates representing *Escherichia coli*, *Klebsiella species*, *Serratia marcescens*, *Staphylococcus aureus* subspecies aureus, coagulase-negative *Staph. species*, *Streptococcus dysgalactiae* subspecies dysgalactiae, *Strep. agalactiae* and *Strep. uberis* obtained from milk samples submitted to the Animal Diagnostic Laboratory for diagnostic bacteriologic testing from January 2006 to December 2010. Antimicrobial susceptibility testing was performed with the Sensititre®automated system using the mastitis plate format. The antibacterial agents included in the selected plate format were penicillin, ampicillin, cephalothin, ceftiofur, penicillin + novobiocin, erythromycin, pirlimycin, tetracycline, and sulfadimethoxine. Logistic regression was used to determine whether percentages of isolates resistant to various antimicrobial agents changed over time. Overall, the results did not indicate increased antimicrobial resistance among mastitis pathogens isolated from milk samples from dairy cows during the study period.

Sodium Fluoroacetate (1080) Intoxication in Sheep

Federico Giannitti[1], Mark Anderson[1], Asli Mete[1], Nancy East[2], Michelle Mostrom[3], Robert Poppenga[1]

[1]California Animal Health and Food Safety (CAHFS) Laboratory System, School of Veterinary Medicine, University of California, Davis, CA; [2]School of Veterinary Medicine, University of California, Davis, CA; [3]Veterinary Diagnostic Laboratory (NDSU-VDL), North Dakota State University, Fargo, ND

Narrative: Sodium fluoroacetate (1080) is an organofluorine chemical toxic to mammals, insects and birds. In the US, 1080 is currently registered for use only in livestock protection collars as a predacide in States that have registrations and US EPA approved certification and training programs; it is not approved for use in California. The present work describes the epidemiological, pathological and toxicological findings in a naturally occurring case of 1080 intoxication in sheep that were strip grazing a municipal landfill site in NE San Joaquin County, CA. On March 13l 2011 a flock of 296 lambs and 149 headlong ewes were moved into a native pasture containing red clover (*Trifolium pratense*), Johnsongrass (*Soghum halepense*), *Lupinus sp.* and *Amsinckia sp*. Within a few hours of moving onto new pasture, 2 ewes were found dead. The next morning 12 ewes were dead and the sheep were moved off the site. Both ewes and lambs exhibited a brief period of disoriented running, breaking through the electric fence followed by apparent blindness, weakness, ataxia and death. Over the next 4 days 63 ewes and 80 lambs died with a peak at 3 days after grazing the suspect pasture (mortality rate: 35.3%). The direct economic loss was approximately $33,000. Four dead animals (2 4-months-old lambs and 2 ewes) were submitted to the CAHFS Laboratory System, Davis for postmortem examination. Grossly in both lambs there was bilateral diffuse pulmonary congestion and edema, the pericardial sacs contained serous clear amber fluid with fibrin clots and there were multifocally extensive areas of epicardial left ventricular pallor and mild petechiation. Grossly the ewes had mild hydrothorax and pericardial effusion with fibrin clots, epicardial petechiae and ecchymotic hemorrhages on the right ventricle extending to the junction with the interventricular septum. In one of them there was also diffuse epicardial hemorrhage in the left auricle, regionally extensive right caudodorsal pulmonary hemorrhage and intraluminal tracheal clotted blood. Microscopically in all 4 cases there was multifocal severe acute to subacute myocardial degeneration and necrosis with neutrophilic and lymphohistiocytic myocarditis. Following negative results for a variety of potential cardiotoxins (oleandrin, strophanthidin, selenium), nitrites/nitrates and cyanide, kidney samples from one of the lambs and one of the ewes were sent to the NDSU-VDL for determination of 1080 by gas chromatography-mass spectrometry. 1080 was detected in both samples at 27.5 ppb and 12.5 ppb, respectively. The positive identification of 1080 in conjunction with the pathologic findings are consistent with 1080 poisoning. 1080 should be considered in the differential diagnoses of myocardial necrosis in sheep. This report demonstrates possible risk associated with contractual targeted grazing of novel areas for vegetation control. The probable source of compound 1080 was likely treated grain used to control burrowing mammals.

Rapid Detection of Influenza A Virus Using the High Affinity Targeting Ligand Oseltamivir

Roman Pogranichniy[1,3], Huiling Wei[1], Youngsoon Kim[2], Bandari Prasad[2], Phil Low[2]

[1]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN; [2]Department of Chemistry, Purdue University, West Lafayette, IN; [3]Animal Diseases Diagnostic Laboratory, Purdue University, West Lafayette, IN

Narrative: Neuraminidase inhibitors, such as oseltamivir, interfere with the release of progeny influenza A virus virions from the surface of infected host cells. They also inhibit viral infection of new host cells, thereby halting the spread of infection in the respiratory tract. We have developed diagnostic methods to detect and capture the influenza virus using oseltamivir (known as Tamiflu) derivatives that bind at nanomolar affinity to neuraminidase of influenza virus group A. In this procedure, oseltamivir was conjugated with bovine serum albumin (BSA) via an amine-terminated PEG linker, and then printed onto a gold-plated chip in linear banding patterns using micro-contact printing methodology. The oseltamivir-patterned chips were incubated with strains of influenza virus H1N1, H3N2 and H5N1 to detect virus binding. The chips were then treated with an oseltamivir-PEG-FITC for fluorescent immunostaining. Examination of the chip surface by fluorescence microscopy confirmed influenza virus retention specific to the stamped periodic pattern of oseltamivir conjugates on the gold chip, with minimal binding to non-functionalized regions of the surface. Furthermore, to examine the sensitivity of the detection strategy, oseltamivir-derivatized chips were exposed to serial influenza virus suspensions ranging from 101-106 pfu/ml. Image analysis by discrete Fourier transformation (DFT) revealed that a patterned distribution of viruses could be observed at concentrations as low as 103 pfu/ml.

Abortions in a University Cow Herd Following Vaccination with a Modified Live Bovine Herpes Virus-1 Vaccine at 7-8 Months Gestation *

Anna Yedinak, Jacqueline Cavender, Donal O'Toole, Myrna Miller

University of Wyoming, Laramie, WY

Narrative: Between December of 2010 and January of 2011, a University of Wyoming herd of Angus-cross heifers experienced abortions comprising a reproductive loss of 25%. This cohort differs from the 2009-2010 heifers in that vaccination for Bovine Herpes Virus-1 (BHV-1) and Bovine Viral Diarrhea Virus (BVDV) with a modified live virus (MLV) multivalent vaccine was given at 7-8 months gestation instead of 6-7 months gestation. Abortions began 32 days post-vaccination with few or no premonitory signs suggesting gestational complication. Most of these abortions were typical for BHV-1 where the characteristic expulsion of an autolytic fetus with serosanguinous effusion into major body cavities. From six aborted fetuses, lesioned liver, spleen, thymus, and brain were fixed in formalin and incubated with polyclonal antibody for BHV-1 or monoclonal antibody for BVDV and analyzed via immunohistochemistry. The analysis revealed BHV-1 in many tissues, predominantly in liver and kidney. It was concluded from the assayed tissues that these abortions were due to BHV-1 and not BVDV or other pathogens. In general, immunohistochemistry is the method used to determine the presence of a BHV-1 infection, including cases of abortion that are a result of the infection. The finding that these abortions result from infection of BHV-1 after vaccination with an MLV vaccine, shows some association between the two, whether it be the vaccine itself, or incomplete protection and infection with field strain BHV-1. This has also been the conclusion of veterinarians in Colorado, Texas, South Dakota and Wyoming who have experienced heifer abortions. The vaccine has been used for 50 years in non-pregnant cows but has only been approved for use in pregnant cows since the 1990s. It became apparent, however, that vaccinating naïve cows in late gestation with a MLV can stress the pregnancy and caused the industry to respond in a way that reduced abortions. A problem of recent increases in abortions in cattle that have been thought to have received appropriately vaccinated with licensed products raise the issue that there is no diagnostic test to determine vaccine from field strain in post-vaccination abortions, including immunohistochemistry. Without this distinction, it becomes difficult to address the problem. Future plans are to sequence vaccine strain virus, the Cooper strain, from which the vaccine was made, as well as fetal samples from the UW herd abortions. Comparisons will provide a better understanding of the attenuation of the MLV vaccines, and possible explanations for the increase in abortions.
Reference: O'Toole D, Miller M, Cavender J, Cornish T. Pathology in Practice: University of Wyoming Heifers and BHV-1. J Am Vet Med Assoc. Under review.

* Graduate Student Poster Presentation Award Applicant

Quantitative Assessment of Adherent Bacteria in Porcine Intestines

Saraswathi Lanka[1], Victor Perez[2], James Pettigrew[3], Carol Maddox[4]

[1]Veterinary Diagnostic Laboratory, University of Illinois, Urbana, IL; [2]Manager Nonruminant Nutrition Research, ADM Alliance Nutrition, Inc, Quincy, IL; [3]Aimal Sciences, University of Illinois, Urbana, IL; [4]Pathobiology, University of Illinois, Urbana, IL

Narrative: The objective of this study was to quantify by real-time quantitative PCR (qPCR), the proportion of adherent *Escherichia coli* populations in the intestines of pigs experimentally infected with F18 *E. coli*. Pure cultures of an enterotoxigenic F18 *E. coli* strain (LT, STB, SLTII), isolated at the University of Illinois Veterinary Diagnostic Laboratory from a field outbreak, were suspended in phosphate buffered saline (PBS) to a concentration of 1010 colony forming units (cfu) per 3ml daily dose. Pigs weaned at 21 days of age were untreated (control), or orally inoculated with three consecutive daily doses of PBS (sham) or F18 *E. coli* (EC) starting at 3 days post weaning. Ileum and colon samples were harvested 5 days or 10 days post inoculation (PI) and mucosa samples were collected by gently rinsing off the ingesta then, removing a $1cm^2$ area of mucosa from the intestine. Total genomic DNA extracted from the mucosa samples was subjected to SYBR green qPCR. Universal primers targeting the16S rRNA gene and primers specifically targeting the gadAB genes and the F18 gene were used to enumerate total bacteria, total coliforms and F18 *E. coli* populations in the mucosal samples respectively by extrapolation from standard curves. Two standard curves were constructed for each set of bacterial primers: (i) using serial dilutions of a known concentration of the F18 *E. coli* (obtained by viable plate count) and (ii) using serial dilutions of a known concentration of F18 *E. coli* genomic DNA (direct measurement of total genomic DNA at 260nm). Primers targeting the villin gene were used to quantify the porcine enterocyte content of the sample by extrapolation from a standard curve constructed using villin primers and total genomic DNA obtained from the mucosal sample of a control pig. Colonization by F18 was ~75-fold higher at day 5 than day 10 while coliform and total bacterial counts were 6.7 and 4.7 fold higher, respectively. There was little difference between the levels of adherent bacteria in the ileum versus the colon. Average coliform counts ranged from 2,000-14,000 bacteria per cm^2 for sham challenged pigs. This qPCR method enabled the investigators to assess and compare colonization of the mucosa by pathogenic versus commensal coliforms or total bacterial flora per standardized area of the mucosal surface as well as their persistence over time.

A Comparison of Biochemical and Histopathologic Staging in Cats with Renal Disease

Shannon McLeland[1], Colleen Duncan[1], Jessica Quimby[2]

[1]Microbiology, Immunobiology and Pathology, Colorado State University, Fort Collins, CO;
[2]Clinical Science, Colorado State University, Fort Collins, CO

Narrative: Feline chronic renal failure (CRF) is a common disease, especially among aged cats. Plasma creatinine and blood urea nitrogen are indirect markers of renal disease but often do not fully elucidate underlying mechanisms of disease. The purpose of this study was to characterize histologic changes present within various stages of renal disease and look for a correlation between clinical and histologic parameters. Cats presenting to Colorado State University Veterinary Teaching Hospital between 2000-2009 with clinical, biochemical, and post-mortem renal histopathology were included in this study. To evaluate if there were an association between the clinical stage, characterization and severity of renal lesions subjective histologic grades of renal tissues were compared to ante-mortem plasma creatinine levels and stage, according to the International Renal Interest Society (IRIS). A total of 56 cats were included in this study representing IRIS stage 1 (n=8), stage 2 (n=19), stage 3 (n=9) and stage 4 (n=20). Of all the components evaluated, severity of interstitial fibrosis, and tubular degeneration were the only lesions that were statistically associated with clinical stage. Progressive renal disease leading to elevations in serum creatinine is best characterized primarily by interstitial fibrosis accompanied by loss of tubules. Insight into the pathogenesis of renal fibrosis may assist in monitoring and management of feline renal disease in the future.

