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, 2012

President	Tim Baszler, Pullman, WA
President-elect	Tom McKenna, Madison, WI
Vice-president	Catherine Barr, College Station, TX
Secretary-Treasurer	John Adaska, Tulare, CA
Immediate Past-President	Craig Carter, Lexington, KY

AAVLD Executive Board, 2012

President	Tim Baszler, Pullman, WA
President-elect	Tom McKenna, Madison, WI
Vice-president	Catherine Barr, College Station, TX
Secretary-Treasurer	John Adaska, Tulare, CA
Northeast	Sandra Bushmich, Storrs, CT
Southeast	Lanny Pace, Pearl, MS
North-central	Pat Halbur, Ames, IA
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
PO Box 1770, Davis, CA 95617
Phone 530-754-9719 Fax 530-752-5680 Email: secretary-treasurer@aavld.org

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. Trista Kang, from Thomson Reuters, helped us navigate the ScholarOne software. 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.

****

2012 AAVLD Program Committee

Tom McKenna, Chair
Catherine Barr, Co-Chair
John Adaska
Tim Baszler
Steve Bolin
Beate Crossely
Francois Elvinger
Scott Fitzgerald
Ashley Hill
Hong Li
Kristy Pabilonia
Amar Patil
Carlos Reggiardo
J. Glenn Songer
David Steffen
Patricia A. Talcott
Kyoung-Jin Yoon

Scientific Session Moderators

Bruce L. Akey
Karyn Bischoff
Suzanne Carman
Beate M. Crossley
Joshua Daniels
Francois C. Elvinger
Scott D. Fitzgerald
Amy Glaser
Ashley Hill
Doreene Hyatt
Arthur (Bill) Layton
Grant Maxie
Michelle Mostrom
Amar Patil
Karen W. Post
M. D. Salman
Deepanker Tewari
Francisco Uzal
Binu Velayudhan
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.

Table of Contents

AAVLD Plenary Session:
Advanced Diagnostic Technologies - Are They Making A Difference?

Saturday, October 20, 2012

Guilford DE

Moderator: Thomas McKenna

7:45 AM **Introduction**

8:00 AM **To MALDI or Not to MALDI**
Carole Bolin, Lori Moon 29

8:35 AM **To Pool or Not to Pool, That is the Question**
Kathy L. Toohey-Kurth 30

9:10 AM **Break**

9:40 AM **Considerations for Performing Luminex® Multiplex Assays in Veterinary Diagnostic Laboratories**
Jane Christopher-Hennings 31

10:15 AM **Diagnostic and Investigational Applications of Metabolic Phenotyping of Bacteria and Cancer Cells**
Luke B. Borst 32

10:50 AM **More Bang For All Those Bucks: How High Resolution Can Equate to Higher Efficiency in the Toxicology Lab**
Michael Filigenzi 33

Symbols at the end of titles indicate the following designations:

AAVLD Trainee Travel Awardee

† Graduate Student Oral Presentation Award Applicant

◊ USAHA Paper

* Graduate Student Poster Presentation Award Applicant

+ AAVLD/ACVP Pathology Award Applicant

Bacteriology 1

Saturday, October 20, 2012
Guilford D

Sponsor: Biovet, Inc.

Moderators: Joshua Daniels, Karen W. Post

1:00 PM **Novel Assay for Detecting *Mycobacterium tuberculosis* Infection in Nonhuman Primates**
Kimberly Luke, JoAnn Yee, Kirk Andrews, Deepak Kaushal, Bill Patterson 36

1:15 PM ***Mycobacterium leprae* Infection in a Nine-banded Armadillo in Florida**
Shipra D. Mohan, Elena Hollender, Teri L. Johnson, Karen McKenzie, Brian Pugh, Gizela Maldonado-Hernández, James Maxwell, Alice Agasan, Susan Kelleher, Danielle Stanek 37

1:30 PM **Petrifilm™ Measurement of Bacteria Counts (SPC) in Pasteurized Waste Milk for Feeding to Dairy Calves and Association of SPC with Time until Feeding, Season and Temperature**
David J. Wilson, Gregory M. Goodell 38

1:45 PM **Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry for Bacterial Identification: Experiences After One Year of Use in a Veterinary Diagnostic Laboratory**
Timothy Frana 39

2:00 PM **Speciation of *Brachyspira spp*. in Poultry Fecal Samples using the GS Junior**
Durda Slavic, Stina Nilsson, Michele Guerin, Genet A. Medhanie 40

2:15 PM **Evaluation of Serodiagnostic Assays for *Mycobacterium bovis* in Elk, White-tailed Deer, and Reindeer in the United States ◊**
Jeffrey T. Nelson, Kathleen Orloski, Audra Lloyd, Mark Camacho, Mark Schoenbaum, Suelee Robbe-Austerman, Bruce V. Thomsen, S. Mark Hall 41

2:30 PM **Development and Evaluation of a New Serological ELISA for Detection of Herds with Swine Dysentery ◊**
Yong Song, Xiaoping Yang, Daniel Zwald, Mario Puerro, Angela Zurfluh, Alex Raeber, David Hampson 42

2:45 PM **Rene Lallier, Biovet**

Symbols at the end of titles indicate the following designations:

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

Epidemiology 1

Saturday, October 20, 2012
Guilford C

Moderators: Francois C. Elvinger, Bruce L. Akey

1:00 PM **Comparison of *M. bovis* Gamma Interferon Test Results Between Tissue Culture Plate and Microtube Methods ◊**
Ashley Hill, Michelle Davidson, Elizabeth Emery 44

1:15 PM **A Preliminary Evaluation of MAPIA as an Ante-mortem Supplemental Test for Use in Suspect Tuberculosis Cattle**
Scott D. Fitzgerald, John Kaneene, Heather Grodi 45

1:30 PM **Systematic Literature Review and Statistical Bayesian Analysis of the Performance Characteristics of Commercially Available Type A Influenza Antigen Capture Tests in Dead Poultry Infected with Highly Pathogenic Avian Influenza**
Todd Weaver, Sasidhar Malladi, Mary J. Folley, Timothy L. Clouse, Kathe Bjork, David A. Halvorson 46

1:45 PM **A Simulation Based Evaluation of the Likelihood of Moving Contaminated Hatching Eggs from a Highly Pathogenic Avian Influenza Infected but Undetected Broiler Breeder House under Active Surveillance**
Sasidhar Malladi, Todd Weaver, Kathe Bjork, David A. Halvorson 47

2:00 PM **Development of a New ELISA Test for Detection of PRRS Antibodies in Swine Oral Fluids**
Sergio Lizano, Andrea Ballagi, Sheri Koller 48

2:15 PM **Detection of PRRSV Antibody in Oral Fluid Specimens from Individual Boars using a Commercial PRRSV Serum Antibody ELISA**
Apisit Kittawornrat, Mark Engle, Jeremy Johnson, John Prickett, Chris Olsen, Trevor Schwartz, Daniel Whittney, Kent Schwartz, Anna Rice, Andrea Ballagi, Sergio Lizano, Chong Wang, Jeff Zimmerman 49

2:30 PM **Ring Test Evaluation for the Detection of PRRSV Antibody in Oral Fluid Specimens using a Commercial PRRSV Serum Antibody ELISA**
Apisit Kittawornrat, Chong Wang, Gary A. Anderson, Andrea Ballagi, Andre Ch. Broes, Suzanne Carman, Kent Doolittle, Judith Galeota, John Johnson, Sergio Lizano, Eric A. Nelson, Devi P. Patnayak, Roman Pogranichniy, Anna Rice, Gail Scherba, Jeff Zimmerman 50

2:45 PM **Seroepidemiology of Equine Leptospirosis Utilizing Diagnostic Laboratory Specimens from 29 States (US) and One Canadian Province ◊**
Craig N. Carter, Noah Cohen, Meg Steinman, Jacqueline Smith, Erdal Erol, Stuart Brown 51

3:00 PM **Spatially Heterogeneous Environmental and Climatic Risk Factors of Canine Leptospirosis**
Ram Raghavan, Gary A. Anderson, Kenneth Harkin 52

Symbols at the end of titles indicate the following designations:

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

Pathology 1

Saturday, October 20, 2012

Auditorium III

Sponsor: Advanced Technology Corporation

Moderators: Francisco Uzal, Grant Maxie

1:00 PM **Introduction by Advanced Technology Corporation**

1:05 PM **Friesian Horses Have High Incidence of Severe Esophageal Disease Associated with Muscular Hypertrophy and Hyperplasia of the Caudal Esophagus # † +**
Misa Komine, Ingeborg Maria Langohr, Matti Kiupel 55

1:20 PM **Evaluating Grading Systems for Accurate Prognisis of Canine Cutaneous Mast Cell Tumors # * † +**
Keiko Y. Petrosky, Rachel Peters, Barbara J. Davis 56

1:35 PM **Freezing or Adding Trypsin Inhibitor to Equine Intestinal Content Extends the Lifespan of Clostridium perfringens Beta Toxin for Diagnostic Purposes # * † +**
Melissa Macias Rioseco, Juliann Beingesser, Francisco Uzal 57

1:50 PM **Enzootic Ataxia in two Katahdin Lambs †**
Stephanie French, Jon S. Patterson, Scott D. Fitzgerald 58

2:05 PM **Skin and Subcutaneous Granulomas in Tuberculosis Test Responder Cattle**
Patricia C. Blanchard, John Adaska, Robert Moeller, Bruce V. Thomsen, Suelee Robbe-Austerman 59

2:20 PM **Intranasal Dust Inoculation of Chronic Wasting Disease in White Tailed Deer (*Odocoileus virginianus*)**
Tracy Nichols, Terry Spraker, Tom Gidlewski, Tara Camenish-Ruby, Mark Zabel, Aru Balachandran, Glenn Telling, Brady Michel, Kurt VerCauteren 60

2:35 PM **Antleromas and Hummel Deer: Lessons on Antlerogenesis from Free-Ranging White-Tailed Deer**
Brandon Munk, Kevin Keel, Angela Ellis, Bambi Clemons, Elina Garrison 61

2:50 PM **A Newly Recognized Feline Infectious Peritonitis-Like Disease in Mink Associated Withan Alphacoronavirus**
Matti Kiupel, Karla A. Fenton, Annabel G. Wise, Steve J. Bursian, Roger K. Maes 62

Symbols at the end of titles indicate the following designations:

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

Toxicology

Saturday, October 20, 2012

Auditorium II

Moderators: Karyn Bischoff, Michelle Mostrom

1:00 PM **Screening and Confirmation of Illicit Drugs in Biological Samples by LC/MS-MS**
Robert H. Poppenga, Gwendolyne Alarcio, Elizabeth R. Tor 64

1:15 PM **Development of a GC/MS Procedure for Detecting Tremetone and Dehydrotremetone in Cases of White Snakeroot Poisoning**
Christina Wilson, Kimberly Meyerholtz, Stephen B. Hooser 65

1:30 PM **Challenges with Vitamin A Quantification in Feeds and Biological Samples**
Gwendolyne Alarcio, John Tahara, Elizabeth R. Tor, Linda Aston, Robert H. Poppenga 66

1:45 PM **Detection of Aflatoxin-DNA Adducts in the Livers of Aflatoxin-treated Rats**
BongSuk Kim, Christina Wilson, Kim Meyerholtz, Stephen B. Hooser 67

2:00 PM **The Use of Iron Quantitation in Dried Blood Spots as a Means of Estimating the Whole Blood Hematocrit of Wild Birds**
Alan Shlosberg, Andreas Lehner, Michael Adkesson, Kirk Stuart, Margaret Johnson, Wilson K. Rumbeiha 68

2:15 PM **Reference Intervals for Inorganic Element Concentrations in Equine Fetal Liver Tissue**
Cynthia L. Gaskill, Lori L. Smith, Michelle N. Helm 69

2:30 PM **Development of Ecofriendly Extraction Processes for Analysis of Aflatoxins and Fumonisins in Corn and Corn Byproducts ◊**
Washington Mutatu, Andreas Lehner, Margaret Johnson, Wilson K. Rumbeiha 70

2:45 PM **Equine Botulism Type A — A Western States Phenomenon # * † ◊**
Stephanie R. Ostrowski, Steven V. Kubiski, Joan Palmero, Christopher M. Reilly, Jamie K. Higgins, Sheri Cronin, Snehal N. Tawde, Beate M. Crossley, Paula Yant, Ray Cazarez, Francisco A. Uzal 71

3:00 PM **Presumptive Diagnosis of Pine Needle Abortion in a Nebraska Cattle Herd using an Isocupressic Acid Metabolite as a Biomarker # * † +**
Douglas Snider, Dale Gardner, Steve M. Ensley, Bruce Janke, Wilson K. Rumbeiha 72

Symbols at the end of titles indicate the following designations:

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

GeneReach

Virology 1
(Molecular Technique Focus)

Saturday, October 20, 2012

Guilford E

Sponsor: GeneReach Biotechnology Corporation

Moderators: Beate M. Crossley, Amar Patil

1:00 PM **Development of a Multiplex Real-Time PCR for the Detection of Bovine Corona and Rota Viruses ◊**
Jennifer Cooper, Meaghan Broman, Francine Cigel, Kathy L. Toohey-Kurth *75*

1:15 PM **Development of a Multiplex Real-time PCR Panel for Detection of Ruminant Endemic Diseases that Mimic Foot-and-Mouth Disease ◊**
Francine Cigel, Jennifer Cooper, Kathy L. Toohey-Kurth *76*

1:30 PM **Development and Performance Evaluation of a Simple Streamlined Method for Bluetongue Virus and Epizootic Hemorrhagic Disease Virus Nucleic Acid Purification, Denaturation, and Detection**
Megan Schroeder, Jennifer Meier, Mangkey Bounpheng, Donna Johnson, Eileen Ostlund, Alfonso Clavijo *77*

1:45 PM **Pockit® Xpress, an Insulated Isothermal PCR (iiPCR) Plaform for On-site Detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)**
Andre Ch. Broes, Simon Chung, Maryse Belanger *78*

2:00 PM **Identification of a Novel Isolate of Type 1A Enterovirus in Bovine Through a Subtractive-Hybridization Approach to Sequence-based Diagnostics**
Roger Barrette, Frederic R. Grau, Fawzi Mohamed, Andrew Fabian, Emily S. O'Hearn, Mary Lou Berninger, Robert Moeller, Marian Van der Schraaf, Fernando Torres-Velez, Michael T. McIntosh *79*

2:15 PM **New Real-Time PCR Assay using Allelic Discrimination for Detection and Differentiation of Equine Herpesvirus-1 Strains with A2254 and G2254 Polymorphisms †**
Kathryn Smith, Yanqiu Li, Patrick Breheny, R. Frank Cook, Pamela J. Henney, Stephen Sells, Stéphane Pronost, Zhengchun Lu, Beate M. Crossley, Peter J. Timoney, Udeni BR Balasuriya *80*

2:30 PM **Optimization and Validation of a High-throughput Real-time RT-PCR Assay for Rapid Detection of Foot-and-Mouth Disease Virus from Bulk Tank Milk**
Amaresh Das, Karissa Casteran, Diane J. Holder, Michael T. McIntosh, Kathy L. Toohey-Kurth, Francine Cigel, Thomas McKenna, Barbara M. Martin, Mangkey A. Bounpheng, Alfonso Clavijo, Tammy Beckham, Jennifer Rinderknecht *81*

2:45 PM **Detection and Differentiation of Equine Herpesvirus 1 by Real-Time PCR and Pyrosequencing**
Yan Zhang, Jing Cui, Jason Herr, Beverly Byrum *83*

3:00 PM **Detecting Canine Distemper Virus by an On-Site Portable PCR Platform – POCKIT**
Fu-Chun Lee, Sih-Ying Wu, Yun-Long Tsai, Ling-Ling Chueh, Bi-Ling Su, Chen Su, Ping-Hua Teng, Hsiao Fen Grace Chang, Pei-Yu Lee, Thomas Wang 83

Symbols at the end of titles indicate the following designations:

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

Thermo SCIENTIFIC

Bacteriology 2

Sunday, October 21, 2012
Guilford E

Sponsor: Thermo Scientific

Moderators: Doreene Hyatt, Deepanker Tewari

8:00 AM **Real-Time PCR Detection of Hemotropic Mycoplasma species in Symptomatic Dairy Cattle from the Midwest United States ◊**
Amanda Kreuder, Adam Herrick, Uri Donnett, Paul Plummer, Jessie D. Trujillo 86

8:15 AM **Detection of *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145 by Antibody Microarray**
Narasimha Hegde, Andrew Gehring, Pina Fratamico, Chitrita DebRoy 87

8:30 AM **Recovery and Identification of *Listeria monocytogenes* During Routine Bacteriology Abortion Screen of Missouri Canine ◊**
Thomas J. Reilly, Michael Calcutt, Irene Ganjam, Sean Spagnoli, K. Kuroki, William H. Fales 88

8:45 AM **Identification of Bacterial Agents Associated with Abortion in Ruminant Species**
Kris Clothier 89

9:00 AM **Diagnostic Investigation of Real-time PCR, Fuorescent Antibody, and Microscopic Agglutination Tests in Cases of Equine Abortion**
Erdal Erol, Neil M. Williams, Stephen Sells, Meg Steinman, Judy Donahoe, Katherine Meares, Alan Loynachan, James M. Donahue, Craig N. Carter 90

9:15 AM **TaqMan Real-time PCR Assay for the Direct Detection of *Campylobacter fetus* subsp. *venerealis* from InpouchTM TF ◊**
Feng Sun, Hemant Naikare, Mangkey A. Bounpheng, Loyd Sneed, Alfonso Clavijo 91

9:30 AM **Break**

10:00 AM **Species Specificity and Molecular Typing of Porcine and Equine *Lawsonia intracellularis* Isolates * † ◊**
Fabio Vannucci, Nicola Pusterla, Samantha Mapes, Yogesh Chander, Molly Kelley, Connie Gebhart 92

10:15 AM **Virulence and Genome Stability of *Lawsonia intracellularis* After Serial Passages *in vitro* * † ◊**
Fabio Vannucci, Dana Beckler, Nicola Pusterla, Samantha Mapes, Yogesh Chander, Connie Gebhart 93

10:30 AM **Seroprevalence of *Coxiella burnetii* in Washington State Domestic Goat Herds # †**
Kerry Sondgeroth, Sara Schlee, Margaret Davis, Andy Allen, James Evermann, Terry McElwain, Timothy V. Baszler 94

10:45 AM **Comparison of Three Common Diagnostic Tests for Leptospirosis # †**
Kenitra Hammac, Gary Haldorson, Thomas E. Besser, Dan S. Bradway, Timothy V. Baszler 95

11:00 AM **Evaluation of Different Fragments of Recombinant *Leptospira* Immunoglobulin-like Protein for Use in a Serologic Test for Equine Leptospirosis**
Weiwei Yan, Patrick L. McDonough, Sean P. McDonough, Bruce L. Akey, Yung-Fu Chang 96

11:15 AM **Isolation of a *Pelistega*-like Organism from a Urethral Swab of a Horse**
Yan Zhang, Jing Cui, Anne Parkinson, Jason Herr, Troy Farrell, Beverly Byrum 97

11:30 AM **Outbreaks of Salmonellosis Associated with Animals**
Yan Zhang, Jing Cui, Anne Parkinson, Mary B. Weisner, Eric Brandt, Rick Bokanyi, Brittany Orlando, Rhiannon Schneider, Jason Herr, Beverly Byrum 98

11:45 AM **Jennifer Lorbach, Thermo Scientific**

Symbols at the end of titles indicate the following designations:

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

Epidemiology 2

Sunday, October 21, 2012

Guilford F

Moderators: Ashley Hill, M.D. Salman

8:00 AM **Effect of Mixed Livestock Production Types on Foot-and-Mouth Disease Outbreaks and Interventions * †**
Sara W. McReynolds, Mike W. Sanderson, Aaron Reeves *101*

8:15 AM **Comparing Vaccine Protocol of Vaccinating Large Feedlots Only to Vaccinating All Livestock Production Systems * †**
Sara W. McReynolds, Mike W. Sanderson, Aaron Reeves *102*

8:30 AM **Canine Dysautonomia in Wyoming: 24 Cases [2004-2012] # †**
Noah C. Hull, Kathryn Drumhiller, Donal O'Toole, Brant Schumaker *103*

8:45 AM **Chimeric protein A/G conjugate for detection of anti-*Toxoplasma gondii* immunoglobulin G in multiple animal species # †**
John J. Schaefer, Holly A. White, Stephanie L. Schaaf, Hussni O. Mohammed, Susan E. Wade *104*

9:00 AM **Sequence Confirmation of *Tritrichomonas foetus* Samples Previously Tested with MagMAX™ Sample Preparation System and VetMAX® *T. foetus* Reagents**
Ivan Leyva Baca, Marilyn Simunich, Lalitha Peddireddi, Richard D. Oberst, Lee J. Effinger, Catherine O'Connell *105*

9:15 AM **Pathogenic Lungworms in Maine Moose: not *Dictyocaulus viviparus* ◊**
Darryl A. Girardin, Sarah Barker, Jana Drury, Lee Kantar, Anne Lichtenwalner *106*

9:30 AM **The Combination of Abundance and Infection Rates of *Culicoides sonorensis* Estimates Risk of Subsequent Bluetongue Virus Infection of Sentinel Cattle on California Dairy Farms ◊**
Christie E. Mayo, Bradley Mullens, Christopher Barker, Alec Gerry, Ian Gardner, James MacLachlan *107*

9:45 AM **Break**

10:15 AM **Evaluation of Bovine Viral Diarrhea Virus Infection in Hunter-harvested White-tailed Deer (*Odocoileus virginianus*) in Georgia using Ear-notch Samples**
Marcia Regina Da Silva Ilha, Michele Coarsey, Lisa Whittington, Sreekumari Rajeev, Sheela Ramamoorthy *108*

10:30 AM **Introduction of the IDEXX Bovine Viral Diarrhea Virus Antigen ELISA (IDEXX BVDV PI X2 Test)**
Anthony Mestek, Katherine Velek, Shona Michaud, Lisa Estey, Sergio Lizano *109*

10:45 AM **Discovery of a Broad Diversity of Mycoplasma species in Companion Animals Utilizing a PanMyco PCR Assay**
Uri Donnett, Adam Herrick, Claudia Baldwin, Peter Nara, Jessie D. Trujillo *110*

11:00 AM **Investigation of *Corynebacterium pseudotuberculosis* cases in Texas Horses from 2005 to 2011**
Barbara Szonyi, Amy K. Swinford, Alfonso Clavijo *111*

11:15 AM **Scoliosis in Three Gopher Tortoises from Baker County, Georgia**
Brandon Munk, Jessica McGuire, Mark G. Ruder, Justin Brown *112*

11:30 AM **Quality Assurance: Sharing the Tools that Support Sound Science**
Rebecca Davies, Shirley D. Johnston *113*

Symbols at the end of titles indicate the following designations:

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

Pathology 2

Sunday, October 21, 2012

Guilford D

Moderators: Scott D. Fitzgerald, Arthur (Bill) Layton

8:00 AM **Two Cases of Chronic Copper Toxicity in Growing Holsteins**
Doug Lyman *116*

8:15 AM **Canine and Feline Yeast Urinary Tract Infections: Agents and Susceptibility to Antifungal Agents**
Barbara Byrne, Eileen M. Samitz, Jodi L. Westropp *117*

8:30 AM **Naked Mole-Rat Skin and Soft Tissue Metastatic Mineralization**
Amanda Crews, Shelley J. Newman, Edward C. Ramsay *118*

8:45 AM **Comparative Virulence of Clinical *Brachyspira spp*. Isolates in Inoculated Pigs**
Eric Burrough, Erin Strait, Joann M. Kinyon, Leslie Bower, Darin Madson, Bailey Wilberts, Kent Schwartz, Timothy Frana, J. Glenn Songer *119*

9:00 AM **Canine Colorectal Polyps and Progression to Malignancy: A Retrospective Study of 100 Canine Colorectal Epithelial Polypoid Tumors (1997-2012) # +**
Brian Butler *120*

9:15 AM **Break**

9:45 AM **Emergency Preparedness in the Necropsy Laboratory: Designing and Conducting a Full-scale Operational Exercise**
Maria Spinato, Jan Shapiro, Margaret J. Stalker, Andrew Brooks *121*

10:00 AM **Gross and Histological Lesions in Northern Elephant Seals (*Mirounga angustirostris*, Gill, 1866), Año Nuevo State Reserve, California 2012**
Terry Spraker, Gene Lyons, Tetiana Kuzmina, Stephen Raverty, Nicole Jaggi, Dan Crocker, Mike Tift *122*

10:15 AM **Pyogranulomatous Dermatitis Associated with *Mycobacterium smegmatis* in a Cat**
Jeffrey R. Hayes, Yan Zhang, Jing Cui, Anne Parkinson, Mary B. Weisner, Beverly Byrum, Joshua Daniels *123*

10:30 AM **Concurrent Vitamin D Toxicosis and Presumptive Proventricular Dilatation Disease in Two Eclectus parrot (*Eclectus roratus*) chicks**
Howard Steinberg, Dominique Keller, Sarah Churgin, Marie E. Pinkerton *124*

10:45 AM **Abortion and Disseminated Infection by *Coccidioides posadasii* in an Alpaca Fetus**
Francisco Uzal, Santiago Diab, Jorge Garcia, Suzanne Johnson, Erin Carlson, Demosthenes Pappagianis, Jana Smith *125*

11:00 AM **Hepatic Encephalomyelopathy in Ten Goat Kids Associated with Congenital Portosystemic Shunting (cPSS) ◊**
Hailu Kinde, Patricia Pessavento, John Adaska, Bradd Barr, Megan Jones, Alexandre P. Loretti, Mark Anderson *126*

Symbols at the end of titles indicate the following designations:

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

Virology 2
(Influenza & Serology)
Sunday, October 21, 2012
Auditorium III

Moderators: Amy Glaser, Suzanne Carman

8:00 AM **Identification of Lymphoproliferative Disease Virus in Wild Turkeys (*Meleagris gallopavo*) in the United States ◊**
Justin Brown, Andrew Allison, Kevin Keel, Trista Welsh, Aly Fadly *129*

8:15 AM **The Role of Enteric Viruses in Light Turkey Syndrome ◊**
Devi P. Patnayak *130*

8:30 AM **Identification of Pandemic H1N1 Influenza Virus in Feral Swine**
Mangkey A. Bounpheng, Amir Nikooienejad, Mohammad Shahrokh, Richard P. Metz, Scott Schwartz, Esmaeil Atashpaz Gargari, Thomas J. Deliberto, Mark W. Lutman, Kerri Pedersen, Luis R. Bazan, Leo Koster, Melinda Jenkins-Moore, Sabrina L. Swenson, Michael Z. Zhang, Tammy Beckham, Charles D. Johnson, Alfonso Clavijo *131*

8:45 AM **Epidemiology of Influenza A Virus (IAV) Circulating in US Swine Between 2004 and 2011**
Jianqiang Zhang, Phil Gauger, Amy Chriswell, Xue Lin, Sara Nezami, Dong Sun, Devon Haney, Wendy Stensland, Aaron Irons, Karen Harmon, Erin Strait, Kyoung-Jin Yoon *132*

9:00 AM **Influenza A Virus Detection in Oral Fluid and Nasal Swabs by a Rapid Antigen Detection Kit in Swine Inoculated with IAV †**
Christa K. Goodell, Apisit Kittawornrat, Yaowalak Panyasing, Chris Olsen, Thomas Overbay, Chong Wang, Rodger Main, Jeff Zimmerman *133*

9:15 AM **Influenza A Virus Detection in Oral Fluid and Nasal Swabs by RT-PCR in Swine Inoculated with IAV †**
Christa K. Goodell, Rolf Rauh, William M. Nelson, Angela Burrell, Catherine O'Connell, Chong Wang, Rodger Main, Jeff Zimmerman *134*

9:30 AM **Influenza A Virus Isolation from Oral Fluid and Nasal Swabs in IAV Inoculated Pigs †**
Christa K. Goodell, Fanghong Zhou, Chong Wang, Kyoung-Jin Yoon, Rodger Main, Jeff Zimmerman *135*

9:45 AM **Break**

10:15 AM **Outbreak of Influenza A (H3N2) in People and Pigs at a County Fair**
Sabrina L. Swenson, Mary Lea Killian, Roman Pogranichniy, Jennifer Strasser, Steve Lindstrom, Pravina Kitikoon, Jennifer House, Stephen D. Lenz, Leo Koster, Bret Marsh, Bill Davis, Lashondra Berman, Amy Vincent, Shawn Richards, Mark Glazier, Sarah M. Tomlinson *136*

10:30 AM **The Effects of Pooling, Swab Type and Transport Conditions on Avian Influenza Virus and Newcastle Disease Virus Detection**
Erica Spackman, Janice C. Pedersen, Enid McKinley *137*

10:45 AM **Modification of the USDA H5 rRT-PCR Assay for Detection of H5N2 Low Pathogenic Avian Influenza Viruses of Mexican Lineage ◊**
Janice C. Pedersen, Mary Lea Killian, Nichole Hines, Beverly Schmitt *138*

11:00 AM **Experimental Co-infection Studies with Avian Influenza Viruses and Newcastle Disease Viruses in Chickens, Turkeys, and Domestic Ducks ◊**
Mary Pantin-Jackwood, Patti Miller, Claudio Afonso, Erica Spackman, Darrell Kapczynski, Eric Shepherd, Diane Smith, Rami Cha, Mariana Sa E Silva, David Swayne 139

11:15 AM **Engineering an Improved Classical Swine Fever ELISA to Detect Antibody in Sera**
Melissa L. Batonick, Gregory Mayr 140

11:30 AM **Monitoring of Four Naturally Infected Horses for Vesicular Stomatitis Antibody**
Dawn Toms, Mindy Powell, Michelle Redlinger, Tamara Beach, Melinda Jenkins-Moore, Tina Buffington, Cindy Harding, Sabrina L. Swenson 141

Symbols at the end of titles indicate the following designations:

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

Virology 3

Sunday, October 21, 2012

Auditorium II

Moderators: Binu Velayudhan, Kyoung-Jin Yoon

8:00 AM **BVD Antigen Detection - Exploring the Diagnostic Gap**
Ini-Isabee Witzel, Hinrich Voges *144*

8:15 AM **HoBi-like Viruses, an Emerging Pestivirus of Cattle**
Julia F. Ridpath, Fernando Bauermann, Aaron Harmon *145*

8:30 AM **Bovine Viral Diarrhea Virus Infections in Pregnant Cattle: Diverse Outcomes of Fetal Infections in a Natural Occurring Herd Disease ◊**
Robert W. Fulton, Grant Rezabek, Julia F. Ridpath *146*

8:45 AM **Comparison of FTA Cards and Whole Blood PCR for Diagnosis of Persistent Infection with Bovine Viral Diarrhea Virus in Cattle #**
Celeste B. Foster, Beate M. Crossley, Bruce R. Hoar *147*

9:00 AM **Effects of Immunoglobulin G as an Inhibitor of Diagnostic Polymerase Chain Reaction (PCR) Demonstrated on the Detection of Bovine Viral Diarrhea Virus in Dairy Calves**
Munashe Chigerwe, Beate M. Crossley *148*

9:15 AM **Experimental Infection of Holstein Calves with Epizootic Hemorrhagic Disease Virus Serotype 7: a Preliminary Study Using a Variety of Inoculation Routes ◊**
Mark G. Ruder, Daniel Mead, David Stallknecht, Deborah Carter, Justin Brown, Maor Kedmi, Eyal Klement, Barbara Drolet, Elizabeth W. Howerth *149*

9:30 AM **Break**

10:00 AM **Tests on Swine Samples of the Dominican Republic Collected in Regions near the Border to Haiti**
Angel Ventura, Wendy Gonzalez, Roger Barrette, Sabrina L. Swenson, Alexa Bracht, Fessica Rowland, Andrew Fabian, Moran Karen, Fawzi Mohamed, Emily O'hearn, Melinda Jenkins-Moore, Dawn Toms, John Shaw, Paula Morales, David Pyburn, Consuelo Carrillo, Gregory Mayr, Michael T. McIntosh, Ming Yi Deng *150*

10:15 AM **Picornavirus Isolated from a Swine Brain in Michigan**
Melinda Jenkins-Moore, Donna Johnson, Mary Lea Killian, Leo Koster, Doreen Cawley, Aaron D. Lehmkuhl *151*

10:30 AM **Oral Fluid Collection in Swine as a Diagnostic Sampling Technique for Foreign Animal Diseases**
Frederic R. Grau, Erin Mulhern, Mangkey A. Bounpheng, Megan Schroeder, Tammy Beckham, Michael T. McIntosh *152*

10:45 AM **Canine Distemper Outbreak in Multiple Pet Store Dogs Linked to High Volume Breeder ◊**
Donal O'Toole, Myrna M. Miller, Jacqueline L. Cavender, Todd Cornish, Donald L. Montgomery, Brant A. Schumaker *153*

11:00 AM **Association between Breed and Antibody Response to Primo-vaccination against Foot and Mouth Disease in Cattle #**
Barbara P. Brito, Sebastian Di Giacomo, Andres Perez, Danilo Bucafusco, Luis L. Rodriguez, Manuel Borca, Mariano Perez *154*

11:15 AM **Detection of Substantial Porcine Group B Rotavirus Genetic Diversity in United States Swine †**
Douglas Marthaler, Kurt D. Rossow, Marie Gramer, James E. Collins, Max Ciarlet, Jelle Matthijnssens *155*

11:30 AM **Molecular Characterization of Canine Parvoviruses Associated with Myocarditis in Puppies * †**
Hai T. Hoang, Kent Schwartz, Kyoung-Jin Yoon *156*

Symbols at the end of titles indicate the following designations:

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

USAHA/AAVLD Joint Plenary Session:
Science, Economics, and Politics, Oh My!

Monday, October 22, 2012
Guilford ABC

Moderator: Richard Breitmeyer

8:00 AM **Introduction**

8:15 AM **Economics of Farm-Level Animal Agriculture in the Future**
John Lawrence 158

9:00 AM **Economy Wide Impact of a Foreign Animal Disease in the United States**
Dermot Hayes, Jacinto Fabiosa, Amani Elobeid, Miguel Carriquiry, Patrick Webb 159

9:45 AM **Break**

10:00 AM **National Institute for Animal Agriculture Forums on One Health Approach to Antibiotic Use in Food Animals**
Leonard Bull 160

10:45 AM **Egg Products Inspection Act Amendments 2012- Proposed Federal Legislation for Egg Farmers**
Gene W. Gregory 161

11:30 AM **Questions**

Symbols at the end of titles indicate the following designations:

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

vmrd Veterinary Medical Research & Development

- Diagnostic Test Kits
  - FA Reagents
  - Antibodies
- Immunology Reagents

vmrd

PO Box 502 • Pullman, WA • 99163
Ph: 509-332-5356 • techesrve@vmrd.com
www.vmrd.com

Poster Session

Friday October 19 – Sunday October 21

Third Floor Prefunction Area

1. **Swinecheck mix-APP®, a New ELISA Tool to Detect Antibodies to the Most Prevalent Serovars of *Actinobacillus pleuropneumoniae* in Swine Sera**
Andre Ch. Broes *165*

2. **The Effects of North American Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)-based Modified Live Vaccines in Preimmunized Sows Artificially Inseminated with European PRRSV Spiked Semen**
Changhoon Park, Kiwon Han, Hwiwon Seo, Yeonsu Oh, Chanhee Chae *166*

3. **Brain Abscess in a White-tailed Deer**
Anwar J. Sarah *167*

4. **Development and Evaluation of a Mobile Diagnostic Computer Application for Veterinary Laboratories**
Jacqueline C. Cassady, Craig N. Carter, Norman Ronald, Michelle L. Arnold, Cynthia L. Gaskill, Jacqueline Smith, Wade A. Northington *168*

5. **The Prevalence of Potentially Pathogenic Bacteria and Fungi in Bobwhite Quail in the Rolling Plains Ecoregion**
Hongwen Su, Shuping Zhang, Jessica McKelvey *169*

6. ***Bartonella bovis* Isolated from a Cow with Endocarditis**
Erdal Erol, Carney Jackson, Ying Bai, Stephen Sells, Steve Locke, Michael Kosoy *170*

7. **Use of Electronic Nose Technology to Identify Cattle Experimentally Infected with *Mycobacterium bovis* By Detection of Unique Volatile Organic Compounds in Breath: Preliminarily Findings**
Christine K. Ellis, Randall Stahl, Pauline Nol, Jack Rhyan, Matthew McCollum, Kurt VerCauteren, Joni Van Sickle, Lyndsey Linke, Roberta Magnuson, M.D. Salman *171*

8. **Effects of Freeze-thaw Cycle on Urine Values from Bottlenose Dolphins (*Tursiops truncatus*)**
Risa Daniels, Cynthia R. Smith, Stephanie Venn-Watson *172*

9. **Dermatitis, Cellulitis and Myositis due to *Serratia marcescens* in a Cat**
Jane Kelly, Thomas J. Baldwin, Aaron Chamberlain *173*

10. **Report of a New Genotype of *Ehrlichia* species from Cattle and Cervids**
Alvin A. Gajadhar, Brad Scandrett, Vladislav A. Lobanov, Batol Al-Adhami, Gary A. Wobeser, John Campbell *174*

11. **Understanding the Factors that Influence Low Pathogenic Avian Influenza Virus Infection in Ducks ◊**
Justin Brown, Monique Franca, Taiana Costa, Camille Lebarbenchon, Deborah Carter, Elizabeth W. Howerth, David Stallknecht *175*

12. **A Validated Assay of Carbamate Pesticides in Feeds, Baits, and Tissues using QuEChERS and Gas Chromatography/Mass Spectrometry**
Wilson K. Rumbeiha, Andreas Lehner, Margaret Johnson, Asley Couch, John Buchweitz *176*

13. Aquatic Animal Research in a Traditionally Terrestrial Animal Environment: Considerations for Success
Jean Kaptur, Richard Clayton *177*

14. Real-time PCR, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma meleagridis*
Pablo Lopez, Kristen Roza, Phyllis Tyrrell *178*

15. Development and Validation of uvrC Gene-based TaqMan Real-time qPCR Assay for Detection of *Mycoplasma bovis*
Hemant Naikare, Daniela Resende Bruno, Debabrata Mahapatra, Alesia Reinisch, Russell Raleigh, Robert W. Sprowls, Tammy Beckham *179*

16. Diagnostic Findings from Common Eider (*Somateria mollissima*) Mortality Events in the Northeastern United States Associated with Wellfleet Bay Virus, a Novel Orthomyxovirus ◊
Jennifer Ballard, Andrew Allison, Justin Brown, Samantha Gibbs, Chris Dwyer, Julie C. Ellis, Sarah Courchesne, Randall Mickley, Kevin Keel *180*

17. Novel Solution for Manual and Automated Identification of Pathogen Nucleic Acids in Various Veterinary Sample Types
Lillian Roth, Denis Fluegge, Sandy Leifholz, Melanie Breitbach, Kathrin Wolf, Dora Quest, Holger Engel *181*

18. Performance Evaluation of Biolog GEN III System for Identification of Bacteria of Veterinary Origins
Hui-Min Hsu, Stan Stankevitch, Ogi Okwumabua *182*

19. Evaluation of the Development of the Bovine Foot in Response to Variation in Management Practices ◊
Julie A. Gard, Debra R. Taylor, Soren P. Rodning, Dewey R. Wilhite, Kent Sanders, Misty A. Edmondson, Thomas Denney *183*

20. Isolation of *Actinobaculum*-like Organism from a Goat Abscess
Yan Zhang, Jing Cui, Anne Parkinson, Troy Farrell, Beverly Byrum *184*

21. Isolation of a *Campylobacter*-like Organism from a Goat Abscess
Yan Zhang, Jing Cui, Anne Parkinson, Troy Farrell, Beverly Byrum *185*

22. Idiopathic Generalized Soft Tissue Mineralization in an Appaloosa Filly # * ◊
Stephanie R. Ostrowski, Alexandre P. Loretti, Snehal N. Tawde, James A. Bergum *186*

23. Avian Encephalomyelitis Virus Outbreak in Pullets from a Colorado Commercial Layer Flock
Chad Frank, Kristy Lynn Pabilonia *187*

24. Botulism Outbreak Associated with Contaminated Soil in Cattle
Jae-Won Byun, Byeongyeal Jung, Hayoung Kim, Albert Byungyun Jeon, Kyunghyun Lee, Ounkyung Moon, Soonseek Yoon, Osoo Lee, Myoungheon Lee, Youchan Bae *188*

25. Development of a Diagnostic Method to Detect Exposure to the Castor Bean Plant
Christina Wilson, Kimberly Meyerholtz, Stephen B. Hooser *189*

26. Development and Validation of a Novel Real-Time RT-PCR Assay to Distinguish Porcine Reproductive and Respiratory Syndrome (PRRS) Virus Subtypes with High Sensitivity
Rolf Rauh, William M. Nelson, Silvia Pillay *190*

27. Pooling of Ear Punch Samples for the Detection of BVDV PI Animals By Real-Time RT-PCR
Quoc Hoang, Darcy Myers, Jeff Zinza, Catherine O'Connell 191

28. Examination of Pet Foods for Three Food Safety Pathogens, a Preliminary Study to Prioritize Future Surveillance Testing
Renate Reimschuessel, Sarah Yachetti, Olgica Ceric, Andriy Tkachenko, April Hodges, Micheal Grabenstein, Tara Doran, Terri McConnell, Kevin Garner, Scott Benko, Frances Mohnke, Karen Pietrzen, Sharon Robeson, Karen Wilson, Ted Gatesy, Gregory Danzeisen, Deepa Mathur, Bryanne Shaw, Grisel Rosario, Barbara Baker, Yong Liu, Maria Ishida, Amy Bryant, Jason Crowe, Maya Achen 192

29. Physical and Histological Characteristics of Chicken Jerky Treats
Renate Reimschuessel, Olgica Ceric, Andriy Tkachenko, Sarah Yachetti, April Hodges, Lee Anne Palmer 193

30. Deployment of a Serological Assay Detecting Foot-and-Mouth Disease virus Antibodies to the National Animal Health Laboratory Network
Stacey M. Bucko, Patricia Glas, Gregory Mayr, Moran Karen, Fernando Torres-Velez, Sarah M. Tomlinson, Thomas Struckmeyer, Barbara M. Martin, Michael T. McIntosh 194

31. Evaluation of an ELISA Assay as an Ancillary Method in a Herd with Natural Tuberculosis Infection
Ignacio Aranaiz, C. Eiras, M. Luaces, J. Fraga, M. Rodriguez, C. Calvo 195

32. Development of an Influenza A Sequencing Workflow on Ion PGM Sequencer for Improved Surveillance
Angela Burrell, Craig Cummings, Wayne Ge, Catherine O'Connell 196

33. PCR-Based Panel Testing for Simultaneous Detection of Bovine Respiratory Pathogens
Jaeik Han, Yongil Cho, Vickie Cooper, Binu T. Velayudhan, Kent Schwartz, Karen Harmon, Wonil Kim, Kyoung-Jin Yoon 197

34. Evaluation of a Panel of Multiplex Real-Time PCR Assays for the Detection of Organisms Associated with Scours in Camelid Feces
Donna M. Mulrooney, Wendy Black, Ling Jin, Kathy O'Reilly 198

35. Integumentary Sensory Organs (ISOs) of the *Alligator mississippiensis* and Intradermal Nevus cells (INC). Is There a Histogenetic and Atavistic Correlation Between Them?
Hector A. Rodriguez-Martinez, Abelardo A. Rodriguez-Reyes, Gizela Maldonado-Hernández, Leonora Chavez-Mercado, Ofelia Perez-Olvera, Alfredo Gómez-Leal, Dolores Ríos y Valles-Valles 199

36. Isolation and Characterization of *Streptococcus suis* from Bovine Clinical Cases
Ogi Okwumabua, Hui-Min Hsu 200

37. Re-evaluation of the IDEXX *Mycobacterium paratuberculosis* Antibody Test to Improve Diagnostic Sensitivity
Hannah Pearse, Christoph Egli 201

38. IDEXX RealPCR Diagnostic Test System for Livestock Applications
Michael Angelichio, Brooke Bivens, Andrea Ballagi, Peter Welles, Valerie Leathers, Christian M. Leutenegger 202

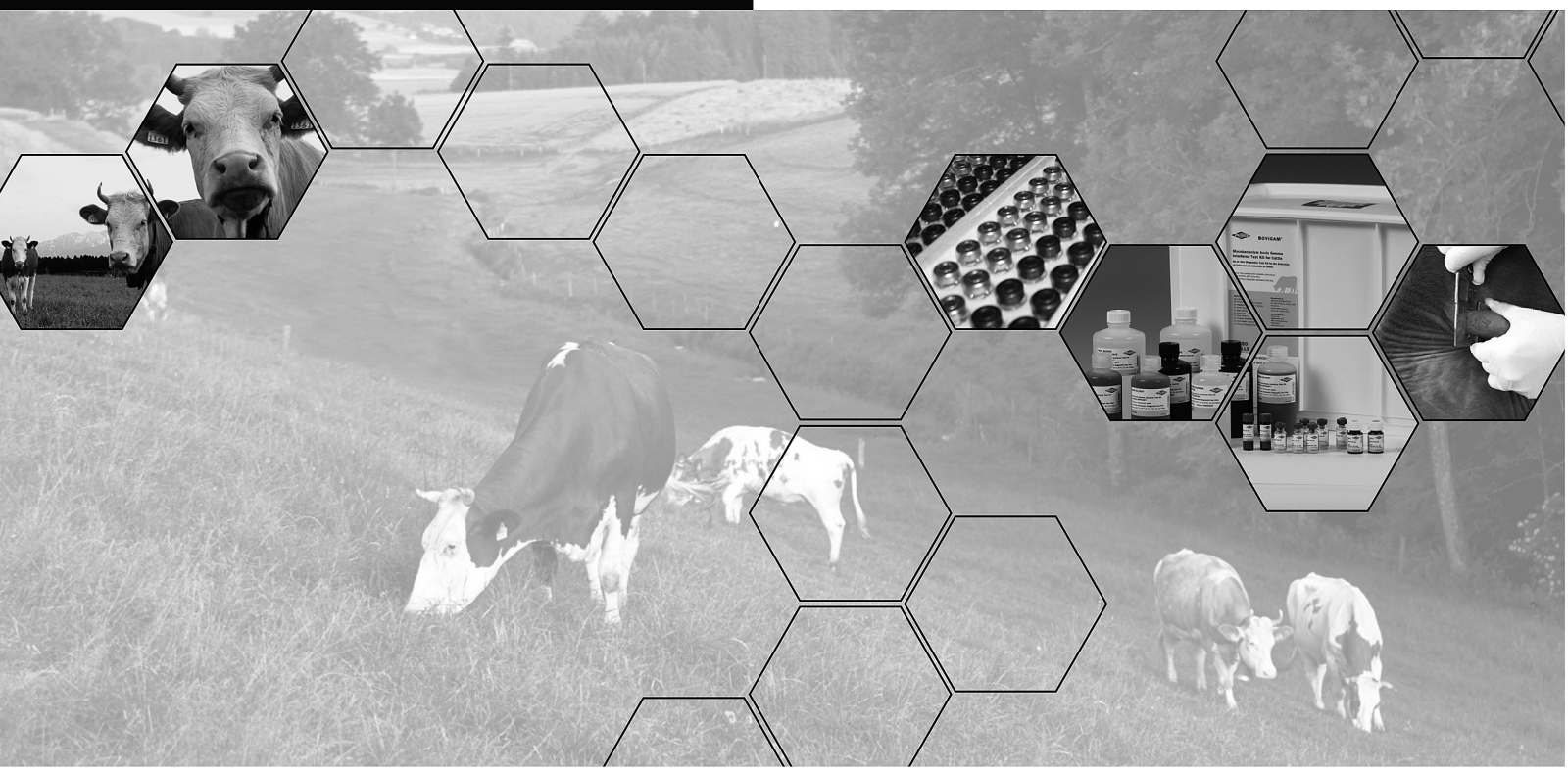
Symbols at the end of titles indicate the following designations:

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

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

AAVLD Plenary Session:
Advanced Diagnostic Technologies - Are They Making A Difference?