Disseminated *Aspergillus versicolor Infection in a Dog*

Shuping Zhang[1], Wayne Corapi[1], Erin Quist[1], Sara Griffin[2], Michael Zhang[3]

[1]Veterinary Pathobiology, Texas A&M University, College Station, TX; [2]Small Animal Clinical Science, Texas A&M University, College Station, TX; [3]Texas Veterinary Medical Diagnostic Laboratory, College Station, TX

Narrative: A two-year-old male castrated German shepherd dog with a clinical history of non-ambulatory paraparesis was presented for necropsy. Major gross lesions included loss of the intervertebral disk at T9-T10 with lysis of the vertebral end plates and resulting joint instability and spinal cord compression; bony proliferation and osteomyelitis throughout sternebrae 2 and 3; and dilated renal pelvises with discrete white foci throughout the kidneys. Histopathologic changes consisted of 1) granulomatous osteomyelitis and diskospondylitis of the vertebrae and intervertebral disks, 2) granulomatous osteomyelitis and vasculitis of the sternebrae, and 3) bilateral granulomatous pyelonephritis and necrotizing vasculitis of the kidneys. All affected tissues contained numerous intralesional fungal hyphae and spores, the morphology of which was compatible with *Aspergillus spp.* Fungal culture of swabs of the sternebrae and vertebrae as well as tissue from the kidney yielded pure growth of Aspergillus versicolor. Morphological identification of the fungus was confirmed by determining the sequence of the internal transcribed spacer (ITS) region of ribosomal DNA. The findings from the present investigation suggest that ***Aspergillus versicolor*** **can cause canine disseminated aspergillosis with characteristics resembling those caused by *Aspergillus terreus*. This is the first documented case of canine *Aspergillus versicolor* infection.**

The importance of Electron Microscopy in the Laboratory Diagnosis of Canine Parvovirus Infections

Shipra Mohan, Travis Heskett, Woody Fraser, Kathy Ball, Annie Yan, J.L. Maxwell, Alice Agasan

Bronson Animal Disease Diagnostic Laboratory, FL-Departmentment of Agriculture, Kissimmee, FL

Narrative: Canine Parvovirus (CPV) enteritis is an acute life threatening infection. It is highly contagious and represents one of the most common causes of acute hemorrhagic diarrhea in pet dogs. Therefore, a quick and reliable diagnosis is important. In the current study, Fluorescent Antibody (FA) staining technique and negative stain Electron Microscopy (EM) were compared to detect the presence of Parvovirus in animal tissues. 18 canine and 6 wild raccoon cadavers were submitted for necropsy at the Bronson Animal Disease Diagnostic Laboratory during the period of 2003-2011. Intestinal tissues were collected and delivered to the Virology lab for testing of Parvovirus. One frozen canine intestinal tissue was directly submitted to Virology lab from a veterinary clinic. Out of 25 tissues submitted 6 were found positive for Parvovirus by EM and negative by FA methods. One specimen was found positive by both methods; one sample was positive by FA, but negative by EM. All 17 tissues were negative for Parvovirus by both methods. Direct FA technique and EM are, in general, rapid and specific, but EM is most helpful for the detection of gastroenteritis viruses and works best if the titer of the virus is at least 10^6 to 10^7 particles per milliliter. Although EM is an older technology and costly to maintain, it is still the only method of choice and primary tool used in the identification of viral agents, especially in gastroenteritis cases. In the present study, we would have missed the diagnosis of the six Parvovirus positive cases, if negative stain electron microscopy was not carried out in addition to FA method. Details will be discussed.

Evaluation of the Diagnostic Efficacy of Recombinant VP2 Protein as an aAlternative to Tissue-Derived IBDV Antigen by Agar Gel Immunodiffusion

Woo-Jin Jeon[1], Eun-Kyoung Lee[1], Kang-Seuk Choi[1], Mi-Ja Park[1], Hoo-Don Joo[2], Jun-Hun Kwon[1]

[1]Veterinary Research, National Veterinary Research & Quarantine Service, Anyang-si;
[2]Jenobiotech Inc., Chuncheon

Narrative: Infectious bursal disease virus (IBDV) causes a highly contagious and immunosuppressive disease of young chickens. Agar gel immunodiffusion (AGID) using bursal antigen extracted from infected chickens has been officially used for diagnosis of IBDV in Korea according to OIE terrestrial manual. With the full enforcement of the Animal Protection Act, the development of an *in vitro* diagnostic reagent that can reduce or replace animals in IBD antigen production is urgently required. In this study, we assessed the diagnostic efficacy of the recombinant VP2 (rVP2) protein of IBDV by comparing it with that of the tissue-derived antigens in AGID assays. The optimal concentration of rVP2 was adjusted to 6 x 105 ELISA units, as was the tissue-derived antigen (4.6±1.0 x 105 ELISA units) by the sandwich ELISA using R63 antibodies. The rVP2 antigen only precipitated IBDV antibodies among 22 different hyper-immune sera against the major avian disease viruses when tested by ELISA and AGID. The AGID using rVP2 antigen detected anti-IBDV antibodies from 6 to 28 dpi (termination of the experiment) in experimentally infected chickens. This result was consistent with that of AGID using IBDV antigen, Virus Neutralization (VN) test and commercial ELISA kit (except for one serum at 6 dpi). Using the serum panels with known VN titers, rVP2 antigen first detected serum VN titre of 3 log2, and all detected serum VN titre of 5 log2 thereafter in AGID. The sensitivity of AGID using rVP2 antigen was the same with that of IBDV antigen when field sera (n=334) tested. Although AGID using the rVP2 antigen could detect maternal antibodies up to 15-day-old chickens (n=20) in a broiler farm, the detection rate of AGID was relatively lower when compared with that of the commercial ELISA kit. The present study indicated that the IBDV whole-virus antigen from IBDV-infected chickens could be replaced by the rVP2 protein as an alternative for AGID.

Pneumonia Cases Associated with *Mycoplasma hyopneumoniae*: an 8 year Retrospective Study, 2003-2010

Joao Carlos Neto, Erin Strait, Neil Boyes, Kent Schwartz, Alex Ramirez

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

Narrative: *Mycoplasma (M.) hyopneumoniae* is the causative agent of porcine enzootic pneumonia and contributes significantly to the occurrence of the porcine respiratory disease complex. This pathogen is spread worldwide and leads to significant economic losses in pig production due to: costs of antibiotic treatment, lower food conversion ratios and decreased average daily weight gain. In order to evaluate the proportion of pneumonia cases associated with *M. hyopneumoniae*, the VDL-ISU database was assessed. A retrospective analysis was carried out using pneumonia cases diagnosed from 2003 to 2010. Pneumonia associated with *M. hyopneumoniae* was defined as a case in which this pathogen was found solely, or in association with other bacteria and/or viruses. In the analysis weather, time, and age effect were used by categorizing periods of year (seasons) and by year by year comparison of proportions. Overall, pneumonia cases associated with *M. hyopneumoniae* represented in average 12.5% (175 out of 1405) of the cases submitted to the histopathology section in the VDL. By adding all the number of cases by month from all the years, a suggestive seasonal effect was found, and the summer and fall held together 67.3% (945 out of 1405) of the pneumonia cases. From 2003 to 2006, the majority of pneumonia cases came from animals in the early finisher (40-50%); on the other hand, from 2007 to 2010, a clearly shift occurred, and the proportion of pneumonia cases increased in the later finishers (40-50%). PRRSV was the pathogen more often associated in *M. hyopneumoniae* associated cases (above 45% in average for all the years) compared to SIV, PCV2, and their different combinations. This retrospective study showed that the *M. hyopneumoniae* has been diagnosed in a fairly steady number of cases over years, and suggested an apparent seasonal and age effect, changing the dynamic of the disease in swine herds, but yet has to be determined.

M. hyosynoviae and *M. hyorhinis*: Do They Require More of Our Attention?

Joao Carlos Neto, Erin Strait, Darin Madson, Kent Schwartz, Phil Gauger, Neil Boyes

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

Narrative: Infectious arthritis has a recognized and major role in causing lameness in pigs in all phases of production. *Mycoplasma hyosynoviae* (Mhs) and *Mycoplasma hyorhinis* (Mhr) are capable of causing arthritis in swine from weaning through finishing phase. There is scarce recently published epidemiological information available, but perceptions are that the role of Mycoplasmas in arthritis has increased over the past years in swine systems within the Midwest U.S. For greater insight into the relationship between clinical disease and agent detection, a record analysis of lameness cases submitted to the Iowa State University Veterinary Diagnostic Laboratory from 2003 to 2010 was performed. All cases of swine lameness were categorized by cause, lesions and age. Of a total of 431 clinical lameness cases examined from 2003 to 2010, 47.5% were diagnosed as infectious arthritis , 16.7% were associated with Mycoplasmas, and 44.9% had a no definitive diagnosis. Of the 221 cases of infectious arthritis, Mhs was associated with 25%, and Mhr was associated with 19%. However, the percent of cases diagnosed with Mycoplasmas arthritis has increased over the past three years, reaching 37% of the total of lameness cases in 2010. Mhr was more frequent in pigs less than 10 weeks of age, but otherwise, Mhs occurred more often in animals older than 10 weeks representing 37% and 68% of the total number of cases over all the years, respectively. The frequency of diagnosis and percent of arthritis cases associated with Mycoplasma has increased over time. Neither prevalence of infection nor of disease in the field can be determined because of the nonrandom sample selection, lack of standardization of sample collection and animal selection, use of antibiotics, and other factors that influence detection and diagnosis. Yet to be developed and/or validated other assays such as IHC and qPCR for a better approach on different specimens selected for diagnosis.

Identification of Lymphoproliferative Disease Virus in Wild Turkeys (*Meleagris gallopavo*) in the Southeastern United States

Justin Brown[1], Andrew Allison[1], Andrew Cartoceti[1,3], Steven Kubiski[1,2], Brandon Munk[1], Nicole Nemeth[1], Kevin Keel[1]

[1]Population Health, Southeastern Cooperative Wildlife Disease Study, Athens, GA; [2]Pathology, Microbiology, and Immunology, College of Veterinary Medicine, University of California-Davis, Davis, CA; [3]New England Wildlife Center, South Weymouth, MA

Narrative: Viral-associated lymphoproliferative neoplasia in domestic poultry is caused by infection with a herpesvirus or three species of retroviruses. Previously, retroviral neoplasms reported in wild upland game birds in the United States of America have typically been associated with reticuloendotheliosis virus infection. Since 2009, lymphoproliferative disease virus (LPDV), a previously exotic virus to the United States, has been identified in six wild turkeys (*Meleagris gallopavo*) from two southeastern states, West Virginia (n=5) and Arkansas (n=1). All infected turkeys were found dead or in moribund condition by state wildlife biologists and subsequently submitted to the Southeastern Cooperative Wildlife Disease Study for diagnostic examination. Lymphoproliferative neoplasms were identified in all six turkeys in various visceral organs, including intestines, liver, kidneys, spleen, pancreas, lungs, adrenal glands, skeletal muscle, esophagus, heart, and air sacs. Using PCR targeting a portion of the gag gene, proviral sequences of LPDV were detected in samples of spleen, lung, heart, and/or liver from each turkey. Additionally, isolation of LPDV from these tissues was attempted by inoculating Pekin duck embryo (PDE) fibroblasts with clarified supernatant. After serial passages, nucleic acid was extracted from cell culture media and gag sequences were amplified by RT-PCR and not standard PCR, indicating the presence of live virus and the apparent in vitro replication of LPDV in PDE cells. Comparative alignment of the partial sequences of the gag gene from the Arkansas and two of the West Virginia isolates demonstrated a 92-94% and a 91-94% shared identity at the nucleotide and amino acid level, respectively. Additionally, comparison of the three North American isolates to the Israeli strain of LPDV identified a 85-86% nucleotide and a 85-87% amino acid identity in their gag sequences. These preliminary phylogenetic analyses of the partial gag sequences of the three North American LPDV isolates identified a monophyletic clade with Old World LPDV, distinct from other avian retroviruses. The cases reported herein are novel as they represent the first reports of LPDV infection in wild turkeys and the first identification of LPDV in North America.

Detection and Isolation of pH1N1 from a Privately Owned Small Swine Herd in Colorado

Kyran Cadmus, Christina Weller, Barbara E. Powers, E. Ehrhart, Kristy Pabilonia

College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Narrative: Lungs and intestines from two piglets (less than one month old) were submitted to the Colorado State University Veterinary Diagnostic Lab in November 2010. The piglets were from a small, privately owned herd and had a history of weight loss and signs of pneumonia. The referring veterinarian reported that, previous to the submission of these samples, other piglets on the farm had died. Gross lesions detected on necropsy included caudoventral pneumonia in both piglets, a section of thickened small intestine in Piglet 1 and peritonitis, an abnormally colored small intestine and an enlarged mesenteric lymph node in Piglet 2. Histopathological lesions in the lungs included multifocal mucosal necrosis and effacement with consolidation by filling of alveolar spaces with neutrophils and histiocytes; loss of alveolar walls in the absence of distinct necrosis; prominent perivascular lymphocytic cuffs; and congestion of alveolar walls. Histopathology on intestine from Piglet 1 suggested enteritis, as evidenced by mucosal crypt epithelia necrosis. Fresh lung was negative for Porcine Reproductive and Respiratory Syndrome by PCR. Aerobic culture of lung swabs was unremarkable. No Salmonella or Clostridium were cultured from small intestinal fecal contents. National Animal Health Laboratory Network (NAHLN) real-time reverse transcription polymerase chain reaction (rRT-PCR) assays designed to detect the 2009 pandemic H1N1 influenza A virus were conducted on RNA isolated from lung homogenates from both piglets. The assays included detection of the matrix gene of influenza A virus and neuraminidase gene specific for 2009 pH1N1 virus. Both samples were positive on both assays. The virus was isolated by inoculation onto Madin-Darby canine kidney (MDCK) cells as well as into specific-pathogen-free embryonated chicken eggs. The virus isolated from cell culture and embryonated eggs was sequenced. Sequence analysis showed 98% homology with 2009 pH1N1 human isolates from across the US and 98% homology against two recent US swine isolates from Nebraska and Minnesota. The owner was employed as a pharmacist and participated in pH1N1 vaccination programs, making occupational exposure to the 2009 pandemic H1N1 virus a possibility and is suspected to be the route of transmission from human to swine.

A Federal and State Transport Plan for Movement of Commercial Turkeys in a High Pathogenicity Avian Influenza Control Area - The FAST Turkeys Plan

Darrell Trampel[1], James Roth[2]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA;
[2]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA

Narrative: The FAST Turkeys Plan is a voluntary program for turkey producers intended to facilitate business continuity during an outbreak of H5 or H7 high pathogenicity avian influenza (HPAI). Components of the FAST Turkeys Plan are described below. **1. Risk Assessment**. A risk assessment of the public health impact of highly pathogenic avian influenza virus (HPAIV) was conducted by FSIS, FDA, and APHIS. If a turkey flock is exposed to HPAIV, there is a 98% probability that the flock would be identified before slaughter based on elevated morbidity and mortality and not enter commerce. On-farm HPAIV testing immediately before slaughter would mitigate 94% of risk of human illness associated with the remaining 2% of flocks. **2. Biosecurity Plans for Commercial Turkey Premises.** Effective biosecurity measures significantly reduce the likelihood that HPAI virus will be introduced onto a turkey farm. Level 1 biosecurity measures are routinely used and should be present on well-managed turkey farms. Level 2 biosecurity measures should be implemented following the diagnosis of highly pathogenic H5 or H7 avian influenza in a region. **3. Location Verification of FAST Turkeys Plan Premises using Global Positioning System (GPS) Coordinates**. **4. Epidemiology Information**. A questionnaire will assist epidemiologists to a) assess risk factors associated with employees, wild birds, and carcass disposal, b) determine how HPAIV may have been carried onto a farm (trace back information), and c) determine where HPAIV may have traveled from a farm (trace forward information). **5. Active Surveillance by Real-Time Reverse Transcriptase Polymerase Chain Reaction (RRT-PCR) Testing.** The absence of infection will be documented by requiring each house on the farm to be tested for 3 consecutive days prior to movement of turkeys and found to be negative by RRT-PCR for HPAIV. **6. Flock Mortality Data and Visual Inspection Prior to Movement.** Prior to moving turkeys, records of daily mortality for the preceding 7 days for each turkey house on the premises must be submitted to the Incident Command. Visual inspection of turkeys in all houses for two consecutive days, including the day before and day of movement, will be required for all premises located in the Control Area (Infected Zone plus Buffer Zone) that wish to move turkeys. **7. Permit Guidance Criteria.** Clear direction is provided for regulatory personnel responsible for issuing permits for movement of turkeys in a Control Area during an outbreak of HPAI so that an informed, risk-based decision can be made.

The Northeast Wildlife Disease Cooperative

Julie Ellis[1], Sarah Courchesne[1], Barbara Davis[1], Maureen Murray[1], Richard A. French[2], Inga Sidor[2], Salvatore "Frasca, Jr."[3], Joan Smyth[3], Michelle Fleetwood[2], Alice D. Roudabush[2], Elizabeth Bunting[4], Bruce Akey[4]

[1]Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA; [2]NH Veterinary Diagnostic Laboratory, University of New Hampshire, Durham, NH; [3]Connecticut Veterinary Medical Diagnostic Laboratory, Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT; [4]Animal Health Diagnostic Laboratory, Cornell University College of Veterinary Medicine, Ithaca, NY

Narrative: The Northeast U.S. is a hotspot for emerging infectious diseases that affect both animals and humans because of its dense human populations and the presence of major ports like Boston and New York, where human and animal travelers pour into the country. West Nile Virus and Lyme Disease were detected here before anywhere else in the country. Eastern Equine Encephalitis and Tularemia persist in wildlife here, claiming both animal and human lives. In spite of our vulnerability, the Northeast does not have a designated wildlife disease laboratory capable of investigating potential disease outbreaks, nor for conducting surveillance to anticipate the emergence of new diseases before they become widespread. To address this gap, we are establishing the Northeast Wildlife Disease Cooperative (NEWDC), a state, federal, and private sector cooperative structure that will provide wildlife health and disease expertise in the Northeast U.S. NEWDC will complement and enhance federal and state wildlife diagnostic efforts already in place by bringing together regional stakeholders and facilitating communications. Wildlife health assessments and diagnostics for live and dead specimens will be conducted by several regional laboratories with expertise in (but, not limited to): terrestrial, freshwater, and marine wildlife pathology; zoonotic diseases; environmental toxicology and immunology; ultrastructural and molecular characterization of pathogens; bioterrorism and informatics. Membership in the NEWDC provides professional training in wildlife diseases, full-service diagnostics, disease fact sheets and other related literature, ready access to professionals and diagnosticians, inclusion in communications regarding regional wildlife disease issues, and assistance in development of research studies.

Isolation of a *Clostridium perfringens* type D Isolate Producing β2 Toxin and Enterotoxin from a Calf

Yan Zhang, Jing Cui, Anne Parkinson, Mary Weisner, Beverly Byrum

ADDL, Ohio Dept of Agriculture, Reynoldsburg, OH

Narrative: *Clostridium perfringens* type D isolates cause natural enterotoxemia in sheep, goats, and occasionally cattle. *C. perfringens* Type D isolates produce two major toxins: α-toxin and ε-toxin. In addition to the major two toxins, some type D organisms isolated from sheep also make β2 toxin or enterotoxin, or both. Here, we report isolation of a *Clostridium perfringens* from a calf that apparently died from enterotoxemia. The calf was 10 days-old and from a 151-head Holstein cattle herd. The herd lost 5 calves during a two week period. All animals died of sudden death. Hemorrhagic small intestine was a common finding for all animals at necropsy. Genotyping indicated that the isolate is a type D *Clostridium perfringens* producing both β2 toxin and enterotoxin. To our knowledge, *Clostridium perfringens* type D isolate that produces both toxins in cattle has not been reported previously.

Differential Shiga Toxin Production among Shiga Toxin-Producing *Escherichia coli*

Chitrita DebRoy, Narasimha Hegde, Elisabeth Roberts, Bhushan Jayarao, Vivek Kapur

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

Narrative: Shiga toxins produced by *Escherichia coli* are responsible for causing diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans. The shiga toxin genes (stx1 and stx2) are carried by prophages that are integrated into *E. coli* genomes. The toxins are expressed and released under stress. Six O groups of shiga toxin producing *E. coli* (STEC), in addition to O157, have been considered important for causing diseases in humans. The objective was to examine if there are differential toxin expressions among the clinical isolates carrying stx genes belonging to different STEC O groups for defining the degree of pathogenicity between the O groups. An ELISA assay was developed by coating 96-well plates with globotriosyl ceramide which is the receptor of shiga toxin. Clinical isolates belonging to O groups O26, O45, O111, O121, O145 and O157 were inoculated into fresh media and induced by ciprofloxacillin (100 ng/mL) or mitomycin C (100 ng/mL) and further incubated at 37oC for 6 h. The cell supernatant was used for the assay. Monoclonal antibodies to Stx1 and Stx2 were used to bind to the receptor-toxin complex. Anti-mouse IgG attached to horseradish peroxidase was added to the complex followed by color reaction that was developed and quantitated by reading at 450 nm. There were differential expressions of shiga toxins among the clinical isolates belonging to different STEC O groups. While some of the strains needed induction for Stx production, others produced the toxin without induction. Further experiments are being conducted to compare the expression among the STEC isolates. It is concluded that the expression of shiga toxins vary considerably and shear presence of shiga toxin genes is not an indicator of pathogenicity of the STEC. This work provides a comparative analysis of association of shiga-toxin gene and its ability to produce toxins among STEC O groups. The ELISA method developed for shiga toxin detection is potentially applicable to screen for STEC strains associated with diverse food materials.

Direct Detection of Dermatophyte Fungi in Clinical Samples Using Real-Time PCR

Feng Sun, Amy Swinford, Alfonso Clavijo

Texas Veterinary Medical Diagnostic Laboratory, College Station, TX

Narrative: Dermatophytes are specialized fungi that can cause skin lesions in humans and animals. Identification of dermatophytes is usually based on morphological characteristics determined by time consuming microscopic and cultural examinations performed by experienced laboratory personnel. Definitive identification is based on microscopic morphology of micro- and/or macroconidia. Although isolation and identification can be specific, it often requires 1-3 weeks to yield positive results. The objective of the present study was to develop a sensitive and specific diagnostic test for the detection and identification of the most common dermatophytes species of veterinary importance directly from clinical samples. To increase the diagnostic sensitivity, accuracy, and turnaround time, a dermatophyte-specific real-time PCR assay with two forward primers, one reverse primer, and one degenerate Taqman probe, within region of 18S ribosomal RNA, ITS1, ITS2, and 5.8S ribosomal RNA, was developed, optimized, and evaluated. The DNA was extracted from dermatophyte isolates and directly from clinical samples including hair, scales, skin, or samples collected using a toothbrush. Nearly 100 samples from species including cats, dogs, cattle, horses and exotic animals were tested. This assay demonstrated potentially greater analytical and diagnostic sensitivity relative to direct culture and microscopic examination. All dermatophyte positives by culture were positive by real-time PCR. However, real-time PCR detected 7 additional samples as positives that were culture negative. The detection limit of the real-time PCR assay was determined using a plasmid containing dermatophyte DNA by diluting 10-fold and 2-fold serial dilutions. The limit of detection for the assay was 16 copies with a cut off of the assay of Ct 38. The duration of the assay from sample preparation to dermatophyte detection is about 4 hours. In conclusion, direct DNA isolation from clinical specimens and the real-time PCR method developed in this study provide a rapid, reproducible, and sensitive tool for detection of major dermatophyte species independent of morphological characteristics.

High-level Azlocillin Against *Pseudomonas aeruginosa* in BD BACTEC™ MGIT™ Para TB System Liquid Cultures *J. Mantlo, R. F. Pfeltz, M. T. Warns, G. L. Campbell* BD Diagnostics, 7 Loveton Circle, Sparks, Maryland, 21152 USA

Matthew Warns, Richard Pfeltz

Research and Development, Becton Dickinson Diagnostic Systems, Sparks, MD

Narrative: Azlocillin (AZ) is in the BD PANTA™ antimicrobial formulation at 4 µg/mL to suppress contaminants in culture media for clinical mycobacteria. Contamination is more challenging from cultured bovine feces for the recovery of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease in ruminants. This study investigated high-level AZ in contamination control formulations for MAP liquid culture with three *Pseudomonas aeruginosa* (PA) isolates of different beta-lactam susceptibilities and one MAP strain. Supplemented BD MGIT™ Para TB Medium tubes were seeded with 800 cfu of MAP ATCC™ 19698 or 5500-7000 cfu of PA ATCC 27853 (beta-lactam susceptible), PA BD 11195 (piperacillin resistant, ceftriaxone susceptible), or PA BD 9905 (piperacillin & timentin resistant). Six antimicrobial formulations were tested, all with the standard 7 µg/mL amphotericin B and 19 µg/mL vancomycin: (1) standard ANV (19 µg/mL nalidixic acid, NAL), (2) NAL-75/CRO-6 (75 µg/mL NAL + 6 µg/mL ceftriaxone), (3) NAL-75/AZ-12.5 (75 µg/mL NAL + 12.5 µg/mL azlocillin), (4) NAL-75/AZ-25, (5) NAL-75/AZ-50, and (6) NAL-75/AZ-100, all conditions in triplicate. After 49 days in the MGIT 960 instrument, detection rates were determined, and times-to-detection (TTDs) analyzed by ANOVA with MINITAB ver. 15.1 statistical software. Detection was 100% for MAP 19698, PA 11195, and PA 9905, but only for PA 27853 with standard ANV. Detection rates for PA 27853 were 33% with NAL-75/CRO-6, 67% with NAL-75/AZ-12.5 or -25, and 0% with NAL-75/AZ-50 or -100. Mean TTDs with standard ANV, NAL-75/CRO-6, and NAL-75/AZ-12.5, -25, -50 and -100 for MAP 19698 were 13.97, 16.67, 15.17, 15.44, 16.11, and 16.34 days respectively. Mean TTDs for PA 11195 & 9905 were 2.01-2.40 days with NAL-75/AZ-50 or -100, significantly longer than the 0.39-0.96 days for other conditions ($P < 0.05$). Standard ANV mean TTD of MAP 19698 was significantly shorter vs. other conditions, but mean TTD with NAL-75/AZ-100 was not significantly different vs. NAL-75/AZ-50 or NAL-75/CRO-6 ($P < 0.05$). Azlocillin at 50 or 100 µg/mL with NAL-75 in BD MGIT Para TB System culture medium was well-tolerated by MAP 19698, which had 100% detection and mean TTDs comparable to the NAL-75/CRO-6 now used for highly contaminated samples. NAL-75/AZ-50 or -100 prevented overgrowth by a beta-lactam susceptible PA but not two resistant strains, for which detection was delayed but not enough to be of practical use. NAL-75/AZ-50 or -100 formulations are of potential use for veterinary labs receiving samples frequently contaminated by beta-lactam susceptible *P. aeruginosa*.

Antimicrobial Resistance and Virulence Genes in *E. coli* Isolates from Diarrheic Piglets *

Jae-Won Byun, Ha-Young Kim, O-Soo Lee, Byeong Yeal Jung

ADDC, NVRQS, Anyang

Narrative: Antimicrobials are widely used for disease control and growth promotion in veterinary fields. The use of them contributes to the development and dissemination of antimicrobial resistance (AMR). Some reports have shown that virulence genes (VGs) of *E. coli* isolates from pigs are associated with AMR. Thus, the aim of this study was to investigate the prevalence of AMR phenotypes, virulence genes of *E. coli* and the association between them.From 2008 to 2010, two hundred and fifteen *E. coli* were collected from diarrheic piglets originating from 152 pig farms throughout Korea. Virulence genes, *F4, F5, F6, F18, F41, LT, STa, STb, STX2e, EAST1, Paa, AIDA-I* and *EAE* were detected by PCR as reported previously (Zhang et al., 2007). Antimicrobial susceptibility testing was carried out using the disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Antimicrobials were amoxicillin, ampicillin, apramycin, ceftiofur, cephalexin, doxycycline, enrofloxacin, florfenicol, flumequine, gentamicin, kanamycin, oxytetracycline, spectinomycin, streptomycin, sulfadiazine and sulfamethoxazole. To calculate the association between VGs and AMR, two-tailed Fisher's exact test were used. P-value of <0.05 were considered significant associations, and in such cases, odds ratio and 95% confidence interval were calculated. Multiple antimicrobial-resistance phenotypes (≥ 5 antimicrobials) were observed in 94% of *E. coli* isolates and the predominant types of resistance were to sulfadiazine (95.5%), streptomycin (90.5%) and oxytetracycline (90.5%). The most prevalent VGs were *EAST1* (43.5%), followed by *LT* (34.6%) and *STb* (34.2%). Resistance to amoxicillin and ceftiofur, was associated with the presence of *STX2e* (95% CI, 2.4-11.6, P = 0.0001) and *F4* (95% CI, 1.6-8.3, P = 0.0016), respectively. *LT* was significantly associated with apramycin (95% CI, 1.9-7.9, P = 0.0007), enrofloxacin (95% CI, 2.3-10.8, P = 0.0002) and flumequine (95% CI, 2.3-20.3, P = 0.0001). The results showed that 94% of *E. coli* isolates from diarrheic piglets were shown to multiple antimicrobial-resistance phenotypes (≥ 5 antimicrobials). The most prevalent VGs were *EAST1* (43.5%), followed by *LT* (34.6%) and *STb* (34.2%). There were clear associations between VGs and several antimicrobials. Therefore, further studies should be done to investigate the prevalence of antimicrobial resistance genes causing AMR and associations between them.