Saturday, October 20, 2012

Guilford DE

Moderator: Thomas McKenna

7:45 AM **Introduction**

8:00 AM **To MALDI or Not to MALDI**
Carole Bolin, Lori Moon 29

8:35 AM **To Pool or Not to Pool, That is the Question**
Kathy L. Toohey-Kurth 30

9:10 AM **Break**

9:40 AM **Considerations for Performing Luminex® Multiplex Assays in Veterinary Diagnostic Laboratories**
Jane Christopher-Hennings 31

10:15 AM **Diagnostic and Investigational Applications of Metabolic Phenotyping of Bacteria and Cancer Cells**
Luke B. Borst 32

10:50 AM **More Bang For All Those Bucks: How High Resolution Can Equate to Higher Efficiency in the Toxicology Lab**
Michael Filigenzi 33

Symbols at the end of titles indicate the following designations:

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

To MALDI or Not to MALDI

Carole Bolin, Lori Moon

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

Narrative: Adoption of new technology in Veterinary Diagnostic Laboratories is an important method to improve quality, accuracy, and timeliness of laboratory results. However, several factors must be considered before adoption of the new technology, including cost/benefit analysis, cost of equipment and service contracts, staff acceptance, staff training, development of new SOP's, education of clients regarding new test methods, etc. A decision to introduce MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectroscopy) methodology to the Bacteriology Laboratory at DCPAH involved consideration of all of these factors and will be used to illustrate the decision making process and some unexpected outcomes.

Speaker Biography: Carole Bolin received her DVM degree from Purdue University in 1982 and her PhD in Veterinary Pathology from Iowa State University in 1986. She began her career at the USDA's National Animal Disease Center in Ames, Iowa, where she served as a Veterinary Medical Officer and Lead Scientist of the Leptospirosis Research Project. Later with USDA, she was appointed as the Research Leader of the Zoonotic Disease Research Unit, with projects including tuberculosis, leptospirosis, brucellosis, and Johnes's disease. After 16 years with USDA, Dr. Bolin joined the faculty at Michigan State University's College of Veterinary Medicine as a Professor and Section Chief of Bacteriology in the Diagnostic Center for Population and Animal Health (DCPAH). At DCPAH, Dr. Bolin commissioned BSL-3 and Select Agent laboratories and established a research and diagnostics program for BSL-3 and Select Agents. She also serves as leader of Laboratory Response Network activities at DCPAH. In April 2008, she was appointed DCPAH Director. Throughout her career, Dr. Bolin's research interests have included zoonotic diseases, vaccine development, vaccine efficacy studies, and development of diagnostic tests. Her research has been funded by USDA, EPA, DHS, NIH, and numerous companies. Her current research involves microbial risk assessment for Francisella tularensis and other Select Agents as part of the EPA/DHS-funded Center for the Advancement of Microbial Risk Assessment.

To Pool or Not to Pool, That is the Question

Kathy L. Toohey-Kurth

[1]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI; [2]Pathobiological Science, School of Veterinary Medicine, University of Wisconsin, Madison, WI

Narrative: Reliable detection of bovine viral diarrhea virus (BVDV) is critical for maintaining the health of a herd. The efficacy of the diagnostic method employed for detection of persistently infected (PI) animals depends upon the assay and the sample type. Assays which are amenable to processing a high volume of samples include antigen capture ELISA, immunohistochemistry (IHC) and reverse transcriptase PCR (RT-PCR). Commonly used sample types for identification of PI animals include ear notches, serum and whole blood. Ear notch samples rapidly gained wide acceptance as the optimal sample for detection of persistent infection because these are easily obtained samples compared to serum and whole blood and because of presumed minimal amounts of maternal derived antibody. Nasal epithelial swab samples are also an easily obtained sample because it is a noninvasive sample which does not rely on a tool that has to be cleaned between animals. Preliminary studies indicate that the nasal swab sample type is more amenable to high throughput PCR than an earnotch sample. Initially, all BVDV testing was performed on single samples. Due to the exceptional sensitivity provided by real-time RT-PCR (rRT-PCR), pooling of 10-100 individual earnotches became a popular sample type because the cost per animal could be decreased significantly. However, the success of pooling depends upon the extraction technique, the number of individual samples pooled as well as the version of real-time PCR assay used. Pooling remains very controversial because of a lack of sensitivity associated with some versions of pooling protocols. As automation and high throughput processes become more available, the question becomes whether the price for individual testing by PCR can be brought closer to the price of pooling or at least that of IHC.To answer this question, novel sample types, platforms and modifications in extraction techniques were evaluated. This presentation will focus on technical and financial aspects of high throughput rRT-PCR for BVDV.

Speaker Biography: Kathy Toohey-Kurth is the Virology Section Chief at the Wisconsin Veterinary Diagnostic Laboratory. She holds a second appointment as a Clinical Professor in the Pathobiological Sciences Department at the UW School of Veterinary Medicine, University of Wisconsin-Madison. She is a Wisconsin native and earned a BS and MS from the Bacteriology Department at the University of Wisconsin-Madison. She worked as a clinical microbiologist prior to training in molecular virology for her PhD at the University of Minnesota Medical School. Dr Kurth continued her training in molecular virology as a post-doctoral fellow at the NIH Rocky Mountain Laboratory. Subsequently she held a Science Fellowship at National Veterinary Services Laboratory and worked at both Ames and Plum Island. At WVDL, Dr. Kurth has applied her clinical and molecular virology expertise to the development and improvement of diagnostic assays for detection of mammalian, avian and aquatic pathogens. She has a specific interest in high throughput and robotic techniques with the goal of improving cost effectiveness while maintaining high quality results. Since 2002, she has been active in the National Animal Health Laboratory Network and participates in the technical working group. This has led to an interest in defining and establishing parameters for molecular assay validation. Dr Kurth co-chairs the AAVLD Laboratory Technology Committee and in this position has led the development of guidelines for performance, validation and interpretation of PCR assays.

Considerations for Performing Luminex® Multiplex Assays in Veterinary Diagnostic Laboratories

Jane Christopher-Hennings

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

Narrative: Luminex multianalyte profiling (xMAP®) assays are applicable for simultaneous detection of multiple analytes in solution (eg. viral and bacterial antigens and antibodies, cytokines, signal transduction molecules, nucleic acids etc.) and are particularly useful for obtaining results from small volumes of sample in a short amount of time. http://www.luminexcorp.com/ The technology involves obtaining or developing ligand (eg. proteins, antibodies, receptors, nucleic acids etc.) labeled fluorescent beads that will bind to a specific analyte in the sample. Each bead is distinguished from the others by varied amounts of two fluorescent dyes in the bead. After binding of the analyte to the bead ligand, a 3rd fluorescent marker is added and if it becomes bound to the analyte, a semi-quantitative measurement of mean fluorescent intensity (MFI) is used to determine if the sample is "positive" or "negative" for the analyte. The Luminex instrument uses two light sources, one that excites the two fluorescent dyes that identify each microsphere particle, and another that excites the reporter dye captured during the assay. Currently, real-time PCR may be able to distinguish between 5 analytes using 5 different fluorophores, however, xMAP may detect 50 (MAGPIX® instrument), 100 (Luminex 100/200™) or 500 (Luminex FLEXMAP 3D®) analytes simultaneously per sample. Many xMAP assays are described for use in human medical laboratories. Recent commercial assays developed for human samples include an "xTAG Respiratory Viral Panel" for detection of 8 viruses and subtypes and an "xTAG Gastrointestinal Pathogen Panel" for detection of 9 bacteria or bacterial toxins, 3 viruses and 3 parasites. Few assays are commercially available for veterinary applications. Therefore, similar to the beginning of PCR based tests being used in veterinary diagnostic laboratories; in-house assays are being developed. Standardization of assays between laboratories will be critical for diagnostics using standard controls. Other basic considerations in development are needed, including defining what the assay will be used for (eg. "fit for purpose") and determining specificity, sensitivity and repeatability, as with any diagnostic test. Luminex based assays, particularly for serologic measurements will require purified proteins for bead coating, and for some antigen detection assays, the continual availability of specific monoclonal antibodies will be necessary. Optimum sample types that are used in veterinary medicine and processing will also need to be considered. Challenges related to the development and routine performance of these assays, along with costs and resources available will be presented for transitioning these assays into veterinary diagnostic laboratories.

Speaker Biography: Dr Christopher-Hennings is a Professor at the Veterinary and Biomedical Sciences Department at South Dakota State University (SDSU). Her research at SDSU started in 1990 where she worked on the initial characterisation of Porcine Reproductive and Respiratory Syndrome (PRRS) Virus, performed C-sections for obtaining gnotobiotic pigs to confirm Koch's postulates in identifying the cause of the disease and developed one of the first PCR based tests that she used in describing the pathogenesis and identification of PRRSV in boars and semen. As a result of that research, the Molecular Diagnostic Section was initiated at SDSU and she has been in charge of that section since its inception, approximately 20 years ago. She received her BS and MS degrees from the University of Wisconsin-Madison and a DVM from the University of Minnesota. She was in private veterinary practice in South Dakota for 6 years and at SDSU in a postdoctoral position for 6 years. Her current research has focused on viral respiratory diseases of swine and development of new veterinary diagnostic technologies, including the latest development of an xMAP® swine cytokine assay which she was invited to present at the 2010 Planet xMAP® Europe meeting in Vienna, Austria and at a national Luminex workshop at Rush University in Chicago, IL in 2011. She is currently working on collaborations with xMAP® serologic profiling in swine.

Diagnostic and Investigational Applications of Metabolic Phenotyping of Bacteria and Cancer Cells

Luke B. Borst

Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Narrative: Foundational to diagnostic microbiology is the identification of bacteria by their differential ability to ferment energy sources and to resist salts or antibiotics. These assays have been incorporated into large scale metabolic profiles which have applications beyond the identification of clinical isolates. The Biolog GenIII MicroPlate™ generates metabolic profiles by detecting the products of metabolism (NADH/NADPH) using a proprietary tetrazolium dye. Color density of elaborated dye is measured in an automated reader (Omnilog®). This system, in which increasing color density directly correlates with substrate metabolism, has varied applications including the routine identification of clinical and research bacterial isolates, the identification of metabolic markers for specific detection of individual strains of bacteria, environmental microbial community profiling, metabolic arrays of bacterial and eukaryotic cells and chemotherapeutic resistance profiling of primary cancer cells. Examples of these various applications will be discussed including: 1) Using metabolic phenotyping to identify specific strains of bacteria. a. Differentiating the modified live vaccine for equine strangles (Pinnacle IN) from wild-type (WT) strains. Diagnostic investigation of nasal swabs and washes from horses with strangles yielded isolates of *Streptococcus equi* ssp *equi* that had a dry (unencapsulated) appearance. These isolates resembled the modified live vaccine (MLV) for equine strangles (Pinnacle IN); however, differentiating the MLV from WT was impossible using routine methods. Mining the data from Biolog GP identification plates revealed multiple metabolic defects in the MLV which allowed for the rapid identification of this strain. b. Differentiating virulent strains of *Enterococcus cecorum* from intestinal commensal strains. *E. cecorum* is an emerging pathogen of poultry which is responsible for global outbreaks of enterococal spondylitis. Data mining the Biolog GenIII identification plates revealed that all isolates recovered from spinal abscesses have a defect in D-mannitol metabolism. 2) Using metabolic phenotyping to investigate carcinogenesis and chemotherapeutic resistance in canine osteosarcoma. a. Metabolism is tightly controlled in normal cells and aberrant metabolism has long been known to be an early indication of carcinogenesis. Using metabolic profiles provided by the Biolog PMM plates, a comprehensive view of the cell's active metabolic pathways can be determined. In addition, we can also measure the functional significance of these pathways under biologically important conditions including hypoxia which has been correlated with increased resistance to standard therapies and increased metastatic potential. This new approach provides functional data to complement both genomic and proteomic approaches.

Speaker Biography: Dr. Luke Borst is a boarded veterinary pathologist with interests in bacterial pathogenesis and molecular mechanisms of neoplastic transformation. Dr. Borst's laboratory is currently working to identify genetic determinants of virulence and antimicrobial resistance in Enterococcus cecorum, an important emerging pathogen of chickens. Dr. Borst lives in Raleigh, NC with his wife, son, 3 dogs, 2 cats and 2 horses.
http://www.cvm.ncsu.edu/dphp/personnel/borst.html

More Bang For All Those Bucks: How High Resolution Can Equate to Higher Efficiency in the Toxicology Lab

Michael Filigenzi

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

Narrative: The requirement to detect minute levels of poisonous compounds in highly complex matrices is one of the major challenges inherent in veterinary analytical toxicology. Advances in analytical instrumentation over the last few decades, particularly in mass spectrometry, have greatly improved our ability to detect toxicants at progressively lower concentrations. Unfortunately, such technological wizardry does not come cheaply – there's no getting away from the fact that modern analytical chemistry is a very expensive undertaking. To further complicate things, we live in a time of strained budgets and minimal resources; labs are required to "do more with less" and efficiency is more important than ever. Opportunities to purchase expensive instruments are rare, and therefore each instrument must be useful for as wide a variety of cases and situations as possible. In this presentation, I'll discuss how a new type of mass spectrometer fits into this scenario. Examples will be shown which demonstrate the potential for expanded capabilities, rapid analyses, and improved efficiency which may be obtained with this new technology. Sadly, its limitations will also be considered.

Speaker Biography: After finishing his undergraduate studies in Psychobiology, Mike Filigenzi somehow found himself working in an analytical chemistry laboratory. While initially dismayed at this turn of events, he soon discovered just how cool mass spectrometers are. He spent the subsequent fifteen years analyzing environmental samples for a wide variety of pollutants using various mass spectrometry techniques. In 2000, he joined the California Animal Health and Food Safety Laboratory where he now works as a group leader in the Toxicology section. Mike specializes in developing mass spectrometry methods for the detection of compounds of interest in veterinary toxicology and food safety.

Bacteriology 1

Saturday, October 20, 2012

Guilford D

Sponsor: Biovet, Inc.

Moderators: Joshua Daniels, Karen W. Post

1:00 PM **Novel Assay for Detecting *Mycobacterium tuberculosis* Infection in Nonhuman Primates**
Kimberly Luke, JoAnn Yee, Kirk Andrews, Deepak Kaushal, Bill Patterson 36

1:15 PM ***Mycobacterium leprae* Infection in a Nine-banded Armadillo in Florida**
Shipra D. Mohan, Elena Hollender, Teri L. Johnson, Karen McKenzie, Brian Pugh, Gizela Maldonado-Hernández, James Maxwell, Alice Agasan, Susan Kelleher, Danielle Stanek 37

1:30 PM **Petrifilm™ Measurement of Bacteria Counts (SPC) in Pasteurized Waste Milk for Feeding to Dairy Calves and Association of SPC with Time until Feeding, Season and Temperature**
David J. Wilson, Gregory M. Goodell 38

1:45 PM **Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry for Bacterial Identification: Experiences After One Year of Use in a Veterinary Diagnostic Laboratory**
Timothy Frana 39

2:00 PM **Speciation of *Brachyspira spp*. in Poultry Fecal Samples using the GS Junior**
Durda Slavic, Stina Nilsson, Michele Guerin, Genet A. Medhanie 40

2:15 PM **Evaluation of Serodiagnostic Assays for *Mycobacterium bovis* in Elk, White-tailed Deer, and Reindeer in the United States ◊**
Jeffrey T. Nelson, Kathleen Orloski, Audra Lloyd, Mark Camacho, Mark Schoenbaum, Suelee Robbe-Austerman, Bruce V. Thomsen, S. Mark Hall 41

2:30 PM **Development and Evaluation of a New Serological ELISA for Detection of Herds with Swine Dysentery ◊**
Yong Song, Xiaoping Yang, Daniel Zwald, Mario Puerro, Angela Zurfluh, Alex Raeber, David Hampson 42

2:45 PM **Rene Lallier, Biovet**

Symbols at the end of titles indicate the following designations:

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

Novel Assay for Detecting *Mycobacterium tuberculosis* Infection in Nonhuman Primates

Kimberly Luke[1], JoAnn Yee[2], Kirk Andrews[3], Deepak Kaushal[4], Bill Patterson[1]

[1]Intuitive Biosciences, Madison, WI; [2]California National Primate Research Center, University of California, Davis, Davis, CA; [3]Oregon National Primate Research Center, Oregon Health and Science Center, Beaverton, OR; [4]Tulane National Primate Research Center, Tulane University, Covington, LA

Narrative: Nonhuman primates (NHPs) used for research purposes are regularly screened for *M. tuberculosis* infection, both as part of transport associated quarantine and for routine colony health maintenance programs. The current recommended screening method relies on the tuberculin skin test (TST) to identify animals with acute or latent *M. tuberculosis* infection; however, this method is labor intensive, takes 3 days to complete, and has known problems with false positive reactors and anergic response by infected animals. More effective testing with higher sensitivity is needed, while remaining economical in material cost and reducing labor required to perform testing on large numbers of animals. This work describes a more accurate antibody detection test for TB surveillance in NHPs by identifying antigens and utilizing the multiplex format of the peptide microarray for simple high throughput antibody detection assay. A large set of immunoreactive antigens was first identified using peptide arrays derived from open reading frames of the *M. tuberculosis* genome, screening serum samples from TST test positive and negative rhesus macaques. The top immunoreactive peptides were chosen for further analysis based on ability to distinguish positive and negative samples by various analysis methods. Next, a large set of positive (both experimental and naturally occurring infections) and negative (by routine TST) serum samples from rhesus and cynomolgus macaques were run on the peptide arrays to determine sensitivity and specificity. Results from further screening at several NHP colonies with historically negative TST are reported here. Overall, this work identifies novel immunoreactive peptide antigens and an antibody detection assay for *M. tuberculosis* that provide high sensitivity and specificity for TB surveillance programs that could be adapted to other species.

Mycobacterium leprae Infection in a Nine-banded Armadillo in Florida

Shipra D. Mohan[1], Elena Hollender[2], Teri L. Johnson[1], Karen McKenzie[1], Brian Pugh[1], Gizela Maldonado-Hernández[1], James Maxwell[1], Alice Agasan[1], Susan Kelleher[3], Danielle Stanek[4]

[1]Department of Agriculture-Animal Industry, Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL; [2]Department of Health, A.G. Holley Hospital, Lantana, FL; [3]Veterinarian, Broward Avian & Exotic Hospital, Coral Spring, FL; [4]Department of Health, State Epidemiology, Tallahassee, FL

Narrative: Abstract: Leprosy (Hansen's disease) is a chronic infectious disease caused by *Mycobacterium leprae*. Though often considered a disease of antiquity, it is still found in tropical and semitropical regions of the world. Humans had been considered main reservoir of *M. leprae*. However, it also occurs naturally among some free-ranging armadillos, and recent reports have postulated an association between *M. leprae* in armadillos and humans. In September, 2011, a two year old female nine-banded female armadillo kept as a pet was brought to a local veterinarian presenting with disseminated subcutaneous nodules on shoulders, thighs and hind limbs. Biopsies were AFB smear + and the State Epidemiologist and Department of Health (DOH) were notified. The armadillo was presented for necropsy at Bronson Animal Disease Diagnostic Laboratory (BADDL) with a strong concern about the possibility of leprosy. Necropsy specimens of the lungs, subcutaneous nodules, liver and intestinal tissues were submitted to bacteriology section of Bronson Laboratory, and also sent to Florida Department of Health, TB Laboratory, Jacksonville, FL (JAX) and to the National Hansen's Disease Program Clinical Laboratory, Baton Rouge, LA. At BADDL, optical microscopy of acid fast stain on all the specimens showed; acid fast bacilli (AFB) in clumps, rounded masses, or groups of bacilli in palisade formation, suggestive of *M.leprae*. At JAX, specimens of lung, subcutaneous nodule and liver were AFB smear 2+, 4+, and 4+ respectively. Nucleic acid amplification (NAA), using Mycobacterium Tuberculosis Direct (MTD®), was performed to differentiate MTb; the results were positive, positive and negative respectively. The NAA test when done on a (+) smear non-respiratory as a sensitivity of 93.1% and a specificity of 97.7% . However, there have been reports of false positive MTDs with *M.celatum*, *M.kansasii* and *M.leprae*. Therefore an in-house real-time PCR test (GeneXpert®) was done. On all 3 specimens MTb was not detected, indicating a non-tuberculous mycobacteria without IS6110 sequencing. A presumptive diagnosis of *M.leprae* was made on the basis of real-time PCR and on the basis of unique characteristic morphology of leprous bacilli on acid fast stain. The National Hansen's Disease Program Clinical laboratory confirmed the diagnosis of leprosy based on pathognomonic lesions in histology samples and PCR results. Interestingly, the *M.leprae* strain identified in the armadillo has also been found in a human Hansen's disease patient residing in East-Central Florida. Details will be discussed.

Petrifilm™ Measurement of Bacteria Counts (SPC) in Pasteurized Waste Milk for Feeding to Dairy Calves and Association of SPC with Time until Feeding, Season and Temperature

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

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

Narrative: Waste milk is often pasteurized before feeding to dairy calves. Primary objectives were evaluation of Petrifilm™-measured milk standard plate counts (SPC) as time passed following on-farm pasteurization and potential differences in SPC over time between seasons and their associated ambient temperatures. A secondary objective was comparison of time after pasteurization until SPC exceeded 20,000cfu/ml, a suggested industry standard for feeding milk to calves, among the seasons. Discard milk was pasteurized at 63°C for 30min on 3 commercial Colorado dairy farms. During each of the 4 seasons, milk was sampled pre-pasteurization, when milk cooled to 49° C post-pasteurization, and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 hr post-pasteurization. One aliquot of each batch was incubated outdoors, another indoors: refrigerated (winter), at room temperature (fall, spring) or incubated at 37°C (summer). At each time point, milk was streaked on Petrifilm™ to determine Aerobic Count SPC. Dilutions of milk in Butterfield's Buffer ranged between undiluted to 10^{-10} depending on logical expectations of SPC for elapsed time and season; no samples had SPC results of TNTC. Pre-pasteurized LogSPC were not different between farms ($P = 0.99$) or seasons ($P = 0.12$, ANOVA). LogSPC decreased significantly after pasteurization ($P < 0.0001$). Spring had the most significant SPC decrease post-pasteurization, 1,749,167 to 1194 cfu/ml ($P < 0.05$, Tukey's). Mean, median indoor and outdoor temperatures respectively were: fall, 23°C, 23°C, 8°C, 8°C; winter, 6°C, 6°C, 8°C, 9°C; spring, 18°C, 17°C, 10°C, 9°C; and summer, 37°C, 37°C, 30°C, 28°C. Indoor temperatures were significantly different among the 4 seasons, and summer had significantly higher outdoor temperatures than the other seasons (all $P < 0.0001$, ANOVA, Tukey's). The final general linear model for factors associated with LogSPC ($R^2 = 0.71$, $P < 0.0001$) included time after pasteurization, summer, and time during summer (interaction) all associated with increased SPC. There was also a farm effect – one farm had faster SPC increase during spring and another farm during summer when compared with other farms. Times until mean seasonal SPC >20,000 cfu/ml were: fall, > 8 hr (between 7796 then and 24hr when reached 2,121,818cfu/ml); winter, > 24hr (at end of 24 hr study, 173cfu/ml); spring, > 12hr (between 1904 then and 24hr when reached 9,234,633cfu/ml); and summer, 4hr (186,800cfu/ml, faster than other seasons, $P <0.0001$). Under conditions during this study year, in the northern temperate climate, milk was safe to feed to calves (defined as SPC < 20,000cfu/ml) for at least 8hr post-pasteurization during fall, 12hr during spring, and 24hr during winter. During summer, if milk was stored outside it could be safely fed for only 3hr after pasteurization. Any milk stored outdoors during summer that remains after the first feeding following pasteurization should be re-pasteurized or discarded.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry for Bacterial Identification: Experiences After One Year of Use in a Veterinary Diagnostic Laboratory

Timothy Frana

College of Veterinary Medicine, Iowa State University, Ames, IA

Narrative: Identification of bacteria using spectra generated from matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) was proposed more than 35 years ago. However, it is only recently that use of this technology has become practical in a diagnostic laboratory setting. MALDI TOF MS offers great promise as a means for quick, easy, and accurate identification of many bacterial organisms. It may actually represent a paradigm shift in the way diagnostic laboratories identify bacteria. Daily use of MALDI TOF MS has provided insights into some of the strengths, limitations and difficulties associated with this methodology. Three examples are provided to show how the MALDI TOF MS system has worked at as a front-line identification system for investigation of microflora from ferrets used in a cystic fibrosis animal model; as an improved method for identifying *Streptococcus suis* from porcine clinical samples; and as an identification method targeting *Bibersteinia trehalosi* in groups of bighorn sheep. Included are comparative identification data of identical isolates by conventional phenotypic and biochemical methods, MALDI TOF MS and 16S ribosomal RNA sequencing. Also discussed will be issues associated with incorporation of MALDI TOF MS into workflow, interpretation of results and additions to a user-defined mass spectrometry database.

Speciation of *Brachyspira spp*. in Poultry Fecal Samples using the GS Junior

Durda Slavic[1], Stina Nilsson[1], Michele Guerin[2], Genet A. Medhanie[2]

[1]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada; [2]Population Medicine, University of Guelph, Guelph, ON, Canada

Narrative: In chickens, *Brachyspira* species are associated with a clinical condition known as avian intestinal spirochaetosis (AIS), a disease characterized by chronic diarrhea, weight loss, low egg production, and fecal-stained eggs. *Brachyspira intermedia* and *Brachyspira pilosicoli* have been shown to play a role in AIS whereas other species of *Brachsypira* associated with AIS cases have uncertain clinical significance. Because *Brachyspira spp*. are difficult to culture, polymerase chain reaction (PCR) based on either 16S rRNA or the nox gene is a preferred detection method. Frequently, however, more than one species of *Brachyspira* can be present in a single sample contributing to diagnostic challenges. A recent study investigating *Brachyspira spp*. in Ontario layer hens established that 63.5% (33/52) of flocks with fecal-stained eggs and 24.3% (9/37) of flocks without fecal-staining of eggs were colonized with *Brachyspira spp*. based on a nox gene-specific real time PCR. Among *Brachyspira spp*.-positive flocks, only 26.2% (11/42) were positive for *B*. *pilosicoli* and all flocks were negative for *B*. *intermedia* and *B*. *hyodysenteriae*. In order to determine which other species of *Brachyspira* were present in Ontario layer hens with fecal-stained eggs, pyrosequencing technology using the GS Junior system was used. This technology allowed simultaneous sequencing of 31 samples, including 3 controls, on the same plate. Initially, a region of the nox gene was amplified using M13 tailed specific primers and a second set of PCRs was carried out to tag amplicons using primers specific for the GS Junior system. After emulsion PCR (emPCR) and pyrosequencing, preliminary sequence data analysis was conducted using the AVA software provided by Roche Applied Science. Sequence data were obtained for all samples sequenced. Overall, *Brachyspira murdochii*, *Brachyspira pulli*, *Brachyspira innocens* and genetic variants of *B*. *intermedia* were detected in 11/28, 9/28, 16/28, and 3/28 samples, respectively. There were four *Brachyspira spp*. detected simultaneously in 1 sample, 3 in 2 samples, 2 in 8 samples and only 1 in 13 samples. Sequences from four samples contained no match to *Brachyspira* reference strains, and it remains to be established if there is a new species of *Brachyspira* present in Ontario layer hens or if this is a sequence artifact. In conclusion, pyrosequencing using GS Junior can be used to detect known and "unknown" species of *Brachyspira* in mixed clinical samples.

Evaluation of Serodiagnostic Assays for *Mycobacterium bovis* in Elk, White-tailed Deer, and Reindeer in the United States ◊

Jeffrey T. Nelson[1], Kathleen Orloski[2], Audra Lloyd[1], Mark Camacho[3], Mark Schoenbaum[2], Suelee Robbe-Austerman[1], Bruce V. Thomsen[1], S. Mark Hall[1]

[1]USDA, APHIS-VS-NVSL, Ames, IA; [2]USDA, APHIS-VS, Fort Collins, CO; [3]USDA, APHIS-VS, Raleigh, NC

Narrative: In 2011, the United States Department of Agriculture conducted a project in which elk (*Cervus elaphus spp.*), white-tailed deer (WTD) (*Odocoileus virginianus*), and reindeer (*Rangifer tarandus*) were evaluated by the single cervical tuberculin test (SCT), comparative cervical tuberculin test (CCT) and serologic tests. The rapid antibody detection tests evaluated were the CervidTB Stat-Pak® (Stat-Pak) and the Dual Path Platform® VetTB (DPP). Blood was collected from presumably uninfected animals prior to tuberculin injection for the SCT. A total of 1,783 animals were enrolled in the project. Of these, 1,752 (98.3%) were classified as presumably uninfected, based on originating from a captive cervid herd with no history of exposure to TB. Stat-Pak specificity estimates were 92.4% in reindeer, 96.7% in WTD and 98.3% in elk and were not significantly different from SCT specificity estimates. Using the DPP in series on Stat-Pak antibody positive samples improved specificity in the three species. Thirty-one animals were classified as confirmed infected, based on necropsy and laboratory results and 27/31 were antibody positive on Stat-Pak for an estimated sensitivity of 87.1%. The study findings indicate that rapid serologic tests used in series are comparable to the SCT and CCT, and may have a greater ability to detect TB infected cervids.

◊ USAHA Paper

Development and Evaluation of a New Serological ELISA for Detection of Herds with Swine Dysentery ◊

Yong Song[1], Xiaoping Yang[2], Daniel Zwald[2], Mario Puerro[2], Angela Zurfluh[2], Alex Raeber[2], David Hampson[1]

[1]School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA, Australia; [2]Prionics AG, Schlieren, Switzerland

Narrative: Swine dysentery (SD) is a major endemic disease in most pig-rearing countries in the world, with evidence of re-emergence in the US and Canada. SD results from infection of the caecum and colon by *Brachyspira hyodysenteriae*, a pathogenic anaerobic intestinal spirochaete, the activity of which causes mucohaemorrhagic colitis. The disease is seen particularly in grower and finisher pigs, and can have a severe impact on production efficiency. In order to develop a serological ELISA, recombinant antigens were produced. These were selected from predicted outer membrane proteins or lipoproteins of *B. hyodysenteriae*, based on bioinformatics analysis of the complete genome sequence of *B. hyodysenteriae* strain WA1. The suitability of the recombinant antigens was further verified by Western blot and ELISA with a range of sera from pigs experimentally challenged with different *B. hyodysenteriae* strains, sera from individual infected pig with a serological conversion to *B. hyodysenteriae*, convalescent-phase sera from pigs recovering from SD, and sera from healthy grower pigs. In order to address potential cross-reactivity with other Brachyspira spp., a panel of sera from pigs infected with B. pilosicoli, B. intermedia and B. innocens/B. murdochii were included in the evaluation. A selected recombinant antigen was then used for the development of an indirect ELISA. Evaluation was performed in Australia with serum samples from healthy finisher pigs at slaughter, including 896 sera from pigs in 18 herds that were considered not to have SD and 355 sera from pigs on six farms that had a previous history of SD. Using the selected cut-off values, all negative herds were correctly identified as negative, and five of the six infected herds were correctly identified. The ELISA was then developed into a commercially available product, the PrioCHECK® Brachyspira Ab porcine ELISA. Sensitivity at the herd level was assessed on 133 samples collected from four herds from Switzerland, Spain and Australia with a history of *B. hyodysenteriae* infection, and all herds were correctly classified as positive. Specificity was tested on 108 samples from four herds from Switzerland, Germany, Spain and Australia and all samples were found negative. These findings demonstrate that the PrioCHECK® Brachyspira Ab porcine ELISA is an efficient tool for identifying infected herds. Its use will reduce the cost of pig production by enhancing identification and control of SD in these herds. Supported in part by the Pork CRC Ltd of Australia.

◊ USAHA Paper

Epidemiology 1

Saturday, October 20, 2012

Guilford C

Moderators: Francois C. Elvinger, Bruce L. Akey

1:00 PM **Comparison of *M. bovis* Gamma Interferon Test Results Between Tissue Culture Plate and Microtube Methods ◊**
Ashley Hill, Michelle Davidson, Elizabeth Emery 44

1:15 PM **A Preliminary Evaluation of MAPIA as an Ante-mortem Supplemental Test for Use in Suspect Tuberculosis Cattle**
Scott D. Fitzgerald, John Kaneene, Heather Grodi 45

1:30 PM **Systematic Literature Review and Statistical Bayesian Analysis of the Performance Characteristics of Commercially Available Type A Influenza Antigen Capture Tests in Dead Poultry Infected with Highly Pathogenic Avian Influenza**
Todd Weaver, Sasidhar Malladi, Mary J. Folley, Timothy L. Clouse, Kathe Bjork, David A. Halvorson 46

1:45 PM **A Simulation Based Evaluation of the Likelihood of Moving Contaminated Hatching Eggs from a Highly Pathogenic Avian Influenza Infected but Undetected Broiler Breeder House under Active Surveillance**
Sasidhar Malladi, Todd Weaver, Kathe Bjork, David A. Halvorson 47

2:00 PM **Development of a New ELISA Test for Detection of PRRS Antibodies in Swine Oral Fluids**
Sergio Lizano, Andrea Ballagi, Sheri Koller 48

2:15 PM **Detection of PRRSV Antibody in Oral Fluid Specimens from Individual Boars using a Commercial PRRSV Serum Antibody ELISA**
Apisit Kittawornrat, Mark Engle, Jeremy Johnson, John Prickett, Chris Olsen, Trevor Schwartz, Daniel Whittney, Kent Schwartz, Anna Rice, Andrea Ballagi, Sergio Lizano, Chong Wang, Jeff Zimmerman 49

2:30 PM **Ring Test Evaluation for the Detection of PRRSV Antibody in Oral Fluid Specimens using a Commercial PRRSV Serum Antibody ELISA**
Apisit Kittawornrat, Chong Wang, Gary A. Anderson, Andrea Ballagi, Andre Ch. Broes, Suzanne Carman, Kent Doolittle, Judith Galeota, John Johnson, Sergio Lizano, Eric A. Nelson, Devi P. Patnayak, Roman Pogranichniy, Anna Rice, Gail Scherba, Jeff Zimmerman 50

2:45 PM **Seroepidemiology of Equine Leptospirosis Utilizing Diagnostic Laboratory Specimens from 29 States (US) and One Canadian Province ◊**
Craig N. Carter, Noah Cohen, Meg Steinman, Jacqueline Smith, Erdal Erol, Stuart Brown 51

3:00 PM **Spatially Heterogeneous Environmental and Climatic Risk Factors of Canine Leptospirosis**
Ram Raghavan, Gary A. Anderson, Kenneth Harkin 52

Symbols at the end of titles indicate the following designations:

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

Comparison of *M. bovis* Gamma Interferon Test Results Between Tissue Culture Plate and Microtube Methods ◊

Ashley Hill, Michelle Davidson, Elizabeth Emery

California Animal Health & Food Safety Laboratory, University of California, Davis, CA

Narrative: In the US, *M. bovis* gamma interferon testing (GIT) is used as a confirmatory test in cows testing positive for bovine tuberculosis on caudal fold testing. In the GIT as historically stipulated by USDA, lymphocytes are stimulated in tissue culture plates, IFN-γ is harvested, and ELISA technology is used to measure IFN-γ produced (Plate method). The harvesting step involves centrifuging 24-well plates and collecting serum using a single-channel pipet, both of which are time consuming, particularly with large numbers of samples. An alternative method involves stimulating lymphocytes in microtubes, rather than tissue culture plates (Microtube method). This shortens the harvesting step by increasing the number of samples centrifuged simultaneously, and by allowing the use of multichannel pipets for serum collection. The Microtube method uses smaller sample volumes for lymphocyte stimulation, potentially producing less IFN-γ and leading to false negative test results. The objective of this project was to compare test results between the Plate and Microtube methods of performing the GIT for *M. bovis*. Samples from 58 Holstein dairy cows from three California dairies currently or historically infected with *M. bovis* were simultaneously tested using both methods. Paired t-tests were used to separately compare the Nil, *M. avium, M. bovis*, Pokeweed, *M. bovis*-Nil, and *M. bovis*-*M. avium* values between the Plate and Microtube methods. 30 of 58 cows tested positive on one or both gamma interferon test methods, and M. bovis was detected postmortem in 29 of these 30 via either culture or PCR. The *M. avium, M. bovis*, and *M. bovis*-Nil OD values produced by the Microtube method were significantly lower than those produced by the Plate method. One sample identified as M. bovis positive via Plate method was negative using the Microtube method. The Microtube method produces less IFN-γ than the Plate method. As a result, the sensitivity of the Microtube method appears lower than that of the Plate method when results from both methods are compared to the same threshold for a positive test. Optimizing an alternative *M. bovis*-Nil threshold for the Microtube method might allow for more efficient performance of GIT without a loss of sensitivity.

◊ USAHA Paper

A Preliminary Evaluation of MAPIA as an Ante-mortem Supplemental Test for Use in Suspect Tuberculosis Cattle

Scott D. Fitzgerald[1, 3], John Kaneene[2, 3], Heather Grodi[3]

[1]Pathobiology & Diagostic Investigation, Michigan State University, Lansing, MI; [2]Center for Comparative Epidemiology, Michigan State University, East Lansing, MI; [3]Diagnostic Center for Population & Animal Health, Michigan State University, Lansing, MI

Narrative: The objective of this study was to make use of bovine tuberculosis suspect cattle from the state of Michigan to validate a multi-antigen print immunoassay (MAPIA) for use on sera to serve as an improved supplementary ante-mortem test to increase specificity of current tuberculosis testing methods. Over a 27 month period, 234 sera were collected and tested by MAPIA method, which was evaluated by four different interpretation criteria. The following antigens were utilized in this study: ESAT-6, ESTA-6/CFP10 fusion protein, Acr1, 38 KDa, 45 KDa, Ag85B, GroEs, MPB59, MPB64, MPB70 and MPB83. MAPIA results were subsequently compared to final mycobacterial culture and PCR results obtained by National Veterinary Services Laboratories, Ames, Iowa, which served as the true indicator of the cattle's tuberculosis infection status. Each criterion and each of the antigens utilized were evaluated for sensitivity, specificity, positive and negative predictive values. Results showed that using any single positive antigen reaction (criterion 1) resulted in the highest sensitivity (97.96%), but low specificity (35.14%). Using any two positive antigen reactions (criterion 2) resulted in high sensitivity (93.88%), but moderate specificity (71.89%). Using any three or more positive antigen reactions (criterion 3) resulted in moderate sensitivity (69.39%), and high specificity (90.27%). Using any strong positive antigen reaction (criterion 4) resulted in intermediate sensitivity (59.18%), and high specificity (88.11%). Of the 11 antigens studied, ESAT-6 and ESAT-6/CFP10 had the highest individual sensitivities, but intermediate specificities, while all other antigens had low to intermediate sensitivities, but all had high specificities. Our conclusions were that utilizing the MAPIA assay ante-mortem as a supplemental test could prove to be a useful epidemiological tool. Depending on the state or herd status, the criterion could be selected to achieve higher sensitivity or higher specificity. A combination of antigens provided the most specific test results. By increasing specificity, substantial savings on cattle indemnities and tuberculosis testing costs could be realized. Finally, if another assay such as the Rapid or Direct Test is approved by the USDA for ante-mortem tuberculosis screening testing, the MAPIA could serve as a very useful supplemental test, since it shares the attributes of being a humoral assay, and would utilize many of the same antigens.