* Graduate Student Poster Presentation Award Applicant

Development of *Actinobacillus pleuropneumoniae* Indirect Enzyme-Linked Immunosorbent Assay Using Recombinant Apx Toxin Antigen

Ji Lee[1], Woo-Chang Kim[2], Aeran Kim[1], Suk Chan Jung[1]

[1]National Veterinary Research & Quarantine Service, Anyang-city; [2]Jenobiotech, Chuncheon

Narrative: *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) is the etiologic agent of pleuropneumonia in pigs. It is one of the important bacterial diseases of the respiratory tract of the pig and occurs in most pig-keeping countries. Early diagnosis and isolation of infected pigs from the herd are most effective in preventing the spread of this disease. Serological testing is the most powerful tool to diagnose subclinical infections due to *A. pleuropneumoniae*. The Apx antigen of *A. pleuropneumoniae* was considered one of the specific toxin antigens. Antibodies against Apx toxin were detected most often in the serum of pigs infected with *A. pleuropneumoniae*. Therefore, it proved to be an excellent marker of APP. In the present study, we developed mutagenic recombinant Apx toxin (r-Apx) protein and validated an indirect ELISA for the detection of serum antibodies against *A. pleuropneumoniae*. N-terminal (1-1,500nt) of APP Apx genes was cloned into a pET100/D-TOPO and transformed in BL21 starTM(DE3). The recombinant proteins were purified and 65kDa protein detected by antiserum against *A. pleuropneumoniae* in western blotting. The purified r- Apx toxin was successfully used in an enzyme-linked immunosorbent assay for detection of antibodies against *A. pleuropneumoniae*. Hyperimmune sera against *A. pleuropneumoniae* were prepared by immunizing pigs known to be free of APP. The negative sera were harvested from known seronegative minipig. All sera were tested with commercial APP ELISA and compared the practical usefulness of ELISA. The r-Apx toxin(65kDa) was strongly bound to the antiserum *A. pleuropneumoniae* in western blotting and was used as APP Apx toxin based ELISA antigen. Sera from uninfected or non-vaccinated SPF mini-pig were used to evaluate specificity of ELISA. The specificity score of the assay was 95%. Antibody conversion in response to *A. pleuropneumoniae* was shown 2 weeks after immunization with inactivated antigen of *A. pleuropneumoniae* and did not react with sera from mock infected animals. The correlation (r2) between APP Apx based ELISA and commercial ELISA was 0.84 and observed similar sero-conversion pattern. In this study, we have successfully demonstrated production of *A. pleuropneumoniae* r- Apx toxin and was successfully used in an enzyme-linked immunosorbent assay for detection of antibodies against *A. pleuropneumoniae*. It improved the reproducibility and simplicity of preparation of an ELISA antigen for diagnosis of *A. pleuropneumoniae* antibodies. Taken together, it is considered an effective tool for maintaining an SPF herd of swine and increasing the productivity of a pig farm.

Disseminated Aspergillosis in a Dog due to *Aspergillus alabamensis*

Eric Burrough[1], Claire Andreasen[2], Timothy Frana[1], Jesse Hostetter[2]

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

Narrative: Disseminated aspergillosis is uncommon in dogs and often associated with *Aspergillus terreus* infection. German shepherd dogs appear predisposed; however, immunosuppression in any breed may increase the potential for disseminated disease. *Aspergillus alabamensis* is a recently described species within the section *Terrei* that is morphologically similar to *A. terreus* but genetically distinct. A 5-year-old, spayed female, English springer spaniel presented to the Lloyd Veterinary Medical Center of the Iowa State University College of Veterinary Medicine with a 7 day history of vomiting, inappetence, and lethargy. Four weeks prior to presenting, the patient was diagnosed with masticatory myositis and started on azathioprine and twice daily prednisone. Abdominal radiographs obtained at presentation revealed moderate ascites, and an ultrasound-guided abdominocentesis was performed. Cytology of the recovered fluid was consistent with a septic exudate containing predominantly neutrophils which occasionally clustered around septate fungal hyphae. Further therapy was not pursued and the patient presented for necropsy where numerous widely scattered pale foci were noted in multiple organs. Histologic evaluation revealed vasculitis and disseminated pyogranulomas in the lung, pleura, heart, thyroid, esophagus, diaphragm, liver, gall bladder, stomach, spleen, small intestine, mesenteric lymph nodes, pancreas, kidney, and adrenal gland. Pyogranulomas often contained numerous conidia, germinating conidia, and fewer short, septate, 4.5 - 5 µm hyphae with parallel walls. Fungal culture of both the ante mortem peritoneal fluid sample and a postmortem liver sample yielded high fungal growth but phenotypic characterization was not definitive. Further analysis by beta-tubulin sequencing identified the isolate as *A. alabamensis*. To the authors' knowledge, this is the first report of disseminated aspergillosis in a dog due to *A. alabamensis* and provides further support for the value of molecular methods in the precise identification of agents noted histologically.

Prevalence of Shiga Toxin Producing *E. coli* in Retail and Game Meat

Chitrita DebRoy[1], Huu Dang[1], Kudakwashe Magwedere[2], Edward Mills[2], Catherine Cutter[3]

[1]Veterinary and biomedical Sciences, Pennsylvania State University, Univeristy Park, PA;
[2]Department of Animal Sciences, Pennsylvania State University, Univeristy Park, PA;
[3]Department of Food Sciences, Pennsylvania State University, Univeristy Park, PA

Narrative: Shiga toxin producing *Escherichia coli* (STEC) belonging to serogroups O26, O45, O103, O111, O113, O121 and O145, in addition to O157, have emerged as important food-borne pathogen of considerable public health significance. The objective of the study was to determine the level of contamination of these STEC O groups in retail ground meat and in game meat. The findings can assist regulatory agencies to consider measures to control contamination of these organisms in meat. Ground meat samples from beef, chicken and pork and game meat from deer, bison, wild boar, elk and rabbit were purchased. Ground meat samples (n=60, subsamples n=300) and game meat samples (n=55, subsamples n=275) were processed for the detection of *Escherichia coli* STEC O groups. Meat sample (5 g) was enriched in Tryptic Soy broth (TSB) containing 8mg/L of novobiocin and 16 mg/L of vancomycin, stomached for 2 min and incubated for 6 h at 37°C. Bile salts (1.5 g/L), rifampicin (2 mg/L) and potassium tellurite (1 mg/L) were added, and incubated for 18 h at 42°C. A positive control sample was prepared per batch where a known STEC strain was inoculated in meat and subjected to the same enrichment. DNA was extracted using Masterpure DNA purification kit and multiplex PCR for all 8 O groups listed above was conducted (DebRoy et al. 2011). Presence of Shiga toxin genes (stx1 and stx2) were tested on samples that exhibited the presence of STEC O groups, using m-PCR optimized in the laboratory. While none of the retail and game meat samples exhibited the presence of O26, 28% of ground beef samples carried *E. coli* O121 and 15% carried O45. Among ground chicken samples 43% samples exhibited presence of O157, 25% carried O45 and 18% carried O103. Of the ground pork samples tested 37% carried O121 and 6% O103. In game meat samples, deer meat and bison carried strains belonging to O45, O103, O111, O113, O121, O145 and O157, although only one sample belonging to O45 from deer carried stx1 genes. None of the retail meat samples carried stx1 or stx2. It is concluded that the meat samples in grocery stores and farmers markets may carry STEC O groups, but they are nonpathogenic and do not carry shiga toxins. Further work needs to be conducted with larger sample size to make definitive conclusions.

Reference: DebRoy C et al. 2011. FBP 8: 651-652.

Specific Detection of Antibodies to *Babesia bigemina* by IFA Using a FITC-Labeled Monoclonal Antibody to Bovine IgG1

Chungwon Chung

VMRD, Inc, Pullman, WA

Narrative: Apicomplexan parasites in the genera Babesia and Plasmodium invade and replicate within host erythrocytes. During this intracellular replication, the erythrocyte membrane experiences diverse metabolic and structural changes. *B. bigemina*-infected erythrocytes, but not uninfected erythrocytes, are reported to bind bovine IgM *in vivo* during acute parasitemia before the occurrence of a specific immune response (Infection and Immunity 66:2922, 1998). Thus, the use of blood smears from infected cattle and from *in vitro* cultures grown with bovine serum as substrates for indirect fluorescent antibody assays (IFA) are not suitable because most FITC-labeled secondary antibodies to bovine immunoglobulins bind to the infected erythrocytes in the absence of primary antibodies to *B. bigemina*. To solve this problem, IFA was performed with blood smears from a *B. bigemina*-infected calf and several secondary reagents consisting of FITC-conjugated antibodies specific to different bovine immunoglobulin isotypes. FITC conjugates made with anti-bovine IgG1 and IgG2 polyclonal antibody and anti-bovine IgG-heavy chain-specific polyclonal antibody bound to infected erythrocytes causing fluorescence in the absence of primary bovine serum. This was also observed with a FITC conjugate made with a bovine IgM-specific monoclonal antibody. However, a FITC conjugate made with monoclonal antibody specific to bovine IgG1 reacted when primary sera from *B. bigemina*-infected cattle were tested and not with sera from uninfected cattle. This IFA conjugate made with bovine IgG1-specific monoclonal antibody results in specific detection of IgG1 antibodies to *B. bigemina* and provides a useful assay for serologic detection of *B. bigemina*-infected animals.

Rapid Confirmation, with Minimal Sample Preparation, of Calcium Oxalate Crystal Deposition in Renal Tissue by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Deon Van der Merwe[1,2], Kelli M. Almes[1,2], Lori Blevins[1]

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

Narrative: Ethylene glycol is the main component of typical automotive antifreeze, and spills are often associated with leaking radiators and servicing of vehicles. Pets readily consume spilled ethylene glycol because it has a sweet taste. Following rapid absorption from the digestive tract, ethylene glycol is converted to glycoaldehydes and organic acids, including oxalic acid, in the liver. Oxalic acid reacts with calcium in the blood and tissues to form insoluble calcium oxalate crystals that are deposited in tissues, particularly renal tissue, and excreted into urine. Calcium oxalate deposits in renal tissue and urine are important diagnostic features of ethylene glycol poisoning. We describe an attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy method for calcium oxalate crystal identification in renal tissue and urine that is simple, rapid and robust. The method allows for the differentiation between calcium oxalate crystals and other crystal types that may be found in the urinary system. Further advantages include non-destructive assessment and small samples size. We demonstrated that the method can be applied to urine, fresh renal tissue, and renal tissue samples that had been fixed in 10% buffered formalin and mounted in paraffin for traditional histological examination.

Development and Validation of a Foot-and-Mouth Disease Virus 3D Protein ELISA for Differentiation Between Infected and Adenovirus-FMD-Vaccinated Bovine.

Brooke Dancho, Abu Sayed, Samia Metwally

Foreign Animal Disease Diagnostic Laboratory, USDA, Greenport, NY

Narrative: There is a high-priority need for an antibody ELISA for early detection of foot-and-mouth disease (FMD) virus infection for use as a companion test to discriminate between infected and adenovirus-FMD-vaccinated animals. This test could be deployed for use in support of a vaccinate-to-live policy as it can discriminate between FMD virus-infected and non-infected animals regardless of their vaccination status. The FMD virus 3D protein was used as the antigen in the assay because: 1) the gene that encodes 3D protein is excluded from the vaccine, 2) 3D protein is the most immunogenic of all of the FMD virus non-structural proteins, and 3) 3D protein antibody response is elicited earliest amongst the FMD virus non-structural proteins. A competitive FMD 3D protein ELISA was developed using baculovirus-expressed 3D protein and an anti-3D protein monoclonal antibody. Positive serum samples were collected from bovine infected with FMD virus serotypes A, O, and Asia 1. A negative serum panel was composed of sera from adenovirus-FMD vaccinated bovine and from diagnostic serum submissions to the Foreign Animal Diagnostic Disease Laboratory at the Plum Island Animal Disease Center. Preliminary bench validation results show that the assay has high sensitivity and specificity.

Comparison of Peptide Cocktails and Purified Protein Derivatives for Use in the Bovigam™ Assay *

Kristin Bass[1,2], Brian Nonnecke[2], Mitchell V. Palmer[2], Tyler Thacker[2], Roland Hardegger[3], Bjoern Schroeder[3], Alex Raeber[3], W. Waters[2]

[1]Immunbiology, Iowa State University, Ames, IA; [2]National Animal Disease Center, Ames, IA; [3]PrionicsAG, Schlieren

Narrative: Currently the Bovigam™ assay is used as an official complementary test within the bovine tuberculosis eradication program. This assay measures Interferon-gamma (IFN-γ) produced by lymphocytes in response to specific antigens. The objectives of the present study were to compare *in vitro* antigen preparations and the kinetics of the IFN-γ response during experimental infection with *Mycobacterium bovis*. Purified protein derivatives (PPDs) derived from *Mycobacterium avium* and *M. bovis* from two manufacturers, CSL and Lelystad, were also evaluated. Liquid and lyophilized preparations of antigens, along with PPDs, were compared using uninfected (control) and cattle experimentally infected with either *M. bovis* 95-1315 or *M. bovis* Ravenel. Prior to infection, responses to PPDs were detected in all cattle with *M. avium* responses exceeding responses to *M. bovis* suggesting prior exposure of cattle to *M. avium* or other non-tuberculosis mycobacteria. Upon *M. bovis* challenge, responses to Lelystad PPDs exceeded respective responses to CSL PPDs as early as three weeks after infection. It was also noted that responses did not differ between the lyophilized and liquid preparations. *M. bovis* infections elicited equal responses to both Peptide Cocktail 11 and ESAT-6/CFP-10 peptide cocktail within three weeks after infection. With no difference between responses to liquid and lyophilized preparations, antigens can be stored in a lyophilized state without diminishing effectiveness of stimulation. Variations in response of bovine lymphocytes to PPDs from different manufacturers should be recognized when implementing changes in antigens (e.g., PPDs) for official bovine TB tests.

* Graduate Student Poster Presentation Award Applicant

Investigating the Spatio-Temporal Epidemiology of *Tritrichomonas foetus* Infection in Texas Bulls Using Diagnostic Laboratory Data #

Barbara Szonyi[1,2], Alfonso Clavijo[1], Indumathi Srinath[2], Renata Ivanek[2]

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

Narrative: Effective control of diseases requires reliable estimates of disease occurrence in populations. However, cross-sectional studies are often cost-prohibitive and unfeasible to carry out in large populations. Under such circumstances, data generated in diagnostic laboratories (DL) may serve as a cost-effective alternative. Nonetheless, veterinary DL data is often not analyzed and interpreted adequately. Texas is the largest cattle producing state that suffers serious economic losses due to abortions caused by *Tritirichomonas foetus*. Our objectives were to 1, estimate the occurrence and spatio-temporal distribution of *T. foetus* infection in Texas; 2, quantify the effect of cattle density on the risk of *T. foetus* infection; and 3, create a risk map for *T. foetus* in Texas, using data generated in a major veterinary DL in Texas. The study population consisted of bulls tested for infection with *T. foetus* in 2010 by the Texas Veterinary Medical Diagnostic Laboratory. Preputial wash samples were transported and cultured. Diagnosis was made by real-time PCR. Data was aggregated at the county level. The scan statistics was used to identify spatial and temporal disease clusters, and a negative binomial model was fit to assess the association between cattle density and the number of *T. foetus* infections per county. Lastly, isopleth risk map was produced to delineate high-risk areas for *T. foetus* using the geostatistical method of kriging. The database included 31,202 test results with a proportion of positives of 3.7%. The scan statistics identified a spatial cluster in southeastern Texas and a temporal cluster in the summer. The number of large herds was positively associated with the risk of *T. foetus* infection while latitude was associated with a decreased risk. Our aim was to improve our understanding of the epidemiology of *T. foetus* in Texas cattle using DL database. The identification of high-risk areas for *T. foetus* in Texas may provide useful information for producers and policy makers about how to improve disease control efforts in the state. This study illustrates the potential of veterinary diagnostic laboratories to support population-based animal health studies. With limited resources to conduct field studies, there is a need to utilize DL data in epidemiologic investigations. At the same time, such data carry inherent biases that should be taken into account by trained epidemiologists. We propose that collaboration between epidemiologists and laboratory diagnosticians greatly enhances the capacity of veterinary diagnostic laboratories to address pressing animal health issues.

AAVLD Trainee Travel Awardee (Epidemiology)

Phenotypic and Genotypic Characterization of *Fusobacterium* Isolates from the Respiratory Tract of Deer

Jason Brooks[1], Bhushan Jayarao[1], Amit Kumar[2], Sanjeev Narayanan[2], Suzanne Myers[1], T. Nagaraja[2]

[1]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA; [2]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS

Narrative: Twenty-eight clinical strains of *Fusobacterium spp*. were isolated from the respiratory tract of cervid species including white-tailed deer, elk, and reindeer at necropsy over a two-year period. Isolates were identified and characterized based on growth and morphologic characteristics, biochemical profiles, antimicrobial susceptibility, PCR detection of known or suspected virulence genes, 16S rDNA sequencing, and detection of leukotoxicity by cell viability assay using flow cytometry. Isolates were identified as *F. varium* (21/28, 75%), *F. necrophorum* subsp. *funduliforme* (5/28, 17.9%), and *F. necrophorum* subsp. *necrophorum* (2/28, 7.1%). Using PCR-based detection of virulence genes, all *F. varium* isolates were negative for the promoter region of the leukotoxin operon of *F. necrophorum* and the hemagglutinin-related protein gene of *F. necrophorum*. Toxicity to bovine polymorphonuclear leukocytes was not observed in any *F. varium* strains. *F. varium* was less susceptible to many antimicrobials compared to *F. necrophorum*. Necropsy examination showed no significant differences in gross or microscopic lesions between *Fusobacterium* species. These data suggest that *F. varium* may be a significant pathogen in deer, may use different virulence factors than *F. necrophorum*, and may require different treatment and prevention methods than used for infections caused by *F. necrophorum*.

Characterization of H5N1 Subtype Highly Pathogenic Avian Influenza Virus Isolated from Poultry and Wild Birds in South Korea, 2010-2011

Hye-Ryoung Kim, Jae-Ku Oem, Hyuk-Man Kwon, In-Soon Roh, Hyun-Mi Kang, O-Soo Lee, You-Chan Bae

National Veterinary Research & Quarantine Service, Anyang

Narrative: In South Korea, we had experienced three outbreaks of H5N1 HPAI virus before 2010. This study was conducted to characterize new H5N1 HPAI virus in 2010~2011 and compare it to the previous HPAI viruses. Oropharyngeal swab, cloacal swab, feces and organ samples from poultry and wild birds were collected to detect HPAI virus all over the country. For virus isolation, 9 to 11-day-old embryonated chicken eggs were used and the isolated viruses were subtyped using RT-PCR. Gene sequencing and phylogenetic analysis were carried out. The intravenous pathogenicity index (IVPI) was confirmed according to the OIE manual. Chickens (SPF white leghorn broiler chickens [*Gallus gallus domesticus*]) were inoculated intranasally with allantoic fluid containing 6.0 logEID50/0.1ml of the first isolates from poultry (A/duck/Korea/Cheonan/2010). On December 7, 2010, H5N1 subtype HPAI virus was isolated from a healthy mallard captured at the Mankyung River in South Korea. And then, from December 2010 to April 2011, a total 53 cases of HPAI were identified in poultry (chickens, quails, pheasant and domestic ducks) and wild birds of various species. A/duck/Korea/Cheonan/2010(H5N1), the first isolates from poultry, was classified into clade 2.3.2 by the phylogenetic analysis of HA gene. This virus was distinct from the HPAI viruses responsible for previous outbreaks in South Korea, A/chicken/Korea/ES/2003 (94.2%), A/chicken/Korea/IS/2006 (93.3%) and A/chicken/Korea/Gimje/2008 (97.3%), whereas closely related (99.2-99.8%) to H5N1 isolates found in Mongolia, China and Russia in 2009 and 2010. The IVPI index was 3.0, meaning that all chickens died within 24 hours. Experimentally infected chickens showed malacia with gliosis, necrosis in spleen and pancreas, which is identical to histopathological lesions of natural infection. The clade 2.3.2 H5N1 strains isolated in Eastern Asia in 2009-2010, have distinctive gene constellation unlike previous clade 2.3.2 viruses. In other words, the phylogenetic evidence suggests that polymerase acidic proteins (PA) of these viruses was similar to that of clade 2.5 HPAI viruses, but the other viral genes were originated from viruses that caused outbreaks in South Korea, Japan and Russia in 2008. The sources of the past three HPAI outbreaks in South Korea were not yet clear, but 2010-2011 outbreak is a good example which is caused by migratory birds.

Identification of Main Biting Midge Species of Korea and Detection of Arboviruses from Them

Jae-Ku Oem[1], Joon-Yee Chung[1], Hye-Ryoung Kim[1], Toh-Kyung Kim[2], Tae-Uk Lee[3], O-Soo Lee[1], You-Chan Bae[1]

[1]Animal Disease Diagnostic Center, National Veterinary Research and Quarantine Service, Anyang; [2]Gyeongnam institute of Livestock and Veterinary Research, Tongyeong; [3]Jeollanamdo Institute of Livestock and Veterinary Science, Gangjin

Narrative: Arthropod-borne viruses (Arboviruses) are transmitted by hematophagous arthropods, such as mosquitoes, Culicoides biting midges, and ticks. The genus Culicoides of the family Cerotopogonidae comprises >1,400 described species and is distributed throughout the world with exception of Antarctica and New Zealand. Culicoides biting midges are known to transmit several diseases caused by viruses. Bluetongue virus and African horse sickness virus are transmitted to ruminants and equids, respectively. The prevalence of the viruses largely depends on vector and host distribution, governed by geographical and climatic factors. Therefore, it is essential for control and prevention of arboviral diseases to identify the principal vectors in specific countries and regions. We undertook this study to identify the main biting midges in Korea, and to detect the arboviruses from them. Culicoides biting midges transmitting arboviruses in three cattle farms were collected in Tongyeong city in Gyeongnam province and Gangjin city in Jeongnam province and Goyang city in Gyeonggi province, from May to October, 2010. Adult Culicoides biting midges were collected once a week by using a light trap. The trap was set between 4:00 and 5:00 p.m. and collected the next morning in 9:00. Collected samples were sent to National Veterinary Research and Quarantine Service (NVRQS) and were sorted into species based on the identification keys of Kitaoka (1984) and were stored at -80° C until virus detection. Samples were pooled based on collection date and species. Arboviruses in Culicoides biting midges were detected by RT-PCR. A total of 21,297 Culicoides biting mideges were collected. *Culicoides punctatus*, *Culicoides arakawae*, *Culicoides oxystoma*, *Culicoides maculatus*, and *Culicoides japonicus* were present at 87.9%, 7.7%, 3.9%, 2.5%, 0.8%, rerspectively. *Culicoides punctatus* was most frequently collected from all three provinces and proved to be the most abundant *Culicoides spp*. in Korea. Identified midges were used for detecting arboviruses. Akabane virus, Aino virus, Chuzan virus and bovine ephemeral fever virus were detected from only *Culicoides punctatus* and *Culicoides arakawae* by RT-PCR . This study is just a start but will elucidate the role of Culicoides biting midges in the transmission of bovine arboviruses in the future. Until now, *Culicoides punctatus* has been the most frequently found Culicoides in the cattle farms in Korea. However, it remains to isolate the arboviruses from Culicoides biting midges and to control and prevent outbreaks of arbovirus diseases in Korea.

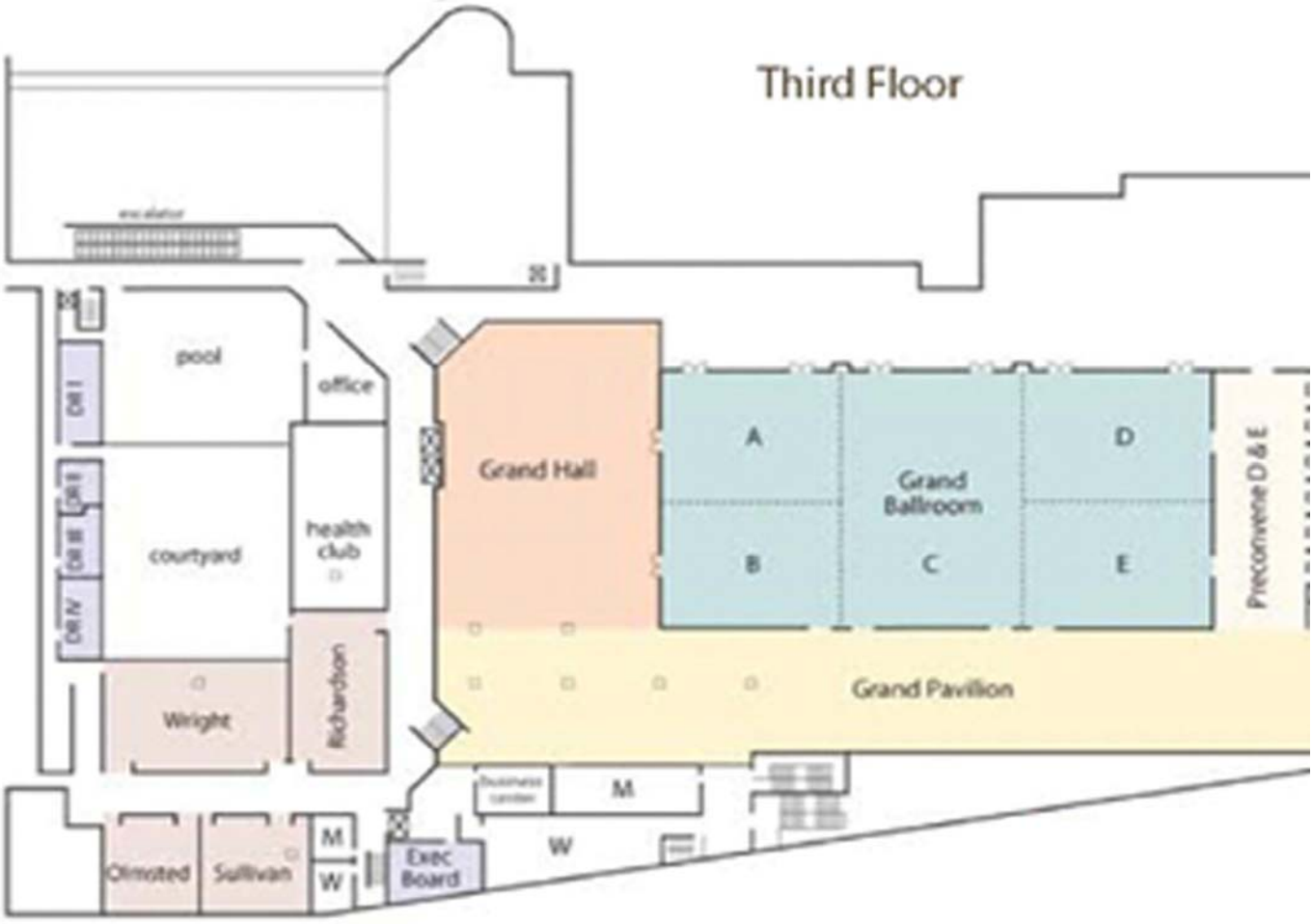
Development of *Mycoplasma hyopneumoniae* Indirect Enzyme-Linked Immunosorbent Assay Using Recombinant P46 Surface Antigen