Systematic Literature Review and Statistical Bayesian Analysis of the Performance Characteristics of Commercially Available Type A Influenza Antigen Capture Tests in Dead Poultry Infected with Highly Pathogenic Avian Influenza

Todd Weaver[1], Sasidhar Malladi[2], Mary J. Folley[1], Timothy L. Clouse[1], Kathe Bjork[1], David A. Halvorson[2]

[1]USDA, Animal and Plant Health Inspection Service, Fort Collins, CO; [2]Veterinary Population Medicine, University of Minnesota, St Paul, MN

Narrative: During a highly pathogenic avian influenza (HPAI) outbreak, active diagnostic surveillance is a key risk mitigation measure used for monitored flocks not known to be infected with HPAI virus. In this case, diagnostic testing is aimed at reducing the likelihood of moving contaminated poultry products from potentially infected but undetected flocks in a control area. Targeted matrix gene real-time reversed transcriptase-polymerase chain reaction (RRT-PCR) testing has been incorporated into draft national HPAI response plans and in business continuity plans across poultry industry sectors as the primary diagnostic test procedure. However, commercially available type A influenza antigen capture immunoassay tests that can be performed at the industry level may be beneficial for supplementary testing in addition to the RRT-PCR testing required for regulatory purposes. In particular, antigen capture tests can be done flock-side with a rapid turnaround time (less than one hour) offering some logistical advantages. Namely, testing can be done closer to the time of product movement. There are some specific issues regarding antigen capture tests, such as widely varying sensitivity estimates when used in the field and a low analytical sensitivity that in turn, can translate into reduced diagnostic sensitivity when testing clinically healthy birds. The objective of this analysis was to estimate the overall diagnostic sensitivity and specificity values for commercially available influenza A antigen capture tests specifically for dead birds clinically infected with HPAI virus, where HPAI test performance is expected to be better due to higher loads of viral shedding. Testing dead bird pools daily or at the time of product movement is one form of targeted active surveillance used in HPAI emergency response plans in the United States. The methodology used is based on Bayesian analysis and estimation of the posterior distribution using Markov chain Monte Carlo simulation methods as implemented in WinBUGS, given the data obtained on test performance through systematic literature review. For robustness, we also compared the results using frequentist maximum likelihood approaches using TAGS software. Because testing multiple birds from a pool of mortality will contribute to overall higher flock sensitivity, the performance characteristics from this study will be useful for further risk analysis for business continuity issues across poultry industry sectors.

A Simulation Based Evaluation of the Likelihood of Moving Contaminated Hatching Eggs from a Highly Pathogenic Avian Influenza Infected but Undetected Broiler Breeder House under Active Surveillance

Sasidhar Malladi[1], Todd Weaver[2], Kathe Bjork[2], David A. Halvorson[1]

[1]Veterinary Population Medicine, University of Minnesota, St Paul, MN; [2]USDA, Animal and Plant Health Inspection Service, Fort Collins, CO

Narrative: The emergency response in the event of highly pathogenic avian influenza (HPAI) outbreak in the United States would include quarantine and movement controls for poultry products. Outbreak control measures may adversely impact business continuity for broiler hatcheries given the limited holding capacity for hatching eggs, and the potential loss of hatchibility with extended holding times. A broiler sector workgroup with participants from the broiler industry, University of Minnesota, and USDA-APHIS-VS was created to provide input into the development of a proactive risk assessment for the movement of broiler hatching eggs during an HPAI outbreak. The key future preventive measures proposed by the workgroup included targeted matrix gene real-time reversed transcriptase-polymerase chain reaction (RRT-PCR) based active surveillance and incorporation of a two day hold after production before the movement of eggs. In addition, two active surveillance protocol options were considered. The first surveillance option required RRT-PCR testing of two pooled samples with 5 oropharyngeal swabs from dead or sick chickens per broiler breeder house taken within one day prior to movement of hatching eggs. In this option, if less than 10 sick or dead birds are present, the swabs from available sick or dead birds should be equally divided between the two pooled samples. In the second surveillance option, a single pooled sample of 5 oropharyngeal swabs from dead or sick birds is tested on two consecutive days prior to movement of hatching eggs. In this paper, we evaluated the likelihood of moving internally contaminated hatching eggs from an infected, but undetected broiler breeder house under the two surveillance protocol options described above. These options include a two day hold time after production before the movement of eggs. A stochastic disease transmission model was used to estimate the HPAI disease mortality and fraction of internally contaminated eggs at various time points post-infection of the breeder house. The transmission model results were then utilized in simulation models of active surveillance protocols. The simulation results using the two surveillance protocol options were similar and indicated a low likelihood of moving contaminated hatching eggs before HPAI infection is detected. Given the similarity in the results, risk managers may choose an appropriate protocol option based on other managerial considerations such as logistics and stakeholder preferences. The results were used in risk evaluation of the spread of HPAI associated with the movement of broiler hatching eggs from breeder houses within control area.

Development of a New ELISA Test for Detection of PRRS Antibodies in Swine Oral Fluids

Sergio Lizano, Andrea Ballagi, Sheri Koller

IDEXX Laboratories, Westbrook, ME

Narrative: Testing of swine oral fluids represents a convenient and cost-effective tool for disease monitoring and surveillance in commercial pig herds. Recently, detection of antibodies to PRRS in oral fluids using an overnight protocol modified from the serum IgG antibody ELISA (HerdChek® PRRS X3 ELISA, IDEXX Laboratories, Inc.) was reported by Kittawornrat et al. (J Vet Diagn Invest. 2012 Mar;24(2):262-9.). In this study, we describe the performance a new ELISA test for same day detection of anti-PRRS IgG antibodies in swine oral fluids. The format of the new PRRS oral fluids ELISA maintains the same protocol workflow as the standard PRRS X3 kit, with the exception that: the sample incubation step is longer (2h vs. 30 min on X3), the original oral fluids sample input is 50µl, as opposed to 2.5µl for the serum assay, and the final dual absorbance read is 450-650nm instead of 650nm. An S/P ≥ 0.4 is considered a positive result. A comparison between the new protocol and the standard overnight protocol (SOP) using a set of reference standards consisting of pooled oral fluids from pigs vaccinated with type 2 PRRS MLV vaccine indicated 100% agreement between the two tests, with average S/P values 1.4 to 1.5-fold higher than SOP, indicating an enhanced sensitivity. The same results were obtained upon analysis of a set of experimentally inoculated pigs as well as field samples obtained from Iowa State University as part of an inter-laboratory reproducibility evaluation of SOP. Moreover, analysis of a temporal series of paired oral fluids and serum collected from individual boars experimentally infected with type I (strain D09-012131) or type II (strain MN-184; GenBank accession no. AY656992) PRRSV for up to 21 days post-infection (DPI) indicated no difference between the days to detection of anti-PRRSV antibodies in serum as in oral fluids, as well as a similar ability to detect both type I and II strains. Finally, an evaluation of pen-based oral fluid samples collected at various prevalences (0%, 4%, 12%, 20%, and 36%) of vaccinated pigs introduced at 14 days post-vaccination into pens of PRRS-negative pigs indicated that the new test detects anti-PRRS antibodies ≥96% of all collection events in pens of at least 20% prevalence, and ≥85% in pens of 12% prevalence. Taken together, these results describe a new sensitive test for anti-PRRS antibody detection aimed to support the emerging use oral fluids for easier and frequent surveillance and monitoring of pig herds.

Detection of PRRSV Antibody in Oral Fluid Specimens from Individual Boars using a Commercial PRRSV Serum Antibody ELISA

Apisit Kittawornrat[1], Mark Engle[3], Jeremy Johnson[1], John Prickett[1], Chris Olsen[1], Trevor Schwartz[1], Daniel Whittney[1], Kent Schwartz[1], Anna Rice[4], Andrea Ballagi[4], Sergio Lizano[4], Chong Wang[1, 2], Jeff Zimmerman[1]

[1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Statistics, Iowa State University, Ames, IA; [3]Health Assurance and Transportation, PIC North America, Hendersonville, TN; [4]Livestock and Poultry Diagnostics Division, IDEXX Laboratories, Westbrook, ME

Narrative: Oral fluid specimens are used in human medicine for detection of a variety of infectious agents, hormones, and drugs. Oral fluid samples are of interest in swine medicine because they are easily collected, yet highly efficacious for the surveillance of PRRSV and other pathogens using PCR-based assays. Recent work showed that a commercial PRRSV serum antibody ELISA (IDEXX Laboratories, Inc., Westbrook, ME, USA) could be adapted to detect PRRSV antibody in oral fluid specimens. The object of this study was to describe the kinetics of the ELISA detectable anti-PRRSV IgG response in oral fluid collected from individually-housed boars. The study was conducted in 72 boars ranging from 6 months to 3.6 years in age. Boars were under the ownership of PIC North America (Hendersonville, TN, USA) and housing, study procedures, and protocols were approved and supervised by the PIC USA Health Assurance and Welfare department. Boars were assigned to three trials (I, II, III). Boars (n = 24) in Trial I were intramuscularly (IM) inoculated with 2 ml of a modified live virus (MLV) vaccine (RespPRRS®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, USA). Boars (n = 24) in Trial II were IM inoculated with 2 ml of a Type 1 PRRSV field isolate. Boars (n = 24) in Trial III were IM inoculated with 2 ml of a PRRSV Type 2 isolate (MN-184). Boars were monitored for 21 days post inoculation (DPI). Oral fluid samples were collected daily using 5/8” 3-strand 100% cotton rope. Serum samples were collected from all boars on DPI -7, 0, 7, 14, 21 and from 4 randomly selected boars on DPIs 3, 5, 10, and 17. Thereafter, serum and oral fluid were assayed for PRRSV antibody using the ELISA protocol appropriate for each sample type (serum or oral fluid). Individual boar oral fluid samples were ELISA positive from DPI 8 to DPI 21. Overall, 96% of the results were in agreement, i.e., 145 oral fluid samples and 150 serum samples were ELISA positive. These data support previous reports on the detection of anti-PRRSV antibody by ELISA in oral fluid and suggest that this approach could be used for disease surveillance in commercial breeding swine populations.

Ring Test Evaluation for the Detection of PRRSV Antibody in Oral Fluid Specimens using a Commercial PRRSV Serum Antibody ELISA

Apisit Kittawornrat[1], Chong Wang[1, 2], Gary A. Anderson[3], Andrea Ballagi[4], Andre Ch. Broes[5], Suzanne Carman[6], Kent Doolittle[7], Judith Galeota[8], John Johnson[1], Sergio Lizano[4], Eric A. Nelson[9], Devi P. Patnayak[10], Roman Pogranichniy[11], Anna Rice[4], Gail Scherba[12], Jeff Zimmerman[1]

[1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA; [2]Department of Statistics, Iowa State University, Ames, IA; [3]Veterinary Diagnostic Laboratory, Kansas State University, Manhattan, KS; [4]Livestock and Poultry Diagnostics Division, IDEXX Laboratories, Inc., Westbrook, ME; [5]Biovet, Inc., Saint-Hyacinthe, QC, Canada; [6]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada; [7]Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; [8]Veterinary Diagnostic Center, University of Nebraska, Lincoln, NE; [9]Animal Disease Research and Diagnostic Lab, South Dakota State University, Brookings, SD; [10]Veterinary Diagnostic Laboratory, University of Minnesota, St Paul, MN; [11]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN; [12]Veterinary Diagnostic Laboratory, University of Illinois, Urbana, IL

Narrative: A commercial PRRS serum antibody ELISA (IDEXX Laboratories, Inc., Westbrook ME USA) was recently adapted to detect anti-PRRSV antibody in oral fluid specimens (Kittawornrat et al., 2012). Based on testing of field and experimental samples, diagnostic sensitivity and specificity was estimated at 94.7% (95% CI: 92.4, 96.5) and 100% (95% CI: 99.0, 100.0), respectively, at a sample-to-positive (S/P) cutoff of ≥ 0.40. The purpose of this study was to evaluate the reproducibility and repeatability of the PRRS oral fluid ELISA in a ring test (check test) format. A total of 263 oral fluid samples were collected, completely randomized, and sent for testing in 12 collaborating diagnostic laboratories. In addition to the set of oral fluid samples, each laboratory received the materials required for conducting the test: ELISA plates (HerdChek® PRRS X3 ELISA, lot #40959-W721), reagents, positive and negative controls, pre-diluted conjugate antibody, and a copy of the standard operating procedure for the PRRS oral fluid IgG ELISA. The laboratories tested the samples and returned the results for analysis. Assay results were analyzed as S/P ratios, with S/P ratios ≥ 0.40 considered positive. Variation in S/P results increased as the concentration of antibody in the sample increased. Overall, this had little impact on categorical results. That is, among the 263 samples tested by the 12 laboratories, 132 samples tested positive in all laboratories; 124 samples tested negative in all laboratories, and 7 samples had discordant results. With the exception of sample #7, a discordant result was reported in each case by only one of the 12 laboratories. Discordant results for sample #7 were reported at 3 laboratories, but this may be explained by the fact that all results for sample #7 clustered close to the 0.40 cutoff. The ring test results showed that the PRRS oral fluid IgG ELISA was highly reproducible across laboratories. These results support the routine use of this test in laboratories providing diagnostic service to pig producers. Thus, herd monitoring based on oral fluid sampling could be one part of a PRRSV control and/or elimination program. Further, the successful adaptation of one assay to the oral fluid matrix suggests that this approach could provide the basis for monitoring specific health and welfare indicators in commercial swine herds using a “pig friendly” approach.

Seroepidemiology of Equine Leptospirosis Utilizing Diagnostic Laboratory Specimens from 29 States (US) and One Canadian Province ◊

Craig N. Carter[1], Noah Cohen[2], Meg Steinman[1], Jacqueline Smith[1], Erdal Erol[1], Stuart Brown[3]

[1]Veterinary Science, University of Kentucky Veterinary Diagnostic Laboratory, Lexington, KY; [2]College of Veterinary Medicine, Texas A&M University, College Station, TX; [3]Ambulatory Medicine, Hagyard Equine Medical Institute, Lexington, KY

Narrative: An epidemiological study was conducted to assess the sero-prevalence of leptospirosis among horses in the US and Ontario, Canada using the microscopic agglutination test (MAT) on residual sera submitted to 30 diagnostic laboratories for Equine Infectious Anemia testing from July, 2010, through April, 2011. All MAT testing was conducted in the University of Kentucky Veterinary Diagnostic Laboratory serology unit. This facility was selected because of the high volume of MAT tests run annually and the expertise of the technical staff with this method. Of the 1,495 horses tested, 561 (38%) were female, 934 (62%) were males (intact or geldings). There were no significant differences in the prevalence of a positive result between sexes for each serovar. Furthermore, 667 (44.6%) were positive (i.e., titer > 1:200) for at least 1 of 6 serovars. The serovar with the highest seroprevalence was Bratislava (31.6%; n=473) followed by Icterohemorrhagica (14.2%; n=216), Canicola (10.2%;n= 153), Grippotyphosa (5.0%n=75), Pomona (3.6%;n=54), and Hardjo (2.9%;n=44). Odds of seropositivity for some serovars differed by regions and states. Horses that were 6-10 years and > 10 years of age were significantly more likely to be positive for serovar Pomona. Finally, although the odds of being seropositive were greater in some breeds for some serovars, breed was not associated with seropositivity after adjusting for age, region, or both. Other epidemiological findings of this study too extensive to list here also will be presented. It was concluded that equine exposure to potentially pathogenic leptospiral organisms is high throughout the US and Ontario, Canada. Furthermore, this exposure may lead to abortion in mares, clinical disease in horses and foals, and may present a risk of zoonotic disease in farm workers and equine veterinarians. Serovars in vaccines generally are not considered to be cross-protective. Assuming this applies to the horse, regional serovar prevalence differences would have to be taken into consideration in the development and administration of a multivalent vaccine.

◊ USAHA Paper

Spatially Heterogeneous Environmental and Climatic Risk Factors of Canine Leptospirosis

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

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

Narrative: Various climatic and environmental variables were evaluated as potential risk factors for canine leptospirosis, further extending from our prior research using cases received at Kansas State Veterinary Diagnostic Laboratory (KSVDL) between 2002 and 2009. Knowing specific risk factors for canine leptospirosis could help form effective preventive strategies such as vaccination and behavior modification. A multitude of factors contribute to this disease in dogs, among which climate, environmental (land-use/land cover) and socio-economic conditions of pet owners are suspected to play a prominent role. Geospatial (GIS, remote sensing) methods were used for risk analysis, and Geographically Weighted Regression (GWR) models were used to identify spatial heterogeneity of risk factors. Ninety-four cases and 185 controls of canine leptospirosis in Kansas and Nebraska region that were found in KSVDL database were selected predominantly based upon polymerase-chain reaction (PCR) test results for known pathogenic leptospires in the urine. Addresses of selected dogs' residences were geocoded using GIS software. Different thematic layers of climate and 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 GWR to identify spatial heterogeneity among risk factors derived. Higher spring precipitation (OR = 1.58, 95% C.I = 1.11, 2.26), higher summer mean temperature (OR = 2.22, 95% C.I = 1.50, 3.30), and medium-intensity urban areas (OR = 1.86, 95% C.I = 1.44, 2.41) were significant risk factors throughout the study region (Kansas, Nebraska) while poverty status (OR = 2.07, 95% C.I = 1.76, 2.45) was a significant risk factor in South Central Kansas. In Central/North Eastern Nebraska, proximity to water features (OR = 0.82, 95% C.I = 0.79, 0.86), and hydrologic density (OR = 2.80, 95% C.I = 1.58, 4.96) were significant risk factor for dogs. The identification of risk factors for canine leptospirosis using geospatial methods could be useful for strategizing prevention methods, and it is likely that these risk factors play a role in the survival and spread of canine leptospirosis in other geographic regions as well. While there may be many risk factors for a given disease, it is evident from this study that not all will have an equal influence throughout the study area. Therefore, it is important to consider different prevention strategies based on spatial heterogeneity of risk factors. Geospatial technology is increasingly being realized as a useful research tool in VDL settings for analyzing case/control retrospective data especially with an environmental perspective.

Pathology 1

Saturday, October 20, 2012

Auditorium III

Sponsor: Advanced Technology Corporation

Moderators: Francisco Uzal, Grant Maxie

1:00 PM **Introduction by Advanced Technology Corporation**

1:05 PM **Friesian Horses Have High Incidence of Severe Esophageal Disease Associated with Muscular Hypertrophy and Hyperplasia of the Caudal Esophagus # † +**
Misa Komine, Ingeborg Maria Langohr, Matti Kiupel 55

1:20 PM **Evaluating Grading Systems for Accurate Prognisis of Canine Cutaneous Mast Cell Tumors # * † +**
Keiko Y. Petrosky, Rachel Peters, Barbara J. Davis 56

1:35 PM **Freezing or Adding Trypsin Inhibitor to Equine Intestinal Content Extends the Lifespan of Clostridium perfringens Beta Toxin for Diagnostic Purposes # * † +**
Melissa Macias Rioseco, Juliann Beingesser, Francisco Uzal 57

1:50 PM **Enzootic Ataxia in two Katahdin Lambs †**
Stephanie French, Jon S. Patterson, Scott D. Fitzgerald 58

2:05 PM **Skin and Subcutaneous Granulomas in Tuberculosis Test Responder Cattle**
Patricia C. Blanchard, John Adaska, Robert Moeller, Bruce V. Thomsen, Suelee Robbe-Austerman 59

2:20 PM **Intranasal Dust Inoculation of Chronic Wasting Disease in White Tailed Deer (*Odocoileus virginianus*)**
Tracy Nichols, Terry Spraker, Tom Gidlewski, Tara Camenish-Ruby, Mark Zabel, Aru Balachandran, Glenn Telling, Brady Michel, Kurt VerCauteren 60

2:35 PM **Antleromas and Hummel Deer: Lessons on Antlerogenesis from Free-Ranging White-Tailed Deer**
Brandon Munk, Kevin Keel, Angela Ellis, Bambi Clemons, Elina Garrison 61

2:50 PM **A Newly Recognized Feline Infectious Peritonitis-Like Disease in Mink Associated Withan Alphacoronavirus**
Matti Kiupel, Karla A. Fenton, Annabel G. Wise, Steve J. Bursian, Roger K. Maes 62

Symbols at the end of titles indicate the following designations:

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

Friesian Horses Have High Incidence of Severe Esophageal Disease Associated with Muscular Hypertrophy and Hyperplasia of the Caudal Esophagus # † +

Misa Komine, Ingeborg Maria Langohr, Matti Kiupel

Michigan State University, Lansing, MI

Narrative: Friesian horses have been perceived to have a high rate of congenital or hereditary diseases including megaesophagus that may lead to choke and death. A retrospective study was performed to characterize the prevalence and pathological commonalities of severe esophageal disease in horses and to compare those in Friesians to others. Necropsy reports of 852 horses, including 17 Friesians, submitted to the DCPAH from October 2005 to December 2011were included in the study. Forty two (4.9%) horses had grossly described esophageal lesions (25 muscular hypertrophy, 7 hemorrhage, 6 megaesophagus, 4 erosion/ulceration, 3 obstruction, 2 tears, 2 secondary neoplastic invasion, 2 lymphoid patches, 1 thin wall and 1 esophagitis). Ten (1.2%) horses died or were euthanized because of severe esophageal diseases (6 megaesophagus, including 2 with tears, 3 esophageal obstruction with food bolus, 1 severe esophagitis). All 6 horses with megaesophagus, including the 2 esophageal tears, were Friesians. The ultimate cause of these lesions was not determined in the original necropsy reports. A detailed review of archived paraffin blocks of all 6 Friesians with severe esophageal lesions was performed. Histologically, the esophagus of 5 of these Friesians had a thickened muscularis interna and externa due to smooth muscle hypertrophy and hyperplasia. There was no evidence of significant fibrosis, degenerative disease or loss of myenteric plexi. Unlike in Friesian, none of the other 4 horses with severe esophageal disease had caudal muscular hypertrophy described. Furthermore, no muscular hypertrophy was reported in the remaining 11 Friesians that had been necropsied for other causes. Based on these data, Friesians have high incidence (35.3%) of severe esophageal disease compared to other horse breeds (0.5%). Interestingly, marked caudal muscular hypertrophy and hyperplasia was observed in 5 of 6 Friesians with megaesophagus and secondary tears. Esophageal idiopathic muscular hypertrophy is generally regarded as incidental finding in aged horses. However, in humans, a hereditary condition characterized by thickening of the esophagus due to circumferential proliferation of smooth muscle, called leiomyomatosis, causes subsequent dysmotility and achalasia. We speculate that caudal muscular hypertrophy and hyperplasia of the esophagus represents a similar hereditary condition in Friesian horses that can cause severe esophageal disease, such as megaesophagus and esophageal tears.

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

Evaluating Grading Systems for Accurate Prognisis of Canine Cutaneous Mast Cell Tumors # * † +

Keiko Y. Petrosky, Rachel Peters, Barbara J. Davis

Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA

Narrative: Cutaneous mast cell tumors (MCT) are common malignant tumors in dogs, composing 10% of all canine tumor diagnoses at Tufts Cummings School of Veterinary Medicine (TCSVM), but commonly used histologic grading systems to assess MCT have limited application with respect to clinical outcome. Recently, a 2-tiered grading system was published to better guide prognosis. Based on the newer criteria, we reclassified MCT with respect to outcome in (n = 402) TCSVM cases over 8 years and in (n = 54) cases with 48-month follow-up. The Student t-test or Mann-Whitney U test was used to determine the significance of association between outcome and histological grade. Significance of association between survival times and histological grade was analyzed using Kaplan-Meier survival curves and appropriate Cox analysis. Although median survival time correlated with the 3-tiered assessments, most tumors (82%) were classified as grade 2 with a wide distribution of survival times. After reclassification of all tumors to either low- or high-grade, survival times remained significantly correlated with tumor grade, including those previously classified as grade 2. Most (94%) of the patients diagnosed with grade 2 tumors reclassified as low-grade and reached the end of the four-year time point; however, the survival time for patients diagnosed with grade 2 tumors reclassified as high-grade varied significantly. We are now evaluating additional biomarkers as tools to predict clinical outcomes. Preliminary studies show that the traditional proliferation markers AgNOR and Ki67 and aberrant localization of KIT may be useful in further describing these high grade tumors.

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

Freezing or Adding Trypsin Inhibitor to Equine Intestinal Content Extends the Lifespan of Clostridium perfringens Beta Toxin for Diagnostic Purposes # * † +

Melissa Macias Rioseco[1], Juliann Beingesser[2], Francisco Uzal[2]

[1]School of Veterinary Medicine, University of California Davis, Davis, CA; [2]Diagnostic Pathology, California Animal Health and Food Safety Laboratory, San Bernardino Branch, University of California Davis, San Bernardino, CA

Narrative: *Clostridium perfringens* type C causes necrotizing enteritis mostly in neonatal animals o f several species, including horses. The virulence of *C. perfringens* type C is mostly mediated by the beta toxin (CPB). This toxin is highly sensitive to the action of trypsin and other proteases, which explains the increased susceptibility of neonatal animals to type C infections. Final confirmation of type C disease diagnosis should be based on detection of CPB in the intestinal content of affected animals. However, because CPB is so sensitive to the action of proteases, it is believed that this toxin persists for only a limited period of time in specimens of intestinal content of animals collected for diagnostic purposes. This study was therefore performed to determine the stability of CPB in intestinal content of horses stored at different temperatures and to evaluate the use of trypsin inhibitor to extend the lifespan of CPB in intestinal content of horses. When the intestinal content of horses that had been spiked with different amounts of CPB was tested by a capture ELISA technique to detect CPB, 319 LD50 of CPB/ml was the lowest amount that could be detected. When the equine intestinal content spiked with the same amount of CPB (319 LD50) and stored at 4°C, CPB was detected by ELISA until day 8 after spiking. Samples spiked with the same amount of CPB and stored at -20°C were positive for at least 5 weeks after spiking. When intestinal samples spiked with 319 LD50/ml of CPB were mixed with 0.1mg/ml or 1.0mg/ml of trypsin inhibitor and stored at 4°C, all the samples were positive for at least 5 weeks after spiking. This study demonstrates that *C. perfringens* CPB present in equine intestinal samples stored at 4°C cannot be detected by ELISA for more than 8 days. Freezing the samples at -20°C or adding trypsin inhibitor before storage at 4°C preserves the lifespan of CPB for at least 5 weeks.

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

Enzootic Ataxia in two Katahdin Lambs †

Stephanie French, Jon S. Patterson, Scott D. Fitzgerald

Pathobiology & Diagnostic Investigation, Michigan State University, Lansing, MI

Narrative: A small Ohio sheep farm experienced a loss of 15 Katahdin lambs over a several-year period with similar clinical histories of paralysis, trembling, and ataxia. Lambs appeared to be normal at birth but became progressively neurologic by 4 to 5 months of age. Two neurologic, female, 4-month-old lambs were submitted to the Diagnostic Center for Population and Animal Health for necropsy. Upon examination, both lambs were trembling, reluctant to stand, and ataxic; when urged to walk, they placed their weight on the cranial tips of their hooves. Gross findings included cestodes in the jejunum of both animals and a few strongyles (*Trichostrongylus*) in the abomasum of one lamb. No gross abnormalities were found regarding the brains, spinal cords, and vertebral columns of the lambs. Histolopathological findings included mild to moderate demyelinization, axonal swelling, and spongiosis primarily within the dorsal lateral funiculi of the cervical and thoracic spine, but affecting all funiculi of the spinal cord to a lesser extent. Skeletal muscle from the rear limbs of one lamb had regions of fatty infiltrate, necrotic and degenerate myofibers, and loss of cross-striations. Skeletal muscle from the rear limbs of lamb B had hypereosinophilic and occasionally plump fibers with centrally located nuclei. The heart of one lamb had multifocal regions of moderate interstitial fibrosis, perivascular and perineuronal lymphoplasmacytic inflammation, variation in myofiber size and staining affinity, loss of cross-striations, nuclear rowing, and occasional pyknotic nuclei. High levels of molybdenum and low levels of copper were noted in the livers of both of the submitted lambs; these findings, in addition to the histopathology and the clinical signs, are consistent with enzootic ataxia. Enzootic ataxia is primarily caused by copper deficiency. Affected lambs can appear normal at birth, but develop progressive neurologic signs within the first few months of life. The cardiomyopathy seen in one lamb and the degenerative myopathy seen in both lambs are consistent with lack of innervation resulting from the hypomyelinogenesis. Some breeds of sheep are more sensitive than others regarding copper deficiency. Recent studies have discovered a link between an excess of fructose in the diet and copper deficiency in rodents; this has not been experimentally reproduced in sheep, yet but may be applicable. This case is complicated by the fact that research also suggests elevations in either molybdenum or high fructose can interfere with copper levels in the diet; both of these factors were present in this herd.

† Graduate Student Oral Presentation Award Applicant

Skin and Subcutaneous Granulomas in Tuberculosis Test Responder Cattle

Patricia C. Blanchard[1], John Adaska[1], Robert Moeller[1], Bruce V. Thomsen[2], Suelee Robbe-Austerman[2]

[1]California Animal Health and Food Safety Laboratory, University of California, Tulare, CA; [2]National Veterinary Services Laboratory, USDA APHIS Veterinary Services, Ames, IA

Narrative: Skin and subcutaneous caseous granulomas with variable numbers of neutrophils and mineralization were found in 70 dairy cows over a 10 year period. The cows originated from 53 premises and were submitted for necropsy due to a reactor or suspect status on a comparative cervical (CCT) or gamma interferon test. Testing was performed as part of *Mycobacterium bovis* infected herd trace out, trace in or for interstate movement. On gross examination lesions were variable size from <1cm solitary to one or more chains of nodules extending up to 20cm in length. Lesions were most commonly in the lower front legs (43), followed by rear legs (11), shoulder (5), neck (3), and 1 each face, thorax and elbow. In five the affected leg was not specified. Histologic lesions consisted of variable amounts of caseous necrosis, giant cells and macrophages forming classic granulomas and some lesions had numerous neutrophils, while others had few and mineralization varied from scant to abundant. Eight animals had acid fast bacilli (AFB) but were culture negative and negative by PCR for *M. tuberculosis* and *M. avium* complex. *Mycobacterium* species but not *M. bovis* or *M. avium* complex was isolated from 8 of the 49 lesions cultured (including 3 pending). These isolates represented five different species. Skin and subcutaneous granulomas histologically have all the characteristics of *Mycobacterium bovis* infections, though *M. bovis* has never been isolated from them nor is skin reported as the sole site of lesions for *M. bovis*. These types of lesions in the skin and subcutaneous tissue are noted in veterinary pathology textbooks as associated with atypical or environmental *Mycobacterium*. They appear to cause a false positive response on tests routinely used for confirmation of *M. bovis* infection in live animals (CCT or gamma interferon).

Intranasal Dust Inoculation of Chronic Wasting Disease in White Tailed Deer (*Odocoileus virginianus*)

Tracy Nichols[2, 1], Terry Spraker[1, 3], Tom Gidlewski[2], Tara Camenish-Ruby[2], Mark Zabel[1], Aru Balachandran[4], Glenn Telling[3], Brady Michel[3], Kurt VerCauteren[2]

[1]College of Veterinary Medicine, Colorado State University, Fort Collins, CO; [2]Wildlife Services, USDA, Fort Collins, CO; [3]Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO; [4]Ottawa Laboratory, Fallowfield, Canadian Food Inspection Agency, Ottawa, ON, Canada

Narrative: Chronic wasting disease (CWD) is a persistent problem in both wild and captive North American cervid populations. This disease continues to spread and cases are found in new areas each year. Indirect transmission can occur via the environment and is thought to occur by the oral and/or intranasal route. Oral transmission has been experimentally demonstrated and although intranasal transmission has been postulated, it has not been tested in a natural host. Prions have been shown to strongly adsorb to clay particles and upon oral inoculation the prion/clay combination exhibits increased infectivity in rodent models. Deer and elk undoubtedly inhale dust while foraging and while performing rut behaviors. We therefore hypothesized that dust particles may be a viable mode of intranasal CWD exposure. To test this hypothesis, CWD-positive brain homogenate was mixed with montmorillonite clay, dried, re-powdered and intranasally inoculated into white tailed deer once a week for 6 weeks. Deer were euthanized at 95, 105, 120 and 175 days post final inoculation and tissues were examined for CWD by immunohistochemistry. Our results demonstrate that CWD can be efficiently intranasally transmitted utilizing montmorillonite dust particles as a carrier and that the intranasal route is a viable route of exposure.

Antleromas and Hummel Deer: Lessons on Antlerogenesis from Free-Ranging White-Tailed Deer

Brandon Munk[1], Kevin Keel[1], Angela Ellis[1], Bambi Clemons[2], Elina Garrison[2]

[1]Population Health, University of Georgia, Athens, GA; [2]Florida Fish and Wildlife Conservation Commission, Gainesville, FL

Narrative: The Southeastern Cooperative Wildlife Disease Study (SCWDS) has examined thousands of white-tailed deer from throughout the southeastern United States and has identified only five bucks with bilateral antler growth disruptions. Failure of antlerogenesis was documented in two white-tailed deer from West Feliciana Parish, Louisiana. Both bucks failed to develop antlers despite the presence of pedicles. Complete failure of antlerogenesis has been reported most frequently in red deer, and attributed to poor nutrition during pedicle formation early in life. Three deer were diagnosed with antler malformations consistent with antleromas. Antleromas are rare in free-ranging deer, but have been reported in white-tailed deer, mule deer, black-tailed deer, Sitka black-tailed deer, and red deer. Their formation has been associated with disruptions in testosterone production due to cryptorchidism, hypogonadism, or toxins. Two of the antleroma deer had lesions consistent with those reported in peer reviewed literature. They were spikes with velveted antlers thickened at their bases by small semi-firm to hard, velveted nodules. However, the third antleroma deer had multilobulated, expansile masses covered in velvet originating from the antler pedicles bilaterally. These masses replaced or displaced the bones and soft tissues of the skull, including temporal, parietal, frontal, maxillary, zygomatic, nasal, and occipital bones, and significant rostral and dorsal displacement of the left eye. They extended through the left cribriform plate and the right petrus temporal bone compressing portions of the brain. Microscopically most regions of this deer's masses were identical to normal growing antler, but some foci had necrosis and hemorrhage suggestive of ischemia or trauma. Antlers develop from the pedicles, permanent bony structures of the frontal bones. The first antlers grow primarily by a modified process of endochondral ossification as direct continuations of the pedicle. The presence of antlerogenic periosteum (AP) is necessary for pedicle and first antler formation, and pedicle periosteum (PP) is necessary for appropriate antler regeneration. Pedicle formation, first antler growth, and antler regeneration cycles are controlled by seasonal variations of circulating testosterone levels. Alterations in seasonal testosterone cycles or damage to the AP or PP will alter or halt normal antler growth and regeneration as seen in the cases presented here.

A Newly Recognized Feline Infectious Peritonitis-Like Disease in Mink Associated Withan Alphacoronavirus

Matti Kiupel, Karla A. Fenton, Annabel G. Wise, Steve J. Bursian, Roger K. Maes

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

Narrative: Coronaviruses have previously been associated with both enteric and systemic infections in several species. An FIP-like disease was recognized microscopically in mink. The objectives of this study were to 1) describe the gross and microscopic lesions associated with an FIP-like disease in mink 2) use immunohistochemistry (IHC), RT-PCR and sequencing to define whether a coronavirus was associated with the lesions and 3) investigate the genetic relationship between this coronavirus and the recently described mink coronavirus (MCoV) associated with enzootic catarrhal gastroenteritis (ECG). Seven mink (*Mustela vison*), from 3 months to 1 year of age, were included in this study. Following microscopic examination, IHC for alphacoronaviruses was performed on select tissues. RNA was extracted from pooled tissue samples obtained from the index case. Initially, a generic coronavirus RT-PCR assay that targets a 179-bp region of open reading frame 1b (ORF1b) was used to test the sample. After confirmation of the presence of coronavirus RNA, a ~700 bp portion of the spike (S) gene was amplified and sequenced using a conserved set of primers designed from published mink and ferret coronavirus S sequences. Gross lesions of the index case were characterized by multifocal coalescing firm, white nodules within the pancreas and throughout the mesentery. Microscopically, the pancreas was characterized by multifocal regions of pyogranulomatous inflammation, necrosis, and fibrosis. Additionally, this mink also had moderate pyogranulomatous meningitis, myocarditis, hepatitis, and necrotizing splenitis. The other 6 cases appeared microscopically similar with pyogranulomatous inflammation involving one or more of the following: spleen, abdominal lymph nodes, liver, and/or mesenteric tissues. Positive IHC labeling for alphacoronavirus antigen was noted in all cases in macrophages within pyogranulomatous lesions. Coronavirus RNA was detected in the index case using the degenerate ORF1b primers. Subsequent RT-PCR and sequencing of a portion of the S gene yielded a unique 651 bp sequence that was analyzed by BLAST against the GenBank database. The newly identified sequence was 89% identical with the analogous sequence of ECG associated MCoV. Other significant homologies included the equivalent porcine and canine coronavirus sequences that were 74-75% identical. A previously unrecognized FIP-like disease was identified in mink. The combined IHC, RT-PCR and sequencing data confirmed an alphacoronavirus associated with the lesions. Sequencing of a portion of the S gene indicated that this virus is genetically most closely related to MCoV. Whether there is consistent genetic fingerprint associated with this novel mink coronavirus that is different from the previously recognized ECG-associated mink coronavirus, needs to be further investigated.

Toxicology

Saturday, October 20, 2012
Auditorium II

Moderators: Karyn Bischoff, Michelle Mostrom

1:00 PM **Screening and Confirmation of Illicit Drugs in Biological Samples by LC/MS-MS**
Robert H. Poppenga, Gwendolyne Alarcio, Elizabeth R. Tor 64

1:15 PM **Development of a GC/MS Procedure for Detecting Tremetone and Dehydrotremetone in Cases of White Snakeroot Poisoning**
Christina Wilson, Kimberly Meyerholtz, Stephen B. Hooser 65

1:30 PM **Challenges with Vitamin A Quantification in Feeds and Biological Samples**
Gwendolyne Alarcio, John Tahara, Elizabeth R. Tor, Linda Aston, Robert H. Poppenga 66

1:45 PM **Detection of Aflatoxin-DNA Adducts in the Livers of Aflatoxin-treated Rats**
BongSuk Kim, Christina Wilson, Kim Meyerholtz, Stephen B. Hooser 67

2:00 PM **The Use of Iron Quantitation in Dried Blood Spots as a Means of Estimating the Whole Blood Hematocrit of Wild Birds**
Alan Shlosberg, Andreas Lehner, Michael Adkesson, Kirk Stuart, Margaret Johnson, Wilson K. Rumbeiha 68

2:15 PM **Reference Intervals for Inorganic Element Concentrations in Equine Fetal Liver Tissue**
Cynthia L. Gaskill, Lori L. Smith, Michelle N. Helm 69

2:30 PM **Development of Ecofriendly Extraction Processes for Analysis of Aflatoxins and Fumonisins in Corn and Corn Byproducts ◊**
Washington Mutatu, Andreas Lehner, Margaret Johnson, Wilson K. Rumbeiha 70

2:45 PM **Equine Botulism Type A — A Western States Phenomenon # * † ◊**
Stephanie R. Ostrowski, Steven V. Kubiski, Joan Palmero, Christopher M. Reilly, Jamie K. Higgins, Sheri Cronin, Snehal N. Tawde, Beate M. Crossley, Paula Yant, Ray Cazarez, Francisco A. Uzal 71

3:00 PM **Presumptive Diagnosis of Pine Needle Abortion in a Nebraska Cattle Herd using an Isocupressic Acid Metabolite as a Biomarker # * † +**
Douglas Snider, Dale Gardner, Steve M. Ensley, Bruce Janke, Wilson K. Rumbeiha 72

Symbols at the end of titles indicate the following designations:

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

Screening and Confirmation of Illicit Drugs in Biological Samples by LC/MS-MS

Robert H. Poppenga, Gwendolyne Alarcio, Elizabeth R. Tor

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

Narrative: A rapid and selective analytical method using liquid chromatography-tandem mass spectrometry for the screening and confirmation of drugs-of-abuse and their common metabolites in urine, serum, stomach contents, and source materials was developed and validated. Screen analytes include tetrahydrocannabinol (Δ9-THC), THC-OH, THC-COOH, amphetamine, methamphetamine, MDMA, methadone, morphine, 6-MAM, heroin, fentanyl, norfentanyl, cocaine and metabolites, ephedrine, LSD, phentermine, nicotine, psilocin, JWH-018, HU-210, and JWH-073. The method detection limits vary among analytes and specimen types but range from 50 to 500 ppb. The screen has been used successfully to confirm or rule out intoxication as illustrated by two cases. Case One: A 10 year-old, male Shih Tzu was presented to our Teaching Hospital with the complaint of anxiety with compulsive pacing of approximately 6 hours duration. The history indicated that the owners occasionally allow the dog to drink coffee, which he likes, and the dog was known to investigate ash trays in the house. On presentation, the dog was anxious, hyperactive, and panting with a temperature of 103.7 and pulse rate of 120. No arrhythmias were auscultated. A urine sample was submitted to the CAHFS' Toxicology Section for drug screening. Amphetamine, methamphetamine, and caffeine (trace amount) were detected by LC/MS/MS. Based upon the relative concentrations of the three analytes, a diagnosis of amphetamine intoxication was made. Case Two: Two dogs, a 9 year-old, female Maltese and an 8 year-old Yorkshire terrier from the same household presented to a local veterinary emergency clinic with signs of lethargy, disorientation, pacing, mydriasis, and abdominal pain. One dog died at the clinic while the other was treated symptomatically and recovered. A urine drug screen at the emergency clinic was positive for methadone. Postmortem samples from one dog and urine, serum, and stomach contents from the dog which survived were submitted for analysis. Methadone was not detected in the urine sample by LC/MS/MS but doxylamine (sold under the tradename Unisom®) was detected. Based upon the detection of doxylamine, presence of clinical signs compatible with an antihistamine overdose, and failure to detect methadone or other illicit drug, a diagnosis of doxylamine intoxication was made. Currently, the incidence of intoxication by drugs-of-abuse in veterinary medicine is unknown. However, based upon discussions with area emergency clinic veterinarians, many dogs present with acute onset of neurological signs that could possibly reflect exposure to such agents. A broad-based, sensitive, and rapid drugs-of-abuse screen can help confirm or rule out an exposure or intoxication to such agents.

Development of a GC/MS Procedure for Detecting Tremetone and Dehydrotremetone in Cases of White Snakeroot Poisoning

Christina Wilson[1, 2], Kimberly Meyerholtz[2], Stephen B. Hooser[1, 2]

[1]Department of Comparative Pathobiology, Purdue University, W. Lafayette, IN; [2]Indiana Animal Disease Diagnostic Laboratory, Purdue University, W. Lafayette, IN

Narrative: The toxicity of white snakeroot (Ageratina altissima) is thought to be attributed to the presence of the toxins tremetone, dehydrotremetone, and 2-oxyangeloyl-tremetone. This plant causes skeletal muscle necrosis and cardiotoxicity in livestock and grazing animals that ingest the plant. Clinical signs of toxicity can include constipation, dyspnea, salivation, acetone odor to the breath and urine (ketosis), muscle tremors or violent trembling, tachycardia or cardiac arrhythmias, and terminal collapse. Diagnosis of white snakeroot toxicosis has been based on characteristic histologic lesions in the myocardium, skeletal muscle, or liver in combination with a clinical history of exposure and/or visual observation of intact plant material in the gastrointestinal tract. In cases in which the clinical history is lacking or identification of plant fragments is difficult, diagnosis can be challenging. Realizing the importance of an analytical test for diagnosing white snakeroot poisoning, a method has been developed to extract and detect tremetone and dehydrotremetone from the plant and from biological samples. Materials and Methods: White snakeroot plants, rumen contents, blood, and serum samples were subjected to organic extraction, concentrated, and analyzed by GC/MS. Organic extractions of liver and kidney samples were loaded onto ENVI-Carb-II/PSA SPE columns, concentrated, and analyzed by GC/MS. GC parameters included: injector port temperature 220C, column flow 1.0 mL/min, column temperature program was initially set at 100C (held 0.10 min) with a 7.1C/min ramp to 300C. The MS ion trap, manifold, and transferline heater were set at 150C, 40C, and 220C respectively. EI-MS scan from 40 to 650 m/z was used to monitor ions. For GC/MS analyses, 1 μL of standards and samples were injected onto a VF-5ms column (30m x 0.25mm, ID; DF = 0.25). Recovery of tremetone and dehydrotremetone was verified using the SPE extraction protocol on the reference standards and the white snakeroot plant extract. Results: Dehydrotremetone and tremetone were detected in the white snakeroot plant. Dehydrotremetone was detected in the liver; however, neither component was recovered in the rumen content. Conclusion: A GC/MS method has been developed to detect tremetone and dehydrotremetone in biological samples. This method can be used to support a diagnosis of white snakeroot poisoning in suspect cases.