Ji Lee[1], Woo-Chang Kim[2], Aeran Kim[1], Suk Chan Jung[1]

[1]National Veterinary Research & Quarantine Service, Anyang; [2]Jenobiotech, Chuncheon

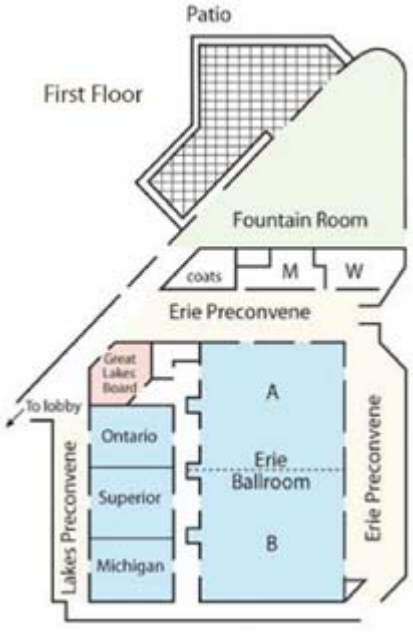
Narrative: Mycoplasmal pneumoniae of swine (MPS) is widespread disease and causes economic losses in the swine industry. *Mycoplasma hyopneumoniae* (*M. hyopnemoniae*) is a causative agent of mycoplasmal pneumoniae of swine (MPS). Early diagnosis and isolation of infected pigs from the herd are most effective in preventing the spread of this disease. The 46 kDa antigen (P46) of MH was considered one of the species-specific surface antigens. In the present study, we developed mutagenic recombinant P46 (r-P46) protein and validated an indirect ELISA for the detection of serum antibodies against *M. hyopnemoniae*. The r-P46 protein expressed in *Escherichia coli* vector containing 6X histidine tag at N-terminal and the mutagenesis P46 gene devoid of its putative promoter and nucleus localization signal sequence (1-36) of *M. hyopnemoniae*. The purified r-P46 was successfully used in an enzyme-linked immunosorbent assay for detection of antibodies against *M. hyopnemoniae*. Hyperimmune sera against *M. hyopnemoniae* were prepared by immunizing pigs known to be free of mycoplasmas. The negative sera were harvested from known seronegative minipig. All sera were tested with commercial MH ELISA and compared the practical usefulness of ELISA. For production of the full-length P46 protein in E. coli, it is necessary to replace TGA with TGG. The resulting plasmid, p-ET-MH P46, was used for expression and purification of r-P46 protein (46kDa). The r-P46 was strongly bound to the antiserum *M. hyopnemoniae* in western blotting and used as MH P46 based ELISA antigen. Sera from uninfected or non-vaccinated SPF mini-pig were used to evaluate specificity of ELISA. The specificity score of the assay was 95%. Seroconversion in response to *M. hyopnemoniae* was shown 3 weeks after immunization with inactivated antigen of *M. hyopnemoniae* and did not react with sera from mock infected animals. The correlation (r2) between MH P46 based ELISA and commercial ELISA was 0.84 and observed similar sero-conversion pattern. In this study, we have successfully demonstrated production of *M. hyopnemoniae* P46 in *E. coli* and r-P46 was successfully used in an enzyme-linked immunosorbent assay for detection of antibodies against *M. hyopnemoniae*. It improved the reproducibility and simplicity of preparation of an ELISA antigen for diagnosis of *M. hyopnemoniae* antibodies. Taken together, it is considered to effective tool for maintaining an SPF herd of swine and increasing the productivity of a pig farm.

Meeting Space Layout



Third Floor Meeting Rooms

Grand Hall
Grand Pavilion
Grand Ballrooms A-E
Richardson
Wright
Executive Board Room
Sullivan
Olmstead
Directors Row I-IV

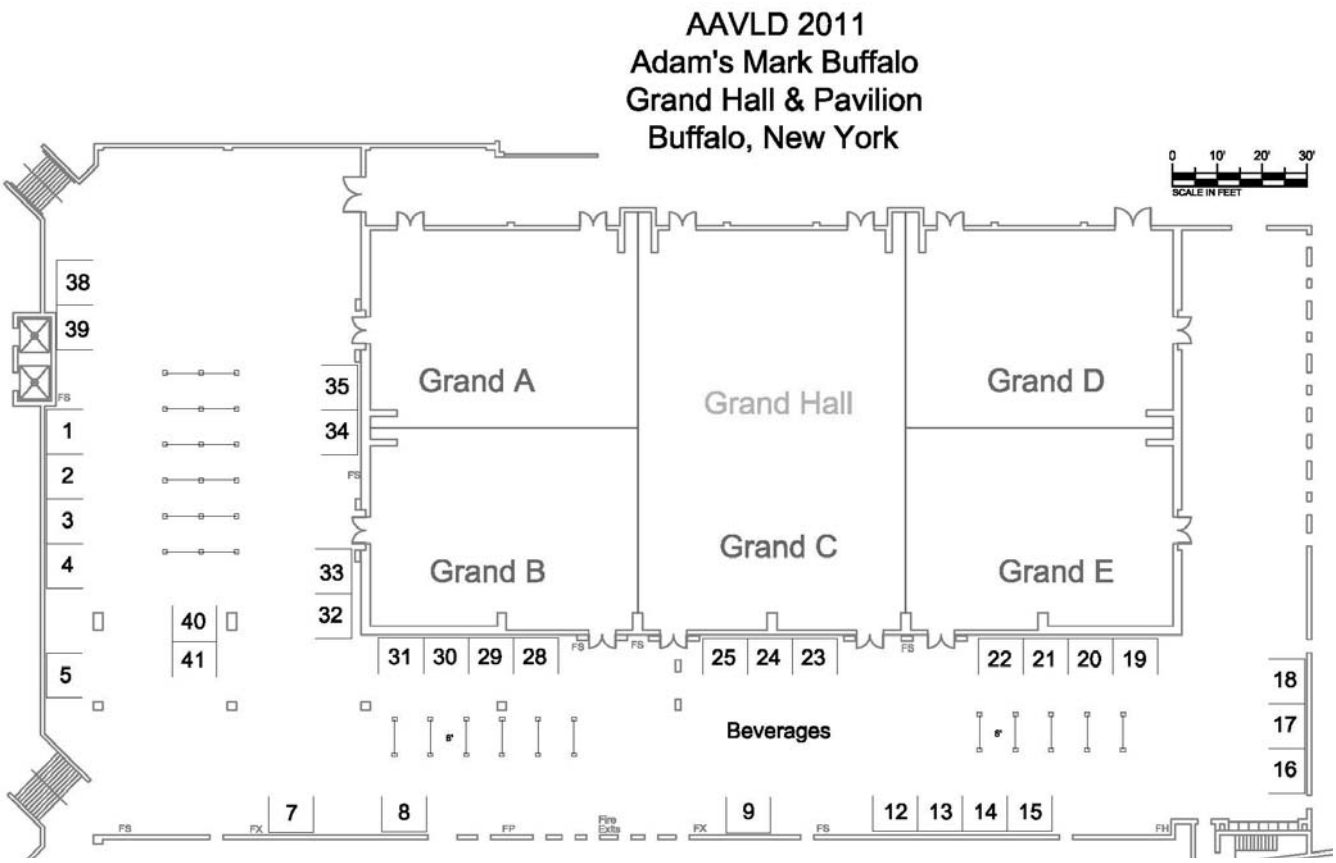


First Floor Meeting Rooms

(Walk passed the restaurant and follow hallway.)

Erie Ballroom A-B
Fountain Room
Great Lakes Boardroom
Michigan
Ontario
Superior

Exhibits and Poster Presentations



Exhibitors

Booth #	Company
1	Biosearch Technologies, Inc.
2	Abaxis, Inc. - Animal Health
3	Anaerobe Systems
4	Progressive Recovery, Inc.
5	Computer Aid, Inc.
7	Advanced Technology Corp.
8	Prionics
9	Tetracore, Inc.
12	VMRD, Inc.
13	VertiQ Software, LLC
14	Bruker Daltonics
15	Ventana Medical Systems, Inc.
16	National Institute for Animal Agriculture

Booth #	Company
21	Safe Path Laboratories, LLC
22	CDC Epi-X
23	Biolog, Inc.
24-25	Life Technologies
28	TREK Diagnostic Systems part of Thermo Fisher Scientific
29	Centaur, Inc.
30	GeneReach Biotechnology
31	Global Vet Link
32-33	IDEXX Laboratories
34-35	Qiagen, Inc.
38	Biovet Inc.
39	CompuGroup Medical
40-41	Synbiotics Corporation

Exhibits and Poster Presentations

Poster Session

3:00 pm Friday, Sep 30, through
2:00 pm Sunday, Oct 2

Saturday, October 1, 2011
3:00-4:00 pm Authors present

Exhibit Times

Saturday, October 1, 2011
9:10 am - 9:40 am
11:30 am - 1:00 pm
2:30 pm - 6:00 pm

Sunday, October 2, 2011
9:30 am - 10:30 am
11:15 am - 2:00 pm

Sponsor Presentations

Saturday – October 2, 2011

Life Technologies	6:00-6:30 pm	Grand Ballroom B	*A look into the now and future of Veterinary Diagnostics* Presented by Life Technologies
Synbiotics Corporation	6:30-7:00 pm	Grand Ballroom B	*Measurement of Porcine Circovirus type 2 (PCV-2) Antibody Titer in Pig Serum using SERELISA® PCV-2* Dr. Chinta Lamichhane, Vice President of Research & Development, Synbiotics Corporation
IDEXX	6:00-6:30 pm	Grand Ballroom E	*The IDEXX Bovine Pregnancy Test, A New Tool To Grow Lab Testing Volumes* Dr. André Fuchs, Senior Director, Worldwide Customer Facing Organization, IDEXX Livestock and Poultry Diagnostics
Prionics	6:00-6:30 pm	Grand Ballroom A	
VADDS	6:30-6:45 pm	Grand Ballroom A	*World-Wide Trends in Veterinary LIMS* Presented by Joseph J. Bove, President, Advanced Technology Corp

American Association of
Veterinary Laboratory Diagnosticians

Exhibit Directory

Buffalo, NY
November 12-14, 2010

Abaxis, Inc

Booth **2**

3240 Whipple Rd
Union City, CA 94587
www.abaxis.com
Contact: 800.822.2947
vetscansales@abaxis.com

Abaxis offers a comprehensive suite of VetScan in-office laboratory equipment for the animal health market. And all of these products deliver the universal VetScan promise of speed, accuracy, simplicity, ergonomics and economics. Your lab could be Better. Actually.

Advanced Technology Corp. VADDS

Booth **7**

79 North Franklin Turnpike, Suite 103
Ramsey, NJ 07446
www.vetstar.com
Contact: Joseph Bove
201.934.7127
jbove@vetstar.com

In a climate of change and uncertainty, VADDS remains the #1 comprehensive LIMS system among public veterinary labs in North America. VADDS is the most widely used LIMS system among AAVLD accredited labs.

New in 2011:

Open Client / Runs in a web browser

Improved Instrument and Data Interfaces

Becoming a world-wide leader in veterinary LIMS with new emphasis on SE Asia and Europe

Advanced Technology Corp. invites you to stop by our booth to learn more about VADDS, our comprehensive, budget-friendly veterinary LIMS system.

We look forward to remaining a leader within the veterinary community, in order to continue to provide the most feature-rich LIMS system at a most affordable price, for many years to come.

Anaerobe Systems

Booth **3**

15906 Concord Circle
Morgan Hill, CA 95037
www.anaerobesystems.com
Contact: Steve Cox
408.782.7557
stevecox@anaerobesystems.com

Anaerobe Systems produces true Pre-Reduced Anaerobically Sterilized (PRAS) plated and tubed media. Benefits of using PRAS media include reduced time handling cultures, substantially increased recovery, and rapid diagnosis. Anaerobe Systems will display our new AS-580 Gloveless Anaerobic Chamber, PRAS culture media, and educational materials for anaerobic bacteriology.

Biolog, Inc.

Booth **23**

21124 Cabot Blvd
Hayward, CA 94545
www.biolog.com
Contact: Yigal Uriel
510.785.2564 x398
yuriel@biolog.com

Biolog is a pioneer in the development of powerful cellular analysis tools for solving critical problems in clinical, pharmaceutical, and biotechnology research and development. In addition to our award winning Microbial Identification Systems, Biolog's unique Phenotype MicroArray technology can be used to assay cells of all types, from microbial to mammalian.

Biosearch Technologies, Inc.