Challenges with Vitamin A Quantification in Feeds and Biological Samples

Gwendolyne Alarcio, John Tahara, Elizabeth R. Tor, Linda Aston, Robert H. Poppenga

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

Narrative: Vitamin A, retinol, a lipid soluble vitamin, plays vital roles in vision, immune system, and cell growth. The liver is the main storage site with greater than 95% being present as retinyl esters, mostly as retinyl palmitate and stearate. Measuring retinol concentration in serum, liver and feed is a common method to assess vitamin A status. Serum samples are typically treated with ethanol and retinol is extracted with a non-polar solvent. Liver and feed samples are commonly saponified to hydrolyze retinyl esters to retinol before extraction. Others analyze retinol and retinyl esters directly, without hydrolysis, and report results as retinol equivalents. However, standards of retinyl esters, with the exception of retinyl palmitate and acetate, are not commercially available. Hydrolysis, therefore, is necessary to analyze liver and feed samples for vitamin A. Liver and feed samples were saponified with potassium hydroxide for 20 minutes at 70°C, in the presence of ascorbic acid, an antioxidant, to hydrolyze retinyl esters to retinol. After hydrolysis, retinol was extracted once with petroleum ether. High variations in retinol concentration were observed in replicate analyses of feed and liver samples. The causes of the high variability, such as sampling of non-homogenous samples, incomplete extraction of retinol, matrix effects, and inefficient and variable hydrolysis were investigated. Experiments with multiple extractions of retinol with petroleum ether show that multiple extractions were no more efficient than a single extraction. Sampling from seven separate and random aliquots of a control bovine liver resulted in a higher inter-assay variability than sampling from a homogenized liver (n=10), which suggests that homogenization of liver from which subsamples are tested increases precision. Lower inter-assay variability of "in-house" spiked chicken feed with retinyl palmitate, in comparison to commercial powdered milk fortified with retinyl palmitate, suggests non-homogenous fortification of feeds. Initial experiments of hydrolyzing 200ug/ml retinyl palmitate in methanol, spiked bovine liver and chicken feed resulted in 50-60% retinol recovery. Hydrolysis times of up to one hour did not affect the recovery of either retinol or retinyl palmitate, suggesting that retinol is stable during hydrolysis. Efforts in determining the cause of these variations and results will be presented.

Detection of Aflatoxin-DNA Adducts in the Livers of Aflatoxin-treated Rats

BongSuk Kim, Christina Wilson, Kim Meyerholtz, Stephen B. Hooser

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

Narrative: Aflatoxin B1 (AFB1) is a fungal toxin produced by *Aspergillus sp.* Ingestion of this toxin in food can cause severe hepatic injury after metabolism and binding of the reactive AFB1 metabolites to intracellular proteins and DNA. Current analytical methods do not detect aflatoxin in animal tissues except at very high concentrations. It is our hypothesis that aflatoxin-DNA adducts can be detected in the livers of rats administered aflatoxin B1 (AFB1) using an antibody to AFB1-DNA adducts. Sprague-Dawley rats were administered AFB1 at 4 mg/kg (ip) or 1.5% DMSO in saline (controls) and were necropsied at 2, 24, or 48h after exposure. In rats treated with AFB1, plasma ALT activities were significantly greater than controls (mean +/- SE = 43 +/- 4 U/L) at 24 and 48h (307 +/- 78 and 698 +/- 242 U/L, respectively). By light microscopy (LM), livers from control rats were morphologically normal. However, LM revealed morphologic changes consistent with apoptosis and hepatoxicity beginning at 2h post-dosing and progressing in severity over 24 and 48h. Liver DNA was extracted and purified. Using a primary antibody to aflatoxin-DNA adducts (6A10 from Pharmingen), a dot blot analysis was performed. A synthetic AFB1-DNA adduct was used as a positive control. Liver DNA from rats treated with AFB1 at 2 and 24h post-treatment, was positive for AFB1-DNA adducts. This initial study indicates that aflatoxin-DNA adducts can be detected in liver DNA from rats treated with AFB1 using an antibody to aflatoxin-DNA adducts.

The Use of Iron Quantitation in Dried Blood Spots as a Means of Estimating the Whole Blood Hematocrit of Wild Birds

Alan Shlosberg[3], Andreas Lehner[2], Michael Adkesson[4], Kirk Stuart[2], Margaret Johnson[2], Wilson K. Rumbeiha[1]

[1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA; [2]Diagnostic Center for Public & Animal Health, Michigan State University, Lansing, MI; [3]Toxicology, Kimron Veterinary Institute, Bet Dagan, Israel; [4]Chicago Zoological Society, Brookfield, IL

Narrative: Dried blood spots (DBS) have recently been used to develop a novel biomonitoring project in wild birds, entitled DABSE (Database of Avian dried Blood Spot Examinations for exposure of wild birds to environmental toxicants and diseases). One of the main aims of DABSE is to give an appraisal of the health status of individual birds by examination of very small, i.e. low impact, volumes (~300μl) of whole blood. At present we are able to determine exposure to the five most harmful groups of environmental contaminants including toxic elements, chlorinated pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and perfluorinated compounds (PFOS and PFOA) at the low parts per billion concentration in DBS. This study was aimed at adding another parameter to the health appraisal of DABSE by an indirect measurement of whole blood hematocrit by examining DBS quantitatively for iron by ICP/MS methodology. The laboratory validation was done by making artificial hematocrits ranging from 15-50% from plasma and washed erythrocytes derived from whole blood of a broiler chicken, a domestic turkey and a domestic duck using a 50 uL DBS. Results from the laboratory validation study showed a strong correlation between artificial hematocrits and iron concentration (R2 = 0.995), indicating that total iron mesurements on DBS can be used to determine hematocrit. Subsequently, we proceeded to determine whether we could accurately predict hematocrit in a field validation study. This involved measurement of DBS iron from 30 wild-trapped Humboldt penguins (*Spheniscus humboldti*), in which hematocrit was measured by standard methods. Using DBS iron concentrations, we calculated a predicted hematocrit for each of the penguins. We then determined the correlation between predicted and measured hematocrits. Results of the field study indicated a strong correlation (R2= 0.74) between predicted and measured hematocrits. Overall, the DBS approach overestimated measured hematocrit by about 5%. This study shows that quantitation of DBS iron can be used to reliably assess hematocrit values in wild birds.

Reference Intervals for Inorganic Element Concentrations in Equine Fetal Liver Tissue

Cynthia L. Gaskill, Lori L. Smith, Michelle N. Helm

Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY

Narrative: Deficiencies or excesses of inorganic elements can be associated with disease and death in equine fetuses and newborn foals. Interpretation of elemental analyses of liver tissue from this group can be problematic because reference intervals have not been established and extrapolation from reference intervals for adult horses may not be appropriate. The purpose of this study was to develop reference intervals for inorganic elements in liver tissue from aborted, stillborn, and neonatal foals that died in central Kentucky. Preliminary data is presented here. Fresh liver tissue was obtained from 137 aborted, stillborn or day-old foals that presented to the University of Kentucky Veterinary Diagnostic Laboratory for postmortem examination from January to May of 2012. Tissues were prepared for analysis by microwave-assisted acid digestion; elemental analyses were performed by inductively coupled plasma mass spectrometry. Cause of death diagnoses were obtained from case reports after postmortem examinations and related testing were completed. Other data collected included gestational age, sex, breed, and weight. Standard descriptive statistics were derived for the concentrations of each element. Normality of distributions was assessed and each data set was evaluated for outliers. Significant outliers were removed. The 10% and 90% percentiles for each element were calculated. Associations between elemental concentrations and other factors were evaluated. All foals/fetuses in this study were third trimester abortions or died the day of birth. Thoroughbreds were over-represented (n=120). Causes of death/abortion included nocardioform placentitis (13), other placentitis causes (19), leptospirosis (11), umbilical cord abnormalities (5), dystocia/perinatal asphyxia (33), flexural limb deformities/scoliosis/other deformities (9), equine herpesvirus (4), septicemia/enteritis (3), other miscellaneous causes (15), and no diagnosis (25). Results for cobalt, cadmium, and chromium were not evaluated because few tissues had values above the minimum levels of quantification (0.025, 0.025, and 0.05ppm, respectively). Arsenic, lead, and thallium were also not evaluated, because no tissues had values above the minimum levels of quantification (0.05ppm). The 10% and 90% percentiles for other elements evaluated were (in ppm wet weight): copper 26.7-108; iron 84.6-279; selenium 0.157-0.326; zinc 14.8-126; molybdenum 0.154-0.355; manganese 0.348-1.30; sodium 1010-1620; magnesium 90.2-136; phosphorus 1530-2520; and potassium 1760-2870. No significant associations were made between elemental concentrations and other factors. Evaluations of additional fetal liver tissues are underway. When all analyses are completed, 90% reference intervals for each element will be calculated and sub-groups evaluated. These reference intervals will help with interpretation of elemental analyses of fetal, stillborn, and newborn equine liver tissue.

Development of Ecofriendly Extraction Processes for Analysis of Aflatoxins and Fumonisins in Corn and Corn Byproducts ◊

Washington Mutatu, Andreas Lehner, Margaret Johnson, Wilson K. Rumbeiha

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

Narrative: Mycotoxin poisoning is of great concern in food production for animal agriculture, and analysis of corn and corn byproducts for mycotoxins is a good preventative approach. Extraction of mycotoxins for analysis using lateral flow devices has traditionally been accomplished with 70% methanol. Unfortunately, methanol is toxic to the environment as well as lab workers. We developed an ecofriendly, aqueous-based extraction method which is also simple, inexpensive, efficient, and reproducible. We tested various solvents which included organic-based solvents comprised of acetonitrile/water mixtures, methanol/water mixtures, ethanol/water mixtures, and aqueous based solvents with SDS, citrate, $NaHCO_3$, Brij-58, cyclodextrin and benzalkonium chloride as additives. The extracts were quantified by LC-MS/MS with ESI-MS detector. Spiking known amounts of mycotoxins in ground corn samples tested the extraction efficiency for each solvent and analyzing certified Trilogy samples checked for accuracy. The results of spiked corn samples (20 ppb total aflatoxins B1 and B2, i.e. AFB1 & AFB2) showed the following order of percent recoveries: acetonitrile/water (78%) > ethanol/water (42%) > methanol/water (30%). The Trilogy sample extraction accuracies were in the order methanol/water (104%) > ethanol/water (99%) > acetonitrile/water (94%). Extraction with aqueous solvents had mixed but interesting results. The SDS, $NaHCO_3$, benzalkonium chloride, and Brij-58 solutions had very suppressive effects on the signal, citrate was mildly suppressive at low concentration and more so at higher concentration, and cyclodextrin offered no advantages in extraction efficiency. The SDS system offered an ecofriendly alternative to the acetonitrile/water mixture regarded as the 'gold standard' in our laboratory, with the 100mM SDS concentration showing higher recoveries than acetonitrile/water. This, however, was determined after carrying out a back titration in which the SDS was precipitated out with saturated sodium chloride. The analysis of the Trilogy samples following back extraction with SDS solutions showed poor recoveries for AFB2 and good (86%) recovery for AFB1 with the 100mM SDS, whereas the 1mM and 10mM concentrations had less than 70% recovery. Similar studies carried out on fumonisins, which also included pure water as a solvent, had the following order in extraction efficiency of fumonisin B1 (FB1) in ground corn and Trilogy samples: acetonitrile/qater (110%) > methanol/water (98%) > ethanol/water (97%) >> deionized water (52%). Similar extractions of FB1 using SDS solutions showed the order of efficiency: acetonitrile/water > 10mM SDS > 1mM SDS > 100mM SDS. In conclusion, our results show that non-toxic solvent combinations such as ethanol/water mixtures and 100mM SDS have been shown to perform well in comparison to 'gold standard' extraction solvents such as acetonitrile/water and methanol/water for both aflatoxins and fumonisins.

◊ USAHA Paper

Equine Botulism Type A — A Western States Phenomenon # * † ◊

Stephanie R. Ostrowski[1], Steven V. Kubiski[2], Joan Palmero[3], Christopher M. Reilly[2], Jamie K. Higgins[3], Sheri Cronin[4], Snehal N. Tawde[1], Beate M. Crossley[1], Paula Yant[1], Ray Cazarez[1], Francisco A. Uzal[1]

[1]California Animal Health & Food Safety Laboratory, UC Davis, Davis, CA; [2]Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, UC Davis, Davis, CA; [3]Department of Medicine and Epidemiology, School of Veterinary Medicine, UC Davis, Davis, CA; [4]Mobile Equine Veterinary Serivices, Willits, CA

Narrative: An outbreak of type A botulism involved four horses in northern California which were fed grass clippings obtained from a nearby park. Within 48 hours, all horses developed a progressive flaccid paralysis syndrome clinically consistent with exposure to pre-formed botulinum neurotoxin (BoNT). All horses exhibited marked cervical weakness (inability to raise their heads to a normal position), and died or required humane euthanasia by 96 hrs. One animal was submitted to the Veterinary Medical Teaching Hospital at the University of California, Davis for diagnostic examination and treatment, and subsequently was presented to the pathology service. At necropsy, edema was observed in the areas of muscle attachment to the nuchal ligament and inguinal fascia; no other lesions were identified. A sample of the feed source (wilted grass clippings) tested positive by the mouse bioassay test for pre-formed BoNT type A. Sporadic cases of equine botulism occurring west of the Mississippi River are more likely to be caused by *Clostridium botulinum* serotypes A or C, rather than type B. The mapped distribution of botulism spore serotypes reported in North America corresponds to epidemiologic reports of the geospatial distribution of botulism serotypes for both human and equine cases. Cervical weakness and edema at the nuchal ligament and inguinal fascia were prominent clinico-pathologic features noted with the current type A outbreak; both have previously been reported as inconsistent findings for type C equine cases. An affordable trivalent (A, B, C) BoNT antiserum product provides therapeutic coverage for all three clinically important *C. botulinum* serotypes. Emphasis should be placed on early case recognition and rapid initiation of treatment with the trivalent antitoxin product, in addition to preventing dietary exposure to BoNT in spoiled forages.

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

Presumptive Diagnosis of Pine Needle Abortion in a Nebraska Cattle Herd using an Isocupressic Acid Metabolite as a Biomarker # * † +

Douglas Snider[1], Dale Gardner[2], Steve M. Ensley[1], Bruce Janke[1], Wilson K. Rumbeiha[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Poisonous Plant Research Laboratory, USDA-ARS, Logan, UT

Narrative: Pine needle abortion is a naturally occurring condition in free range bovine caused by the consumption of pine needles from *Pinus ponderosa* and select Cupressaceae species. A presumptive diagnosis of pine needle abortion has relied on the detection of isocupressic acid (ICA) which is only present in serum for hours post exposure. Recent studies using oral dosing of ICA, *Pinus ponderosa* needles, and *Juniperus comminus* needles to cattle produced detectable amounts of agathic acid (AA), dihydroagathic acid (DHAA), and tetrahydroagathic acid (THAA) in the serum for up to sixty hours. There are no previous publications indicating these stable ICA metabolites cross the placenta or are detectable in fetal tissues. There still remains a need for development of diagnostic tests for pine needle abortion in the most commonly submitted abortion sample – a whole fetus. Therefore, we evaluated the presence of AA, DHAA, and THAA from two aborted fetuses with a recent history of pine needle consumption by the dam. The fetal thoracic fluid and stomach contents were collected from two aborted bovine fetuses with a recent herd history of pine needle consumption. These samples were evaluated using high pressure liquid chromatography (HPLC) for the presence of AA, DHAA, and THAA. These ICA metaboltes were evaluated in fetal fluids from nearly fifty additional abortion cases submitted to the diagnostic laboratory for routine evaluation. Results indicated THAA was present in the fetal thoracic fluid and fetal stomach contents of two aborted fetuses from the pine needle consuming herd from Nebraska. By contrast, no ICA metabolites were detected in the fetal fluids of the nearly fifty other aborted fetuses. A presumptive diagnosis of pine needle abortion was applied to this case due to the presence of THAA in fetal tissues, negative *Leptospira spp*. polymerase chain reaction, negative bovine viral diarrhea virus immunohistochemistry (IHC), negative bovine herpes virus IHC, negative *Campylobacter spp*. culture, the absence of significant bacterial growth from tissues, and the absence of significant lesions during gross and microscopic evaluation. Additional work is warranted to evaluate the limit of detection and subsequent sensitivity of the THAA HPLC diagnostic test for screening fetal tissues and fluids for pine needle abortion. THAA is detectable in the fetal thoracic fluid and stomach contents of aborted fetuses following consumption of pine needles by the dam. Therefore, the detection of THAA in fetal fluids is a novel approach to the diagnosis of pine needle abortion in cattle.

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

GeneReach

Virology 1
(Molecular Technique Focus)

Saturday, October 20, 2012
Guilford E

Sponsor: GeneReach Biotechnology Corporation

Moderators: Beate M. Crossley, Amar Patil

1:00 PM **Development of a Multiplex Real-Time PCR for the Detection of Bovine Corona and Rota Viruses ◊**
Jennifer Cooper, Meaghan Broman, Francine Cigel, Kathy L. Toohey-Kurth *75*

1:15 PM **Development of a Multiplex Real-time PCR Panel for Detection of Ruminant Endemic Diseases that Mimic Foot-and-Mouth Disease ◊**
Francine Cigel, Jennifer Cooper, Kathy L. Toohey-Kurth *76*

1:30 PM **Development and Performance Evaluation of a Simple Streamlined Method for Bluetongue Virus and Epizootic Hemorrhagic Disease Virus Nucleic Acid Purification, Denaturation, and Detection**
Megan Schroeder, Jennifer Meier, Mangkey Bounpheng, Donna Johnson, Eileen Ostlund, Alfonso Clavijo *77*

1:45 PM **Pockit® Xpress, an Insulated Isothermal PCR (iiPCR) Plaform for On-site Detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)**
Andre Ch. Broes, Simon Chung, Maryse Belanger *78*

2:00 PM **Identification of a Novel Isolate of Type 1A Enterovirus in Bovine Through a Subtractive-Hybridization Approach to Sequence-based Diagnostics**
Roger Barrette, Frederic R. Grau, Fawzi Mohamed, Andrew Fabian, Emily S. O'Hearn, Mary Lou Berninger, Robert Moeller, Marian Van der Schraaf, Fernando Torres-Velez, Michael T. McIntosh *79*

2:15 PM **New Real-Time PCR Assay using Allelic Discrimination for Detection and Differentiation of Equine Herpesvirus-1 Strains with A2254 and G2254 Polymorphisms †**
Kathryn Smith, Yanqiu Li, Patrick Breheny, R. Frank Cook, Pamela J. Henney, Stephen Sells, Stéphane Pronost, Zhengchun Lu, Beate M. Crossley, Peter J. Timoney, Udeni BR Balasuriya *80*

2:30 PM **Optimization and Validation of a High-throughput Real-time RT-PCR Assay for Rapid Detection of Foot-and-Mouth Disease Virus from Bulk Tank Milk**
Amaresh Das, Karissa Casteran, Diane J. Holder, Michael T. McIntosh, Kathy L. Toohey-Kurth, Francine Cigel, Thomas McKenna, Barbara M. Martin, Mangkey A. Bounpheng, Alfonso Clavijo, Tammy Beckham, Jennifer Rinderknecht *81*

2:45 PM **Detection and Differentiation of Equine Herpesvirus 1 by Real-Time PCR and Pyrosequencing**
Yan Zhang, Jing Cui, Jason Herr, Beverly Byrum *82*

3:00 PM **Detecting Canine Distemper Virus by an On-Site Portable PCR Platform – POCKIT**
Fu-Chun Lee, Sih-Ying Wu, Yun-Long Tsai, Ling-Ling Chueh, Bi-Ling Su, Chen Su, Ping-Hua Teng, Hsiao Fen Grace Chang, Pei-Yu Lee, Thomas Wang 83

Symbols at the end of titles indicate the following designations:

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

Development of a Multiplex Real-Time PCR for the Detection of Bovine Corona and Rota Viruses ◊

Jennifer Cooper[1], Meaghan Broman[1], Francine Cigel[1], Kathy L. Toohey-Kurth[1, 2]

[1]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI; [2]Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

Narrative: A multiplex real time RT-PCR (rRT-PCR) panel for the detection of bovine corona and rota (type A) viruses has been developed at the Wisconsin Veterinary Diagnostic Laboratory (WVDL). Assays in the panel were validated individually as well as in multiplex format using Ambion Path-ID Multiplex One-Step RT-PCR Kit with samples prepared by making a 10% tissue or feces slurry in Phosphate Buffered Saline (PBS) and homogenized using ceramic beads. Samples were evaluated by PCR and compared to electron microscopy and PCR performed at another laboratory. The WVDL corona virus assay showed high sensitivity and specificity using primers directed to the spike gene as evidenced by detection of 40/40 negative samples and 15/15 positive samples. The bovine rotavirus assay showed high sensitivity and high specificity using primers directed to the RNA polymerase gene as evidenced by detection of 26/26 negative samples and 30/30 positive samples. An internal control reaction was also included in the multiplex assay in order to monitor for inhibition in every sample. To further improve cost effectiveness and to avoid the potential for over-loading the sample homogenates, new sampling procedures were also evaluated. The alternative sampling method comprised of swabs dipped into feces or mucosal intestinal tissue and then vortexed in 1 ml PBS. Comparable sensitivity and specificity was obtained using the swab as compared to the homogenate. In addition to the swab comparison, a pooling strategy was evaluated as a potential cost savings for clients. Positive pools (n=41) consisted of at least one positive sample with a maximum of 3 samples per pool. Comparable specificity was found. However, sensitivity decreased with pools containing individual fecal samples with a cycle threshold value greater than or equal to 35. The rRT-PCR multiplex assay developed at WVDL has increased the sensitivity of corona and rotavirus detection compared to electron microscopy. Modifying our sample preparation from homogenates to swabs results in a savings of approximately 40% per sample to WVDL while not significantly compromising sensitivity. The pooling strategy will be more economical for the client but results in a loss of sensitivity.

◊ USAHA Paper

Development of a Multiplex Real-time PCR Panel for Detection of Ruminant Endemic Diseases that Mimic Foot-and-Mouth Disease ◊

Francine Cigel[1], Jennifer Cooper[1], Kathy L. Toohey-Kurth[1, 2]

[1]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI; [2]Pathobiological Sciences, University of Wisconsin, Madison, WI

Narrative: The Wisconsin Veterinary Diagnostic Laboratory previously developed a real-time PCR panel for rapid detection and concurrent confirmation of ruminant endemic diseases that mimic foot-and-mouth disease (FMD). This presentation describes the conversion of that panel to a multiplexing format with use of an internal control and includes the following viruses that are causative agents of diseases that must be considered in a differential diagnosis: bovine viral diarrhea virus (BVDV), bovine herpesvirus 1 (BHV-1), bovine herpesvirus 2 (BHV-2), bovine herpesvirus 4 (BHV-4), bluetongue virus (BTV), Epizootic hemorrhagic disease (EHD), Malignant catarrhal fever (MCF), Contagious ecthyma (ORF), and bovine papular stomatitis virus (BPSV). The FMD mimic panel detects, but does not differentiate the following serotypes: BVDV 1a, 1b, 2; BTV 2, 10, 11, 13, 17; and EHD 1, 2. Conversion of the panel to a multiplex format was initiated to increase efficiency and decrease cost. In addition, an exogenous internal control was included to monitor potential PCR inhibition and extraction success. To test performance of the assays, nucleic acid from viral stocks or clinical samples was purified using the MagMAX™-96 viral RNA isolation kit (Life Technologies) and a Kingfisher 96 magnetic particle processor. The nucleic acid was then serially diluted in triplicate and the limit of detection (LOD) and amplification efficiency were determined for each assay. Singleplex assays using ABI Taqman Universal Master Mix, Invitrogen Superscript III Platinum One-Step qRT-PCR System or TaqMan One-Step RT-PCR Master Mix Reagents were compared with assays using Ambion Ag-Path ID Multiplex One-Step RT-PCR chemistry. Primer and probe concentrations were modified as necessary until all assays demonstrated LODs comparable to the original data and an assay efficiency of ≥90%. Two to four assays were then grouped into multiplexed assays such that a clinical sample would unlikely be positive for more than one target in the multiplex reaction. An internal control PCR was included in two of the multiplex reaction groupings. Results show that LODs and PCR efficiencies were comparable using the multiplex format. In summary, the panel in a multiplex format will provide a more efficient and cost effective approach than singleplex assays for differential diagnoses in clinical samples with potential foot and mouth disease. Also, the internal control is an added benefit for the prevention of false negatives due to inhibition or extraction failures.

◊ USAHA Paper

Development and Performance Evaluation of a Simple Streamlined Method for Bluetongue Virus and Epizootic Hemorrhagic Disease Virus Nucleic Acid Purification, Denaturation, and Detection

Megan Schroeder[1], Jennifer Meier[1], Mangkey Bounpheng[1], Donna Johnson[2], Eileen Ostlund[2], Alfonso Clavijo[1]

[1]Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, TX; [2]Diagnostic Virology Laboratory, National Veterinary Services Laboratory, Ames, IA

Narrative: Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) are members of the Reoviridae family and are transmitted by biting *Culicoides* midges. BTV causes disease in cattle and other ruminants, resulting in significant economic loss due to treatment costs, production losses, and trade restrictions of infected animals. EHDV associated disease in cattle is less prominent, however, it has emerged as a major economic threat to the white-tailed deer (WTD) industry in many states, often causing severe debilitation and death in affected animals. The incursion of a new serotype of the virus (EHDV-6) into US is raising additional concerns about the future economic impact of this virus on the WTD industry. The potential emergence of exotic serotypes of BTV and EHDV emphasizes the need for robust detection of all known strains and differential diagnosis. For this purpose, a streamlined workflow consisting of an automated nucleic acid purification and denaturation method and multiplex one-step RT-qPCR for the simultaneous detection of all serotypes of BTV and EHDV was developed using previously published BTV[1] and EHDV[2] signatures. The denaturation of double stranded (ds) BTV and EHDV RNA was incorporated into the automated nucleic acid purification process thus eliminating the separate step of dsRNA denaturation (i.e., DMSO, MMOH, or betaine, or high temperature) commonly used for enhanced PCR sensitivity. The workflow analytical sensitivity, based on Probit analysis, was < 200 BTV and EHDV target copies per reaction. The performance of this workflow was assessed by comparison with nested RT-PCR assays for BTV and EHDV conducted at the NVSL using 125 samples (originated from Texas Veterinary Medical Diagnostic Laboratory). NVSL and TVMDL results showed high agreement (Cohen's Kappa 0.86-0.89, using NVSL method as the reference standard) and support the use of this workflow for concurrent detection of BTV and EHDV in the same reaction. Approximately 1850 samples consisting of bovine, ovine, caprine, cervine blood, tissue, and semen have been tested and 251 positives (~13.5% positive rate) were identified, specifically, 72 BTV only positives, 119 EHDV only positives, and 60 BTV and EHDV positives. Interestingly, BTV/EHDV co-infections were observed at a significant rate (24% (60/251) of all positives); this observation may indicate opportunities for potential interaction between closely related orbiviruses and may be important for understanding disease clinical presentations. References 1. Hofmann, M. et al. (2008). Bluetongue disease reaches Switzerland. *Schweiz Arch Tierheilkd*. 2008 Feb;150(2):49-56. German. 2. Clavijo, A. et al. 2010. An improved real-time polymerase chain reaction for the simultaneous detection of all serotypes of Epizootic hemorrhagic disease virus. *J Vet Diagn Invest*. 2010 Jul;22(4):588-93.

Pockit® Xpress, an Insulated Isothermal PCR (iiPCR) Plaform for On-site Detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

Andre Ch. Broes[1], Simon Chung[2], Maryse Belanger[1]

[1]Biovet, Inc., Saint-Hyacinthe, QC, Canada; [2]GeneReach Biotechnology Corp., Taichung City, Taiwan

Narrative: PRRS is one of the most economically important infectious diseases of swine worldwide. Rapid diagnosis of new PRRSV infections is critical for effective control of the disease. In remove areas diagnosis may be abnormally long due to delays in shipping samples to a laboratory. Efficient tools allowing on-site virus detection would thus represent an important improvement in the diagnosis of PRRSV infections. Several tools such as immunochromatographic strip tests (ICST), Loop Mediated Isothermal Amplification (LAMP) or real-time polymerase chain reaction (PCR) platforms have been proposed for that purpose. However they are either too expensive, lack practicability, or have poor diagnostic performances. Recently a new qualitative PCR detection platform based on the concept of insulated isothermal PCR (iiPCR) has been developed. This platform can detect both DNA and RNA. The nucleic analyser is supplied in a hard-shell suitcase package along with a mini-centrifuge and micropipettes (Pockit® Xpress). The analyzer is equipped with a chip to collect optical data, interpret them, and display test results on an integrated screen. The nucleic acid extraction kit supplied separately is spin-column based and is simple and fast. All specific reagents are lyophilized and stable at 40-80C. Equipment and reagents are relatively inexpensive. They can be used in a broad range of ambient temperatures. Assays are easy and fast to perform (30 minutes for nucleic acid extraction and 1 hour for amplification). We did evaluate the diagnostic performances of the Pockit® Xpress and its companion reagents for the detection of PRRSV type 2 strains. We compared them with a commercial extraction kit (QIAamp viral RNA) and an in-house TaqMan® reverse transcriptase real-time PCR assay. For test sensitivity we used serum samples representing a broad range of type 2 virus clusters. Pockit® appeared very user friendly and demonstrated excellent diagnostic sensitivity and specificity. Detailed data will be presented at the meeting. References Anonymous. Pockit. www.iipcr.com Chang HF, Tsai YL, Tsai CF, Lin CK, Lee PY, Teng PH, Su C, Jeng CC. A thermally baffled device for highly stabilized convective PCR. *Biotechnol J*. 2012 Jan 13

Identification of a Novel Isolate of Type 1A Enterovirus in Bovine Through a Subtractive-Hybridization Approach to Sequence-based Diagnostics

Roger Barrette[1], Frederic R. Grau[1], Fawzi Mohamed[1], Andrew Fabian[1], Emily S. O'Hearn[1], Mary Lou Berninger[1], Robert Moeller[2], Marian Van der Schraaf[3], Fernando Torres-Velez[1], Michael T. McIntosh[1]

[1]USDA APHIS VS NVSL Foreign Animal Disease Diagnostic Laboratory, Plum Island Animal Disease Center, Greenport, NY; [2]California Animal Health and Food Safety Laboratory, UC Davis, Tulare, CA; [3]USDA APHIS Veterinary Services, California and Nevada, Tulare, CA

Narrative: Next generation sequencing is a powerful tool for detection and characterization of pathogens. This technology is rapidly reducing the sequencing cost per base while simultaneously increasing amount of sequence data produced. Next generation sequencing is particularly well suited to screening complex mixtures of DNA, which can be exploited for pathogen identification. However, such DNA mixtures are often biased to host derived, rather than pathogen derived, nucleic acid. This host bias can greatly reduce the number of sequence reads that are relevant to the target of interest. By generation of random cDNA libraries from samples and hybridization to host cellular total RNA tagged by ligation to a biotin-oligonucleotide complex and immobilized by binding to Streptavidin paramagnetic beads, we have developed a novel subtracted hybridization method that may be used to enrich for pathogen cDNA from a given diagnostic specimen. To test this method, samples from cattle exhibiting clinical signs similar to foot-and-mouth disease (FMD) were cultured in primary lamb kidney cells (LK cells) and a monkey kidney cell line (Vero cells) for isolation of potential viruses. FMD virus was rapidly ruled out by conventional diagnostic methods including real-time RT-PCR of the original and cultured samples; however, electron microscopy of LK and Vero cell cultured samples revealed picornavirus-like particles. LK cultured material was subsequently subjected to random cDNA amplification, host subtraction and next-generation sequence analysis using a Roche 454 GS Junior. Total cDNA was simultaneously sequenced to compare the effectiveness of the new method. Results from the host subtracted cDNA library resolved 74% of the genome of a novel isolate of Enterovirus within the Type 1A enterovirus grouping. In contrast, 454 sequencing of the total cDNA library resolved only 34% genome for the newly identified virus. Phylogenetic analysis revealed the isolate to be most closely related to a recently described Bottlenose Dolphin Enterovirus with 84% nucleotide identity. Conventional sequence analysis was employed to validate the 454 sequence data. This work illustrates the utility of next generation sequencing and host or background subtraction in sequence-based diagnostics including the detection and simultaneous characterization of new viral species.

New Real-Time PCR Assay using Allelic Discrimination for Detection and Differentiation of Equine Herpesvirus-1 Strains with A2254 and G2254 Polymorphisms †

Kathryn Smith[1], Yanqiu Li[1], Patrick Breheny[2], R. Frank Cook[1], Pamela J. Henney[1], Stephen Sells[3], Stéphane Pronost[4], Zhengchun Lu[1], Beate M. Crossley[5], Peter J. Timoney[1], Udeni BR Balasuriya[1]

[1]Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY; [2]Department of Biostatistics, University of Kentucky, Lexington, KY; [3]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY; [4]Frank Duncombe Laboratory, University of Caen Lower Normandy, Caen, France; [5]California Animal Health and Food Safety Laboratory, University of California, Davis, CA

Narrative: A single nucleotide polymorphism (A2254 or G2254) in open reading frame 30 (ORF30) has been linked to the neuropathogenic phenotype of equine herpesvirus -1 (EHV-1). Identification of this polymorphism led to the development of an allelic discrimination, real-time PCR (rPCR) assay (E2) to distinguish between potential neuropathogenic and non-neuropathogenic EHV-1 strains. Although this rPCR assay can detect and genotype EHV-1 strains, subsequent studies have demonstrated it lacks adequate sensitivity for the routine detection of viral nucleic acid in clinical specimens. This prompted the development of a new allelic discrimination EHV-1 rPCR assay (E1) that involved redesigning primers and probes to ORF30. Viral DNA purified from serial 10-fold dilutions (10-1 -10-8) of tissue culture fluid, containing either EHV-1 A2254 or G2254 strain, were used to ascertain the analytical sensitivity of each rPCR assay. The E1 and E2 rPCR assays were also evaluated using 76 archived EHV isolates, and 433 clinical specimens from cases of suspected EHV-1 infection. Nucleotide sequence analysis of ORF30 was used to confirm the presence of EHV-1 and characterize the genotype (A2254 or G2254) in all archived isolates plus 168 of the clinical samples. The E1 assay was ten times more sensitive than E2, with a lower detection limit of 10 infectious virus particles. Furthermore, all A2254 and G2254 genotypes along with three cases of dual infection (A2254+G2254) were correctly identified by E1, whereas E2 produced 20 false dual-positive results with only one confirmed A2254+G2254 genotype. Based on these findings, E1 offers greater sensitivity and accuracy for the detection and A/G2254 genotyping of EHV-1, making this improved rPCR assay a very valuable diagnostic tool for investigating outbreaks of EHV-1 infection.

† Graduate Student Oral Presentation Award Applicant

Optimization and Validation of a High-throughput Real-time RT-PCR Assay for Rapid Detection of Foot-and-Mouth Disease Virus from Bulk Tank Milk

Amaresh Das[1], Karissa Casteran[1], Diane J. Holder[1], Michael T. McIntosh[1], Kathy L. Toohey-Kurth[2], Francine Cigel[2], Thomas McKenna[2], Barbara M. Martin[3], Mangkey A. Bounpheng[4], Alfonso Clavijo[4], Tammy Beckham[4], Jennifer Rinderknecht[5]

[1]Proficiency and Validation Services, Foreign Animal Disease Diagnostic Laboratory, National Veterinary Science Laboratory, Plum Island Animal Disease Center, Orient, NY; [2]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI; [3]National Animal Health Laboratory Network, National Veterinary Science Laboratories, Ames, IA; [4]Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, TX; [5]National Center for Foreign Animal and Zoonotic Disease Defense, Texas A&M University, College Station, TX

Narrative: Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals that can spread by infected animal products such as milk. In the event of an outbreak, there will be a need for a surveillance plan involving bulk tank milk (BTM) testing to facilitate the continuity of business for the dairy industry. Earlier studies (Blackwell and Hyde, 1976; Reid et al., 2006) indicate that as many as 104 infectious FMD virus (FMDV) particles per ml may be shed in the milk of an infected cow, suggesting that a single infected animal from a dairy herd milking into a bulk tank may be detectable by RT-PCR. In this study we report optimization of a high-throughput real-time RT-PCR assay (rRT-PCR) for rapid detection of FMDV in BTM samples. Using a safe recombinant FMD surrogate virus, a high-throughput nucleic acid purification method using the MagMAX™-96 viral RNA isolation kit (Life Technologies) was developed at the Wisconsin Veterinary Diagnostic Laboratory. The methodology was transferred to Foreign Animal Disease Diagnostic Laboratory for further optimization. For further evaluation, an exogenous non-competitive internal positive control (XIPC) RNA was added to the sample lysis buffer to monitor the extraction process and to detect any potential PCR inhibitors present in the milk samples. Detection of viral RNA and XIPC was performed by a multiplex rRT-PCR assay using Taqman fluorescent probes, specific to the targets (FMDV and XIPC). The cycle threshold (Ct) values of the FMDV RNA extracted from serial dilutions of live virus in Eagles Minimal Essential Medium, bulk tank Jersey milk (high fat content) and bulk tank Holstein milk (low fat content) were found to be very similar, indicating no inhibition in the detection of FMDV. There was also no inhibition in the detection of FMDV or XIPC in fresh, frozen, virus spiked or archived milk from experimentally infected animals. The amplification efficiency of the rRT-PCR assay was determined after amplification of the FMDV RNA serially diluted in PurNA (total RNA from Jersey and Holstein milk) and TE buffer as diluents and the amplification efficiencies were determined to be between 90 and 112%. These results demonstrate robust performance of this procedure for detection of FMDV in milk. Further evaluations using experimentally and naturally infected FMDV-infected milk samples are in progress.

Detection and Differentiation of Equine Herpesvirus 1 by Real-Time PCR and Pyrosequencing

Yan Zhang, Jing Cui, Jason Herr, Beverly Byrum

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

Narrative: Equine herpesvirus type 1 (EHV-1) is a member of the genus Varicellovirus, subfamily Alphaherpesvirinae, in the family Herpesviridae. EHV-1 is prevalent in most horse populations worldwide. Clinical signs include respiratory disease in young horses, abortion in pregnant mares, neonatal death in foals, and occasionally myeloencephalopathy (equine herpesvirus myeloencephalopathy: EHM). The disease has serious economic consequences for the equine industry. Recently, the emergence of a neurologic strain of EHV-1 is of major concern due to the increasing frequency of EHM associated with this strain. A molecular epidemiological study conducted by Nugent et al. demonstrated that a single point mutation of a nucleotide at position 2254 in the viral DNA polymerase gene from guanine to adenine, resulted in an amino acid change at position 752 from asparagine to aspartic acid. This point mutation has been strongly associated with the neuropathogenicity of the virus. Because the clinical course of infection with EHV-1 can vary from subclinical to severe EHM, rapid,reliable detection and differentiation of neurotropic strains of EHV-1 is essential. Traditionally, the diagnosis of EHV-1 infection has been based primarily based on serologic assays, virus isolation, and PCR. Realtime PCR assays for detection and differentiation of EHV-1 have been developed, however, the specificity of those assays has been inconsistent. There is a real need for a reliable molecular assay for EHV-1 diagnosis that is able to detect and differentiate between the neurologic and non-neurologic strains of EHV-1. In the present study, a real-time RT-PCR assay targeting EHV-1 DNA polymerase gene was developed. The assay is specific for both neurotropic and non-neurotropic strains of EHV-1 using four different platforms; the LightCycler, the ABI 7500 fast, Smartcycler, and Realplex. Pyrosequencing was used to provide sequence information on the amplified DNA product to confirm the PCR results and to provide more detailed differentiation between neurotropic strains and non-neurotropic strains of EHV-1. To date, this is the first study reported in which pyrosequencing was applied for EHV-1 identification using a less expensive diagnostic approach. Results from this study indicate that this procedure is suitable for detection and differentiation of EHV-1 in samples of equine origin.

Detecting Canine Distemper Virus by an On-Site Portable PCR Platform – POCKIT

Fu-Chun Lee, Sih-Ying Wu, Yun-Long Tsai, Ling-Ling Chueh, Bi-Ling Su, Chen Su, Ping-Hua Teng, Hsiao Fen Grace Chang, Pei-Yu Lee, Thomas Wang

GeneReach Biotechnology Corporation, Taichung City 407, Taiwan

Narrative: Canine infectious respiratory disease (CIRD) is a complex infection that occurs worldwide predominantly in shelters and boarding kennel environments, and several bacterial and viral micro-organisms have been associated with outbreaks of CIRD, including canine distemper virus (CDV). Although highly sensitive and specific reverse transcription polymerase chain reaction (RT-PCR) assays are available for CDV detection to facilitate CIRD management, the requirement of complicated procedures and a costly device has limited the application of the techniques. In this study, a portable POCKIT system based on TaqMan probe chemistry and insulated isothermal polymerase chain reaction (iiPCR) technologies was developed to detect CDV from clinical samples on site within 1.5 hour. After optimizing the conditions of primers and probes, the CDV POCKIT system assay can detect CDV specifically, and showed no cross reaction with other common canine pathogens, including parvovirus, coronavirus, adenovirus, *Babesia, Ehrlichia, Hemotropic mycoplasma* and *Toxoplasma*. Moreover, the limit of detection of CDV POCKIT assay was equivalent to that of reverse transcription-nested PCR (RT-nPCR) and real-time RT-PCR (~101 copies of the target RNA/reaction). A total of 105 clinical specimens collected from 44 CDV-suspected dogs were tested simultaneously by CDV POCKIT and RT-nPCR assays and showed 97% agreement. The POCKIT system comes in as a carry-on hard-shell suitcase package including an iiPCR instrument, a mini-centrifuge and two micro pipettes. The reagents are lyophilized in a unit-dose format for room temperature shipping and long term stability storage. The system can take up to eight samples per run. The total run time from sample to result is less than 1.5 hours. Taken together, with its high sensitivity and specificity, ease-of-use and short-turn-around-time, POCKIT can provide kennel and shelter environments a powerful tool for CIRD management.