Booth **1**

81 Digital Drive
Novato, CA 94949-5728
www.biosearchtech.com
info@biosearchtech.com
Contact: Marc Beal
800.436.6631 (U.S. and Canada)
+1.415.883.8400 (World wide)
Marcb@biosearchtech.com

Biosearch Technologies, Inc., located in northern California, is an ISO 9001:2008 certified manufacturer of sophisticated oligonucleotides for real-time qPCR applications. Since its founding in 1993, Biosearch has been at the forefront of advancing nucleic acid technology with the invention of CAL Fluor® and Quasar® fluorophores, and the Black Hole Quencher® (BHQ®) series of dyes – the industry standard in full spectrum quenchers for real time qPCR.

Biosearch supplies businesses with custom oligos for all stages of product development from design through commercialization. A vertically integrated structure allows Biosearch to precisely control the raw materials and manufacturing process, reducing costs and turnaround time.

Biosearch produces a variety of probe formats, including Dual-Labeled BHQ® Probes, BHQplus™ Probes, Molecular Beacons, and Scorpions® Primers. Picking the right probes and primers is made easy through Biosearch's free RealTimeDesign™ software, a web-based qPCR assay design application for gene expression analysis, SNP genotyping, and multiplexed qPCR.

Biovet

Booth **38**

9025 Penn Avenue South
Minneapolis, MN 55431
www.biovet-inc.com
Contact: Stephanie Unverferth or Sheila Braun
877,824.6838
biovetusa@biovet-inc.com

Biovet Inc. develops, manufactures and markets animal diagnostic kits. Additionally, Biovet provides animal health and agro-industry specialists with a unique expertise in the diagnostic field. Biovet operates certified laboratories offering a complete array of innovative diagnostic services for veterinarians. The US head office is located in Minneapolis, MN. Biovet employs more than 50 people including 15 scientists. The company is active internationally particularly in North America and Europe.

Bruker Daltonics

Booth **14**

40 Manning Road
Billerica, MA 01821
www.bdal.com
Contact: Nancy Salt
978.663.3660 x1492
nrs@bdal.com

Bruker Daltonics is a leading provider of analytical systems for emerging diagnostic applications. Led by innovative, easy-to-use and cost effective systems for Microbial Identification, the industry leading MALDI Biotyper produces identifications in minutes for a broad range of bacteria and yeast with minimal reagents from primary culture.

CDC/EPI-X

Booth **22**

1600 Clifton Road NE M/S D-40
Atlanta, GA 30333
https://expi.cdc.gov
Contact: Amanda Evanson
404.639.5049
aevanson@cdc.gov

Epi-X is CDC's secure, web-based communications and alerting system that keeps frontline public health officials informed about developing health threats. The system rapidly establishes secure communication channels between its users, providing a safe way to exchange confidential information. *Epi-X* helps public health officials stay informed, inform others, and share expertise.

Centaur, Inc.

Booth **29**

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

Please stop by Centaur, Inc.'s booth to see what new products we are able to introduce of those that are currently awaiting USDA approval. We intend to add to our existing participation in the equine market with our equine infectious anemia and foal IgG tests, the TB market for non-human primates, the small animal market with our heartworm test, and our multi-species blood analyte tests.

Centaur continues to produce a number of pharmaceutical products under FDA guidelines in categories that include sanitizers and disinfectants (one of which is our DEA approved 7% iodine formulation), skin and wound products, bulk liquids to treat metabolic disorders, to serve as laxatives, to preserve tissues and to serve as delivery vehicles for custom compounded medicinals. In addition, Centaur has expanded to include contract manufacturing of FDA and non-regulated medicinals for select customers.

CompuGroup Medical

Booth **39**

228 Business Center Drive
Reistertown, MD 21136
www.CGMus.com
Contact: Kristin Brillantes
800.359.0911
kbrillantes@antekhealthware.com

CompuGroup Medical is a solution-focused Healthcare IT company whose suite of products improves operational efficiency, patient outcomes, and financial results for health care providers in over 25 countries for 375,000 healthcare professionals. CompuGroup medical employs of 3,100 dedicated employees to manage the wide array of Healthcare needs in the constantly changing technology environment of the 21st century.

LabDAQ Laboratory Information System is an award winning software application, with 2,500 installations worldwide, over 30 of which are in independent Veterinary laboratories. LabDAQ has been designed to meet the needs of a veterinary laboratory setting. The LIS manages multiple species and reference ranges based on those species as well as track multiple pets to owners. LabDAQ is designed to streamline the Pre-Analytic, Analytic and Post-Analytic processes in the Veterinary laboratory.

Computer Aid, Inc.

Booth **5**

470 Friendship Road, Suite 300
Harrisburg, PA 17111
www.compaid.com
Contact: John Kucek
717.651.3000
john_kucek@compaid.com

Computer Aid, Inc. (CAI) is a $300 million Information Technology (IT) firm based in Pennsylvania. CAI specialized in Application Development & Application Maintenance. In 2009 the National Agribusiness Technology Center (NATC) named USALims and USAHerds "Best Available Technology" for enhanced protection of America's food supply. Developed by the Pennsylvania Department of Agriculture, these software applications assist farmers and food safety regulators in keeping our food supply safe and disease free. These products are being "marketed" by the NATC under the "brand" name of AgraGuard. CAI is a certified vendor to install, maintain and enhance the AgraGuard suite of applications. USALIMS is a web-based smart client animal health laboratory tool that features automated client report generation, distribution and auditing, advanced search options, NAHLN interface, and client account and billing management. USAHERDS is a web-based system for complete disease program management, animal traceability, LIMS integration, radius reporting, and license management.

GeneReach Biotechnology Corporation

Booth **30**

No 19, Kyuan Second Rd
Central Taiwan Science Park
Taichung, 407 Taiwan ROC
www.genereach.com
Contact: Simon Chung
886.4.2463.9869
sales@genereach.com

GeneReach Biotechnology is a worldwide biotechnology company dedicated to bringing the innovation to global health management. By developing, manufacturing and marketing products for applied nucleic acid detection technology, we offer disease detection platforms, including equipment and reagents, to multiple industries such as aquaculture, agriculture, livestock, companion animals, and in vitro diagnostic industries. We developed and manufacture the world's one and only OIE-certified PCR diagnostic system, IQ2000. Our quality system is in compliance with ISO9001 and ISO13485.

GeneReach has developed POCKIT, a portable PCR platform based on the principle of insulated isothermal PCR. It comes in as a carry-on hard-shell suitcase package for room temperature shipping. The system can take up to eight samples per run. The total run time is less than 1.5 hours. With its high sensitivity and specificity, ease-of-use and short-turn-around-time, POCKIT can provide farmers and field consultants a powerful tool for veterinary disease management.

GlobalVetLINK

Booth **31**

2520 N Loop Drive, Suite 7100
Ames, IA 50010
www.globalvetlink.com
Contact: Kaylen Henry
515.296.4191
khenry@globalvetlink.com

Company Representatives: Kevin Maher and Kaylen Henry

GlobalVetLINK is the nation's leader in electronic animal health documentation. Since 2001, they have offered digital EIA certificates, certificates of veterinary inspection and online laboratory/veterinarian connectivity to clients across the country.

GlobalVetLINK is easy to use, offers 24/7 database availability and real-time access to test results and animal health records. The certificates are auto-matically sent to the appropriate state animal health officials, eliminating the need for veterinarians and laboratories to send paper copies. This system enables GVL's clients to utilize "best practices", save time and create additional revenue. Veterinarians can grant access for animal owners to retrieve certificates through MyVetLINK.com, drastically increasing the speed of certificate distribution. EIA and health certificates generated through GlobalVetLINK are accepted to move animals into all 50 states and 3 U.S. territories.

IDEXX Laboratories

Booths **32-33**

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

About IDEXX Livestock and Poultry Diagnostics
IDEXX Livestock and Poultry Diagnostics is the worldwide leader in diagnostic tests for livestock and poultry. For over 25 years, animal health professionals have turned to IDEXX for proven diagnostics, information-technology and equipment solutions. The IDEXX portfolio addresses more than 50 pathologies effecting bovine, small ruminant, porcine, poultry and equine populations. For more information, please contact us at phone: (800) 548-9997 or (207) 556-4300; email: LPDweb@idexx.com; or visit our website at www.idexx.com/production.

Life Technologies

Booth **24-25**

2130 Woodward Street
Austin, TX 78744
www.lifetechnologies.com/animalhealth
Contact: Kirk Adams
512.651.0200
kirkadams@lifetech.com

We know in the production animal health industry, the results you report can have a profound impact on your customer's business and on your reputation. False positives can cause problems for the producer; false negatives can be catastrophic for the industry. That's why Life Technologies is focused on providing products that help to instill confidence. Committed to the animal health industry, we provide products and services designed to help you adapt and succeed in an evolving environment. Our molecular testing tools include automated sample prep, superior molecular reagents and master mixes, and easy-to use instruments for PCR analysis.
Find the confidence you're looking for—explore molecular technology for animal health at booth 24-25.

National Institute for Animal Agriculture

Booth **16**

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

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

NIAA's purpose is to provide a source for individuals, organizations, and the entire animal agriculture industry to receive information, education and solutions for animal agriculture challenges. NIAA accomplishes this by coordinating and promoting industry programs and materials that assist animal agriculture professionals in addressing emerging and current issues.

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

Prionics USA, Inc.

Booth **8**

9810 Hupp Drive
La Vista, NE 68128
www.prionics.com
Contact: Tom Kellner
402.212.5126
thomas.kellner@prionics.com

Based in Zurich, Switzerland, Prionics is one of the world's leading providers of farm animal diagnostic solutions and is a recognized center of expertise in BSE and prion diseases. Founded in 1997, Prionics researches and markets innovative diagnostic solutions for major farm animal diseases; thereby making a major contribution to the protection of consumer health.

In 2005, Prionics acquired Pfizer Animal Health's diagnostic portfolio and, in 2006, entered into a strategic partnership with the Animal Science Group of the University of Wageningen (Netherlands). With the acquisition in 2009 of the tuberculin business of former Lelystad Biologicals, Prionics is one of largest providers of bovine TB diagnostic solutions worldwide. Winner of the Swiss Economic Award for "Company of the year" (2002) and the European Biotech Award for "Excellence in Biotech Business" (2004), in 2006 Prionics was nominated as the world's best animal health company.

The Company operates R&D facilities in Switzerland and the Netherlands and has regional hubs in key markets such as Germany, Italy, the Netherlands, and the USA. Prionics is also represented by distribution partners around the world.

Progressive Recovery, Inc. (PRI)

Booth **4**

700 Industrial Drive
Dupo, IL 62239
www.pri-bio.com
www.progressive-recovery.com
Contact: Shanon Jones
618.286.5000
sjones@progressiverecovery.com

Progressive Recovery, Inc. is a global company, providing your single source for engineering and manufacturing of Biowaste / Effluent Decontamination Systems and Caustic Digester Units for carcass disposal. PRI's systems represent the final boundary in sterilization treatment of liquid and solid wastes before discharge to the environment.

Located in Dupo, Illinois, PRI has more than 25 years experience in modular equipment design and fluid processing. Our unique EDS designs have been used in BSL-4, BSL-3 AG, and BSL-3 Enhanced and BSL-3 facilities since 1999.

PRI has introduced the TTD (Thermal Tissue Digester) in 2011, which offers much flexibility for waste streams including a dehydration mode to solve BOD sewer issues with previous digester designs. Various capacity versions are available from 300lbs to 10,000lbs per cycle. The TTD also significantly reduces caustic chemical usage, the most expensive part of operational costs.

Quality equipment, manufacturing excellence, and engineering support are recognized with PRI's name and history.. www.pri-bio.com

Qiagen, Inc.

Booth **34-35**

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

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

Safe Path Laboratories, LLC

Booth **21**

5909 Sea Lion Place, Suite D
Carlsbad, CA 92010
www.safepath.com
Contact: Dave Lambillotte
760.929.7444 x307
dave@safepath.com

SafePath Laboratories is a USDA Biologics licensed manufacturing facility that also operates under FDA GMP's. SafePath laboratories, located in Carlsbad, California, manufactures companion animal assays on 96 well ELISA and Direct Fluorescent Antigen platforms for use in veterinary reference laboratories. We also manufacture rapid, point of care tests on a lateral flow platform for veterinary clinic and reference laboratory use. Companion animal tests currently offered include canine heartworm antigen, canine and feline giardia antigen, feline leukemia virus antigen, and giardia/cryptosporidium direct fluorescent assay.

Synbiotics Corporation

Booth **40-41**

12200 NW Ambassador, Suite 101
Kansas City, MO 64163
www.synbiotics.com
Contact: Ronan Molloy
800.228.4305
ronanm@synbiotics.com

Synbiotics Corporation is a Pfizer wholly owned subsidiary. We are a leading developer, manufacturer and marketer of veterinary diagnostics and services for veterinary practices, livestock and poultry producers, reference laboratories and animal breeders world-wide. We are the leader in animal diagnostics of zoonotic pathogens such as tuberculin, influenza, brucellosis, leishmania and ehrlichia. Our core competencies include immunology, immunodiagnostics and reproduction.

Our mission is to serve mankind through products and services that contribute to the production of a healthy and abundant protein food supply and to a long and rich bond with our companion animals.

Our sales, service and marketing teams are unparalleled in knowledge of our products, interpretation of diagnostic results and understanding the needs of our customers. Our research and development organization includes university and industry collaborations. Both our manufacture facilities in San Diego, California and Lyon, France are permitted by the USDA and are ISO 9001 certified.

Tetracore, Inc.