Thermo SCIENTIFIC

Bacteriology 2

Sunday, October 21, 2012
Guilford E

Sponsor: Thermo Scientific

Moderators: Doreene Hyatt, Deepanker Tewari

8:00 AM **Real-Time PCR Detection of Hemotropic Mycoplasma species in Symptomatic Dairy Cattle from the Midwest United States ◊**
Amanda Kreuder, Adam Herrick, Uri Donnett, Paul Plummer, Jessie D. Trujillo 86

8:15 AM **Detection of *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145 by Antibody Microarray**
Narasimha Hegde, Andrew Gehring, Pina Fratamico, Chitrita DebRoy 87

8:30 AM **Recovery and Identification of *Listeria monocytogenes* During Routine Bacteriology Abortion Screen of Missouri Canine ◊**
Thomas J. Reilly, Michael Calcutt, Irene Ganjam, Sean Spagnoli, K. Kuroki, William H. Fales 88

8:45 AM **Identification of Bacterial Agents Associated with Abortion in Ruminant Species**
Kris Clothier 89

9:00 AM **Diagnostic Investigation of Real-time PCR, Fuorescent Antibody, and Microscopic Agglutination Tests in Cases of Equine Abortion**
Erdal Erol, Neil M. Williams, Stephen Sells, Meg Steinman, Judy Donahoe, Katherine Meares, Alan Loynachan, James M. Donahue, Craig N. Carter 90

9:15 AM **TaqMan Real-time PCR Assay for the Direct Detection of *Campylobacter fetus* subsp. *venerealis* from InpouchTM TF ◊**
Feng Sun, Hemant Naikare, Mangkey A. Bounpheng, Loyd Sneed, Alfonso Clavijo 91

9:30 AM **Break**

10:00 AM **Species Specificity and Molecular Typing of Porcine and Equine *Lawsonia intracellularis* Isolates * † ◊**
Fabio Vannucci, Nicola Pusterla, Samantha Mapes, Yogesh Chander, Molly Kelley, Connie Gebhart 92

10:15 AM **Virulence and Genome Stability of *Lawsonia intracellularis* After Serial Passages *in vitro* * † ◊**
Fabio Vannucci, Dana Beckler, Nicola Pusterla, Samantha Mapes, Yogesh Chander, Connie Gebhart 93

10:30 AM **Seroprevalence of *Coxiella burnetii* in Washington State Domestic Goat Herds # †**
Kerry Sondgeroth, Sara Schlee, Margaret Davis, Andy Allen, James Evermann, Terry McElwain, Timothy V. Baszler 94

10:45 AM **Comparison of Three Common Diagnostic Tests for Leptospirosis # †**
Kenitra Hammac, Gary Haldorson, Thomas E. Besser, Dan S. Bradway, Timothy V. Baszler 95

11:00 AM **Evaluation of Different Fragments of Recombinant *Leptospira* Immunoglobulin-like Protein for Use in a Serologic Test for Equine Leptospirosis**
Weiwei Yan, Patrick L. McDonough, Sean P. McDonough, Bruce L. Akey, Yung-Fu Chang 96

11:15 AM **Isolation of a *Pelistega*-like Organism from a Urethral Swab of a Horse**
Yan Zhang, Jing Cui, Anne Parkinson, Jason Herr, Troy Farrell, Beverly Byrum 97

11:30 AM **Outbreaks of Salmonellosis Associated with Animals**
Yan Zhang, Jing Cui, Anne Parkinson, Mary B. Weisner, Eric Brandt, Rick Bokanyi, Brittany Orlando, Rhiannon Schneider, Jason Herr, Beverly Byrum 98

11:45 AM **Jennifer Lorbach, Thermo Scientific**

Symbols at the end of titles indicate the following designations:

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

Real-Time PCR Detection of Hemotropic Mycoplasma species in Symptomatic Dairy Cattle from the Midwest United States ◊

Amanda Kreuder[2], Adam Herrick[1], Uri Donnett[1], Paul Plummer[2], Jessie D. Trujillo[1]

[1]Center for Advanced Host Defenses, Immunobiotics and Translational Comparative Medicine, Iowa State University, Ames, IA; [2]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA

Narrative: *Mycoplasma wenyonii*, previously *Eperythrozoon wenyonii*, is a non-culturable hemotropic Mycoplasma that infects cattle. In the United States, *M. wenyonii* has been thought to be of low pathogenicity, and reports of clinical disease are rare. An investigation into this organism was initiated in response to an outbreak of clinical disease in multiple dairy cows exhibiting signs previously reported in cattle infected with *M. wenyonii*, including hindlimb edema and reduced milk production. Blood smears from symptomatic cattle were consistent with *M. wenyonii* infection, however, PCR detection was recommended for confirmation. Several previously published qPCR assays for the detection of two bovine hemoplasma species, *Mycoplasma wenyonii* and *Candidatus* Mycoplasma haemobos, were validated in our laboratory for sensitivity and specificity and utilized to detect these hemotropic Mycoplasma species (Meli et., al 2010, 48(10) pg 3563 Journal of Clinical Microbiology). Serial samples from symptomatic and normal cattle demonstrated a high prevalence with cyclicity of hemoplasma species detection in this herd. Normal and symptomatic cattle demonstrated equally high prevalence of *C.*M. haemobos, and, to our knowledge, this is the first utilization of real time PCR for the detection of *C.*M. haemobos detection in the United States. Symptomatic animals had a higher prevalence of *M. wenyonii* than clinically normal herdmates and tended to be more likely to have dual infections with both *M. wenyonii* and *C.*M. haemobos. This work suggests that *M. wenyonii* can cause persistent infection in US cattle, warranting further investigation into the significance of this disease, including its pathogenesis and ecology. Additionally, we demonstrate the necessity of PCR for the sensitive and accurate detection of non-culturable hemotropic Mycoplasma species for investigational studies and diagnostics.

◊ USAHA Paper

Detection of *Escherichia coli* Serogroups O26, O45, O103, O111, O121, and O145 by Antibody Microarray

Narasimha Hegde[1], Andrew Gehring[2], Pina Fratamico[2], Chitrita DebRoy[1]

[1]Veterinary and Biomedical Science, E coli Reference Center, Pennsylvania State University, University Park, PA; [2]Eastern Regional Research Center, USDA ARS, Wyndmoor, PA

Narrative: Shiga toxin producing *Escherichia coli* (STEC) serogroups O26, O45, O103, O111, O121, and O145 have been identified as the "top six" non-O157 STEC by the US. Centre for Disease Control and Prevention as causative agents of diseases with high morbidity and mortality. Food Safety and Inspection Services (FSIS) has recently declared these O groups as adulterants in meat. While there are methods available for identification of O157, no analogous detection procedures are yet available for identification of these serogroups other than by conventional serotyping and PCR assays. The objective was to develop an antibody based microarray to detect the six STEC O groups, O26, O45, O103, O111, O121, and O145 using polyclonal antibodies specific for each O group. Rapid and easy detection and identification methods are crucial for controlling the pathogens to improve food safety and public health. We have recently developed both ELISA assays and flow cytometric methods to detect non-O157 STEC O groups using polyclonal antibodies to specific O groups. Using the same polyclonal antibodies we have developed microarrays for rapid detection of the strains. The antibodies against the six STEC O groups were printed on Super Epoxy glass slides to capture cells. Heat inactivated lipopolysaccharide (LPS) of all six STEC O groups were also printed as positive and negative controls. Bacterial cells (105) were allowed to bind to respective antibodies on the glass slide. After a brief wash, Zenon-labeled (fluorophore) antibodies against each of these O groups were added and the reaction allowed to continue for another 1 h at 37^{o}C, washed with phosphate-buffered saline and read for fluorescent signal under a microscope. The antibody microarray developed for six STEC O groups could detect the reference strains and clinical samples belonging to the target O group. The assay was validated by spiking ground beef samples with different concentrations of a strain belonging to one of the six target O groups following enrichment as per FSIS protocol. The sensitivity of the assay was found to be 1-10CFU/ml. The antibody microarray for each O group was specific for the O group tested and did not cross react with 173 different known *E. coli* O groups or other members of Enterobacteriaceae. The antibody microarray assays described are rapid, sensitive, specific and easy to use that ideally lends themselves applicable to diagnostics for rapid detection of STEC O groups.

Recovery and Identification of *Listeria monocytogenes* During Routine Bacteriology Abortion Screen of Missouri Canine ◊

Thomas J. Reilly[1, 2], Michael Calcutt[2], Irene Ganjam[1], Sean Spagnoli[1, 2], K. Kuroki[1, 2], William H. Fales[1, 2]

[1]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO; [2]Department of Veterinary Pathobiology, University of Missouri, Columbia, MO

Narrative: *Listeria monocytogenes* is a Gram-positive motile facultative anaerobe that inhabits a broad ecologic niche. It can be found in meat and vegetables, is a transient inhabitant of the gastrointestinal tract, and is a cause of septicemia, abortion, and central nervous tract infections in both animals and humans. During a recent routine bacteriological abortion screen of three canine tissues (liver, lung, and placenta), heavy growth of small translucent hemolytic colonies was noted in all samples examined. Results from initial bacteriological testing showed these organisms to be Gram-positive, motile, catalase-positive rods. Further phenotypic testing, employing the ThermoFisher-Trek-Sensititer AP90® Gram-positive identification panel identified the organisms as *Listeria monocytogenes*. Such findings were consistent with initial bacteriological findings and while not commonly noted, were consistent with a potential cause of canine abortion. Genotypic characterization of the isolated organisms showed that (i) ~1250 base pairs of the 16S ribosomal RNA genes were 100% identical to those of over thirty *L. monocytogenes* strains in the current GenBank databases; (ii), the 16S-23S rDNA intergenic spacer region exhibited complete identity to multiple *L. monocytogenes* isolates and characteristic differences to the equivalent locus from other taxa of the Listeria genus and (iii), the hly gene, encoding the Listeriolysin O toxin, was identical to other *L. monocytogenes* hly genes. In addition, upon histopathological evaluation, large numbers of bacteria adhered to the placenta and had strongly positive immunoreactivity for Listeria antigen in immunohistochemically-stained sections. Interestingly while *L. monocytogenes* is a known cause of canine abortions, it is not a common finding and while the "picture" presented here appears "classic," the isolated strain was CAMP-negative and identified by the Biomerieux API®Listeria system as *Listeria innocua*. Additional genotypic characterization of this isolate are currently in progress

◊ USAHA Paper

Identification of Bacterial Agents Associated with Abortion in Ruminant Species

Kris Clothier

California Animal Health and Food Safety Lab, UC Davis, Davis, CA

Narrative: Bacterial abortion is an economic concern in ruminants. Detection of the causative agent can be difficult due to the potential for an extended time from fetal death to expulsion, to the condition of the fetus at examination, or to the presence of opportunistic organisms. Selection of the most appropriate specimen for testing can have a direct impact on the successful identification of these agents. To address this question, a review of 1352 (972 bovine, 271 caprine, and 109 ovine) abortion cases submitted for bacterial diagnostics to the California Animal Health and Food Safety Lab System (CAHFS) over a five year period of time was performed. A definitive bacterial diagnosis was obtained for 22.9%, 45.2% and 66.7% of bovine, caprine, and ovine cases, respectively. *Campylobacter sp.* was the most frequently cultured bacterial agent in all three species (1.5% in cattle, 1.5% in goats, 9.2% in sheep.) In cattle, *C. fetus* ssp *fetus* (22.2%), *C. fetus* ssp *venerealis* (27.8%), and *C. jejuni* ssp *jejuni* (16.7%) were recovered at nearly identical rates. Additionally, *C. hyointestinalis* was recovered in 11.1% of abortion samples. In sheep and goats, *C. jejuni* ssp *jejuni* accounted for 92.9% of Campylobacter associated with abortion. Samples collected from the abomasum had the greatest Campylobacter recovery. *Listeria spp.* (1.2% in cattle) was cultured from liver and placenta; and other presumed opportunists associated with septicemia in the fetus, including Salmonella (3.0% in cattle), *Staphylococcus aureus* (1.2% in cattle), and *E. coli* (0.5% in cattle, 0.6% in sheep) were most commonly recovered from liver and lung tissues. Other bacterial agents, such as *Leptospira spp.* (1.0% in cattle, 3.7% of sheep), *Chlamydia/Chlamydophila spp.* (2.8% in goats, 1.8% in sheep), *Coxiella burnetii* (5.2% of goats, 2.3% of sheep), and Epizootic Bovine Abortion, attributed to a novel deltaproteobacterium, required ancillary diagnostic such as immunoflorescent antibody (IFA) testing or immunohistochemical (IHC) staining. More definitive reviews of the fetal locations most likely to result in bacterial identification can improve the odds of recovering bacterial agents associated with abortion in ruminants.

Diagnostic Investigation of Real-time PCR, Fuorescent Antibody, and Microscopic Agglutination Tests in Cases of Equine Abortion

Erdal Erol, Neil M. Williams, Stephen Sells, Meg Steinman, Judy Donahoe, Katherine Meares, Alan Loynachan, James M. Donahue, Craig N. Carter

Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY

Narrative: Leptospirosis is an important cause of equine abortion. From a total of 339 equine fetuses necropsied, 21 cases were diagnosed as leptospiral abortion at the University of Kentucky Veterinary Diagnostic Laboratory between September 1, 2011, and April 10, 2012. Twenty abortion cases occurred between November 1, 2011, and February 20, 2012, almost exclusively in the last trimester. A *Leptospira spp*. fluorescent antibody (FA) test, microscopic agglutination test (MAT) and real-time PCR assay targeting the LipL32 gene were compared, for the first time, in fetal specimens (placenta, kidney, liver and heart blood) and maternal serum in order to determine the best assay(s), to diagnose leptospiral abortions. Real-time PCR detected 21 of 21 cases, whereas MAT and FA detected 19 and 18 cases, respectively. Combination of MAT and FA detected 20 cases correctly. In two leptospirosis cases detected by real-time PCR, antibodies in fetal heart blood were undetectable by MAT, suggesting the possibility of acute infection with inadequate time for the fetus to develop antibodies. Comparing tissues, placental and kidney specimens yielded somewhat similar Ct values (except in one case) by real-time PCR assay, whereas kidney was the most sensitive specimen for the FA test. Based on the MAT results, in 18 out of 19 cases, the predominate titer was to serovar Pomona (ranging 1:100 to 1:204,800 in fetuses) even though some mares had high cross-reacting titers against serovar Grippotyphosa. The results indicate that real-time PCR is an effective method for the diagnosis of leptospiral abortion cases in horses.

TaqMan Real-time PCR Assay for the Direct Detection of *Campylobacter fetus* subsp. *venerealis* from InpouchTM TF ◊

Feng Sun[1], Hemant Naikare[2], Mangkey A. Bounpheng[1], Loyd Sneed[1], Alfonso Clavijo[1]

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

Narrative: Bovine genital campylobacteriosis, caused by *Campylobacter fetus* subsp. *venerealis* (CFV), is a highly contagious sexually transmissible disease. Infection with this agent may lead to serious reproductive problems including sterility and abortion. Bovine genital campylobacteriosis is listed as category B notifiable disease in OIE, and is considered to have significant economic and public health implications, particularly with respect to the international trade of animals and animal products. Quick and sensitive identification of this agent is becoming very important to the cattle industries worldwide. Culture is normally a routine identification method for CFV, however, it is limited by several factors, such as medium selection, transport time from sampling to processing and growth conditions. In addition, the differentiation between CFV and *Campylobacter fetus* subsp. *fetus* (CFF) by culture still remains challenging. The objective of the present study was to develop a rapid, sensitive, and specific diagnostic test for the direct detection and identification of *Campylobacter fetus* subsp. *venerealis* using the InpouchTM TF system (Biomed Diagnostics) used for *Trichomonas foetus* collection. Using the same InpouchTM TF to directly detect both *Trichomonas foetus* and CFV will save money, time, and labor. A unique set of primers and a TaqMan probe for *Campylobacter fetus* subsp. *venerealis* specific PCR assay were designed from insertion sequence ISCfe1, tnpA gene, tnpB gene, metT gene, and smtA gene of CFV referenced Genbank database. The real time PCR (qPCR) assay was developed, optimized and its performance was evaluated by comparing with the conventional PCR (Hum et al., *Aust. Vet. J.* 1997). Limit of detection of the qPCR assay was found to be ~ 587 copies. The genomic DNAs from a total of 510 InpouchTM TF preputial wash specimens were extracted using the Kingfisher 96 MagMax nucleic acid extraction kit (Ambion). One hundred twenty specimens were found to be CFV positive by the qPCR assay. Fifty-one samples were then selected for sequencing in order to confirm the specificity of the qPCR assay. Sequencing results confirmed all samples as *Campylobacter fetus* subsp. *venerealis*. In addition, no cross-reactivity was found with forty-two close related bacterial pathogens as no amplification occurred with the genomic DNAs from these bacteria. In conclusion, the TaqMan real-time PCR method and direct DNA extraction from InpouchTM TF prepucial wash samples provides a rapid, reliable, and sensitive tool for direct detection of *Campylobacter fetus* subsp. *venerealis* from clinical samples.

◊ USAHA Paper

Species Specificity and Molecular Typing of Porcine and Equine *Lawsonia intracellularis* Isolates * † ◊

Fabio Vannucci[1], Nicola Pusterla[2], Samantha Mapes[2], Yogesh Chander[1], Molly Kelley[1], Connie Gebhart[1]

[1]Veterinary and Biomedical Science, University of Minnesota, St. Paul, MN; [2]Department of Veterinary Medicine and Epidemiology, University of California, Davis, CA

Narrative: *Lawsonia intracellularis* is the causative agent of proliferative enteropathy (PE), an endemic disease in pigs and an emerging concern in horses. Enterocyte hyperplasia is a common lesion in every case but there are differences regarding clinical and pathological presentations among affected species. The objective of this study was to evaluate the susceptibilities of pigs and horses to *L. intracellularis* infection using porcine and equine isolates and compare the molecular typing of these and other *L. intracellularis* isolates. Twelve foals were divided into three groups (n=4/group) and infected with a porcine or an equine isolate and saline solution (control group). An identical experimental design was applied to 18 pigs divided into three groups (n=6/group). The animals were monitored regarding clinical signs, fecal shedding of *L. intracellularis* and humoral serological response during 56 days post-infection (PI). The variable number tandem repeat (VNTR) profiles of both porcine and equine isolates from this experiment and from three pig herds, 14 horse sites, and various other animal species were determined. Fecal shedding and serologic response were higher and longer in foals infected with the equine isolate compared with foals infected with the porcine isolate or with the negative-control group. One equine-isolate infected foal developed severe clinical signs and was euthanized 24 days PI. Typical lesions and marked presence of Lawsonia antigen was identified by IHC. Similarly, reduced average daily gain and diarrhea were observed in pigs infected with the porcine isolate. Only porcine isolate-infected pigs demonstrated proliferative lesions associated with the presence of specific Lawsonia antigen by IHC. Additionally, these animals showed higher and longer shedding of bacteria in the feces and serologic response compared with equine isolate-infected pigs. The VNTR typing profiles were conserved within outbreaks in horse and pig farms and slight variations were observed between porcine and equine isolates from different geographic locations. Moderate variation in VNTR types were found between isolates from horse and other animal species, with the most marked differences were found compared to pig isolates. Marked clinical signs, longer periods of bacterial shedding and stronger immune responses were observed in animals infected with species-specific isolates supporting our hypothesis that host susceptibilities can be driven by the origin of the bacterial isolate. The molecular typing results will further enhance our understanding of the transmission dynamics and epidemiology of PE within and between host species. Currently, the whole genome sequencing of the porcine and equine isolates used is being conducted in order to associate these phenotypic characteristics with potential genomic variations.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Virulence and Genome Stability of *Lawsonia intracellularis* After Serial Passages *in vitro* * † ◊

Fabio Vannucci[1], Dana Beckler[2], Nicola Pusterla[3], Samantha Mapes[3], Yogesh Chander[1], Connie Gebhart[1]

[1]College of Veterinary Medicine, University of Minnesota, Saint Paul, MN; [2]Gut Bug, Inc., Fergus Falls, MN; [3]Department of Veterinary Medicine and Epidemiology, University of California, Davis, CA

Narrative: *L. intracellularis* is an obligate intracellular bacterium and causative agent of porcine proliferative enteropathy. Clinical disease has been reproduced using pure cultures after up to 13 passages in cell culture. Non-pathogenic strains have been obtained through multiple passages; however, there is no information regarding the number of passages necessary to attenuate a pathogenic isolate. The present study evaluated the susceptibility of pigs to *L. intracellularis* after 10, 20 and 40 passages in vitro. The whole genome sequencing of a pathogenic isolate (passage 10) was compared with the homologous non-pathogenic isolate (passage 60). Twenty four 3-week-old pigs were divided into four groups (n=6/group). Three groups were infected with a pure culture of *L. intracellularis* on passage 10, 20 or 40 and one group with placebo. Regardless of cell passage, the challenge doses were standardized to 109 bacterial organisms per pig. The animals were monitored for clinical signs, fecal shedding and serological response during 28 days post-inoculation (PI). Two animals from each group were euthanized on days 14, 21 and 28 PI. The levels of infection were graded by immunohistochemistry (IHC) based on the amount of positive labeled antigen in the intestinal epithelium. The whole genome of this isolate was sequenced using high-throughput Illumina® technology. The genome comparisons between *L. intracellularis* isolate on passages 10 and 60 were performed using Sequencher® 5.0 and Tablet® software. This bioinformatics tools allowed the visualization of genomic variations present in the bacterial chromosome and its three plasmids. Animals infected with passages 10 and 20 demonstrated proliferative lesions associated with the presence of Lawsonia-specific antigen in the intestinal epithelium. Passage 40-infected pigs did not show proliferative lesions or presence of Lawsonia-antigen at any time point. There was no significant difference in the magnitude and duration of fecal shedding between animals infected with passages 10 and 20. However, a significant ($p<0.05$) lower amount and much shorter period of *L. intracellularis* DNA shedding was identified in the feces of pigs infected with passage 40. Additionally, serological IgG responses were observed in passages 10 and 20-infected but not in passage 40-infected animals. Based on these results, complete attenuation was observed to occur between 20 and 40 cell passages in vitro. We believe this information will be valuable for future experimental models and for studying the mechanisms involved in the attenuation of *L. intracellularis* virulence. The comparative wide genome analysis showed a deletion of 18,088 bp in the non-pathogenic homologous *L. intracellularis* isolate passed 60 times in vitro. This region comprises 15 protein-encoded genes including prophage DLP12 integrase. The identification of immunogenic proteins encoded within this region may be useful for differentiation of infected and vaccinated animals.

* Graduate Student Poster Presentation Award Applicant
† Graduate Student Oral Presentation Award Applicant
◊ USAHA Paper

Seroprevalence of *Coxiella burnetii* in Washington State Domestic Goat Herds # †

Kerry Sondgeroth[1, 2], Sara Schlee[1], Margaret Davis[2], Andy Allen[3], James Evermann[1, 3], Terry McElwain[1, 2], Timothy V. Baszler[1, 2]

[1]Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman, WA; [2]Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [3]Veterinary Clinical Sciences, Washington State University, Pullman, WA

Narrative: A caprine herd seroprevalence of *C. burnetii* infection was determined by passive surveillance of domestic goats in Washington State, US. Serum samples (n=1891) from 107 herds in 31 counties were analyzed for *C. burnetii* antibodies using a commercially available Q-Fever antibody ELISA test kit. The sera were submitted to the Washington State University, Washington Animal Disease Diagnostic Laboratory for routine herd serologic screening over an approximate one year period from November, 2010, through November, 2011. To target testing of goats without clinical disease (healthy animal survey), only submissions representing goat herds of 5 or greater were included. A cluster sampling approach was used to determine sample size. The results identified *C. burnetii* antibodies in 9.9% of samples tested (188/1891), 8.4% of goat herds tested (9/107), and 25.8% of counties tested (8/31). Within herd seroprevelance in positive counties ranged from 3.4% to 75%. Counties with seropositive goats were represented in the major geographically distinct areas of the state (west, central and east). To our knowledge this is the first county specific, statewide study of *C. burnetii* goat herd seroprevalence in Washington State. The findings provide baseline information for future epidemiology, herd management and public health investigations of Q-fever.

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

Comparison of Three Common Diagnostic Tests for Leptospirosis # †

Kenitra Hammac, Gary Haldorson, Thomas E. Besser, Dan S. Bradway, Timothy V. Baszler

Veterinary Microbiology and Pathology, Washington State University, Pullman, WA

Narrative: Diagnosis of leptospirosis can be challenging due to nonspecific clinical signs in a wide range of hosts and diagnostic tests that may be subjective or produce inconsistent results. Observation of discordant test results in WADDL led to development of a retrospective study to compare three common diagnostic tests for leptospirosis: fluorescent antibody (FA), immunohistochemistry (IHC) and polymerase chain reaction (PCR). The tests were applied to the same set of cases in order to define patterns of inconsistencies as a first step toward determining a more consistent diagnostic approach. Fifty seven cases were selected based on the availability of fixed paraffin-embedded tissues for IHC and PCR, including 27 FA positive cases and 30 FA negative cases. Pairwise comparisons of the FA, IHC and PCR test results were evaluated by Kappa and Fisher's Exact tests. There was fair to substantial agreement between IHC and PCR, but only slight to moderate agreement between FA and either IHC or PCR. Many FA positive cases were negative by IHC and PCR, and four FA negative cases tested positive by IHC or PCR. In addition, IHC evaluations of the same cases by different pathologists frequently resulted in different interpretations. These findings clearly reflect shortcomings of the subjectively read tests (FA and IHC), and suggest that PCR may be the most reliable approach to leptospirosis diagnosis.

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

Evaluation of Different Fragments of Recombinant *Leptospira* Immunoglobulin-like Protein for Use in a Serologic Test for Equine Leptospirosis

Weiwei Yan[1], Patrick L. McDonough[1], Sean P. McDonough[2], Bruce L. Akey[1], Yung-Fu Chang[1]

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

Narrative: Leptospirosis is a worldwide zoonosis. The prevalence of leptospiral infection in horses may be greater than that for other species, but most of the presenting signs are nonspecific creating a major obstacle for the clinical diagnosis of disease. *Leptospira* immunoglobulin (Ig)-like protein (Lig protein) is a novel family of surface-associated proteins shown to be present exclusively in pathogenic but not saprophytic *Leptospira* species. Lig proteins are expressed during host infection and appear to induce strong antibody responses in infected animals. In present research, we truncated and expressed the conserved region of the Lig protein into 7 fragments comprising the 1st to 3rd (Lig1-3), 4th to 7th(Lig4-7), 4th(Lig4), 4.5th to 5.5th(Lig4.5-5.5), 5.5th to 6.5th(Lig5.5-6.5), 4th to 5th(Lig4-5) and 6th to 7th(Lig6-7) Lig domains of *L. interrogans* serovar *pomona*. All these 7 trunked recombinant Lig proteins were screened by slot shaped dot blot assay for the diagnosis of equine leptospirosis. Serum samples that were either positive or negative in the leptospiral microscopic agglutination test (MAT) to the most common serovars causing equine leptospirosis, including *L. interrogans* serovar *pomona*, *L. kirschneri* serovar *grippotyphosa*, and *L. interrogans* serovar *icterohaemorrhagiae*, which were used in the study. Our results showed that Lig4-7 was the best candidate antigen for use in the slot shaped dot blot assay. Based on the screening results, Lig4-7 was selected and evaluated as an indirect ELISA antigen for detection of *Leptospira* antibodies in equine sera. This assay was evaluated with equine sera (n=60) that were MAT-negative and sera (n= 220) that were MAT-positive to the 5 serovar that most commonly cause equine leptospirosis. The indirect ELISA results showed that at a single serum-dilution of 1:250, the sensitivity and specificity of ELISA were 80.2% and 87.5% respectively compared to microscopic agglutination test (MAT). In conclusion, an indirect ELISA was developed utilizing a recombinant protein fragment comprising 4th to 7th (Lig4-7) of Lig protein in serodiagnosis of equine leptospirosis. This ELISA assay was found to be sensitive, specific and in agreement with the standard MAT.

Isolation of a *Pelistega*-like Organism from a Urethral Swab of a Horse

Yan Zhang, Jing Cui, Anne Parkinson, Jason Herr, Troy Farrell, Beverly Byrum

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

Narrative: A urethral swab sample from an 8-month-old Hackney horse was submitted to the Ohio ADDL for an export screen for *Taylorella equigenitalis*. After three days of incubation, a few suspect colonies resembling *T. equigenitalis* appeared. The organism grew on blood agar but not on MacConkey agar. It was Gram negative, rod-shaped, and non-spore forming, and positive for oxidase and catalase. *T. equigenitalis* specific PCR was positive. DNA sequencing analysis of the 16S rRNA gene demonstrated that the organism was likely to be a new species that is most closely related to *Pelistega europaea* (95% identical) and distantly related to *T. equigenitalis*.

Outbreaks of Salmonellosis Associated with Animals

Yan Zhang[1], Jing Cui[1], Anne Parkinson[1], Mary B. Weisner[1], Eric Brandt[2], Rick Bokanyi[2], Brittany Orlando[3], Rhiannon Schneider[4], Jason Herr[1], Beverly Byrum[1]

[1]Ohio Department of Agriculture, Animal Disease Diagnostic Laboratory, Reynoldsburg, OH; [2]Ohio Department of Health, Bureau of Public Health Laboratory, Reynoldsburg, OH; [3]College of Public Health, The Ohio State University, Columbus, OH; [4]Department of Entomology, Pennsylvania State Univeristy, University Park, PA

Narrative: Salmonellosis is an infection caused by *Salmonella* species. In humans, the infection is characterized by diarrhea, fever, and abdominal cramps lasting 4 to 7 days. However, in a severe case, a patient needs to be hospitalized due to systemic septicemia. Here, we report the isolation and characterization of *Salmonella* organisms from animals that were linked to two multistate outbreaks in humans.

Epidemiology 2

Sunday, October 21, 2012

Guilford F

Moderators: Ashley Hill, M.D. Salman

8:00 AM **Effect of Mixed Livestock Production Types on Foot-and-Mouth Disease Outbreaks and Interventions * †**
Sara W. McReynolds, Mike W. Sanderson, Aaron Reeves *101*

8:15 AM **Comparing Vaccine Protocol of Vaccinating Large Feedlots Only to Vaccinating All Livestock Production Systems * †**
Sara W. McReynolds, Mike W. Sanderson, Aaron Reeves *102*

8:30 AM **Canine Dysautonomia in Wyoming: 24 Cases [2004-2012] # †**
Noah C. Hull, Kathryn Drumhiller, Donal O'Toole, Brant Schumaker *103*

8:45 AM **Chimeric protein A/G conjugate for detection of anti-*Toxoplasma gondii* immunoglobulin G in multiple animal species # †**
John J. Schaefer, Holly A. White, Stephanie L. Schaaf, Hussni O. Mohammed, Susan E. Wade *104*

9:00 AM **Sequence Confirmation of *Tritrichomonas foetus* Samples Previously Tested with MagMAX™ Sample Preparation System and VetMAX® *T. foetus* Reagents**
Ivan Leyva Baca, Marilyn Simunich, Lalitha Peddireddi, Richard D. Oberst, Lee J. Effinger, Catherine O'Connell *105*

9:15 AM **Pathogenic Lungworms in Maine Moose: not *Dictyocaulus viviparus* ◊**
Darryl A. Girardin, Sarah Barker, Jana Drury, Lee Kantar, Anne Lichtenwalner *106*

9:30 AM **The Combination of Abundance and Infection Rates of *Culicoides sonorensis* Estimates Risk of Subsequent Bluetongue Virus Infection of Sentinel Cattle on California Dairy Farms ◊**
Christie E. Mayo, Bradley Mullens, Christopher Barker, Alec Gerry, Ian Gardner, James MacLachlan *107*

9:45 AM **Break**

10:15 AM **Evaluation of Bovine Viral Diarrhea Virus Infection in Hunter-harvested White-tailed Deer (*Odocoileus virginianus*) in Georgia using Ear-notch Samples**
Marcia Regina Da Silva Ilha, Michele Coarsey, Lisa Whittington, Sreekumari Rajeev, Sheela Ramamoorthy *108*

10:30 AM **Introduction of the IDEXX Bovine Viral Diarrhea Virus Antigen ELISA (IDEXX BVDV PI X2 Test)**
Anthony Mestek, Katherine Velek, Shona Michaud, Lisa Estey, Sergio Lizano *109*

10:45 AM **Discovery of a Broad Diversity of Mycoplasma species in Companion Animals Utilizing a PanMyco PCR Assay**
Uri Donnett, Adam Herrick, Claudia Baldwin, Peter Nara, Jessie D. Trujillo *110*

11:00 AM **Investigation of *Corynebacterium pseudotuberculosis* cases in Texas Horses from 2005 to 2011**
Barbara Szonyi, Amy K. Swinford, Alfonso Clavijo *111*

11:15 AM **Scoliosis in Three Gopher Tortoises from Baker County, Georgia**
Brandon Munk, Jessica McGuire, Mark G. Ruder, Justin Brown *112*

11:30 AM **Quality Assurance: Sharing the Tools that Support Sound Science**
Rebecca Davies, Shirley D. Johnston *113*

Symbols at the end of titles indicate the following designations:

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

Effect of Mixed Livestock Production Types on Foot-and-Mouth Disease Outbreaks and Interventions * †

Sara W. McReynolds[1], Mike W. Sanderson[2], Aaron Reeves[3]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS; [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS; [3]Animal Population Health Institute, Colorado State University, Fort Collins, CO

Narrative: The central United States (US) has a large livestock population including cattle, swine, sheep, and goats. Simulation models were developed to assess the impact of livestock herd types and vaccination on Foot-and-Mouth Disease (FMD) outbreaks using the North American Animal Disease Spread Model (NAADSM), a spatially explicit, stochastic infectious disease model. Based on data from the US Department of Agriculture National Agricultural Statistic Service, a simulated population of livestock operations was generated. The population included 151,620 herds defined by latitude and longitude, production type, and herd size. The population was of herds that were all FMD susceptible except for a single 17,000 head feedlot selected as the initial latently infected herd. Direct and indirect contact rates between herds were based on survey data of livestock producers in Kansas and Colorado or estimated from expert opinion. The livestock producer survey indicated a significant proportion of mixed beef-swine herds (approximately 8% of herds) not accounted for in previous models. As such, scenarios were simulated in two populations, one without beef-swine herds and one with beef-swine herds. Simulated vaccination protocols included vaccination zones of 10 km vs. 50 km, and vaccination trigger of 10 herds compared to 100 herds. Scenarios were simulated in each population with either no vaccination or a vaccination ring around each infected premise. The results of the scenarios were compared to assess the effect of mixed beef-swine herds on the impact of the outbreak and the effect of vaccination. The scenarios that included the mixed beef-swine population had similar results to those that did not include the mixed population. Results demonstrated that the inclusion of mixed beef-swine herds had minimal impact on the median duration of disease, and the total number of herds and animals destroyed. While inclusion of mixed production types could increase the contact rates between different livestock production types, this did not seem influential for the modest proportion of mixed production types in these scenarios where the outbreak began in a large feedlot. Modeling of FMD outbreaks may be robust to small misspecification of production types. Additional scenarios to further evaluate the effect of mixed production types under different starting conditions are needed to better estimate their effects.

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

Comparing Vaccine Protocol of Vaccinating Large Feedlots Only to Vaccinating All Livestock Production Systems * †

Sara W. McReynolds[1], Mike W. Sanderson[2], Aaron Reeves[3]

[1]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS; [2]Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS; [3]Animal Population Health Institute, Colorado State University, Ft. Collins, CO

Narrative: The central United States (US) has a large livestock population including cattle, swine, sheep, and goats. Simulation models were developed to assess the impact of livestock herd types and vaccination on Foot and Mouth Disease (FMD) outbreaks using the North American Animal Disease Spread Model (NAADSM), a spatially explicit, stochastic infectious disease model. Based on data from the US Department of Agriculture National Agricultural Statistic Service, a simulated population of livestock operations was generated. The population included 151,620 herds defined by latitude and longitude, production type, and herd size. For the simulations, a single 17,000 head feedlot was selected as the initial latently infected herd in an otherwise susceptible population. Direct and indirect contact rates between herds were based on survey data of livestock producers in Kansas and Colorado or estimated from expert opinion. Scenarios were simulated for different vaccination protocols compared to depopulation only. Ring vaccination of herds was triggered around infected herds. Feedyards >3,000 head had the highest vaccination priority. Simulated vaccination protocols included low and high vaccine capacity based on results from a livestock producer survey and expert opinion, vaccination zones of 10km vs. 50km, and vaccination trigger of 10 herds compared to 100 herds. There was a decrease in the magnitude of outbreaks in scenarios where vaccination was combined with depopulation compared to scenarios with only depopulation. The size of the vaccination zone had a substantial effect on the outbreak, while vaccine capacity and vaccination trigger had a smaller effect. The length of the outbreak and the number of herds destroyed increased with decreasing vaccination zone diameter, vaccine capacity and increased number of herds infected at initiation of vaccination. The results of the scenarios were compared to assess the effect of vaccination on the duration of the outbreak and the number of animals destroyed during the outbreak. Increased size of the vaccination zone during an outbreak may lead to decreased length of the outbreak and number of herds destroyed. Increasing the vaccination capacity had a smaller impact on the outbreak and may not be feasible if vaccine production and delivery is limited. In these scenarios, feedyards >3,000 head had the highest vaccination priority, and even with larger vaccine capacity few other production types were vaccinated in some scenarios. Additional scenarios to further evaluate the effect of vaccination priorities are needed to better estimate the effects of vaccination.

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

Canine Dysautonomia in Wyoming: 24 Cases [2004-2012] # †

Noah C. Hull, Kathryn Drumhiller, Donal O'Toole, Brant Schumaker

Department of Veterinary Sciences, University of Wyoming, Laramie, WY

Narrative: Canine dysautonomia (CD) is a sporadic, generally fatal autonomic neurological disorder of dogs in the United States and Western Europe, including the United Kingdom. The first reported case in the US occurred in 1989 in Wyoming, and was subsequently published in 1991. Most US cases have been recognized in Missouri, Kansas, Colorado, Nebraska, southern Illinois, the Texas panhandle, and western Kentucky. The cause of CD is unknown. Progression is rapid (several days – 2 weeks), with euthanasia or death in >90% of dogs. We report 24 cases of histologically-confirmed CD. Most affected dogs were <24 months old (median = 7 months; youngest: 2 months; oldest: 48 months). Vomiting and ileus on clinical presentation in many cases suggested enteric foreign body obstruction, resulting in exploratory surgery with negative results. Clinical progression involved loss of anal tone and bladder control, mydriasis, prolapsed 3rd eyelid, decreased tear production and, in dogs with extended survival times, posterior paresis. All confirmed cases of CD were confined to the eastern half of the Wyoming, affecting 11 of the state's 23 counties. CD in Wyoming typically affected ranching or working dogs that were largely unsupervised outdoors and had access to cattle and cattle pastures. Two multi-case households were found. One episode affected three animals (all histologically confirmed) with an additional two suspected but histologically unexamined cases over a 20-year period. All dogs in the household were Irish Setters. A second property had two histologically confirmed cases over a 4-month period. All dogs in this household were Pit Bull Terriers. None of the dogs in these episodes were genetically related. Multiple owners reported recent ingestion of soil or recent digging/turning of soil (construction/agriculture) coinciding with the time of clinical onset. Exposure to soil by ingestion or recent excavation is a reported risk factor in other states. An environmental toxin (biological or synthetic) is a plausible etiologic factor. Two samples of soil from one multi-case household and the local environment were analyzed using the BioLog© identification system. None of the bacterial species identified in soil were known to produce neurotoxins. Future directions of study include 16S rRNA extraction to evaluate GI flora of affected animals versus controls and 16S rRNA extraction to evaluate soil samples taken from other affected properties.

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

Chimeric protein A/G conjugate for detection of anti-*Toxoplasma gondii* immunoglobulin G in multiple animal species # †

John J. Schaefer, Holly A. White, Stephanie L. Schaaf, Hussni O. Mohammed, Susan E. Wade

Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY

Narrative: Serological testing for toxoplasmosis diagnosis remains the method of choice in human medicine due to the accessibility of the requisite sample, the difficulty in predicting the parasite's location in the host for direct detection and the availability of established commercial methods. In veterinary medicine, though the first two conditions are unchanged, there is a need for commercially produced test methods that are validated for *Toxoplasma gondii* detection across the range of animal species that can serve as intermediate hosts. The development of a serological method for animals would allow the diagnosis of toxoplasmosis in individual animals and a higher throughput method for population level toxoplasmosis surveys. The incorporation of a non-species specific chimeric protein A/G conjugate into an anti-*Toxoplasma* IgG enzyme linked immunosorbent assay (ELISA) is described. Serum from potential intermediate hosts was reevaluated using this method and compared to earlier testing using an established agglutination procedure. Very good agreement between the two tests was noted ($\kappa = 0.81$), establishing the method as a useful option for veterinary diagnostic testing.

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

Sequence Confirmation of *Tritrichomonas foetus* Samples Previously Tested with MagMAX™ Sample Preparation System and VetMAX® *T. foetus* Reagents

Ivan Leyva Baca[1], Marilyn Simunich[2], Lalitha Peddireddi[4], Richard D. Oberst[4], Lee J. Effinger[3], Catherine O'Connell[1]

[1]Aninal Health, Life Technologies, Austin, TX; [2]Animal Health Laboratory, Idaho State Department of Agriculture, Boise, ID; [3]Animal Health & ID Division, Department of Agriculture, Salem, OR; [4]College of Veterinary Medicine, College of Veterinary Medicine, Kansas State University, Manhattan, KS

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. Thus, the implementation of an accurate diagnostic test is fundamental to maintain a clean herd and a successful breeding program. Increased interest in implementing a confirmatory test has been identified in private and government diagnostic labs; this interest has led to the development of a sensitive test for *T. foetus* qPCR. Suspect samples are those tested by qPCR that show signal with (Ct ≥38). Normally a suspect sample needs to be re-tested by qPCR in order to confirm a positive or negative result. In the current study, the specificity of the VetMAX®*T. foetus* reagents was investigated on bull smegma samples obtained from several regions of the United States. Thus the aim of this study was to implement DNA sequencing technology as a confirmatory test for *T. foetus* positive and suspect samples tested with the MagMAX™ and amplified with VetMAX® *T. foetus* reagents. Sample identification was conducted with the collaboration of 5 feeder diagnostic state or university labs (CA, KS, NM, and two TX). Samples from 176 positive and 610 negative bulls were collected and individually processed for DNA extraction with the MagMAX™ pathogen RNA/DNA kit using cultured media and tested with VetMAX® *T. foetus* reagents. Sequencing results showed that all 176 positive samples; with Ct values ranging from 17.49 to 39.53 (including 4 samples with Ct values in the suspect range), were confirmed as *T. foetus*; therefore, the VetMAX® *T. foetus* reagents were proven to be highly specific. 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.

Pathogenic Lungworms in Maine Moose: not *Dictyocaulus viviparus* ◊

Darryl A. Girardin[1], Sarah Barker[2], Jana Drury[1], Lee Kantar[3], Anne Lichtenwalner[1]

[1]Animal and Veterinary Science, University of Maine, Orono, ME; [2]Aquaculture Research Institute, University of Maine, Orono, ME; [3]Inland Fish and Wildlife, State of Maine, Bangor, ME

Narrative: Lungworms (presumably *Dictyocaulus spp.*) along with heavy winter tick (*Dermacentor albipictus*) infestation, have been reported to be associated with mortality in young moose in the Northeastern US. However, the pathogenicity of these lungworms, as well as definitive species identification, has not been well established. In Maine during 2011-12, six cases of young moose with heavy infestations of lungworm were necropsied in our laboratory. Clinical manifestations of lungworm infections in young moose included severe irregular dorso-caudal lobular congestion contrasting with lobular ischemic necrosis, manifesting as a "checkerboard" appearance of the lung lobes. Numerous coiled slender white nematodes, 0.5-1 inch in length, were seen in the airways. Histologic findings in the lungs included severe lobular chronic/active interstitial inflammation, extensive interstitial and peribronchial fibrosis, with partial obliteration of the alveoli. Cut sections of nematodes were visible within the interstitial tissues. Multifocal areas of bronchial mural fibrosis, mucosal hypertrophy and luminal "plugging" were seen. Adjacent lung lobules were either slightly hyperinflated and relatively normal in appearance, or severely and extensively congested within the vascular spaces, with some hemorrhage into the interstitial spaces. These lesions were seen in young moose with high numbers of both attached (feeding) and of unattached winter ticks. To investigate the species of lungworm, we collected lungworm samples from deceased Maine moose obtained as clinical cases or during the legal hunt. Eviscerated lungs were evaluated by pouring saline solution into the trachea, massaging the lungs, and then filtering the recovered saline with muslin. Lungworm adults were visually identified, measured and photographed for morphological identification, and preserved in ethanol or isopropyl for genomic DNA extraction. In some animals, fecal samples were also evaluated for lungworm ova. In order to speciate the lungworms, we developed a PCR assay based on the Dictyocaulus ribosomal internal transcribed spacer region 2 (ITS-2). The ITS-2 sequence from 3 adult worms, collected from two heavily infected Maine moose, were cloned and sequenced. The ITS-2 sequence from these 3 worms were 99% similar to each other. Compared to other known sequences of *Dictyocaulus spp.* from the NCBI database, the ITS-2 sequences from Maine isolates were closely homologous (92-96%) to *Dictyocaulus eckerti* and to three isolates reported to be found in red deer in New Zealand, but only 77% homologous to *Dictyocaulus viviparous*. Phylogenetic analysis suggests *D. eckerti* and these isolates share a common ancestor, but *D. eckerti* has since diverged. Based on these preliminary findings, we hypothesize that Maine moose are host to a previously unreported species or subspecies of Dictyocaulus, closely related to *Dictylocaulus eckerti*.

◊ USAHA Paper

The Combination of Abundance and Infection Rates of *Culicoides sonorensis* Estimates Risk of Subsequent Bluetongue Virus Infection of Sentinel Cattle on California Dairy Farms ◊

Christie E. Mayo, Bradley Mullens, Christopher Barker, Alec Gerry, Ian Gardner, James MacLachlan

Veterinary Medicine: Pathology, Microbiology & Immunology, UC Davis, Davis, 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). 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. 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. Given recent changes in the global distribution of BTV infection, we initiated an epidemiological study of BTV infection in California. The objective of the current study was to evaluate the interaction of population dynamics of *C. sonorensis* midges with the seasonal occurrence of BTV infection of cattle at individual dairy farms in the northern Central Valley of California. Specifically, we determined the seasonal patterns of abundance and infection rates of vector *C. sonorensis* midges at each farm, as estimated using different insect trapping methods (CO_2 baited traps equipped with and without UV light, and mechanical aspiration directly from cows using a modified hand-held household vacuum). Further, we determined the serotypes of BTV present at each farm using a sensitive quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) assay for both midges and sentinel cattle. Evaluation of midges for BTV infection rates indicated the number of serotypes circulating differed markedly among the individual farms. More serotypes of BTV were present in midges than in sentinel cattle at individual farms where BTV circulated, and the virus was detected at each farm in midges prior to detection in cattle. BTV infection rates were remarkably lower amongst female *C. sonorensis* midges collected by CO_2 traps with UV light than among midges collected by the other trapping methods. BTV infection rates of *C. sonorensis* midges that were detected earlier in the season than sentinel cattle in addition to the plurality of serotypes on individual dairy farms suggests that the midge vector constitutes a reservoir of genetically divergent BTVs that potentially sustain the virus in seasonally endemic areas. The data suggests infection prevalence may be misrepresented when UV light traps alone, a method most often used in routine vector (*C. sonorensis*) surveillance. In summary, findings from this study confirm the importance of using sensitive surveillance methods for both midge collection and virus detection.

◊ USAHA Paper

Evaluation of Bovine Viral Diarrhea Virus Infection in Hunter-harvested White-tailed Deer (*Odocoileus virginianus*) in Georgia using Ear-notch Samples

Marcia Regina Da Silva Ilha, Michele Coarsey, Lisa Whittington, Sreekumari Rajeev, Sheela Ramamoorthy

Tifton Veterinary Diagnostic & Investigational Laboratory, UGA, Tifton, GA

Narrative: Several recent experimental studies have indicated that bovine viral diarrhea virus (BVDV) can be transferred back and forth between cattle and white-tailed deer (WTD) and among WTD. Surveys for BVDV in wild WTD in other states indicated low prevalence (less than 1%). A study was conducted to evaluate the occurrence of BVDV infection in WTD in the state of Georgia using ear notches from hunter-harvested deer during the hunting season of 2010-2011. Ear samples were collected at multiple game management areas throughout the state. Data on sex, age and county of origin was recorded. During September to December of 2010, 367 samples of ear from free ranging WTD were collected from 37 counties in Georgia, mainly from southern areas. The samples were from 178 (48.5%) female deer, 187 (51%) male deer, and two (0.5%) of unknown sex. The age varied from 6 months to 6.5 years. The age was not recorded in 34 animals (9.3%). Within the animals with known ages, 42% of the deer sampled were under 2 years. Samples of ear notches were placed in 1.5ml of phosphate buffer solution (PBS) and processed for antigen capture enzyme-linked immunosorbent assay (AgELISA). A second sample of similar size was fixed in neutral-buffered 10% formalin and embedded in paraffin blocks within 24 to 48 hours for further immunohistochemistry (IHC). The remaining sample was frozen for virus isolation (VI). All ear notches were individually tested for BVDV by AgELISA using a commercially available kit (IDEXX) according with manufacturer's instructions. Routine BVD immunohistochemistry and virus isolation were performed in samples with suspect results for BVDV by AgELISA. Three ear samples out of 367 were within the suspect range of the assay by AgELISA. One animal was a 1.5 year old male deer and the other two deer were females with ages of 3.5 and 5.5 years. All 3 samples were negative by IHC and VI. In conclusion, although a few of our samples were suspect for BVDV, we could not further confirm the presence of the virus within the deer population studied. Although the results from this preliminary study in the population sampled may not support the hypothesis that WTD could be a potential reservoir for the BVDV in the state of Georgia, low prevalence of the disease in WTD is a possibility. Future studies targeting a larger population may be helpful to determine if BVDV is present in WTD in this region.

Introduction of the IDEXX Bovine Viral Diarrhea Virus Antigen ELISA (IDEXX BVDV PI X2 Test)

Anthony Mestek, Katherine Velek, Shona Michaud, Lisa Estey, Sergio Lizano

IDEXX Laboratories, Inc., Westbrook, ME

Narrative: Bovine viral diarrhea virus (BVDV) is an infectious disease of cattle that can cause significant economic losses within the livestock industry. Cattle that are persistently infected (PI) with BVDV constitute one of the mechanisms by which BVDV spreads among cattle herds. Detection and elimination of PI animals is a necessary component of an effective biosecurity program for controlling BVDV. IDEXX Laboratories produces a USDA-licensed ELISA test for the detection of BVDV, and has recently validated changes designed to enhance the performance and usability of the test. The purpose of this study was to evaluate test method repeatability, diagnostic sensitivity/specificity for all sample claims (small/large ear notch and serum), and genotype detection capability.

Discovery of a Broad Diversity of Mycoplasma species in Companion Animals Utilizing a PanMyco PCR Assay

Uri Donnett[1], Adam Herrick[1], Claudia Baldwin[2, 3], Peter Nara[1, 4], Jessie D. Trujillo[1, 5]

[1]The Center for Advanced Host Defenses, Immunobiotics and Comparative Translational Medicine, Iowa State University College of Veterinary Medicine, Ames, IA; [2]The Maddie's Shelter Medicine Program®, Iowa State University College of Veterinary Medicine, Ames, IA; [3]Department of Veterinary Clinical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA; [4]Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA; [5]Department of Veterinary Microbiology and Preventative Medicine, Iowa State University College of Veterinary Medicine, Ames, IA

Narrative: Approximately 200 species of Mycoplasma exist and many are considered host species adapted. Atypical Mycoplasma infections in humans, presumably acquired through zoonotic transmission, question this traditional assumption of host specificity. *M. felis* and *M. cynos* are pathogens of Feline Upper Respiratory Disease (FURD) and Canine Infectious Respiratory Disease (CIRD). Little is known about the prevalence of other Mycoplasma species in companion animals. Molecular diagnostics commonly rely on single pathogen, probe based qPCR. Although sensitivity and specificity are favored with these assays, this specificity prevents detection of unknown or atypical species. Molecular assays with broader Mycoplasma species detection capabilities are necessary for greater understanding of Mycoplasma ecology. We have developed a PanMyco PCR assay for detection and differentiation of Mycoplasma species. This assay uses a single set of primers and SYBR green dye. Species identity is determined by amplicon melt temperature, size, sequence, and High Resolution Melt analysis. This assay is being employed to determine the prevalence and association of Mycoplasma species in companion animal disease. Animal populations include shelter cats, cats visiting the ISU Lloyd Veterinary Medical Center, and canine samples submitted for diagnostic evaluation to the ISU VDL. Clinical disease is scored in cats. Samples collected include conjunctival, pharyngeal, and nasal swabs and occasional bronchoalveolar lavage fluid. In 53 felines (case-control), we detected nine species of Mycoplasma (*M. felis, M. gateae/canadense/arginini, M. canis, M. hyorhinis, M. buccale/spumans, M. hominis, M. bovis, M. bovoculi*, and *M. maculosum/leopharyngis*), with six previously unreported in cats (underlined). Mycoplasma prevalence is 51% with 28% of Mycoplasma positive cats having FURD. We detected two or more Mycoplasma species in eight cats. In 26 canines (symptomatic), we detected eight species of Mycoplasma (*M. canis, M. spumans, M.cynos, M. maculosum, M. bovis, M. canadense/gateae/arginini, M. hyosynoviae*, and *M. edwardii*) out of 20 positive samples with two previously unreported in dogs. We detected two or more Mycoplasma species in three canines. Five Mycoplasma species detected are zoonotic (*M. felis, M. maculosum, M. argnini, M. bovis* and *M. canis*) with *M. bovis* being detected in cats and dogs for the first time to our knowledge. *M. hominis*, a known cause with human Pelvic Inflammatory Disease, was detected from a cat. Through the utilization of the PanMyco PCR assay in a relatively small subset of companion animals, we document a broad diversity of Mycoplasma species previously unknown to these hosts, demonstrating cross species transmission of many Mycoplasma species. This ongoing study provides much need information, related to Mycoplasma ecology, necessary to aid in diagnosis, treatment, and prevention of disease in companion animals and humans.

Investigation of *Corynebacterium pseudotuberculosis* cases in Texas Horses from 2005 to 2011

Barbara Szonyi, Amy K. Swinford, Alfonso Clavijo

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

Narrative: *Corynebacterium pseudotuberculosis* is a gram-positive bacterium which is the causative agent of various diseases in goats, sheep, cattle, and horses. In horses, the disease is commonly called pigeon fever, but is also known as dryland distemper, as it is commonly associated with areas that have low annual rainfall. In recent years, Texas has seen a dramatic increase in the number of clinical cases of pigeon fever. Equine *C. pseudotuberculosis* cases submitted over the past 7 years to the Texas Veterinary Medical Diagnostic Laboratories (TVMDL) were analyzed with the following objectives: 1) to estimate the occurrence and spatio-temporal distribution of *C. pseudotuberculosis* infection in horses in Texas over the years 2005 to 2011; 2) to investigate annual trends of *C. pseudotuberculosis* cases in horses in Texas; and 3) to create a risk map for *C. pseudotuberculosis* in horses in Texas. The study population consisted of horses that tested culture-positive for *C. pseudotuberculosis* between 01/01/2005 and 12/31/2011 at TVMDL. Data was aggregated at the county level. The horse population in each county was assumed to be the population at risk. The Poisson and Bernoulli models of the scan statistics were fitted to identify space-time disease clusters. Empirical Bayesian smoothing was performed on the crude cumulative incidence estimates for the year 2011, followed by isopleth risk mapping to delineate high-risk areas using the geostatistical method of kriging. Cases increased tenfold between 2005 and 2011, with an average annual increase of 177%. The cumulative incidence ranged from 9.3 to 99.5 per 100,000 horses at risk in 2005 and 2011, respectively. Two consistent annual peaks in the number of cases were observed over the years 2009-2011, with the first annual peak in June and a second peak in December. The scan statistics identified a primary space-time cluster in central Texas in 2011 ($p<0.0001$ and relative risk of 9.2). The results of isopleth risk mapping were consistent with the cluster detection method in identifying a high-risk area in central Texas. In addition, high risk areas were detected in the Panhandle and areas of northern Texas. The epidemiological investigation confirmed anecdotal reports from veterinarians that increasing numbers of pigeon fever cases have been diagnosed in the Texas horse population over the past years. While submission bias cannot be ruled out as the cause of the observed increase, it is plausible that changing climatic conditions lead to the emergence of this disease in the Texas horse population.

Scoliosis in Three Gopher Tortoises from Baker County, Georgia

Brandon Munk[1], Jessica McGuire[1], Mark G. Ruder[2], Justin Brown[1]

[1]Population Health, University of Georgia, Athens, GA; [2]United States Department of Agriculture, Agriculture Research Service, Manhattan, KS

Narrative: Scoliosis was diagnosed in three of nine gopher tortoises (*Gopherus polyphemus*) from Baker County, Georgia that were submitted to the Southeastern Cooperative Wildlife Disease Study in 2011 and 2012 for postmortem examination. One tortoise had moderate scoliosis with two opposing curves forming an S-shape. Two other tortoises had mild scoliosis both with single, small C-shaped curves. The tortoises had other primary diseases including trauma, bacterial pneumonia, sepsis, a ruptured urinary bladder, and upper respiratory tract disease. Scoliosis is a congenital deformity with multiple possible etiologies. Chemical teratogens, suboptimal moisture or temperature during incubation, and nutritional deficits have all been linked to skeletal deformities in chelonians, though other causes may exist. Gopher tortoises are species of concern and throughout their range are listed as either "Threatened" or "Candidate" species by the U.S. Fish and Wildlife Service. The presence of scoliosis in three of nine (33.3%) gopher tortoises examined from one population may be indicative of an underlying disease process that could have population implications. Studies are currently underway to determine the true incidence of scoliosis in this population, whether it differs from other populations of gopher tortoises or related tortoises, and whether there is a specific cause for these deformities.

Quality Assurance: Sharing the Tools that Support Sound Science

Rebecca Davies[1], Shirley D. Johnston[2]

[1]Veterinary Diagnostic Laboratory, University of Minnesota College of Veterinary Medicine, St. Paul, MN; [2]Found Animals Foundation, Michelson Prize & Grants Program, Los Angeles, CA

Narrative: Diagnostic Laboratories that are accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) have successfully implemented comprehensive quality assurance (QA) programs that support their ability to meet or exceed the standards of the World Organization for Animal Health (Office International des Epizooties) (OIE) described in the OIE Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases, 2008. The QA programs integrated in these accredited laboratories support scientific excellence through the emphasis on consistent and required documentation of procedures, equipment management, personnel training and competency, approach to non-conforming work and a required focus on data integrity and traceability. It is worthwhile to consider whether some of the QA tools in use by our accredited laboratories could be adapted for voluntary adoption by other scientific teams in order to promote the sound science they perform. Research that leads to the availability of new diagnostic tests, drugs and therapeutics is often performed in a non-regulated environment where mature quality systems that support data integrity and overall research management are not likely present. Typically, the benefits of QA are not realized in these research environments because quality systems are not required, there is a lack of awareness and training and the necessary infrastructure does not exist. Frequently, this type of research is being performed in institutions where AAVLD accredited QA programs are operational and could be used to address some of these gaps. In addition, voluntary adoption of simple QA best practices could potentially speed up the adoption of new diagnostic tests developed in research units of accredited diagnostic laboratories. Our objective was to create simple research management tools, make them readily available, and monitor the number of access events as an indicator of voluntary use. In collaboration with a research sponsor (Found Animals Foundation, FAF) a Research Quality Assurance toolkit was designed for use by any scientist and made freely available from the FAF website. The FAF commissioned this toolkit as a strategy for encouraging research applicants and grantees to adopt quality management practices to promote data traceability and facilitate research reconstruction. This QA toolkit will be described and metrics data related to website accessions and investigator interviews at FAF site visits will be presented. The production of a simple and voluntary toolkit is an example of one strategy for promoting quality in research and training and for furthering the AAVLD vision of providing quality service, service development and scientific expertise.

Pathology 2

Sunday, October 21, 2012

Guilford D

Moderators: Scott D. Fitzgerald, Arthur (Bill) Layton

8:00 AM **Two Cases of Chronic Copper Toxicity in Growing Holsteins**
Doug Lyman *116*

8:15 AM **Canine and Feline Yeast Urinary Tract Infections: Agents and Susceptibility to Antifungal Agents**
Barbara Byrne, Eileen M. Samitz, Jodi L. Westropp *117*

8:30 AM **Naked Mole-Rat Skin and Soft Tissue Metastatic Mineralization**
Amanda Crews, Shelley J. Newman, Edward C. Ramsay *118*

8:45 AM **Comparative Virulence of Clinical *Brachyspira spp*. Isolates in Inoculated Pigs**
Eric Burrough, Erin Strait, Joann M. Kinyon, Leslie Bower, Darin Madson, Bailey Wilberts, Kent Schwartz, Timothy Frana, J. Glenn Songer *119*

9:00 AM **Canine Colorectal Polyps and Progression to Malignancy: A Retrospective Study of 100 Canine Colorectal Epithelial Polypoid Tumors (1997-2012) # +**
Brian Butler *120*

9:15 AM **Break**

9:45 AM **Emergency Preparedness in the Necropsy Laboratory: Designing and Conducting a Full-scale Operational Exercise**
Maria Spinato, Jan Shapiro, Margaret J. Stalker, Andrew Brooks *121*

10:00 AM **Gross and Histological Lesions in Northern Elephant Seals (*Mirounga angustirostris*, Gill, 1866), Año Nuevo State Reserve, California 2012**
Terry Spraker, Gene Lyons, Tetiana Kuzmina, Stephen Raverty, Nicole Jaggi, Dan Crocker, Mike Tift *122*

10:15 AM **Pyogranulomatous Dermatitis Associated with *Mycobacterium smegmatis* in a Cat**
Jeffrey R. Hayes, Yan Zhang, Jing Cui, Anne Parkinson, Mary B. Weisner, Beverly Byrum, Joshua Daniels *123*

10:30 AM **Concurrent Vitamin D Toxicosis and Presumptive Proventricular Dilatation Disease in Two Eclectus parrot (*Eclectus roratus*) chicks**
Howard Steinberg, Dominique Keller, Sarah Churgin, Marie E. Pinkerton *124*

10:45 AM **Abortion and Disseminated Infection by *Coccidioides posadasii* in an Alpaca Fetus**
Francisco Uzal, Santiago Diab, Jorge Garcia, Suzanne Johnson, Erin Carlson, Demosthenes Pappagianis, Jana Smith *125*

11:00 AM **Hepatic Encephalomyelopathy in Ten Goat Kids Associated with Congenital Portosystemic Shunting (cPSS) ◊**
Hailu Kinde, Patricia Pessavento, John Adaska, Bradd Barr, Megan Jones, Alexandre P. Loretti, Mark Anderson *126*

Symbols at the end of titles indicate the following designations:

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

Two Cases of Chronic Copper Toxicity in Growing Holsteins

Doug Lyman

Wisconsin Veterinary Diagnostic Laboratory, Madison, WI

Narrative: It is thought that bovines are relatively resistant to copper toxicity, but recent reports and the two cases reported here would seem to suggest that bovines, at least young, growing animals, are more susceptible than previously appreciated. In case 1, a group of 200-250kg Holstein heifers was fed a ration (as individual components) consisting of corn, oats, hay, and pellets for an unknown duration, presumed to be two to several months, at which time several stopped eating, became lethargic and died within 2-3 days of onset. Eventually, 8 of 40 died. Clinical examination revealed chocolate-colored blood (methemoglobinemia), mild icterus, and "elevated liver enzymes" (details not available). Field necropsy revealed "generalized icterus and orange-brown, coarsely granular liver". Histopathology of the liver revealed extensive hepatic necrosis, hepatocyte vacuolation, portal fibrosis, marked biliary hyperplasia, mitotic hepatocytes, and distension of portal macrophages and Kupffer cells by light brown, granular material that stained positive (brick red) for copper with rhodanine. Renal lesions included interstitial hemorrhages and moderate deposition of brown, granular material that stained positive for iron in cortical and medullary tubules. Liver analysis revealed 440ug/g wet weight of copper. Published range for toxicity is 250-800ug/g wet weight. Case 2 involved a group of 25, 150kg Holstein steers, from which two died with similar symptoms and findings. These cases suggest that young, growing Holsteins are more susceptible to chronic copper toxicity than previously thought.

Canine and Feline Yeast Urinary Tract Infections: Agents and Susceptibility to Antifungal Agents

Barbara Byrne[1, 2], Eileen M. Samitz[2], Jodi L. Westropp[3]

[1]Pathology, Microbiology, Immunology, University of California, Davis, CA; [2]William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA; [3]Medicine and Epidemiology, University of California, Davis, CA

Narrative: A retrospective study was initiated in order to determine the species of yeast associated with urinary tract infections (UTIs) in our canine and feline hospital population and to describe the antifungal susceptibility patterns observed. Patient records and microbiology reports from 1992 to 2012 were searched in order to detect patients with a urine or bladder culture positive for yeast. Twenty-nine patients, 21 dogs and 8 cats, were identified with a total of 30 yeast isolates. Twenty-two samples were collected via cystocentesis, 5 via catheterization, 1 at necropsy, and 1 was unspecified. *Candida albicans* was isolated most frequently (13/29 patients). *C. glabrata* was also often observed (11/29 patients). There were 4 isolations of *C. tropicalis* and 1 each of *Candida spp*. and *Trichosporon mucoides*. Antifungal susceptibility testing was carried out on a total of 14 isolates: 7 *C. glabrata*, 6 *C. albicans*, and 1 *C. tropicalis*. In general, the isolates were susceptible to a variety of antifungal agents, although resistance to itraconazole (4 isolates) and dose-dependent susceptibility to fluconazole (3 isolates) was detected in *C. glabrata*. Two of 4 isolates from patients with recurrent/persistent infection showed increased minimum inhibitory concentrations to azole antifungal drugs when compared to initial testing suggesting acquisition of resistance (1 *C. glabrata* and 1 *C. albicans*). Identification of isolated yeast to the species level is important for empirical therapy as some resistance to azoles was seen in *C. glabrata*. Additional susceptibility testing may be indicated in patients with persistent/recurrent infections.

Naked Mole-Rat Skin and Soft Tissue Metastatic Mineralization

Amanda Crews, Shelley J. Newman, Edward C. Ramsay

Department of Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN

Narrative: Skin and/or soft tissue metastatic mineralization is occurring in naked mole-rats (NMRs), *Heterocephalus glaber*, at the Knoxville Zoological Gardens (KZG). Zoological parks around the country are also seeing similar metastatic mineralization in their NMRs. The objective of this report is to characterize lesions grossly and histologically, and discuss diet changes, results of monitoring and draining of lesions, and, briefly, the theoretical pathogenesis for the development of these lesions. Pathologic lesions in KZG NMR necropsy and biopsy cases show a distribution varying from focal to multifocal and disseminated dermal to visceral foci. Grossly, lesions are irregularly round, multifocal to coalescing, white to tan, chalky, firm to fluctuant nodules that range in size from 1 mm to 5 cm in diameter. Histologically, nodules are small to large lakes of deeply amphophilic material (mineral) that are surrounded by minimal to moderate granulomatous to pyogranulomatous inflammation. We theorize the development of mineralization seen in the NMRs from KZG is due to excess levels of vitamin D in their captive diets and specifically the rodent pelleted feed. In the wild, NMRs are underground dwellers and are naturally deficient in the prohormone cholecalciferol (vitamin D3), which is a precursor of 1, 25 dihydroxyvitamin D3 (calcitriol), and have no obvious dietary source of vitamin D. However, even with an impoverished vitamin D status, studies have shown that naturally vitamin D-deficient NMRs are able to independently maintain mineral homeostasis without appreciable levels of cholecalciferol. Over the past year at the KZG with diet changes and occasional drainage of larger lesions in NMRs, we have seen partial to complete regression of skin mineralization.

Comparative Virulence of Clinical *Brachyspira spp*. Isolates in Inoculated Pigs

Eric Burrough[1], Erin Strait[1], Joann M. Kinyon[1], Leslie Bower[1], Darin Madson[1], Bailey Wilberts[2], Kent Schwartz[1], Timothy Frana[1], J. Glenn Songer[3, 1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA; [2]Veterinary Pathology, Iowa State University, Ames, IA; [3]Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA

Narrative: Classical swine dysentery is associated with the presence of the strongly beta-hemolytic *Brachyspira hyodysenteriae*; however, multiple *Brachyspira spp*. can colonize the porcine colon. Recently, several *Brachyspira spp*. not identified as *B*. *hyodysenteriae* by genotypic and/or phenotypic methods have been isolated from the feces of pigs with clinical disease typical of swine dysentery. In this study, eight clinical isolates, including five strongly beta-hemolytic and three weakly beta-hemolytic *Brachyspira*, and a reference strain of *B*. *hyodysenteriae* (B204) were inoculated into pigs (n = 6 per isolate) to compare pathogenic potential following oral inoculation. Results revealed that strongly beta-hemolytic isolates induced significantly greater typhlocolitis than those that are weakly beta-hemolytic, regardless of the genetic identification of the isolate, and that strongly beta-hemolytic isolates detected as '*Brachyspira sp*. SASK30446' and *B*. *intermedia* by PCR produced lesions similar to those caused by *B*. *hyodysenteriae*. These results suggest that phenotypic culture characteristics of *Brachyspira spp*. may be a more sensitive indicator of potential to induce dysentery-like disease in pigs than molecular identification alone based on currently available PCR assays. Additionally, culture of mucosal scrapings obtained at necropsy was more sensitive than direct PCR on the same samples for detection of *Brachyspira spp*.

Canine Colorectal Polyps and Progression to Malignancy: A Retrospective Study of 100 Canine Colorectal Epithelial Polypoid Tumors (1997-2012) # +

Brian Butler

Biomedical Sciences, Cornell, Ithaca, NY

Narrative: Colorectal cancer (CRC) is the third most common form of human cancer and the second leading cause of cancer-related deaths in developed Western countries. Canine CRC shares features of this human disease. Importantly, dogs are one of few species that exhibit a spectrum of colorectal neoplasia with progression to malignancy similar to humans. Few studies have documented the malignant transformation of canine colorectal polypoid tumors, and very little is understood about the clinicopathologic features of this disease. The objective of this study was to identify the histopathological features of canine colorectal polypoid tumors that correlate with malignant transformation. Five distinct stages of tumor development were identified in a retrospective study of 100 epithelial polypoid tumors. Results indicated that tumor size, degree of epithelial dysplasia, and mitotic rate were reliable indicators of early malignant transformation, and nearly 70% of canine polypoid tumors displayed evidence of malignant transformation at the time of surgical removal. Findings from this study provided support for the hypothesis that benign polypoid tumors in dogs have pre-malignant potential, which is consistent with the current understanding of CRC in humans. Screening of dogs for the early detection and removal of colorectal polypoid tumors may therefore be beneficial in the prevention of canine CRC.

AAVLD Trainee Travel Awardee (Pathology)
+ AAVLD/ACVP Pathology Award Applicant

Emergency Preparedness in the Necropsy Laboratory: Designing and Conducting a Full-scale Operational Exercise

Maria Spinato[1], Jan Shapiro[2], Margaret J. Stalker[1], Andrew Brooks[2]

[1]Animal Health Laboratory - Guelph, University of Guelph, Guelph, ON, Canada; [2]Animal Health Laboratory - Kemptville, University of Guelph, Kemptville, ON, Canada

Narrative: In this era of heightened awareness of the risks of transboundary disease (TD) incursions, emergency planning has become an important strategic goal of veterinary diagnostic laboratory operations. Whereas strict biosecurity controls have been implemented in the virology and molecular diagnostic laboratories involved in testing for emerging or transboundary diseases, necropsy labs remain a problematic site for potential exposure and spread of disease. A producer experiencing high mortality on farm is likely to bring a carcass to a necropsy lab for testing. Should this animal be infected with a TD, the biocontainment and notification procedures implemented in the first few hours following a tentative diagnosis by the pathologist would assist in containing the disease, and limit potential spread to the farms of other clients using diagnostic laboratory facilities. The Animal Health Laboratory (AHL) at the University of Guelph developed an operations-based exercise to test and validate its standard operating procedure (SOP) for management of a suspect TD case in the necropsy laboratory. This event was held in conjunction with a broader provincial TD exercise examining the interactions among the Canadian Food Inspection Agency (CFIA) and various swine industry organizations involved in responding to a suspected outbreak of 'standard swine fever'. Independent observers at both the Guelph and Kemptville locations were provided with an evaluation guide that outlined specific objectives, expected actions and desired timelines. Additional qualitative evaluations were elicited from participating AHL and CFIA staff during a post-exercise 'hot wash'. The exercise was deemed a success at both AHL necropsy laboratories. Evaluators concluded that participants were well-prepared, the specific objectives of testing and validating the TD SOP were fully met, and all tasks were completed within the suggested time limits. Recommendations for improvement included revision and expansion of decontamination and disinfection procedures, minor facility modifications, and additional physical resources. Full-scale operational exercises are critical to maintaining a high level of emergency preparedness, as they provide laboratory staff with an opportunity to practice procedures in real time, and facilitate gap analysis. Moreover, these simulations offer opportunities to interact with local stakeholders and animal health partners, thereby improving co-ordination during an actual emergency.

Gross and Histological Lesions in Northern Elephant Seals (*Mirounga angustirostris*, Gill, 1866), Año Nuevo State Reserve, California 2012

Terry Spraker[1], Gene Lyons[2], Tetiana Kuzmina[3], Stephen Raverty[5], Nicole Jaggi[6], Dan Crocker[7], Mike Tift[4]

[1]College of Veterinary Medicine, Colorado State University, Fort Collins, CO; [2]Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY; [3]Institute of Zoology, NAS of Ukraine, Kiev, Ukraine; [4]Department of Biology, Sonoma State University, Rohnert Park, CA; [5]Diagnostic Pathology, Animal Health Center, Abbotsford, BC, Canada; [6]School of Veterinary Medicine, University of California, Davis, Davis, CA; [7]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Santa Cruz, CA

Narrative: The cause of death of northern elephant seals (*Mirounga angustirostris*, Gill, 1866) inhabiting rookeries on the mainland at Año Nuevo State Reserve in central California, were investigated in 2012. This population of elephant seals went through a serious bottle neck in the early 1900's due to humans killing them for their blubber/oil. These animals have little genetic diversity; however their population is expanding. Necropsies were done on 21 freshly dead pups over a three week period during the last week of January and first two weeks in February. Ages of pups were stillbirths to approximately 2 week. Pups were grouped into four categories according to body condition: excellent, good, fair and poor/emaciated. Histological examination was done on all pups. Histological lesions found in these pups: Excellent body condition - 2 pups: blunt trauma (2), pneumonia (1), enterocolitis (1), multifocal hepatitis (1), necrotizing lymphadenitis (1), bile duct hyperplasia/dysplasia (1); Good body condition - 7 pups: blunt trauma (5), fetal pneumonia (1), bile duct hyperplasia/dysplasia (1), suppurative meningoencephalitis (1), renal cortical dysplasia (1), drowning (2) and mild hydrocephalus (2); Fair body condition - 6 pups: bite wound/cellulitis (1), blunt trauma (4) and pneumonia (1); Poor/Emaciation body condition - 6 pups: bite wound with cellulitis (3), blunt trauma (2), pneumonia (1) and large open fontanels (1). Usually more than one histological lesion was found in each pup. Six congenital anomalies were found; three considered to be fatal and three non-fatal.

Pyogranulomatous Dermatitis Associated with *Mycobacterium smegmatis* in a Cat

Jeffrey R. Hayes[1], Yan Zhang[1], Jing Cui[1], Anne Parkinson[1], Mary B. Weisner[1], Beverly Byrum[1], Joshua Daniels[2]

[1]Pathology Section, Ohio Animal Disease Diagnostic Laboratory, Reynoldsburg, OH; [2]Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, OH

Narrative: A biopsy sample collected from a chronic draining wound at the left axilla region of a 10-year-old neutered male DSH cat was submitted to the diagnostic laboratory for histopathology, aerobic and anaerobic culture. Nocardia or Actinomyces organisms were suspected based on acid fast staining. Histopathology revealed marked, multifocal to coalescing pyogranulomatous dermatitis, cellulitis and panniculitis. The dermis and subcutis were markedly expanded by an inflammatory infiltrate that consisted of poorly organized aggregates of large epithelioid macrophages arranged as variably sized nodules and as sheets, admixed with moderate to large numbers of non-degenerate neutrophils and low numbers of lymphocytes and plasma cells. Infrequent binucleate and multinucleated giant cells were also present. Nodular perivascular infiltrates of lymphocytes and plasma cells were present in the deep subcutis, and scattered irregular infiltrates of similar cells extend into the subjacent panniculus carnosus. Multiple round to oval, variably sized, clear vacuole-like structures were present within the exudate. Very few acid fast bacilli were observed within macrophages of the dermal infiltrate. Few colonies of pure growth of rapid growing *Mycobacterium sp.* were isolated after 72 hours of aerobic culture. A diagnosis of mycobacterial dermatitis was confirmed by sequencing of the 16S rRNA gene from the isolated colonies, which indicated a 100% nucleotide identity with the *M. smegmatis* strain ATCC 19420. References: 1. Alander-Damsten YK, Brander EE, Paulin: 2003, Panniculitis, due to Mycobacterium smegmatis, in two Finnish cats. J Feline Med Surg 5(1):19-26. 2. Gin PE, Joanne EK, Mansell KL, Rakich PM: 2007, Skin and appendages. In: Jubb, Kennedy and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 687-691. Elsevier Limited, Philadelphia, PA. 3. Govendir M, Hansen T, Kimble B, Norris JM, Baral RM, Wigney DI, Gottlieb S, Malik R: 2011, Susceptibility of rapidly growing mycobacteria isolated from cats and dogs, to ciprofloxacin, enrofloxacin and moxifloxacin. Vet Microbiol 147(1-2):113-8. Epub 2010 Jun 22. 4. Gross TL, Ihrke PJ, Walder EJ, Affolter VK: 2005, Infectious nodular and diffuse granulomatous and pyogranulomatous diseases of the dermis. In: Skin Diseases of the Dog and Cat, 2nd ed., pp. 283-287. Blackwell Science Ltd, Ames IA. 5. Malik R, Wigney DI, Dawson D, Martin P, Hunt GB, Love DN: 2000, Infection of the subcutis and skin of cats with rapidly growing mycobacteria: a review of microbiological and clinical findings. J Feline Med Surg 2(1):35-48.

Concurrent Vitamin D Toxicosis and Presumptive Proventricular Dilatation Disease in Two Eclectus parrot (*Eclectus roratus*) chicks

Howard Steinberg[1], Dominique Keller[2], Sarah Churgin[2], Marie E. Pinkerton[1]

[1]Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI; [2]Department of Surgical Sciences, University of Wisconsin-Madison, Madison, WI

Narrative: Two hand-fed 3-month-old Eclectus parrot (*Eclectus roratus*) clutch-mates, male and female, presented for evaluation due to failure to thrive. The chicks were parent-reared until seven weeks of age, then fed a commercial hand-feeding formula used with success in previous clutches. One to two weeks later, the chicks presented to the referring veterinarian due to poor weight gain and delayed crop emptying; both were emaciated and the male had a weak grip. Both chicks were treated with enrofloxacin and nystatin; nystatin was discontinued when the female began to regurgitate. When their condition did not improve, the chicks presented to UW Veterinary Care (UWVC) with severe dehydration, emaciation, stunted feather development, and mental dullness. The male had a weak grip and walked on his hocks. Plasma biochemistries and complete blood count of the male chick showed severe hyperuricemia (>25 mg/dL), elevated AST, hypercalcemia, and leukopenia. Ancillary tests were not performed on the female chick due to financial constraints. Both chicks were hospitalized and treated with subcutaneous fluids, gavage feeding, antibiotics, itraconazole, and cisapride. The chicks' condition briefly improved, however the female died 4 days after presentation. The male was discharged 5 days following presentation after hyperuricemia improved, and treatment was continued at home, but the chick returned to UWVC the day following discharge after clinical signs worsened. Plasma biochemistries revealed a dramatic worsening of hyperuricemia (55.5 mg/dL), hypoalbuminemia, hyperkalemia, and hyponatremia. Euthanasia was elected. On necropsy, both chicks were emaciated with a markedly dilated and thin-walled proventriculus. The kidneys were enlarged, pale tan and finely stippled with pinpoint white gritty foci. The female's oral and nasal cavity was obstructed by feed material and the crop was markedly distended with similar material; asphyxiation may have contributed to death. Histologically, both chicks had marked renal tubular necrosis, mineralization, and interstitial fibrosis. Multiple organs had mild to marked mineralization, including lung, heart, and proventricular mucosa. Histologic lesions were consistent with vitamin D toxicosis. The feed company reported high vitamin D levels in the batch of formula used for hand-feeding. PCR for avian bornavirus on frozen brain tissue in the female chick was positive. While gross lesions were consistent with proventricular dilatation disease (PDD), no histologic lesions of PDD in the brain or gastrointestinal ganglia were seen. Clinical signs were attributed to vitamin D toxicosis and renal failure; gross lesions and positive bornavirus PCR are suggestive of but not definitive for PDD in the absence of histologic lesions.

Abortion and Disseminated Infection by *Coccidioides posadasii* in an Alpaca Fetus

Francisco Uzal[1], Santiago Diab[1], Jorge Garcia[1], Suzanne Johnson[2], Erin Carlson[2], Demosthenes Pappagianis[2], Jana Smith[3]

[1]California Animal Health & Food Safety Laboratory, University of California, Davis, San Bernardino, CA; [2]School of Medicine, UCDavis, Davis, CA; [3]Private Practitioner, Southern California, Somis, CA

Narrative: Coccidioidomycosis is a fungal disease caused by either *Coccidioides immitis* or *Coccidioides posadasii*. Congenital *Coccidioides spp*. infections followed by abortion have been rarely described in humans and in a mare, but no congenital infection or abortion has been described in camelids. An aborted 9-month gestation alpaca fetus with the placenta was submitted for necropsy. Multiple organs of the fetus and the placenta presented pyogranulomas. Multifocally, within these pyogranulomas, there were large numbers of round, 60-100um fungal spherules (sporangia) with a 4-5um thick refractile and hyaline double wall. Sporangia contained flocculent basophilic to amphophilic material and, rarely, multiple 5-7μm endospores. Frequently, the spherules were seen within the cytoplasm of giant cells. The spherules stained positive with GMS and PAS. DNA isolated from placenta and lung was evaluated for *Coccidioides spp*. using real-time PCR, which produced a positive result for both tissues. Conventional nested PCR was used to amplify a region of the ribosomal DNA followed by sequencing and comparison with known strains of *C. immitis* and *C. posadasii*. The sequence obtained was most similar to that of *C. posadasii*. Complement fixation for *Coccidioides spp*. on the dam serum was positive; by quantitative immunodiffusion this animal had a titer of 1:256, which is interpreted as an indicator of disseminated coccidioidomycosis. The dam was euthanized, and necropsy revealed pyogranulomas in multiple organs. Most of the granulomas in the dam contained only occasional fungal spherules similar to those described in the fetus. PCR on the dam's tissues is under way. Additional abortion work-up performed on the fetus, placenta and dam's serum was non-diagnostic. It is most likely that the infection was transmitted to the fetus from the dam via placental circulation. This seems to be the first confirmed case of *C. posadasii* infection in animals in California and the first case of congenital coccidioidomycosis in an alpaca.

Hepatic Encephalomyelopathy in Ten Goat Kids Associated with Congenital Portosystemic Shunting (cPSS) ◊

Hailu Kinde[1], Patricia Pessavento[2], John Adaska[3], Bradd Barr[4], Megan Jones[5], Alexandre P. Loretti[4], Mark Anderson[4]

[1]California Animal Health and Food Safety Laboratory System (CAHFS) San Bernardino Branch, School of Veterinary Medicine, UC Davis, San Bernardino, CA; [2]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, UC Davis, Davis, CA; [3]CAHFS Tulare Branch, School of Veterinary Medicine, UC Davis, Tulare, CA; [4]CAHFS Davis, School of Veterinary Medicine, UC Davis, Davis, CA; [5]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA

Narrative: Ten goat kids (2 live and 8 dead) of various breeds, ages between 1.5 and 5 months, and a body mass ranging from 3.67 to 18 kg were submitted for necropsy at the California Animal Health and Food Safety Laboratory System (CAHFS), or the Veterinary Medical Teaching Hospital (VMTH), School of Veterinary Medicine, University of California, Davis between 1999 and 2011. The history included two or more of the following clinical signs: ataxia, circling, blindness, seizures, teeth grinding, opisthotonus, paddling, general weakness, and ill thrift. Results of serum bile acids test from 2 animals were 134 and 209 μmol/l (reference intervals: 0-50 μmol/l). Gross necropsy revealed that animals were in poor to fair body conditions and had minimal fat reserve. Liver weights from 3 animals were 76 g (2% of the body weight), 280 g (1.8%), and 300 g (1.9%). Histologically, in all animals there was bilateral and symmetric spongy degeneration throughout the cerebrum, midbrain, cerebellum, brainstem, and spinal cord, more prominently at the white/grey matter junction. In three goats, proliferation of Alzheimer type II astrocytes were noted in the cerebral cortex and adjacent cerebral white matter. Histological lesions in the liver of these animals included atrophy of the hepatic parenchyma, small hepatocytes, increased numbers of arteriolar profiles, oval cell hyperplasia, and hypoplasia or absence of portal veins, and were consistent with congenital portosystemic shunting (cPSS). The clinical and pathological findings in all 10 goats were consistent with hepatic encephalopathy. Spongy degeneration of the CNS in these cases resulted from liver failure due to cPSS. cPSS should be considered in the differential diagnosis of young goats with a history of weakness, ill thrift, and neurological signs.

◊ USAHA Paper

Virology 2
(Influenza & Serology)
Sunday, October 21, 2012
Auditorium III

Moderators: Amy Glaser, Suzanne Carman

8:00 AM **Identification of Lymphoproliferative Disease Virus in Wild Turkeys (*Meleagris gallopavo*) in the United States ◊**
Justin Brown, Andrew Allison, Kevin Keel, Trista Welsh, Aly Fadly *129*

8:15 AM **The Role of Enteric Viruses in Light Turkey Syndrome ◊**
Devi P. Patnayak *130*

8:30 AM **Identification of Pandemic H1N1 Influenza Virus in Feral Swine**
Mangkey A. Bounpheng, Amir Nikooienejad, Mohammad Shahrokh, Richard P. Metz, Scott Schwartz, Esmaeil Atashpaz Gargari, Thomas J. Deliberto, Mark W. Lutman, Kerri Pedersen, Luis R. Bazan, Leo Koster, Melinda Jenkins-Moore, Sabrina L. Swenson, Michael Z. Zhang, Tammy Beckham, Charles D. Johnson, Alfonso Clavijo *131*

8:45 AM **Epidemiology of Influenza A Virus (IAV) Circulating in US Swine Between 2004 and 2011**
Jianqiang Zhang, Phil Gauger, Amy Chriswell, Xue Lin, Sara Nezami, Dong Sun, Devon Haney, Wendy Stensland, Aaron Irons, Karen Harmon, Erin Strait, Kyoung-Jin Yoon *132*

9:00 AM **Influenza A Virus Detection in Oral Fluid and Nasal Swabs by a Rapid Antigen Detection Kit in Swine Inoculated with IAV †**
Christa K. Goodell, Apisit Kittawornrat, Yaowalak Panyasing, Chris Olsen, Thomas Overbay, Chong Wang, Rodger Main, Jeff Zimmerman *133*

9:15 AM **Influenza A Virus Detection in Oral Fluid and Nasal Swabs by RT-PCR in Swine Inoculated with IAV †**
Christa K. Goodell, Rolf Rauh, William M. Nelson, Angela Burrell, Catherine O'Connell, Chong Wang, Rodger Main, Jeff Zimmerman *134*

9:30 AM **Influenza A Virus Isolation from Oral Fluid and Nasal Swabs in IAV Inoculated Pigs †**
Christa K. Goodell, Fanghong Zhou, Chong Wang, Kyoung-Jin Yoon, Rodger Main, Jeff Zimmerman *135*

9:45 AM **Break**

10:15 AM **Outbreak of Influenza A (H3N2) in People and Pigs at a County Fair**
Sabrina L. Swenson, Mary Lea Killian, Roman Pogranichniy, Jennifer Strasser, Steve Lindstrom, Pravina Kitikoon, Jennifer House, Stephen D. Lenz, Leo Koster, Bret Marsh, Bill Davis, Lashondra Berman, Amy Vincent, Shawn Richards, Mark Glazier, Sarah M. Tomlinson *136*

10:30 AM **The Effects of Pooling, Swab Type and Transport Conditions on Avian Influenza Virus and Newcastle Disease Virus Detection**
Erica Spackman, Janice C. Pedersen, Enid McKinley *137*

10:45 AM **Modification of the USDA H5 rRT-PCR Assay for Detection of H5N2 Low Pathogenic Avian Influenza Viruses of Mexican Lineage ◊**
Janice C. Pedersen, Mary Lea Killian, Nichole Hines, Beverly Schmitt *138*

11:00 AM **Experimental Co-infection Studies with Avian Influenza Viruses and Newcastle Disease Viruses in Chickens, Turkeys, and Domestic Ducks ◊**
Mary Pantin-Jackwood, Patti Miller, Claudio Afonso, Erica Spackman, Darrell Kapczynski, Eric Shepherd, Diane Smith, Rami Cha, Mariana Sa E Silva, David Swayne 139

11:15 AM **Engineering an Improved Classical Swine Fever ELISA to Detect Antibody in Sera**
Melissa L. Batonick, Gregory Mayr 140

11:30 AM **Monitoring of Four Naturally Infected Horses for Vesicular Stomatitis Antibody**
Dawn Toms, Mindy Powell, Michelle Redlinger, Tamara Beach, Melinda Jenkins-Moore, Tina Buffington, Cindy Harding, Sabrina L. Swenson 141

Symbols at the end of titles indicate the following designations:

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

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

Justin Brown[1], Andrew Allison[2, 1], Kevin Keel[1], Trista Welsh[1], Aly Fadly[3]

[1]Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, GA; [2]Cornell University, Baker Institute for Animal Health, Ithaca, NY; [3]United States Department of Agriculture - Agricultural Research Service, Avian Disease and Oncology Laboratory, East Lansing, MI

Narrative: Viral-associated lymphoproliferative neoplasia in domestic poultry is caused by infection with a herpesvirus (Marek's disease virus) or three species of retroviruses [Reticuloendotheliosis virus (REV), Avian leukosis/sarcoma virus, and lymphoproliferative disease virus (LPDV)]. Previously, retroviral neoplasms reported in wild upland game birds in the US have typically been associated with REV infection. Since 2009, LPDV, a virus previously believed to be exotic to the US, has been identified in 10 wild turkeys (*Meleagris gallopavo*) submitted to the Southeastern Cooperative Wildlife Disease Study for diagnostic examination. These birds were collected in six states, including West Virginia (n=5), North Carolina (n=1), Missouri (n=1), Georgia (n=1), and Arkansas (n=1). Infected turkeys were found dead or in moribund condition. Proviral sequences of LPDV were detected in various tissue samples from each turkey using PCR targeting a portion of the gag gene. Based on gross and microscopic lesions, lymphoproliferative disease associated with LPDV infection was determined to be the primary cause of mortality in five of the turkeys, with proliferating mononuclear cells identified in various visceral organs and tissues, including skin, intestines, liver, kidneys, spleen, pancreas, lungs, adrenal glands, skeletal muscle, esophagus, heart, and air sacs. Other primary causes of morbidity and/or mortality were determined in the remaining five turkeys. To follow up on these clinical cases, tissues collected from hunter-killed turkeys from multiple states were tested for LPDV, as described above, and additional positive turkeys were identified in Colorado (n=1) and South Carolina (n=36). Genetic comparisons of sequences obtained from tissues from all LPDV-positive turkeys demonstrated a high level of diversity, with nucleotide divergence ranging up to 15%. Notably, phylogenetic analysis of gag sequences from a subset of turkeys from South Carolina were shown to cluster independently from all other North American LPDV strains and formed a monophyletic group with the prototype Israeli strain, suggesting these viruses may represent an evolutionary bridge between the Old and New World viruses. 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. Current research efforts are underway to better understand the epidemiology, natural history, and significance of this virus to wild and domestic galliforms, including 1) active surveillance of hunter-killed turkeys, 2) genetic characterization of North American strains, 3) experimental challenge studies in domestic turkeys, and 4) evaluation of LPDV replication in cell culture systems.