Booth **9**

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, Dr. Beverly Mangold, Tracy Fecteau, 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 *Classical swine fever virus* (CSFV), in addition to specific detection reagents for *Porcine reproductive and respiratory syndrome virus* (PRRSV), *Foot-and-mouth disease virus* (FMDV), *West Nile virus*, *African swine fever virus* (ASFV) and others. We are also proud to announce the TCOR4 – an all-new real-time PCR thermocycler. Featuring four sample wells and multiplex capability, it is small, highly portable, completely self contained, and has an 8-hour (rechargeable) battery life.

TREK Diagnostic Systems, now Thermo Scientific

Booth **28**

982 Keynote Circle, Suite 6
Cleveland, OH 44131
www.trekds.com
Contact: 1.800.871.8909
info@trekds.com

TREK Diagnostic Systems, now Thermo Scientific, is a dedicated microbiology company, providing innovative, automated products to the microbiology laboratory. TREK's products provide cost-effective improvements in workflow efficiencies, ergonomics, and test result performance. Signature products include the VersaTREK® Automated Microbial Detection System, Sensititre ARIS® 2X Microbiology System, and the Vizion® Digital Imaging System.

Ventana Medical Systems, Inc.

Booth **15**

1910 Innovation Park Drive
Tucson, AZ 85755
www.ventana.com
Contact: Megan Forzano
520.241.6029
Megan.forzano@ventana.roche.com

The VENTANA DISCOVERY product line provides best in-class instrumentation & reagents to the life sciences research market. Structured within the broader Translational Diagnostics (TDx) organization, the mission of the DISCOVERY business team is to foster strategic partnerships with key market influencers that support high medical value innovations in IHC, ISH, FISH, and IF research, ultimately advancing medical science.

The DISCOVERY business is organized to serve a broad range of customers, including pharmaceutical & biotech companies, contract/clinical research organizations (CROs), government research laboratories, and key opinion leaders (KOLs). The DISCOVERY series integrated systems, including instrumentation, reagents, and software, offer the following benefits:

- Automation and optimization capabilities.
- End-user application support.
- Reproducibility, sensitivity, and high performance.

All reagents are manufactured and quality-tested within our Tucson-based pilot plant. DISCOVERY instruments feature significantly advanced software & protocol development flexibility, enabling researchers to test the boundaries of tissue-based research, all while retaining reproducibility through standardization & automation.

VertiQ Software LLC

Booth **13**

18525 Sutter Blvd, Suite 280
Morgan Hill, CA 95037
www.vertiq.com
Contact: Paula Lomanto
408.778.0608
paula@vertiq.com

VertiQ (the leader in Medical Examiner case management software) has developed a special LIMS system for animal disease and diagnostic laboratories. Designed and in use by San Diego County ADDL, the system is based on the same toolkit that has been used successfully for many years for Medical Examiners and forensic laboratories.

A key component of the system is the ease of customization. Every laboratory works in a different fashion from other agencies in their field so customizing a system to fit the specific internal needs of the laboratory is very important.

VQ-LIMS-ADDL includes the following modules: Administrative (including Billing and Accounts Receivable), Necropsy and Laboratory Functions. It also includes a tickler system, internal communications, task management, reports, barcode chain of custody, image and document linking. Pricing upon request.

VMRD, Inc.

Booth **12**

4641 Pullman Albion Road
Pullman, WA 99163
www.vmrd.com
Contact: Michelle Nay
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, Washington 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.

SAGE

Booth **17**

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.

AAVLD/USAHA Upcoming Meetings

2012: October 17-24
Greensboro, North Carolina

2013: October 17-23
San Diego, California

IDEXX Livestock and Poultry Diagnostics

For nearly three decades, laboratories, veterinarians and producers have made decisions more confidently using IDEXX diagnostic tests and technologies. We don't take that trust for granted—which is why we work so hard to earn it.

May we help you make more confident decisions?

For more information, contact your IDEXX representative or visit idexx.com/livestockpoultry.

IDEXX

VLA SCIENTIFIC®

Products and Services for Scientists around the World

To contact VLA Scientific

Tel: +44 (0)1932 357641 Fax: +44 (0)1932 357701
Email: vlascientific@vla.defra.gsi.gov.uk

or see our website
www.vlascientific.com

expert science ● excellent service

Digital Solutions for Animal Health

515.296.0860 • GlobalVetLINK.com

GlobalVetLINK offers:

- Easy & efficient batch resulting
- Secure 24/7 online accessibility
- Automatic submission to state veterinarians

10 YEARS • 50 STATES • 100 LABS

In a climate of change and uncertainty,
VADDS remains the #1
comprehensive LIMS system
among public veterinary labs in
the US & Canada.

- #1 LIMS supplier among AAVLD accredited labs
- Interfaces with Vetstar Hospital Mgt System
- Expanded presence in Europe and SE Asia
- Simple NAHLN Interface
- Comprehensive Budget-Friendly Solution
- New Version Runs in Web Browser!

79 North Franklin Turnpike
Ramsey, NJ 07446
p 201-934-7127
www.vetstar.com
vadds@vetstar.com

PRIONICS USA Inc.
9810 HUPP DRIVE
LA VISTA – NE 68128
USA
PHONE +1 (888) 205-0807
FAX +1 (402) 991-1392
INFO@PRIONICS.US
WWW.PRIONICS.US

A world free of TB

PRIONICS IS THE WORLD LEADING COMPANY IN VETERINARY TB DIAGNOSTICS

Prionics provides the complete diagnostic solution for the detection, eradication and monitoring of bovine TB:

Field Testing: Skin test with Lelystad Tuberculin PPD
Laboratory Testing: BOVIGAM® IFNγ test for in vitro diagnosis

For more information please check our website at: www.prionics.com

Lastname	Firstname	Session Title	Page #
Fortney	William	Bacteriology Scientific Session 2	77
Fosgate	Geoffrey	TB-Johnes Special Scientific Session	50
Fossler	Charles	TB-Johnes Special Scientific Session	50
Foster	Kelly	Bovine Virus Diarrhea Special Scientific Session	91
Foster	Kelly	Bovine Virus Diarrhea Special Scientific Session	90
Foster	Kelly	Bovine Virus Diarrhea Special Scientific Session	88
Frana	Timothy	AAVLD Posters	181
Frana	Timothy	Bacteriology Scientific Session 2	79
Frana	Timothy	Bacteriology Scientific Session 2	80
Frasca, Jr.	Salvatore	AAVLD Posters	174
Fraser	Woody	AAVLD Posters	167
French	Richard A.	AAVLD Posters	174
Fuller	Alex	Virology Scientific Session 2	131
Fyock	Terry	TB-Johnes Special Scientific Session	51
Gabor	Melinda	Virology Scientific Session 2	127
Gabriel	Mourad	Toxicology Scientific Session	57
Galik	Patricia	Bovine Virus Diarrhea Special Scientific Session	92
Gardner	Ian	TB-Johnes Special Scientific Session	47
Gardner	Ian	Epidemiology Scientific Session	105
Gardner	Michael	Bacteriology Scientific Session 2	78
Gary	Joy	Pathology Scientific Session 2	109
Gaskill	Cynthia	Toxicology Scientific Session	56
Gauger	Phil	AAVLD Posters	170
Gerdin	Jodie	Pathology Scientific Session 1	37
Gerry	Alec	Epidemiology Scientific Session	105
Getchell	Rodman	Epidemiology Scientific Session	106
Giannitti	Federico	AAVLD Posters	161
Giannitti	Federico	Pathology Scientific Session 1	41
Giannitti	Federico	AAVLD Posters	158
Giray	Cem	Epidemiology Scientific Session	106
Givens	Daniel	Bovine Virus Diarrhea Special Scientific Session	89
Givens	Maurice Daniel	Bovine Virus Diarrhea Special Scientific Session	93
Givens	Daniel	Bovine Virus Diarrhea Special Scientific Session	94
Givens	Maurice Daniel	Bovine Virus Diarrhea Special Scientific Session	92
Goodell	Greg	Bovine Virus Diarrhea Special Scientific Session	87
Goodwin	Andrew	Epidemiology Scientific Session	106
Graham	Tanya	Pathology Scientific Session 1	42
Graham	Tanya	AAVLD Posters	146
Grause	Juanita	Bacteriology Scientific Session 1	33
Gravelyn	Tara	TB-Johnes Special Scientific Session	48
Gray	Gregory	Pathology Scientific Session 2	115
Green	Jessica	Virology Scientific Session 2	132
Griffin	Sara	AAVLD Posters	166
Griffith	Ronald	AAVLD Posters	154

Lastname	Firstname	Session Title	Page #
Li	Shubo	Bacteriology Scientific Session 1	32
Li	Hong	Pathology Scientific Session 2	113
Lim	Ailam	Bacteriology Scientific Session 2	78
Lin	Xue	Virology Scientific Session 2	125
Lindpaintner	Klaus	Bacteriology Scientific Session 1	31
Linter	Valerie	AAVLD Posters	160
Linter	Valerie	AAVLD Posters	157
Linter	Valerie	AAVLD Posters	156
Locke	Stephen	Bacteriology Scientific Session 2	82
Looney	Andrea	Pathology Scientific Session 1	37
Low	Phil	AAVLD Posters	162
Lowe	James	Epidemiology Scientific Session	101
Lu	Huaguang	Virology Scientific Session 1	67
Lubbers	Brian	Bacteriology Scientific Session 2	77
Lukens	Patricia	AAVLD Posters	147
Macedo	Nubia	Bacteriology Scientific Session 2	85
MacLachlan	James	Epidemiology Scientific Session	105
Maddox	Carol	Bacteriology Scientific Session 2	81
Maddox	Carol	AAVLD Posters	164
Madson	Darin	AAVLD Posters	170
Magwedere	Kudakwashe	AAVLD Posters	182
Mahmood	Taghreed	Bovine Virus Diarrhea Special Scientific Session	91
Main	Rodger	Epidemiology Scientific Session	99
Main	Rodger	Epidemiology Scientific Session	102
Makolinski	Kathleen	Pathology Scientific Session 1	37
Mansell	Joanne	Pathology Scientific Session 2	120
Mansfield	Kristin	Pathology Scientific Session 2	112
Mansfield	Kristin	Virology Scientific Session 1	64
Marley	M. Shonda	Bovine Virus Diarrhea Special Scientific Session	93
Marley	M. Shonda	Bovine Virus Diarrhea Special Scientific Session	94
Marley	M. Shonda	Bovine Virus Diarrhea Special Scientific Session	92
Marolf	Angela	Pathology Scientific Session 2	111
Marsh	Tom	AAVLD/USAHA Joint Plenary	138
Martin	Nicole	Pathology Scientific Session 1	37
Martin	Barbara M.	Virology Scientific Session 1	69
Martin	Barbara M.	AAVLD Posters	147
Matthews	Tammy	AAVLD Posters	160
Matthews	Tammy	AAVLD Posters	157
Matthews	Tammy	AAVLD Posters	156
Maxwell	J.L.	AAVLD Posters	167
Mayo	Christie	Epidemiology Scientific Session	105
McAdams	Susan	TB-Johnes Special Scientific Session	51
McDonough	Sean P.	Pathology Scientific Session 1	37
McElwain	Terry	AAVLD Posters	147

Lastname	Firstname	Session Title	Page #
Oberst	Richard	Virology Scientific Session 2	131
Oberst	Richard	Bovine Virus Diarrhea Special Scientific Session	90
Oberst	Richard	Bovine Virus Diarrhea Special Scientific Session	88
O'Connell	Catherine	Virology Scientific Session 2	126
O'Connell	Catherine	AAVLD Posters	151
O'Connell	Catherine	Bovine Virus Diarrhea Special Scientific Session	88
O'Connell	Catherine	AAVLD Posters	149
Odemuyiwa	Solomon	Pathology Scientific Session 1	39
Oem	Jae-Ku	AAVLD Posters	190
Oem	Jae-Ku	AAVLD Posters	189
Okafor	Chika	TB-Johnes Special Scientific Session	48
Okafor	Chika	TB-Johnes Special Scientific Session	49
Oliveira	Simone	Epidemiology Scientific Session	102
Oliveira	Simone	Bacteriology Scientific Session 2	85
Olsen	Chris	Epidemiology Scientific Session	99
Olsen	Chris	Epidemiology Scientific Session	100
Olsen	Chris	Epidemiology Scientific Session	101
Olson	Kenneth	Virology Scientific Session 2	135
O'Neill	Kevin	Virology Scientific Session 2	123
Opriessnig	Tanja	Virology Scientific Session 2	125
Opriessnig	Tanja	Virology Scientific Session 2	123
Opriessnig	Tanja	Bacteriology Scientific Session 2	84
Osterstock	Jason	TB-Johnes Special Scientific Session	50
Ostlund	Eileen	Virology Scientific Session 2	134
O'Toole	Donal	AAVLD Posters	163
Otterson	Tracy	AAVLD Posters	153
Pabilonia	Kristy	AAVLD Posters	172
Paddock	Zachary	Bacteriology Scientific Session 1	32
Pagan	Israel	Virology Scientific Session 1	65
Palmer	Mitchell V.	AAVLD Posters	186
Panyasing	Yaowalak	Epidemiology Scientific Session	99
Panyasing	Yaowalak	Epidemiology Scientific Session	100
Panyasing	Yaowalak	Epidemiology Scientific Session	101
Park	Seong	TB-Johnes Special Scientific Session	50
Park	Mi-Ja	AAVLD Posters	168
Parker	Jerry	AAVLD Posters	158
Parkinson	Anne	AAVLD Posters	175
Parlane	Natalie	AAVLD Plenary Session	26
Parrish	Colin	Virology Scientific Session 1	65
Patnayak	Devi P.	Virology Scientific Session 1	68
Patterson	Sheila	Bacteriology Scientific Session 2	81
Peddireddi	Lalitha	Bovine Virus Diarrhea Special Scientific Session	89
Peddireddi	Lalitha	Bovine Virus Diarrhea Special Scientific Session	91
Peddireddi	Lalitha	Bovine Virus Diarrhea Special Scientific Session	90