◊ USAHA Paper

The Role of Enteric Viruses in Light Turkey Syndrome ◊

Devi P. Patnayak

Veterinary Population Medicine, Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN

Narrative: Turkey flocks with Light Turkey Syndrome (LTS) are described as having market age turkeys that are lower in body weight than the standard breed characteristics. Two studies were conducted to determine the possible role of enteric viruses in LTS. For surveillance study, fecal samples were collected at 2, 3, 5 and 8 weeks of age from four LTS and two non-LTS turkey flocks in Minnesota. Of the 80 pools from LTS flocks, 40 (50%) were positive for astrovirus type 2 while rotavirus was detected in 6 (7.5%) pools. In addition, 11 pools (13.8%) contained a combination of astrovirus and rotavirus while 1 pool (1.2%) had mixed infection with astrovirus and reovirus. In the experimental study, 2-week-old turkey poults were divided into groups A and B with 35 poults in each. Poults in group A were inoculated orally with a 10% fecal suspension from LTS flocks while phosphate buffered saline (PBS) was given to group B poults. Birds in both groups were monitored for clinical signs, feed consumption and shedding of enteric viruses. Birds in group A shed astrovirus, rotavirus and reovirus in their droppings until 9 weeks of age. Birds in group B (the control group) shed only astrovirus and not rota- or reovirus. After 9 weeks of age, most of the birds in both groups were virus negative. Significantly lower weight gain was seen in experimental group A birds at seven weeks of age and this lower weight gain continued until 20 weeks of age. Feed consumption was also lower in this group than in the control group. These findings suggest that viral enteritis at an early age may set up conditions for the development of light turkey syndrome in adult turkeys.

◊ USAHA Paper

Identification of Pandemic H1N1 Influenza Virus in Feral Swine

Mangkey A. Bounpheng[1], Amir Nikooienejad[2], Mohammad Shahrokh[2], Richard P. Metz[2], Scott Schwartz[2], Esmaeil Atashpaz Gargari[2], Thomas J. Deliberto[3], Mark W. Lutman[3], Kerri Pedersen[3], Luis R. Bazan[4], Leo Koster[5], Melinda Jenkins-Moore[5], Sabrina L. Swenson[5], Michael Z. Zhang[1], Tammy Beckham[1], Charles D. Johnson[2], Alfonso Clavijo[1]

[1]Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, TX; [2]Genomics and Bioinformatics, Texas AgriLife Research, College Station, TX; [3]USDA, Fort Collins, CO; [4]USDA, San Diego, TX; [5]National Veterinary Services Laboratories, USDA, Ames, IA

Narrative: Feral swine are considered invasive in the United States and play a role in the spread and emergence of diseases, including zoonotics. The US feral swine population (in 38 states) estimate is approximately 5 million, and the Texas Parks and Wildlife Department estimates the Texas population makes up more than half of the US population. The 2009 pandemic influenza A H1N1 (pH1N1) virus was detected in humans in the US in April, 2009, and was reported in more than 200 countries, resulting in over 18,000 deaths world-wide by August, 2010. Shortly after the human infections, pH1N1 infections in domestic swine were identified in Canada in May, 2009, and have since been reported in more than 20 countries. The maintenance of pH1N1 in domestic swine subsequently resulted in an increasing number of reassortants with endemic swine influenza virus worldwide. The identification of these reassortants and potential further reassortments with other influenza viruses prompted implementation of swine influenza surveillance programs in many countries. We report the first case of pH1N1 virus in feral swine in the US through USDA Wildlife Services' National Wildlife Disease Program surveillance activities in Texas. Two samples were identified as pH1N1 by reverse transcriptase quantitative PCR (RT-qPCR) and successfully virus isolated in culture. Full genome Sanger and Illumina next generation sequencing (NGS) of virus isolates confirmed pH1N1 lineage. Unbiased whole genome amplification successfully amplified Influenza RNA from the virus isolate and respective diagnostic samples for the multiplex NGS. In summary, we identified the first pH1N1 strain in feral swine in the US., demonstrated the use of unbiased whole genome amplification for multiplex NGS sequencing, and developed a bioinformatics pipeline for NGS data analysis; this feasibility data provides foundation for unbiased universal pathogen detection (surveillance and syndromic applications). In addition, feral swine have behavior and habitat preferences that bring them into contact with wild waterfowl, domestic swine, poultry, and humans and make them possible vectors for virus transfer. This study also highlights the importance of educating hunters and others who come in contact with feral swine on the risk of zoonotic diseases and ways to reduce the risk of exposure to these agents.

Epidemiology of Influenza A Virus (IAV) Circulating in US Swine Between 2004 and 2011

Jianqiang Zhang, Phil Gauger, Amy Chriswell, Xue Lin, Sara Nezami, Dong Sun, Devon Haney, Wendy Stensland, Aaron Irons, Karen Harmon, Erin Strait, Kyoung-Jin Yoon

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

Narrative: Contemporary IAVs circulating in US swine are H1N1, H1N2, and H3N2 subtypes. Phylogenetically H1 viruses can be classified into one of 4 clusters (α, β, γ, and δ), while H3 viruses can be clustered as I, II, III or IV. In early spring 2009, a novel pandemic H1N1 virus (H1N1pdm09) emerged in humans and subsequently spread to pigs. The H1N1pdm09 virus has NA and M genes of Eurasian lineage that was not detected in US swine before. Understanding the current status and trends of various clusters of IAV is important for control and prevention of IAV infection in US swine. We surveyed and analyzed HA sequences and NA subtypes of 906 IAVs detected in swine submissions to Iowa State University Veterinary Diagnostic Laboratory between 2004 and 2011. The majority of IAVs in swine were H1. Phylogenetically, very few H1 viruses belonged to the α-cluster. H1β viruses predominated (67-45%) in 2004-2006 and then decreased to 3% in 2011, whereas H1γ viruses predominated in 2007 (49%) and then remained consistent at 34-38% from 2008-2011. Prior to 2009, no H1N1pdm09 viruses were detected; however, in 2010, 35% of the H1 viruses were H1N1pdm09 and this number declined to 11% in 2011 with exclusion of reassortant viruses containing genes from the H1N1pdm09 virus. H1δ viruses were detected starting from 2005 and tended to increase over time. The H1δ1 viruses were almost exclusively H1N2 and had a sharp increase in 2011; H1δ2 viruses were mostly H1N1 during 2005-2007 but gradually replaced by H1N2 during 2008-2011. During 2004-2011, cluster IV H3 viruses predominated among the H3 viruses. To further understand the possible reassortment between H1N1pdm09 and endemic IAVs in swine, 215 IAV isolates (176 H1 and 39 H3) obtained in 2011 under the SIV surveillance program were sequenced for HA, NA and M genes. Of the H1 viruses (H1N1 and H1N2), 10% had H1N1pdm09 HA, NA and M genes. Surprisingly, additional 52% of the H1 viruses had only the H1N1pdm09 M gene. Among the H3N2 viruses, 56% contained the H1N1pdm09 M gene. Surveillance data also indicated viruses with HA, NA and M genes closely related to an H3N2 variant virus which emerged in the fall of 2011 resulting in 13 human infections were detected in the swine population. In summary, IAVs circulating in US swine continue to change. Reassortment between H1N1pdm09 virus and endemic swine IAVs had increased significantly since 2011 and greatly contributed to diverse influenza virus ecology in US swine.

Influenza A Virus Detection in Oral Fluid and Nasal Swabs by a Rapid Antigen Detection Kit in Swine Inoculated with IAV †

Christa K. Goodell[1], Apisit Kittawornrat[1], Yaowalak Panyasing[1], Chris Olsen[1], Thomas Overbay[3], Chong Wang[1, 2], Rodger Main[1], 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]Abaxis, Inc., Union City, CA

Narrative: The detection of IAV in swine populations using nasal swabs is labor intensive and insensitive in non-febrile pigs[1]. Oral fluid samples have been an excellent surveillance sample for several swine respiratory viruses[2,3,4]. The objective of this study was to compare the detection of IAV in nasal swabs (NS) and pen-based oral fluid (OF) from experimentally inoculated swine over time using the VetScan® Rapid Test. Eighty two piglets were isolated for 30 days and confirmed negative for PRRSV, *Mycoplasma hyopneumoniae*, and IAV infections. A subset (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health). Thereafter, pigs were intratracheally inoculated with one of two IAV viruses (A/Swine/OH/511445/2007 γ H1N1 or A/Swine/Illinois/02907/2009 Cluster IV H3N2) or remained negative controls. Virus isolates were kindly provided by Dr. Amy Vincent (USDA, NADC, Ames, IA) and Dr. Marie Gramer (University of Minnesota, St. Paul, MN), respectively. Pen-based OF samples were collected daily DPI 0-16. Individual NS were collected daily DPI 0-6, then DPI 8, 10, 12, 14, 16. Samples were randomized and tested for IAV using a 15 minute antigen detection assay (VetScan® Rapid Test, Abaxis Inc.). Only samples collected on DPIs 0 to 10 were tested. There were no differences in detection between serotypes, therefore results were summarized by sample matrix (OF or NS). No false positives were observed with the Rapid Test. There were minimal differences in rate and duration of detection between NS and OF in unvaccinated pigs by either serotype. Sensitivity of IAV detection in OF DPI 0-5 improved if the assay was read at 30 rather than 15 minutes (AUC= 0.752 vs. AUC = 0.701) and was equivalent to NS (p = 0.74). Detection of IAV in OF was inhibited by vaccination. The VetScan® Rapid Test could be a useful pen-side test for the detection of IAV antigen during acute infection using either OF or NS. The study was supported by Pork Checkoff funds through the National Pork Board (#09-193). VetScan® AIV Rapid Test kits were kindly provided by Abaxis, Inc. References: 1. Olsen C et al., 2006. In: Straw B et al, (eds). Diseases of Swine (9th ed.). pp. 469-482. 2. Hoffmann P et al., March 8-11, 2008. 38th Annual Meeting of the AASV, 301-302. 3. Prickett et al., 2008. *J Vet Diagn Invest* 20,156-163. 4. Prickett et al., 2008. *J Swine Health Prod* 16(2), 86-91.

† Graduate Student Oral Presentation Award Applicant

Influenza A Virus Detection in Oral Fluid and Nasal Swabs by RT-PCR in Swine Inoculated with IAV †

Christa K. Goodell[1], Rolf Rauh[3], William M. Nelson[3], Angela Burrell[4], Catherine O'Connell[4], Chong Wang[1, 2], Rodger Main[1], 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]Tetracore, Inc., Rockville, MD; [4]Life Technologies, Inc., Austin, TX

Narrative: The detection of influenza A virus (IAV) in swine populations using nasal swab specimens is labor intensive and relatively insensitive in non-febrile pigs[1]. Oral fluid samples have been shown to be an excellent surveillance sample for several swine respiratory viruses[2,3,4]. Therefore, the objective of this study was to compare the rate of detection of IAV by RT-PCR in nasal swabs vs. pen-based oral fluid from experimentally inoculated swine over time. Eighty two piglets were isolated for 30 days and confirmed negative for PRRSV, *Mycoplasma hyopneumoniae*, and IAV infections. A subset (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health). Thereafter, pigs were intratracheally inoculated with one of two IAV viruses (A/Swine/OH/511445/2007 γ H1N1 or A/Swine/Illinois/02907/2009 Cluster IV H3N2) or remained negative controls. The virus isolates were kindly provided by Dr. Amy Vincent (USDA, NADC, Ames, IA) and Dr. Marie Gramer (University of Minnesota, St. Paul, MN), respectively. Pen-based oral fluid (OF) samples were collected daily on DPI 0-16. Individual nasal swabs (NS) were collected daily on days post inoculation (DPI) 0-6, then DPI 8,10,12,14,16. Samples were randomized and then tested for IAV using a matrix screen RT-PCR. False positive PCRs were reported in both OF (n = 1) and NS (n = 3) samples. A pen was classified NS positive if ≥ 1 pig in a pen was NS RT-PCR positive. Using this convention, NS and OF RT-PCR testing results were equivalent through DPI 8, with more OF-positive pens thereafter. Vaccination reduced the duration of detection of IAV in both OF and NS, although RT-PCR positive NS and OF were detected through DPI 14. RT-PCR testing of pen-based OF was equivalent to, or better than, detection using NS at the pen level. Oral fluid is a valid and useful sample type for the detection of IAV by RT-PCR in both unvaccinated and vaccinated pigs for at least 14 days post infection. The study was supported in part by Pork Checkoff funds distributed through the National Pork Board (#09-193). References: 1. Olsen C et al., 2006. In: Straw B et al, (eds). Diseases of Swine (9th ed.). pp. 469-482. 2. Hoffmann P et al., March 8-11, 2008. 38th Annual Meeting of the AASV, 301-302. 3. Prickett et al., 2008. *J Vet Diagn Invest* 20,156-163. 4. Prickett et al., 2008. *J Swine Health Prod* 16(2), 86-91.

† Graduate Student Oral Presentation Award Applicant

Influenza A Virus Isolation from Oral Fluid and Nasal Swabs in IAV Inoculated Pigs †

Christa K. Goodell[1], Fanghong Zhou[1], Chong Wang[1, 2], Kyoung-Jin Yoon[1], Rodger Main[1], 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

Narrative: The detection of IAV in swine populations using nasal swab specimens is labor intensive and relatively insensitive in non-febrile pigs[1]. As an alternative, oral fluid samples have been shown to be an excellent surveillance sample for several swine respiratory viruses[2,3,4]. The objective of this study was to compare the rate of virus isolation of IAV in nasal swabs and pen-based oral fluid from experimentally inoculated swine over time. Eighty two piglets were isolated for 30 days and confirmed negative for PRRSV, *Mycoplasma hyopneumoniae*, and IAV infections. A subset (n = 28) was vaccinated twice with a commercial multivalent vaccine (FluSure XP™, Pfizer Animal Health). Thereafter, pigs were intratracheally inoculated with one of two IAV viruses (A/Swine/OH/511445/2007 γ H1N1 or A/Swine/Illinois/02907/2009 Cluster IV H3N2) or remained negative controls. The virus isolates were kindly provided by Dr. Amy Vincent (USDA, NADC, Ames, IA) and Dr. Marie Gramer (University of Minnesota, St. Paul, MN), respectively. Pen-based oral fluid (OF) samples were collected daily on days post inoculation (DPI) 0-16. Individual nasal swabs (NS) were collected daily on DPI 0-6, then DPI 8, 10, 12, 14, 16. Samples were randomized and submitted for IAV detection by virus isolation (VI) at the Iowa State University Veterinary Diagnostic Laboratory. Results: To compare OF and NS results, a pen was classified NS "positive" if any pig in the pen was NS VI positive. Differences were noted between the number of VI positive pens (NS vs OF) detected for each serotype by DPI, but over time, differences were seen due to vaccination status and sample matrix. One false positive VI was reported in a NS sample. In unvaccinated pigs, there was no difference in the duration of detection by VI between NS and OF (DPI 6) regardless of serotype. Detection of IAV by VI on both OF and NS was inhibited by vaccination. Thus, pen-based OF is a valid and useful sample type for VI in unvaccinated pigs for at least 6 days post infection. The study was supported in part by Pork Checkoff funds distributed through the National Pork Board (#09-193). References: 1. Olsen C et al., 2006. In: Straw B et al, (eds). Diseases of Swine (9th ed.). pp. 469-482. 2. Hoffmann P et al., March 8-11, 2008. 38th Annual Meeting of the AASV, 301-302. 3. Prickett et al., 2008. *J Vet Diagn Invest* 20,156-163. 4. Prickett et al., 2008. *J Swine Health Prod* 16(2), 86-91.

† Graduate Student Oral Presentation Award Applicant

Outbreak of Influenza A (H3N2) in People and Pigs at a County Fair

Sabrina L. Swenson[1], Mary Lea Killian[1], Roman Pogranichniy[2], Jennifer Strasser[4], Steve Lindstrom[5], Pravina Kitikoon[6], Jennifer House[7], Stephen D. Lenz[3], Leo Koster[1], Bret Marsh[4], Bill Davis[5], Lashondra Berman[5], Amy Vincent[6], Shawn Richards[7], Mark Glazier[7], Sarah M. Tomlinson[1]

[1]Diagnostic Virology Laboratory, NVSL, Ames, IA; [2]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN; [3]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN; [4]Indiana Board of Animal Health, Indianapolis, IN; [5]Centers for Disease Control and Prevention, Atlanta, GA; [6]National Animal Disease Center, USDA, Ames, IA; [7]Indiana State Department of Health, Indianapolis, IN

Narrative: On July 11, 2012 a fair veterinarian was requested to examine an ill pig in the show barn. The following day additional pigs were reported as listless, anorexic, and febrile (up to 107°F). The Board of Animal Health was notified of the situation on July 12th. Approximately 280 pigs were in attendance at the fair, which ran July 8th-14th. The majority of the pigs arrived at the fairgrounds on July 8th. Nasal and oropharyngeal samples were randomly collected from 12 pigs dispersed throughout the swine barn. Each pig represented a different herd and was chosen to represent a range of clinical signs. Samples were sent to the State Animal Disease Diagnostic Laboratory (a participating National Animal Health Laboratory Network Laboratory) where they were tested by the matrix rRT-PCR for the presence of influenza A. All 12 samples tested positive. The samples were tested by H and N subtyping rRT-PCRs and all 12 were identified as H3N2. Samples were forwarded to the National Veterinary Services Laboratories (NVSL) where rRT-PCR testing determined that 12/12 samples contained the matrix gene from the 2009 pandemic H1N1 influenza virus. Four samples with the strongest Ct's were sequenced using the Ion Torrent whole genomic sequencer. Sequence was obtained for all eight genes for three of the samples and multiple genes for the fourth sample. Diagnostic specimens were collected from people reporting influenza-like illness that had been in contact with pigs at the fair. PCR testing at the State Department of Health Laboratory indicated a presumptive influenza A H3N2v. Samples were forwarded to the Centers for Disease Control and Prevention (CDC) for analysis. Sequence was obtained directly from the clinical specimens. Comparison of the sequence obtained by NVSL on the pig samples and CDC on the human specimens indicated virtually identical sequence, demonstrating virus transmission between people and pigs. Influenza H3N2 viruses with the pandemic matrix have been found in pigs in multiple states based on swine influenza surveillance testing by State veterinary diagnostic laboratories. Swine influenza is endemic in pig populations throughout the world. Transmission of influenza virus has been documented to occur from pigs to people and vice versa. This report demonstrates the importance of influenza surveillance in livestock and people and the need for strong ties between public health and animal agriculture at the local and Federal levels.

The Effects of Pooling, Swab Type and Transport Conditions on Avian Influenza Virus and Newcastle Disease Virus Detection

Erica Spackman[1], Janice C. Pedersen[2], Enid McKinley[1]

[1]Southeast Poultry Research Laboratory, USDA-ARS, Athens, GA; [2]National Veterinary Services Laboratory, USDA-APHIS, Ames, IA

Narrative: A crucial aspect of achieving high diagnostic test accuracy is sample collection and transport. In order to optimize avian influenza virus (AIV) detection in poultry we have evaluated swab pooling, different swab material types, and transport conditions. Swab pooling was evaluated with Newcastle disease virus (avian paramyxovirus-1 (APMV-1)) in addition to AIV. Parameters for each method which are currently used were compared with potential improvements that are expected to be practical both economically and logistically. Swab pooling is used to save time and money when multiple animals are tested from the same flock or premise. Currently pools of 5 are common for poultry testing however 11 total swabs (2-3 pools) are required to meet the statistical recommendations of the National Poultry Improvement Plan, therefore it would be more efficient for the commercial industry to pool all swabs from the same flock. However, pooling 11 swabs does have cost and logistical disadvantages for the diagnostic lab (pros and cons will be discussed). Detection of virus by rRT-PCR, virus isolation, and for AIV, commercial antigen detection immunoassays were compared with pools of 5 or 11 swabs, or a single swab (oro-pharyngeal and cloacal). To simulate clinical samples swabs were collected from chickens experimentally exposed to A/chicken/VA/SEP-67/2002 H7N2 LPAIV or the Roakin strain of APMV-1. Virus detection by all methods with 1, 5 or 11 swab pools for either AIV or APMV-1 was similar. A second parameter that was evaluated was swab material type. Currently plastic shafted swabs with a wound nylon tip are most common in the US, however some new swabs types, nylon flocked and urethane foam, are expected to have superior capture and release characteristics, thus improving virus detection. Importantly they cost the same as nylon wound swabs. Using oropharyngeal and cloacal swabs collected from chickens experimentally exposed to H7N2 LPAIV, we found that flocked swabs were significantly better ($P<0.05$) to wound nylon swabs and that foam swabs were also better for virus recovery, particularly with cloacal swabs. Finally, we compared: collecting a sample and leaving the swab in the vial during transport with collecting the samples, expressing the swab contents into the vial and then removing the swab prior to transport. This work was just completed therefore the results are still pending.

Modification of the USDA H5 rRT-PCR Assay for Detection of H5N2 Low Pathogenic Avian Influenza Viruses of Mexican Lineage ◊

Janice C. Pedersen, Mary Lea Killian, Nichole Hines, Beverly Schmitt

Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Ames, IA

Narrative: The fifth and final stage of assay validation as outlined by the World Organization for Animal Health (OIE) is the monitoring and maintenance of validation criteria, which includes evaluating the specificity of primers and probes for new lineages of viruses as well as mutations that have occurred due to genetic drift or shift and errors in RNA synthesis. The current United States Department of Agriculture (USDA) official H5 avian influenza (AI) real-time RT-PCR (rRT-PCR) assay has been used for the detection of low pathogenic (LP) and highly pathogenic H5 AI in commercial, live bird market, and backyard poultry, as well as wild bird surveillance, since 2006. The assay is a semi-multiplex method with 2 forward primers, one targeted specifically for detection of European and Asian lineages of H5 and a second targeted to North American (NA) lineages of H5 AI. Assay evaluation and interlaboratory collaboration for harmonization of AI diagnostic tools identified a lineage of LPAI that is not detected by the current USDA H5 rRT-PCR assay. Mutations resulting from immunological pressure from an ongoing vaccination program in the Mexican (MX) poultry industry have resulted in a new lineage of H5 LPAI. Sequence alignments were conducted with H5N2 viruses isolated from poultry in Mexico, as well as NA and Asian H5 AIV for modification of the USDA H5 rRT-PCR assay. In reference to the MX H5 LPAI, the NA forward primer has 3 mismatches in the middle region of the primer located between nucleotides (nt) 8 and 13. The NA probe has four mismatches in the first 12 nucleotides including the first nt while the NA reverse primer does not contain any mismatches with the MX viruses analyzed. A new forward primer and probe have been designed for detection of the MX lineage of H5 and are currently being validated for modification of the USDA H5 rRT-PCR assay. Both the new and old primers and probes are located in the H2 region of the HA gene. Primer and probe specificity was evaluated by single-nucleotide polymorphism analysis with 37 Mexican and 362 American H5 viruses and with H1-H16 subtypes of AI as well as near-neighbor agents. Analytical sensitivity and specificity testing data will be presented.

◊ USAHA Paper

Experimental Co-infection Studies with Avian Influenza Viruses and Newcastle Disease Viruses in Chickens, Turkeys, and Domestic Ducks ◊

Mary Pantin-Jackwood, Patti Miller, Claudio Afonso, Erica Spackman, Darrell Kapczynski, Eric Shepherd, Diane Smith, Rami Cha, Mariana Sa E Silva, David Swayne

Southeast Poultry Research Laboratory, USDA-ARS, Athens, GA

Narrative: Co-infections of poultry with Newcastle disease viruses (NDVs) and avian influenza viruses (AIVs) present a problem both from the clinical point of view and the diagnosis of these viruses. Little has been done to understand the interactions between these two viruses when infecting poultry. Exposure to NDV, either live vaccines or field strains, is nearly unavoidable for commercial and non-commercial poultry worldwide, so co-infections with avian influenza viruses are expected to occur. The goal of this study was to examine the interaction between NDV and AIV in infected poultry species. We conducted experiments in which we infected chickens, turkeys and domestic ducks with lentogenic, mesogenic or velogenic strains of NDV, and with low pathogenicity (LP) or high pathogenicity (HP) AIV, as relevant to specific ecosystems, by giving one of the viruses first or by giving them simultaneously. Pathogenesis (clinical signs, lesions), presence of the viruses in tissues, duration and titer of virus shedding for each virus, transmission to contact birds, and seroconversion to both viruses were evaluated. Chickens co-infected with a lentogen NDV vaccine strain (LaSota) and a LPAIV (H7N2) responded similarly to infection as chickens infected with the viruses given separately. In turkeys, infection with the LPAIV interfered with the NDV infection, especially if given first. Interestingly, chickens inoculated with a more virulent NDV virus (a mesogenic strain, Pigeon/84) were refractory to infection with a HPAIV (H5N2) if given three days after NDV infection (at the peak of virus replication in tissues). Similarly, previous infection of domestic ducks with a velogenic NDV or a LPAIV (H7N8) interfered with infection with the other virus. In conclusion, previous or simultaneous infection of NDV and AIV can affect the replication dynamics and the disease caused by these viruses in poultry. The information obtained from these studies helps in understanding the interaction of these viruses in the field and improves the diagnosis of these viruses.

◊ USAHA Paper

Engineering an Improved Classical Swine Fever ELISA to Detect Antibody in Sera

Melissa L. Batonick, Gregory Mayr

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

Narrative: The United States has been free of classical swine fever virus (CSFV) since 1978. The US employs a serological surveillance program to ensure early detection of CSF antibody in case of an outbreak. CSFV has high protein sequence homology with the other members of the pestivirus family, including bovine viral diarrhea virus (BVDV) and border disease virus (BDV), which can also both infect pigs. Currently, a commercially available E2-coated blocking ELISA is being used for CSF antibody detection. This assay has relatively high sensitivity and specificity yet still allows for 1% false positive results due to cross reactivity with antibodies against BVDV and BDV. While a 1% false positive rate seems low, the current follow-up effort involved in confirmatory testing is time consuming and labor intensive. We are engineering a more specific CSF blocking ELISA by testing a panel of monoclonal antibodies (mAbs) that have previously been shown to be specific for CSFV. We have identified three mAbs that, when used with recombinant E2 protein as the coating antigen, have increased specificity for CSF as compared to two commercially available kits. In an attempt to further enhance the specificity of the assay, we sub-cloned a highly immunogenic domain of the CSFV E2 protein into a baculovirus expression system. The specificity of the 100 amino acid protein and the full length recombinant E2 protein as the coating antigen in a blocking ELISA are compared. The most specific and sensitive antigen and mAb combination will be fully validated with both laboratory and field samples. This improved CSF antibody ELISA will lead to faster results and have a cost-savings impact on the national surveillance program.

Monitoring of Four Naturally Infected Horses for Vesicular Stomatitis Antibody

Dawn Toms, Mindy Powell, Michelle Redlinger, Tamara Beach, Melinda Jenkins-Moore, Tina Buffington, Cindy Harding, Sabrina L. Swenson

National Veterinary Services Laboratories, USDA-VS, Ames, IA

Narrative: The National Veterinary Services Laboratories purchased a total of four horses that were naturally infected with vesicular stomatitis virus (VSV) New Jersey serotype during the 2004 and 2005 VSV outbreaks. The horses were kept on site and are bulk bled (500mls) on a monthly schedule for antibody analysis and reagent production. The monthly blood sample was processed and tested in three antibody tests. The tests included the complement fixation test (CF), the virus neutralization test (VN), and a competitive enzyme linked immunoassay (cELISA). All four horses had significant titers on the CF and VN test upon arrival and were positive on the cELISA. Over time the titers on the VN have dropped, but remain high, and three of the four horses remain positive on the cELISA. Three of the four horses are now negative on the CF, with one horse showing a periodic titer of 1:5. This report shows that naturally infected horses can, and will, remain positive on the serologic tests used for the detection of VSV many years after exposure.

Virology 3

Sunday, October 21, 2012

Auditorium II

Moderators: Binu Velayudhan, Kyoung-Jin Yoon

8:00 AM **BVD Antigen Detection - Exploring the Diagnostic Gap**
Ini-Isabee Witzel, Hinrich Voges *144*

8:15 AM **HoBi-like Viruses, an Emerging Pestivirus of Cattle**
Julia F. Ridpath, Fernando Bauermann, Aaron Harmon *145*

8:30 AM **Bovine Viral Diarrhea Virus Infections in Pregnant Cattle: Diverse Outcomes of Fetal Infections in a Natural Occurring Herd Disease ◊**
Robert W. Fulton, Grant Rezabek, Julia F. Ridpath *146*

8:45 AM **Comparison of FTA Cards and Whole Blood PCR for Diagnosis of Persistent Infection with Bovine Viral Diarrhea Virus in Cattle #**
Celeste B. Foster, Beate M. Crossley, Bruce R. Hoar *147*

9:00 AM **Effects of Immunoglobulin G as an Inhibitor of Diagnostic Polymerase Chain Reaction (PCR) Demonstrated on the Detection of Bovine Viral Diarrhea Virus in Dairy Calves**
Munashe Chigerwe, Beate M. Crossley *148*

9:15 AM **Experimental Infection of Holstein Calves with Epizootic Hemorrhagic Disease Virus Serotype 7: a Preliminary Study Using a Variety of Inoculation Routes ◊**
Mark G. Ruder, Daniel Mead, David Stallknecht, Deborah Carter, Justin Brown, Maor Kedmi, Eyal Klement, Barbara Drolet, Elizabeth W. Howerth *149*

9:30 AM **Break**

10:00 AM **Tests on Swine Samples of the Dominican Republic Collected in Regions near the Border to Haiti**
Angel Ventura, Wendy Gonzalez, Roger Barrette, Sabrina L. Swenson, Alexa Bracht, Fessica Rowland, Andrew Fabian, Moran Karen, Fawzi Mohamed, Emily O'hearn, Melinda Jenkins-Moore, Dawn Toms, John Shaw, Paula Morales, David Pyburn, Consuelo Carrillo, Gregory Mayr, Michael T. McIntosh, Ming Yi Deng *150*

10:15 AM **Picornavirus Isolated from a Swine Brain in Michigan**
Melinda Jenkins-Moore, Donna Johnson, Mary Lea Killian, Leo Koster, Doreen Cawley, Aaron D. Lehmkuhl *151*

10:30 AM **Oral Fluid Collection in Swine as a Diagnostic Sampling Technique for Foreign Animal Diseases**
Frederic R. Grau, Erin Mulhern, Mangkey A. Bounpheng, Megan Schroeder, Tammy Beckham, Michael T. McIntosh *152*

10:45 AM **Canine Distemper Outbreak in Multiple Pet Store Dogs Linked to High Volume Breeder ◊**
Donal O'Toole, Myrna M. Miller, Jacqueline L. Cavender, Todd Cornish, Donald L. Montgomery, Brant A. Schumaker *153*

11:00 AM **Association between Breed and Antibody Response to Primo-vaccination against Foot and Mouth Disease in Cattle #**
Barbara P. Brito, Sebastian Di Giacomo, Andres Perez, Danilo Bucafusco, Luis L. Rodriguez, Manuel Borca, Mariano Perez *154*

11:15 AM **Detection of Substantial Porcine Group B Rotavirus Genetic Diversity in United States Swine †**
Douglas Marthaler, Kurt D. Rossow, Marie Gramer, James E. Collins, Max Ciarlet, Jelle Matthijnssens *155*

11:30 AM **Molecular Characterization of Canine Parvoviruses Associated with Myocarditis in Puppies * †**
Hai T. Hoang, Kent Schwartz, Kyoung-Jin Yoon *156*

Symbols at the end of titles indicate the following designations:

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

BVD Antigen Detection - Exploring the Diagnostic Gap

Ini-Isabee Witzel, Hinrich Voges

Animal Health, Livestock Improvement Corporation, Hamilton, New Zealand

Narrative: Early detection and removal of persistently infected (PI) calves is important for bovine viral diarrhea virus (BVDv) control. However, virus detection may be hampered in neonatal calves by maternal antibodies that 'shield' circulating viral particles, leading to false negative test results. This effect is particularly evident during virus isolation (VI) and NS2-3 antigen (Ag) ELISA testing using blood samples. RT-PCR and IHC are essentially unaffected by colostral antibodies, but these methods remain costly or complex. In recent years, validation work carried out on IDEXX's BVD Erns AgELISA has suggested an insignificant diagnostic gap using tissue punches. This cheap and convenient test method is now widely used as a BVDv diagnostic for cattle of all ages. In spring, 2011, we had the opportunity to monitor calves from 24 beef heifers experimentally challenged with BVDv during their first trimester. Our aim was to assess the performance of the BVD Erns AgELISA in newborn calves and specifically to test the hypotheses that: 1.) there is no diagnostic gap if the test is performed on ear tissue punches, and 2.) the diagnostic gap in serum tests lasts less than 5 weeks. Blood serum and ear punch samples were collected on the day of birth, then 1, 2, 4, 6, and 8 days later. Sampling continued at approximately weekly intervals until 5+ weeks of age. Serum IgG levels were measured to confirm colostrum intake. BVD AgELISA and RT-PCR were carried out on all samples, while antibody levels were monitored using IDEXX BVD antibody ELISA and VNT. Serial screening confirmed twelve PI calves (including one PI abortus). Transient infections (TI) were detected in four of the remaining twelve 'normal' calves. Three TI calves appeared to be congenitally infected with evidence of prolonged infection lasting several weeks, although cell-free viremia was suppressed. The serum RT-PCR was 100% sensitive for PI detection. RT-PCR on tissue punches proved particularly efficient at detecting congenital infections and extended TIs, yet RNA detection failed completely in a small number of samples. While most PI calves were test-positive by BVD Erns AgELISA on serum and tissue punches before the age of 2 weeks, antigen remained undetectable in some calves for extended periods. By day 38, all PI samples tested positive by AgELISA. Our study revealed a similar diagnostic gap for both serum and tissue punches in neonatal calves by BVD Erns AgELISA. Test sensitivity ranged from approximately 20% in 1-2 day old calves to 100% by week 6 among the study group. We hope to explore test performance of neonatal calf screening tools for BVD PI detection in the field with a more extensive trial amongst dairy herds during the 2012 calving season.

HoBi-like Viruses, an Emerging Pestivirus of Cattle

Julia F. Ridpath[1], Fernando Bauermann[2], Aaron Harmon[3]

[1]Ruminant Diseases and Immunology, NADC/ARS/USDA, Ames, IA; [2]Veterinary Sciences, University of Santa Maria, Santa Maria, Brazil; [3]Novartis Animal Health, Larchwood, IA

Narrative: Currently there are four recognized species within the pestivirus genus. Phylogenetic analysis has identified four additional species. Listed in chronological order of discovery these are, Giraffe (isolated from an outbreak of mucosal-like disease in giraffes in the Nanyuki District of Kenya), HoBi (first isolated from fetal bovine serum originating in Brazil and later from samples originating in Southeast Asia), Pronghorn (isolated from an emaciated blind pronghorn antelope in U.S.), and Bungowannah (isolated following an outbreak in pigs, resulting in still birth and neonatal death, in Australia). Viruses from "HoBi virus like" putative species (also known as HoBi-like viruses, atypical pestiviruses or BVDV3) have been isolated from cattle or bovine products originating in South America, Southeast Asia and Europe. In a series of studies we observed the following. Clinical presentation following acute infection of cattle with a HoBi-like virus was very similar to field strains of noncytopathic BVDV and includes low-grade pyrexia and reduction in circulating lymphocytes. Detection of a HoBi-like virus using a commercially available antigen capture enzyme-linked immunosorbent assays (ELISA) kit, based on the detection of the pestiviral protein Erns, was statistically similar to that of BVDV1 and BVDV2. In contrast, reverse transcription polymerase chain reaction (RT-PCR) tests using the panpestivirus primers had lower sensitivity for the detection of HoBi-like strains than the four recognized species of pestivirus. Two commercial ELISA kits designed to detect antibodies against BVDV, missed 22.2% and 77.7% of serum samples containing low to moderate levels of HoBi virus neutralizing antibodies. Sera of cows vaccinated using killed or modified live vaccine containing either BVDV1 and BVDV2 antigens had low neutralizing activity against two HoBi-like viruses, indicating that these vaccines provided limited protection against infection with a HoBi-like virus. It appears that exposure to different pestivirus species can be monitored by serology. Animals exposed to BVDV1 or BVDV2, either by natural exposure or vaccination, have a higher serum antibody titer against BVDV1 or BVDV2 than other pestiviruses. Similarly, animals exposed to a HoBi-like virus will have a higher titer against another HoBi-like virus than against other pestiviruses. We propose using differential serology to survey pestivirus exposure in US cattle. To date, HoBi-like viruses have not been isolated from animals residing in Africa, North America or Australia suggesting that wildlife and domestic ruminants in these continents are naïve and vulnerable to infection. Introduction of these viruses into naïve populations could have serious economic impact.

Bovine Viral Diarrhea Virus Infections in Pregnant Cattle: Diverse Outcomes of Fetal Infections in a Natural Occurring Herd Disease ◊

Robert W. Fulton[1], Grant Rezabek[2], Julia F. Ridpath

[1]Veterinary Pathobiology, Oklahoma State University, Stillwater, OK; [2]Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stilllwater, OK; [3]National Animal Disease Center, USDA-ARS, Ames, IA

Narrative: Bovine viral diarrhea virus (BVDV) affects cattle and clinical forms are varied, including fetal infections. Fetal outcomes depend on stage of fetal development. Outcomes include fetal malformations, abortions, stillbirths, and calves born persistently infected (PI). A herd owner purchased two cows pregnant and nursing calves in December, 2010. One cow (#31) delivered a calf in 2011 that was test positive in spring 2012 as yearling (#52) by skin test (ear notch) immunohistochemistry (IHC) for BVDV. Another 11 cows nursing small calves (assumed open) were purchased in March, 2011, and commingled with the first two cows. Vaccination status was not known, nor were cattle tested for BVDV at purchase. A breeding bull purchased in June, 2011, tested negative for BVDV by skin test IHC. On January 3, 2012, one cow (#33) aborted, and on February 5, another cow (#50) aborted with a suspect fetal anomaly. A third cow (#46) was believed to have aborted in 2012. The aborted fetuses from cows #33 and #46 were not collected for diagnostic testing. The fetus from #50 was approximately 6.5 months, consistent with breeding by the bull purchased in June, 2011. Necropsy examination confirmed multiple congenital anomalies including arthrogryposis, kyphosis, scoliosis, polydactylism, and cardiac overriding aorta. Many cases have genetic basis in the Angus breed, and arthrogryposis multiplexa (AM) and contractural arachnodactyly (CA) were suspected. BVDV was in the differential diagnosis, and BVDV fluorescent antibody testing was positive in the liver and kidney. Tissue homogenates of lung, liver, and kidney were positive for BVDV2 by PCR. The homogenates grown on MDBK cells were positive for BVDV2 by PCR and were subtyped as BVDV2a. Subsequently, 13 cows, 5 calves and 2 yearlings were tested using IHC, and were negative except for the yearling, #52, born in 2011. Serum from this calf and cell culture fluids were PCR positive for BVDV2. This virus has been submitted for subtyping. The dam of the calf with the congenital deformities was negative by the skin test IHC. Fetal tissue (liver/kidney) from the calf with the congenital deformities was submitted for AM and CA genetic testing, and was negative for AM and CA. This case illustrates issues for the clinician and diagnostician: (1) genetic based anomalies are possible, yet infections and toxin-based etiologies must be considered, (2) BVDV fetal infections are varied as illustrated with abortions, anomalies and PI calves all being possible, and (3) biosecurity measures are not always followed nor known by the owner.

◊ USAHA Paper

Comparison of FTA Cards and Whole Blood PCR for Diagnosis of Persistent Infection with Bovine Viral Diarrhea Virus in Cattle #

Celeste B. Foster[1], Beate M. Crossley[2], Bruce R. Hoar[3]

[1]School of Veterinary Medicine, University of California, Davis, CA; [2]California Animal Heath and Food Safety, University of California, Davis, CA; [3]Western Institute for Food Safety and Security, University of California, Davis, CA

Narrative: Infection with bovine viral diarrhea virus (BVDV) causes disease in cattle worldwide resulting in economic losses. Persistently infected (PI) animals serve as a major viral reservoir as these animals shed virus throughout their lives. Identification and removal of PI animals from herds is paramount to successful control and eradication efforts. This study aims to simplify the collection and submission process for BVDV testing by validating the use of Flinders Technology Associates (FTA) paper for blood collection for subsequent RT-PCR. Using FTA cards as sample matrix allows storage and transport of samples at room temperature, and allows sampling in remote areas where no cold chain can be implemented. For this study, a pen of PI animals was sampled and a group of BVDV negative animals were sampled and comparisons between nasal swabs and blood samples on FTA cards versus simultaneously collected EDTA blood were performed. Additional to the detection of BVDV, viral typing was successfully conducted using blood collected in this manner. In a test of 92 beef animals, 100% correlation in PCR test result (positive or negative) was found between the results of whole blood RT-PCR and RT-PCR of blood from FTA papers and 98.6% correlation between the results of RT-PCR of blood and nasal swabs for the previously positive samples. Evaluation of the PCR assay cycle threshold (CT) showed a greater number of cycles for the FTA samples to reach the CT as compared to whole blood however, Bland-Altman plots revealed good agreement between the two assays with 95% agreement intervals of 7.31 and 14.93 for FTA blood and nasal swabs respectively. Thus, FTA papers may be used for blood or nasal secretion collection and storage for later RT-PCR for BVDV, which will allow for increased efforts at detection of PI animals and elimination of this important virus.

AAVLD Trainee Travel Awardee (Virology)

Effects of Immunoglobulin G as an Inhibitor of Diagnostic Polymerase Chain Reaction (PCR) Demonstrated on the Detection of Bovine Viral Diarrhea Virus in Dairy Calves

Munashe Chigerwe[1], Beate M. Crossley[2]

[1]Department of Medicine and Epidemiology, University of California, Davis, CA; [2]California Animal Health and Food Safety Laboratory, University of California, Davis, CA

Narrative: Objective: To investigate if presence of colostral derived immunoglobulin G (IgG) in blood is an inhibitor of diagnostic PCR for detection of bovine viral diarrhea virus (BVDV). Animals: 11 precolostral and 11 postcolostral blood in EDTA anticoagulant and serum samples from 11 Holstein calves. Procedures: Serum and blood in EDTA anticoagulant samples were collected from calves prior to ingestion of colostrum. Calves were fed 3 L of colostrum once, by oroesophageal tubing. Post-colostral, blood and serum samples were collected at 48 hours of age. Serum IgG concentrations were determined in the precolostral and postcolostral serum samples using radial immunodiffusion. The blood samples (precolostral and postcolostral) were spiked with BVDV virus and two diagnostic PCR extraction methods were applied to each sample. The amplification efficiencies of the two PCR methods on the precolostral and postcolostral EDTA blood samples were evaluated. Results: Two of the 11 calves had inadequate passive transfer of colostral immunoglobulins at 48 hours of age based on the serum IgG concentrations. All blood samples from calves were negative for BVDV prior to the spiking with the virus. There was no difference in amplification efficiency in precolostral and postcolostral samples, using the two different extraction methods among the three different virus concentrations evaluated. Conclusions and Clinical Relevance: The results of this study suggest that IgG is not an inhibitor of PCR used for detection BVDV in cattle. The two extraction methods for BVDV used in this study are acceptable for PCR detection of BVDV in cattle.

Experimental Infection of Holstein Calves with Epizootic Hemorrhagic Disease Virus Serotype 7: a Preliminary Study Using a Variety of Inoculation Routes ◊

Mark G. Ruder[1, 3], Daniel Mead[1], David Stallknecht[1], Deborah Carter[2], Justin Brown[1], Maor Kedmi[4], Eyal Klement[4], Barbara Drolet[3], Elizabeth W. Howerth[2]

[1]Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, GA; [2]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA; [3]Arthropod-Borne Animal Diseases Research Unit, USDA-ARS, Manhattan, KS; [4]Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel

Narrative: Infection of cattle with epizootic hemorrhagic disease (EHD) viruses (EHDV) is frequently subclinical but reports of EHD in cattle have increased in recent years. In 2006, a widespread EHDV-7 epizootic caused disease and economic loss in the Israeli dairy industry. EHDV-7 is exotic to North America, but previous studies show that white-tailed deer are potential hosts and *Culicoides sonorensis*, a North American vector of EHDV, is a competent vector. Our primary objective was to infect cattle with EHDV-7 and attempt to replicate disease observed in Israel. A sub-objective was to evaluate cattle with low titer viremia (<102.3 TCID50/ml) as a source of virus to feeding *C. sonorensis*. Seven, two-month-old Holstein calves were used. The virus was provided by the Institute for Animal Health, Pirbright Laboratory and was originally isolated from a cow in Israel. Three inoculation methods were used (two calves/method): group 1, baby hamster kidney (BHK) cell culture supernatant by intradermal (ID) and subcutaneous (SC) injection (1.5 ml/route; 107.12 TCID50); group 2: BHK supernatant by ID, SC, and intravenous (IV) injection (0.67 ml/route; 107.12 TCID50); and group 3: transmission by laboratory infected *C. sonorensis*. A negative control received non-infected BHK supernatant similar to group 2. Animals were monitored daily and blood collected on 0, 3, 5, 7, 10, 13, and 18 days post infection (dpi) for virus isolation and titration, serology, and complete blood count. On dpi 18, *C. sonorensis* were fed on four calves and processed in pools of five for virus isolation 10 days post feeding. All calves had detectable viremia by 3 dpi through 18 dpi (end of study). Peak viremia occurred 7-10 dpi (102.63-103.5 TCID50/ml). No differences in virus kinetics were observed between inoculation groups. Calves seroconverted by 10 dpi. Group 2 calves developed a transient fever (103.9 and 104.7 °F) on 1dpi and again 5-9 dpi (103.3-104.4 ° F). No other clinical abnormalities were observed. Midges were fed on four calves on 18 dpi (viremia <102.3 TCID50/ml). None of the 124 midge pools processed were positive by virus isolation. This study demonstrates US-origin cattle are susceptible to infection with EHDV-7 by multiple inoculation methods; however, similar to other studies, overt disease consistent with field reports was not replicated experimentally. Midges that fed on calves with low-titer viremia did not become infected; however, only 620 midges were processed, so these animals should not be excluded as a potential source of virus to biting midges.

◊ USAHA Paper

Tests on Swine Samples of the Dominican Republic Collected in Regions near the Border to Haiti

Angel Ventura[1], Wendy Gonzalez[1], Roger Barrette[2], Sabrina L. Swenson[3], Alexa Bracht[2], Fessica Rowland[2], Andrew Fabian[2], Moran Karen[2], Fawzi Mohamed[2], Emily O'hearn[2], Melinda Jenkins-Moore[3], Dawn Toms[3], John Shaw[4], Paula Morales[4], David Pyburn[5], Consuelo Carrillo[2], Gregory Mayr[2], Michael T. McIntosh[2], Ming Yi Deng[2]

[1]Ministry of Agriculture, Santo Domingo, Dominican Republic; [2]NVSL, USDA/APHIS/VS, Greenport, NY; [3]NVSL, USDA/APHIS/VS, Ames, IA; [4]US Embassy, USDA/APHIS/IS, Santo Domingo, Dominican Republic; [5]Swine Health Program, USDA/APHIS/VS, Des Moines, IA

Narrative: The Dominican Republic (DR) and Haiti share the island of Hispaniola, and reportable trans-boundary animal diseases have been introduced between the two countries historically. Outbreaks of severe teschovirus encephalomyelitis in pigs began occurring in Haiti in February, 2009, and porcine teschovirus type 1 (PTV-1) was isolated from brain samples of sick pigs. Results of sequence and phylogenetic analyses on the polyprotein of PTV strains indicate that the Haitian isolate is most closely related to other PTV-1 strains, including the strain Konratice which was isolated in Czechoslovakia from pigs with porcine viral encephalomyelitis (Teschen disease). One of two healthy pigs inoculated with the Haitian PTV-1 isolate showed typical clinical signs of teschovirus encephalomyelitis, including paralysis of the hindquarters in an experiment at the National Veterinary Services Laboratories, Ames, IA. A field and laboratory study of April, 2010, indicated that multiple disease agents, including PTV-1, classical swine fever virus (CSFV), porcine circovirus type 2 (PCV-2), porcine reproductive and respiratory syndrome virus (PRRSV), and swine influenza virus (SIV) were circulating in the swine population in Haiti including regions close to the border with the DR. This study was conducted in August, 2010, to determine if swine teschovirus encephalomyelitis is endemic in regions of the DR near the border to Haiti and to identify the other viral disease agents present there. Serum samples were collected from 109 pigs including those clinically suspected of having PTV infection, as well as apparently healthy pigs from 36 swine premises. Seven pigs with central nervous system signs were euthanized and necropsied, and brains, spinal cords, and tonsils of these animals were collected. Among 109 serum samples tested, 65 (59.6%) were positive for antibodies to PCV-2, and 51 (46.8%) were positive for antibodies to CSFV. Fifty-four of the 109 serum samples were tested for antibodies to other agents. Among the 54 samples, 20 (37.0%) were seropositive to PTV-1, 17 (31.5%) tested seropositive to SIV H3N2, 12 (22.2%) were seropositive to SIV H1N1, and 1 (1.9%) was seropositive to PRRSV. PTV-1 sero-positives were scattered in all study regions. Six of 7 brains and 6 of 7 spinal cords were positive in reverse transcription-polymerase chain reaction for PTV. Genome sequencing on the Dominican PTV and phylogenetic analysis on the polyprotein of PTV strains indicate that the sequence of the Dominican PTV is virtually identical to the Haitian isolate and closely related to other PTV-1 strains in the world.

Picornavirus Isolated from a Swine Brain in Michigan

Melinda Jenkins-Moore[1], Donna Johnson[1], Mary Lea Killian[1], Leo Koster[1], Doreen Cawley[2], Aaron D. Lehmkuhl[1]

[1]National Veterinary Services Laboratories, USDA, Ames, IA; [2]Michigan Area Office, East Lansing, MI

Narrative: On November 2, 2011, a sow was observed with neurologic symptoms and hypersalivation at a Michigan slaughter plant. Brain tissue samples were collected and submitted fresh to the National Veterinary Services Laboratories (NVSL) for analysis. Sections of cerebrum, cerebellum, and brainstem were fixed in formalin upon receipt at the NVSL and processed routinely for histologic examination. No gross brain lesions were apparent. Microscopic changes were extremely mild and included multifocal hemorrhage and necrosis in the brainstem, and neuronal satellitosis in the cerebrum. Brain tissue homogenate was inoculated onto cell monolayers: primary swine kidney (pSK), porcine kidney (PK-15), swine testicle (ST), and Vero. The monolayers were held at 37C for seven days and observed regularly for cytopathic effect (CPE). No CPE was observed in the inoculated monolayers. After seven days the monolayers were frozen at -70C and subpasssaged onto fresh cell monolayers. CPE was observed five days post inoculation in the pSK cell monolayers. Attempts to identify the isolate by a polyvalent immunofluorescence assay (IFA) specific for 17 common swine viruses and a Seneca Valley-like virus IFA were unsuccessful. Cells were stained by IFA with polyvalent porcine antiserum (porcine teschovirus 1-7 (PTV), porcine sapelovirus (PSV), pseudorabies virus (PRV), encephalomyocarditis virus (EMCV), porcine reovirus, hemagglutinating encephalomyocarditis virus (HEV), porcine adenovirus, transmissible gastroenteritis virus (TGE), swine influenza virus(SIV), porcine parvorvirus, and porcine rotavirus) and a Seneca Valley-like virus (SVV) antiserum. The cell culture isolate was then examined by electron microscopy (EM) and picornavirus-like particles were observed. The isolate was sequenced using an Ion Torrent and was 95% homologous to published SVV sequence. This report demonstrates the importance of utilizing classic methods of virology, the use of primary cell cultures, EM, and modern molecular technologies to identify novel viruses.

Oral Fluid Collection in Swine as a Diagnostic Sampling Technique for Foreign Animal Diseases

Frederic R. Grau[1], Erin Mulhern[1], Mangkey A. Bounpheng[2], Megan Schroeder[2], Tammy Beckham[2, 3], Michael T. McIntosh[1]

[1]USDA APHIS VS NVSL Foreign Animal Disease Diagnostic Laboratory, Plum Island Animal Disesae Center, Greenport, NY; [2]Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, TX; [3]National Center of Excellence for Foreign Animal and Zoonotic Disease Defense, College Station, TX

Narrative: Foreign animal disease (FAD) surveillance in livestock within the United States has traditionally relied on sampling individual animals. Oral fluid collection in swine by way of introducing a chewing rope into a pen may allow for easy sampling of multiple animals or entire herds. This low tech, inexpensive and non-invasive technique has been demonstrated to detect endemic diseases caused by agents such as Influenza A, Porcine Circovirus type 2 and Porcine Reproductive and Respiratory Syndrome Virus and may be useful in FAD surveillance. Oral fluids absorbed in rope can be tested for antibody and/or antigens specific to swine pathogens using serological or molecular techniques. In collaboration with the FAZD and TVMDL, oral fluids from rope samples are being evaluated for Foot-and-Mouth Disease Virus (FMDV), Classical Swine Fever Virus (CSFV) and African Swine Fever Virus (ASFV) detection as an appropriate sample type for a new multiplex real-time PCR assay for endemic and foreign animal diseases in swine. The study design consists of repeated experiments in which animals were directly inoculated with FMDV 01 Brugge, CSFV Haiti, CSFV Brescia, ASFV Lisbon 60' or ASFV Georgia. Oral fluid and oral swabs were collected throughout the course of each experimental infection and tested by realtime RT-PCR using GeneAmp (Applied Biosystems) and Path ID Multiplex (Applied Biosystems) chemistries for FMDV and CSFV detection, and by realtime PCR using a Taqman EZ kit (Applied Biosystems) for ASFV detection. Preliminary data show that FMDV can be detected as early as one day post inoculation (DPI) and one day prior to the presentation of clinical signs. CSF was detected at three DPI for the Brescia strain and five DPI for the less virulent Haiti strain. In at least one study, CSFV was detected in oral fluids one day earlier than in oral swabs. ASF was detected as early as five DPI, and both CSFV and ASFV were detected coincident with the onset of clinical signs. Results between all assays used revealed disease agent detection rates that were comparable between oral fluids collected from rope and individual animal swabs. Data will be compared for performance traits in a feasibility study on a multiplex Taqman-based assay to detect FADs in oral fluids that may serve as a companion to a multiplexed Taqman-based assay for endemic diseases of swine.

Canine Distemper Outbreak in Multiple Pet Store Dogs Linked to High Volume Breeder ◊

Donal O'Toole, Myrna M. Miller, Jacqueline L. Cavender, Todd Cornish, Donald L. Montgomery, Brant A. Schumaker

Wyoming State Veterinary Laboratory, Laramie, WY

Narrative: Canine distemper is uncommon in the pet trade in the United States, due in large part to effective vaccines against canine distemper virus. This is a report of distemper affecting 24 young dogs of multiple breeds shortly after sale by 2 pet stores in Wyoming in August–October, 2010. Cases were diagnosed over 37 days. It was the largest outbreak of distemper in pet dogs recognized by the Wyoming State Veterinary Laboratory over the past 20 years. Diagnosis was established by a combination of fluorescent antibody staining (FA), reverse transcriptase polymerase chain reaction (RT-PCR), virus isolation, negative stain electron microscopy, and necropsy/histopathology. A two-step approach was used to screen high risk dogs by FA of conjunctival swabs, followed by RT-PCR of swabs or buffy coat samples from FA-negative dogs. The approach kept kept costs low and encouraged submissions. Canine distemper virus hemagglutinin gene sequences were obtained from two affected dogs from each of the stores. They were identical. The sequences were distinct from those in an unrelated case of canine distemper occurring contemporaneously in a distempered Wyoming dog from an Indian reservation. Sequences were deposited in the National Center for Biotechnology Information database as one accession (GenBank JF283477; pet store dogs), along with those from the unrelated reservation case (JF283476; Wind River Reservation). The authors are curious to know whether other diagnostic laboratories recognized the former strain associated with distemper in recently purchased dogs in 2010. The breeding property from which dogs originated was quarantined by the Kansas Animal Health Department. Dogs intended for sale were tested for distemper. Distemper was diagnosed on site in November, 2010. At that point 1,466 dogs were euthanized to eliminate dispersal of distemper via commercial channels. The investigation underscores risks inherent in large-scale dog breeding where vaccination and biosecurity practices are suboptimal. Practical steps to diagnose, prevent and control canine distemper in high volume breeding facilities are suggested.

◊ USAHA Paper

Association between Breed and Antibody Response to Primo-vaccination against Foot and Mouth Disease in Cattle #

Barbara P. Brito[1, 4], Sebastian Di Giacomo[2], Andres Perez[1, 3], Danilo Bucafusco[2, 3], Luis L. Rodriguez[4], Manuel Borca[4], Mariano Perez[2, 3]

[1]Center for Animal Disease Modeling and Surveillance, Department of Medicine and Epidemiology, University of California- Davis, Davis, CA; [2]Instituto de Virología, Centro de Investigaciones en Ciencias Veterinarias y Agronómicas (CICVyA), Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina; [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; [4]Plum Island Animal Disease Center, Agricultural Research Service, USDA-ARS, Greenport, NY

Narrative: Foot and mouth disease (FMD) is one the most important animal diseases of livestock. In recent years, social, economic, and environmental concerns have increasingly favored the use of vaccination as the default control measure along with movement restrictions and, in many cases, culling of infected and sometimes in-contact herds. Recent development of effective FMD virus (FMDV) diagnostic test differentiating infection from vaccination has added another advantage to the use of vaccine. Genetic characteristics of the host are known to be an important factor in the immune response for a number of diseases and conditions, and have been investigated, mainly, in specific immune responses elicited by discrete FMDV-derived peptide sequences. However, and although much attention has been given to the quantification of the immune response raised by the vaccine to homologous and heterologous strains, little is known about the host-specific variation in the response. Here, we measured neutralizing antibodies against three FMDV strains (O1/Campos, A24/Cruzeiro, and A/Arg/01) 45 days post vaccination and quantified the breed- and individual-specific variation in the response. A multivalent commercial vaccine was used to immunize 377 naïve 4-7 month-old-calves from four dairy farms in Argentina. Liquid phase blocking-ELISA was used to determine antibody titer. The Pearson correlation of antibody responses was computed to estimate the relationship between each two serotype responses in each calf. Immune response were significantly ($P<0.05$) correlated between virus strains. Hierarchical mixed regression models were formulated separately for each strain to estimate the association of the immune response with sire and sire breed. Herd was included as a random factor to control for lack of independence of within-herd observations. Sire's breed was significantly ($P<0.05$) associated with the immune response for the three FMDV strains ($P=0.02$, 0.01 and 0.04 for O1/Campos, A24/Cruzeiro, and A/Arg/01 respectively). Calves from Jersey sires' antibody titers were 0.45, 0.42, and 0.35 O/D lower than calves from Holstein sires. Results suggest that neutralizing antibody response to FMDV vaccination was associated with breed effect, whereas no intra-breed significant variation was detected.

AAVLD Trainee Travel Awardee (Virology, Epidemiology)

Detection of Substantial Porcine Group B Rotavirus Genetic Diversity in United States Swine †

Douglas Marthaler[1], Kurt D. Rossow[1], Marie Gramer[1], James E. Collins[1], Max Ciarlet[2], Jelle Matthijnssens[3]

[1]Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, MN; [2]Vaccines and Diagnostics, Novartis, Cambridge, MA; [3]Microbiology and Immunology, Rega Institute for Medical Research, Leven, Belgium

Narrative: Rotavirus (RV) is an important cause of gastrointestinal disease in animals and humans. While group A rotavirus (RVA) is considered the most important RV group, four other RV groups have been identified in pigs (RVB, RVC, RVE and RVH). Small intestines from nine piglets with enteritis revealed lesions typical of RV enteritis using histological methods and RV particles detected by electron microscopy, but were negative for rotavirus RVA and RVC by RT-PCR. Subsequently, a RVB specific VP7 RT-PCR was developed, and identified RVB in 46.8% of 173 samples tested thereafter, with the majority (86.4%) having mixed RV infections. RVA/RVB/RVC co-infections were detected at a higher rate (24.3%) than previously reported. Our results revealed a broad genetic diversity of porcine RVB strains, suggesting RVB may be a common and pre-existing cause of enteritis in pigs. RVB infection in pigs is common throughout North America and is associated with clinical disease and tissue lesions typical of the other RV groups. Diagnostic screening for gastroenteritis in pigs should include assays that at the least, look for group A, B and C rotaviruses.

† Graduate Student Oral Presentation Award Applicant

Molecular Characterization of Canine Parvoviruses Associated with Myocarditis in Puppies * †

Hai T. Hoang, Kent Schwartz, Kyoung-Jin Yoon

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

Narrative: Canine parvovirus type 2 (CPV2) is a significant viral pathogen of dogs, particularly puppies. The virus is a non-enveloped DNA virus belonging to the family Parvoviridae and is known to be hardy in the environment. To date, 3 variants of CPV2 have been identified based on the genetic and antigenic difference of VP2 gene: 2a, 2b and 2c. CPV2a and CPV2b are antigenically more similar to the original CPV2 than is CPV2c. In the United States, CPV2b and CPV2c are dominant variants causing parvoviral diseases in dogs. CPV2 can cause three different pathologic forms of disease in dogs: a) gastrointestinal form; b) cardiac form; and c) neurologic form. The enteric form is most commonly associated with CPV2 infection. There have been occasional reports of parvoviral cardiac form; however, these cases were not well characterized for the virus on a molecular basis. Here we report cases of parvoviral myocarditis in a kennel setting with molecular characterization of the virus. Two unrelated 8- and 9-week-old puppies originating from different breeders were submitted to Iowa State University Veterinary Diagnostic Laboratory. The 8-week-old pup was found dead and the 9-week-old pup exhibited respiratory distress immediately prior to death. Grossly, both puppies had a varying degree of pulmonary edema and congestion. Mild cardiomegaly and foci of pallor was present in heart from both puppies. Microscopically, multifocal myocardial necrosis with mineralization was observed in the hearts from both. Mononuclear cellular infiltration was also noted. In one pup, myocardial necrosis was accompanied by fibrosis. *Escherichia coli* (smooth/mucoid) and *Bordetella bronchiseptica* cultured from one of the 2 lung but were not deemed significant. Heart and lung samples were negative for canine herpesvirus, canine distemper virus, canine adenoviruses and influenza virus by frozen tissue section FA tests and/or real-time PCRs. Parvovirus DNA was detected in the hearts from both puppies, suggesting that CPV infection was responsible for myocarditis. Sequencing for the VP2 gene revealed that CPV identified in one of the 2 heart samples was type 2, which was unexpected as type 2 is believed to have been replaced by type 2a or 2b. The CPV identified in the other heart could not be typed because it had amino acid substitutions at important antigenic sites as compared to other CPV2's sequences available in GenBank. These observations suggest that CPV2 causing myocarditis may be genetically different from those causing enteric disease.

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

USAHA/AAVLD Joint Plenary Session:
Science, Economics, and Politics, Oh My!

Monday, October 22, 2012
Guilford ABC

Moderator: Richard Breitmeyer

8:00 AM **Introduction**

8:15 AM **Economics of Farm-Level Animal Agriculture in the Future**
John Lawrence 158

9:00 AM **Economy Wide Impact of a Foreign Animal Disease in the United States**
Dermot Hayes, Jacinto Fabiosa, Amani Elobeid, Miguel Carriquiry, Patrick Webb 159

9:45 AM **Break**

10:00 AM **National Institute for Animal Agriculture Forums on One Health Approach to Antibiotic Use in Food Animals**
Leonard Bull 160

10:45 AM **Egg Products Inspection Act Amendments 2012- Proposed Federal Legislation for Egg Farmers**
Gene W. Gregory 161

11:30 AM **Questions**

Symbols at the end of titles indicate the following designations:

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

Economics of Farm-Level Animal Agriculture in the Future

John Lawrence

College of Agriculture and Life Sciences and Extension and Outreach, Iowa State University, Ames, IA

Narrative: Growing world corn demand for feed and fuel has resulted in higher and more volatile prices for corn. Livestock and poultry industries in the US and globally that developed during a time of plentiful and relatively low cost grain now face significant financial challenges as costs have risen faster that revenues. Consumer-level prices adjust when supplies of meat, milk and eggs decline, but the supply reductions have not been fully reflected in the stores or have been complicated by shifting levels of imports and exports or drought driven decisions. Ultimately, the supply of animal agriculture products will be smaller due to the higher feed costs than would have been the case had world grain prices remained at the pre-2006 levels. Livestock and poultry producers have few options to address the higher feed costs. First, is to hope for a change in energy and/or trade policy to reduce the competition for grain. Second, is to expand demand for their product. Checkoff programs have and continue to work on domestic demand and additional growth has come from exports. Like policy decisions, factors that impact trade are often beyond the producer's control. The third option has been the basis for survival in commodity agriculture and that is for producers to lower their cost of production through improved biological and economic efficiency. Improved efficiency and lower costs of production require renewed innovation and research in technology and production systems. The majority of technologies and production systems used today were developed during a time of lower priced energy, grains and labor. These conditions do not exist today and are not likely to exist in the future. Why would the optimal system for $2/bushel corn be the optimal system for $6/bushel corn? While it is important to recognize that relative prices matter more than absolute prices, producers, the industry and researchers should re-evaluate current beliefs and systems in the new paradigm. A new paradigm where the energy costs for transportation and fertilizer are significant, consumers are more diverse in their preferences and spending regarding food choices; where labor for production systems is often less available, less experienced and more costly than before and where off-shore production is increasingly competitive.

Speaker Biography: John is the Associate Dean, Extension Programs and Outreach in the College of Agriculture and Life Sciences and Director, Agriculture & Natural Resources Extension at Iowa State University. In this position he leads the extension and outreach programs to farmers, agribusiness and natural resource managers in the state of Iowa. Previously, John was the Extension Livestock Economist and Professor, Department of Economics and Director of the Iowa Beef Center. John has written extensively for professional and trade journals, extension publications, and given over 700 formal presentations in 24 states and 5 Canadian provinces, Mexico, Korea, The Netherlands, and Australia. In 2009 he was named by Iowa Farm Today magazine as one of the 25 most influential people in Iowa Agriculture over the past 25 years, 1984-2009.

Economy Wide Impact of a Foreign Animal Disease in the United States

Dermot Hayes[1], Jacinto Fabiosa[1], Amani Elobeid[1], Miguel Carriquiry[1], Patrick Webb[2]

[1]Center for Agricultural and Rural Development, Iowa State University, Ames, IA; [2]Science and Technology, National Pork Board, Des Moines, IA

Narrative: This report uses the CARD FAPRI model to evaluate the economy wide impacts of a disease outbreak that eliminates US pork and beef exports simultaneously and pork exports alone. In either case industry losses are enormous and spread well beyond the pork and beef sectors. Revenues fall significantly for poultry, corn and soybean producers and employment in rural areas is negatively impacted as the US pork and beef sectors are forced to downsize. Revenue losses in the combined US pork and beef industries fall by an average of $12.9 billion per year. The removal of this level of value added activity is equivalent to the loss of as many as 58,000 full time jobs. The report uses option prices to calculate the likelihood of a price impact of the magnitude reported here. This suggests a less than one percent possibility of an outbreak of this severity. Multiplying the probability of an outbreak times the reduction in pork industry net revenues over variable costs in the event of an outbreak, suggests that the annual benefit of eliminating the possibility of this outcome would be worth $137 million.

Speaker Biography: Dr. Patrick Webb is the director of swine health programs at the National Pork Board, where he joined in 2005. He is responsible for the Pork Checkoff efforts in animal identification, pre-harvest traceability and foreign animal disease planning, preparedness and response. Dr. Webb has worked as a private veterinary practitioner in food animal practice in rural Iowa and for Iowa's Department of Agriculture and Land Stewardship as foreign animal disease program coordinator, where he developed the department's emergency preparedness plan for animal disease disasters. Prior to joining the National Pork Board Dr. Webb works as private consultant and has worked to develop foreign animal disease plans at the State and Federal levels. Throughout his career Dr. Webb has worked extensively on emergency preparedness and planning at the local, state and federal level s and has developed and delivered numerous educational programs directed at training producers, veterinarians, county emergency managers and first responders on how to respond to foreign animal disease disasters. Dr. Webb received both his DVM and BS in animal science degrees from Iowa State University and has completed Foreign Animal Disease Diagnostician School. He is a member of the American Association of Swine Veterinarians, Iowa Veterinary Medical Association and the AVMA.

National Institute for Animal Agriculture Forums on One Health Approach to Antibiotic Use in Food Animals

Leonard Bull

Antibiotic Council, NIAA, New Haven, VT

Narrative: The use of antibiotics in animal agriculture is frequently heard and read about in the news media. Because of a general lack of understanding around this topic and often misleading reference or inference by the media, there is increasing concern and confusion among the general public. This is especially important in a time when consumers are increasingly interested in where their food comes from and how it is produced. The concern about antibiotic use in animals primarily revolves around sub therapeutic use and its potential contribution to pathogen resistance to antibiotics . Unfortunately, the issue is frequently biased or oversimplified by the media. The National Institute for Animal Agriculture (NIAA): www.animalagriculture.org organized and hosted a forum in 2011 to bring together representatives from both the animal and human health professions to discuss what is known and what is not known about antibiotic use and the impact of use on antibiotic resistance in pathogens of both humans and animals. That highly successful forum, in which there was a significant exchange of factual, sound information around the entire topic, resulted in a summative White Paper (highlights to be presented— see NIAA website; has had nearly 250,000 views and is available in English, Spanish and Portuguese), and set the stage for a follow up forum in 2012 to focus on a discrete, substantive plan of action. All agree that antibiotic use must be judicious and managed in a careful manner. Everyone has a stake in engaging in meaningful dialogue and creating successful strategies to preserve antibiotic efficacy as a critical global public health tool. The forum, to be held November 13-15 in Columbus, Ohio, will build upon information and consensus from the previous forum. That venue was selected because it offers a unique collection of seven colleges and schools related to both animal and human health within the Ohio State University campus network in Columbus. This will allow NIAA to efficiently draw participation not only from a national and international audience, but from the local community that represents the entire scope of interest in this topic! The global perspective that is built into the program is critical to the discussion, especially from parts of the world where antibiotic use is regulated differently than in the United States. A high priority in the planning of this forum will be focused participation by attendees in the discussions. Details will be presented.

Speaker Biography: Dr. Leonard S. Bull Leonard Bull (Len) is Emeritus Professor of Animal/Poultry Science and Emeritus Associate Director of the Animal and Poultry Waste Management Center at NCState University. He has BS and MS degrees in Dairy Science/Animal Nutrition from Oklahoma State University and a PhD degree from Cornell University with a major in Animal Nutrition and minors in Veterinary Physiology and Biochemistry. His research interests are energy and protein metabolism, ruminant nutrition and animal waste management, and has over 250 publications including a book on Nutritional Energetics. He has held faculty positions at the universities of Maryland, Kentucky and Maine, was Chair of Animal Science at the University of Vermont and was Head of Animal Science at NCState. At NCState he held administrative positions as: Assistant Dean/Director of Agricultural Research and International Agriculture Programs; Associate Vice Chancellor for International Programs; Founder/Director, Center for Global Competitiveness and Associate Director/COO, Animal and Poultry Waste Management Center. Len is Past President of: American Society of Animal Science; American Registry of Professional Animal Scientists; Registry of Environmental and Agricultural Professionals; Vice President, World Association for Animal Production; Chair/CEO, National Institute for Animal Agriculture; Chair/Executive Director, North Carolina State Animal Response Team; Director, American Dairy Science Association; Co-Chair, Southern Agriculture and Forestry Energy Resources alliance; member, Steering Committee and Carbon Reduction Committee, 25x25; director, North Carolina Green Power Commission. As Chair of NIAA he led the development of the efforts associated with antibiotic use in animal agriculture. Retired, Len is Chair,Vermont Agricultural and Forest Products Development Board; consultants on animal agriculture, renewable energy and environmental issues; is Vice Chair of Diplomacy Matters, LLC, (Washington D.C), facilitating linkages between U.S. business and foreign embassies and is advisor to the World Heritage Animal Genetic Resources Institute. Len and wife Angela live in New Haven, Vermont.

Egg Products Inspection Act Amendments 2012- Proposed Federal Legislation for Egg Farmers

Gene W. Gregory

United Egg Producers, Alpharetta, GA

Narrative: United Egg Producers was one of the first, if not the first, among animal agriculture to develop a voluntary animal welfare program based upon the recommendations of an independent scientific committee. Despite these advancements in animal welfare, egg farmers faced state ballot initiatives brought forth by the Humane Society of the United States (HSUS) to force egg production into a cage-free industry. After seeing five (5) states with conflicting laws that will impair our ability to distribute eggs across state lines, we determined for the benefit of egg farmers, our customers and consumers that we needed to find a way to end the conflict with HSUS and to advance the welfare of hens even further with enriched colony cages. UEP reached an agreement in July 2011 to join with HSUS in seeking federal legislation that would preempt conflicting state laws and enact a national standard that would transition the industry to enriched colony cage housing over an 18-year period. We proposely are proposing that a 40-year old eg law, the Egg Products Inspection Act, be amended to include all the points agreed upon between UEP and HSUS.

Speaker Biography: Gene Gregory entered the egg business in 1960 with Corn Belt Hatcheries in Illinois. Within a few years he became Vice President and General Manager of the company that hatched 4 million day old chicks per year, owned and marketed the eggs from 500,000 laying hens, feed manufacturing and poultry house construction company. After having served on the Board of Directors for United Egg Producers, he decided to change his career and became the Midwest regional membership director for UEP in 1982. In 1992 he moved to Georgia and became Senior Vice President and was promoted to President and CEO in February 2007.

Poster Session

Friday October 19 – Sunday October 21

Third Floor Prefunction Area

1. **Swinecheck mix-APP®, a New ELISA Tool to Detect Antibodies to the Most Prevalent Serovars of *Actinobacillus pleuropneumoniae* in Swine Sera**
Andre Ch. Broes *165*

2. **The Effects of North American Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)-based Modified Live Vaccines in Preimmunized Sows Artificially Inseminated with European PRRSV Spiked Semen**
Changhoon Park, Kiwon Han, Hwiwon Seo, Yeonsu Oh, Chanhee Chae *166*

3. **Brain Abscess in a White-tailed Deer**
Anwar J. Sarah *167*

4. **Development and Evaluation of a Mobile Diagnostic Computer Application for Veterinary Laboratories**
Jacqueline C. Cassady, Craig N. Carter, Norman Ronald, Michelle L. Arnold, Cynthia L. Gaskill, Jacqueline Smith, Wade A. Northington *168*

5. **The Prevalence of Potentially Pathogenic Bacteria and Fungi in Bobwhite Quail in the Rolling Plains Ecoregion**
Hongwen Su, Shuping Zhang, Jessica McKelvey *169*

6. ***Bartonella bovis* Isolated from a Cow with Endocarditis**
Erdal Erol, Carney Jackson, Ying Bai, Stephen Sells, Steve Locke, Michael Kosoy *170*

7. **Use of Electronic Nose Technology to Identify Cattle Experimentally Infected with *Mycobacterium bovis* By Detection of Unique Volatile Organic Compounds in Breath: Preliminarily Findings**
Christine K. Ellis, Randall Stahl, Pauline Nol, Jack Rhyan, Matthew McCollum, Kurt VerCauteren, Joni Van Sickle, Lyndsey Linke, Roberta Magnuson, M.D. Salman *171*

8. **Effects of Freeze-thaw Cycle on Urine Values from Bottlenose Dolphins (*Tursiops truncatus*)**
Risa Daniels, Cynthia R. Smith, Stephanie Venn-Watson *172*

9. **Dermatitis, Cellulitis and Myositis due to *Serratia marcescens* in a Cat**
Jane Kelly, Thomas J. Baldwin, Aaron Chamberlain *173*

10. **Report of a New Genotype of *Ehrlichia* species from Cattle and Cervids**
Alvin A. Gajadhar, Brad Scandrett, Vladislav A. Lobanov, Batol Al-Adhami, Gary A. Wobeser, John Campbell *174*

11. **Understanding the Factors that Influence Low Pathogenic Avian Influenza Virus Infection in Ducks ◊**
Justin Brown, Monique Franca, Taiana Costa, Camille Lebarbenchon, Deborah Carter, Elizabeth W. Howerth, David Stallknecht *175*

12. **A Validated Assay of Carbamate Pesticides in Feeds, Baits, and Tissues using QuEChERS and Gas Chromatography/Mass Spectrometry**
Wilson K. Rumbeiha, Andreas Lehner, Margaret Johnson, Asley Couch, John Buchweitz *176*

13. Aquatic Animal Research in a Traditionally Terrestrial Animal Environment: Considerations for Success
Jean Kaptur, Richard Clayton *177*

14. Real-time PCR, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma meleagridis*
Pablo Lopez, Kristen Roza, Phyllis Tyrrell *178*

15. Development and Validation of uvrC Gene-based TaqMan Real-time qPCR Assay for Detection of *Mycoplasma bovis*
Hemant Naikare, Daniela Resende Bruno, Debabrata Mahapatra, Alesia Reinisch, Russell Raleigh, Robert W. Sprowls, Tammy Beckham *179*

16. Diagnostic Findings from Common Eider (*Somateria mollissima*) Mortality Events in the Northeastern United States Associated with Wellfleet Bay Virus, a Novel Orthomyxovirus ◊
Jennifer Ballard, Andrew Allison, Justin Brown, Samantha Gibbs, Chris Dwyer, Julie C. Ellis, Sarah Courchesne, Randall Mickley, Kevin Keel *180*

17. Novel Solution for Manual and Automated Identification of Pathogen Nucleic Acids in Various Veterinary Sample Types
Lillian Roth, Denis Fluegge, Sandy Leifholz, Melanie Breitbach, Kathrin Wolf, Dora Quest, Holger Engel *181*

18. Performance Evaluation of Biolog GEN III System for Identification of Bacteria of Veterinary Origins
Hui-Min Hsu, Stan Stankevitch, Ogi Okwumabua *182*

19. Evaluation of the Development of the Bovine Foot in Response to Variation in Management Practices ◊
Julie A. Gard, Debra R. Taylor, Soren P. Rodning, Dewey R. Wilhite, Kent Sanders, Misty A. Edmondson, Thomas Denney *183*

20. Isolation of *Actinobaculum*-like Organism from a Goat Abscess
Yan Zhang, Jing Cui, Anne Parkinson, Troy Farrell, Beverly Byrum *184*

21. Isolation of a *Campylobacter*-like Organism from a Goat Abscess
Yan Zhang, Jing Cui, Anne Parkinson, Troy Farrell, Beverly Byrum *185*

22. Idiopathic Generalized Soft Tissue Mineralization in an Appaloosa Filly # * ◊
Stephanie R. Ostrowski, Alexandre P. Loretti, Snehal N. Tawde, James A. Bergum *186*

23. Avian Encephalomyelitis Virus Outbreak in Pullets from a Colorado Commercial Layer Flock
Chad Frank, Kristy Lynn Pabilonia *187*

24. Botulism Outbreak Associated with Contaminated Soil in Cattle
Jae-Won Byun, Byeongyeal Jung, Hayoung Kim, Albert Byungyun Jeon, Kyunghyun Lee, Ounkyung Moon, Soonseek Yoon, Osoo Lee, Myoungheon Lee, Youchan Bae *188*

25. Development of a Diagnostic Method to Detect Exposure to the Castor Bean Plant
Christina Wilson, Kimberly Meyerholtz, Stephen B. Hooser *189*

26. Development and Validation of a Novel Real-Time RT-PCR Assay to Distinguish Porcine Reproductive and Respiratory Syndrome (PRRS) Virus Subtypes with High Sensitivity
Rolf Rauh, William M. Nelson, Silvia Pillay *190*

27. Pooling of Ear Punch Samples for the Detection of BVDV PI Animals By Real-Time RT-PCR
Quoc Hoang, Darcy Myers, Jeff Zinza, Catherine O'Connell *191*

28. Examination of Pet Foods for Three Food Safety Pathogens, a Preliminary Study to Prioritize Future Surveillance Testing
Renate Reimschuessel, Sarah Yachetti, Olgica Ceric, Andriy Tkachenko, April Hodges, Micheal Grabenstein, Tara Doran, Terri McConnell, Kevin Garner, Scott Benko, Frances Mohnke, Karen Pietrzen, Sharon Robeson, Karen Wilson, Ted Gatesy, Gregory Danzeisen, Deepa Mathur, Bryanne Shaw, Grisel Rosario, Barbara Baker, Yong Liu, Maria Ishida, Amy Bryant, Jason Crowe, Maya Achen *192*

29. Physical and Histological Characteristics of Chicken Jerky Treats
Renate Reimschuessel, Olgica Ceric, Andriy Tkachenko, Sarah Yachetti, April Hodges, Lee Anne Palmer *193*

30. Deployment of a Serological Assay Detecting Foot-and-Mouth Disease virus Antibodies to the National Animal Health Laboratory Network
Stacey M. Bucko, Patricia Glas, Gregory Mayr, Moran Karen, Fernando Torres-Velez, Sarah M. Tomlinson, Thomas Struckmeyer, Barbara M. Martin, Michael T. McIntosh *194*

31. Evaluation of an ELISA Assay as an Ancillary Method in a Herd with Natural Tuberculosis Infection
Ignacio Aranaiz, C. Eiras, M. Luaces, J. Fraga, M. Rodriguez, C. Calvo *195*

32. Development of an Influenza A Sequencing Workflow on Ion PGM Sequencer for Improved Surveillance
Angela Burrell, Craig Cummings, Wayne Ge, Catherine O'Connell *196*

33. PCR-Based Panel Testing for Simultaneous Detection of Bovine Respiratory Pathogens
Jaeik Han, Yongil Cho, Vickie Cooper, Binu T. Velayudhan, Kent Schwartz, Karen Harmon, Wonil Kim, Kyoung-Jin Yoon *197*

34. Evaluation of a Panel of Multiplex Real-Time PCR Assays for the Detection of Organisms Associated with Scours in Camelid Feces
Donna M. Mulrooney, Wendy Black, Ling Jin, Kathy O'Reilly *198*

35. Integumentary Sensory Organs (ISOs) of the *Alligator mississippiensis* and Intradermal Nevus cells (INC). Is There a Histogenetic and Atavistic Correlation Between Them?
Hector A. Rodriguez-Martinez, Abelardo A. Rodriguez-Reyes, Gizela Maldonado-Hernández, Leonora Chavez-Mercado, Ofelia Perez-Olvera, Alfredo Gómez-Leal, Dolores Ríos y Valles-Valles *199*

36. Isolation and Characterization of *Streptococcus suis* from Bovine Clinical Cases
Ogi Okwumabua, Hui-Min Hsu *200*

37. Re-evaluation of the IDEXX *Mycobacterium paratuberculosis* Antibody Test to Improve Diagnostic Sensitivity
Hannah Pearse, Christoph Egli *201*

38. IDEXX RealPCR Diagnostic Test System for Livestock Applications
Michael Angelichio, Brooke Bivens, Andrea Ballagi, Peter Welles, Valerie Leathers, Christian M. Leutenegger *202*

Symbols at the end of titles indicate the following designations:

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

Swinecheck mix-APP®, a New ELISA Tool to Detect Antibodies to the Most Prevalent Serovars of *Actinobacillus pleuropneumoniae* in Swine Sera

Andre Ch. Broes

Biovet Inc., Saint-Hyacinthe, QC, Canada

Narrative: *Actinobacillus pleuropneumoniae* (APP) is still an important swine respiratory pathogen in many countries worldwide. A remarkable feature of this organism is that its virulence greatly varies depending on the isolates. This results in a variety of clinical situations varying from subclinical infections to acute mortalities. Interestingly, the virulence of a given isolate correlates well with the serovar in a given geographical location. Fifteen APP serovars based on capsular polysaccharide (CPS) antigens have been identified so far. Among them, serovars 1, 5 and 7 in North America and 2, 4 and 9 in Europe are the most virulent, even if other serovars such 8 and 15 may occasionally cause significant losses. In addition to CPS antigens, APP isolates are also characterized by somatic (O-LPS) antigens. O-LPS antigens consist in the long chain of the lipopolysaccharides (LC-LPS). O-LPS antigens corresponding to serovars 2, 5, 10, 12, 13, and 14 are unique. By contrast those corresponding respectively to serovars 1, 9 and 11, 3-6-8 and 15, as well as 4 and 7 are similar. Due to virulence variability the control of APP mainly focus on the most virulent serovars. The most convenient tool to monitor APP infections in swine populations is serology. Numerous serological assays for detecting APP serovar specific antibodies have been developed. The most sensitive and specific one is the indirect ELISA using highly purified LC-LPS as antigen. LC-LPS ELISA can be used to identify antibodies specific to serovars O-LPS 2, 5, 10, 12, 13, and 14 as well as antibodies to serovars O-LPS 1-9-11, 3-6-8-15, and 4-7. Seven ELISA kits using LC-LPS antigens have been commercially available for a while (Swinecheck APP®, Biovet). They allow detecting antibodies against all the 15 APP serovars except 13 and 14. In order to reduce the cost of testing we undertook developing assays using combinations of LC-LPS antigens. At this occasion we noticed that combining LC-LPS antigens was hampered by competition between antigens during ELISA plate coating and that combining more than two antigens usually resulted in loss of sensitivity. So far we have succeeded in combining antigens 1-9-11 and 2, 2 and 3-6-8-15, 4-7 and 5, 10 and 12. Each mix-antigen assay has test sensitivity and specificity quite similar to those of the corresponding individual assays. Biovet is now offering several mix-antigen plates which can be incorporated in customized kits (Swinecheck mix-APP®). These kits represent an interesting tool to screen for antibodies against the most prevalent APP serovars. Positive samples may be further characterized using the relevant individual tests.

The Effects of North American Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)-based Modified Live Vaccines in Preimmunized Sows Artificially Inseminated with European PRRSV Spiked Semen

Changhoon Park, Kiwon Han, Hwiwon Seo, Yeonsu Oh, Chanhee Chae

Seoul National University, Seoul, Republic of Korea

Narrative: The European porcine reproductive and respiratory syndrome virus (PRRSV) emerged in Asian countries including Korea. In Korea, it is common to vaccinate sows with the North American PRRSV-based modified live vaccine as recommended by the manufacturer because the commercial European PRRSV-based vaccine is not available yet. However, it is unclear whether preimmunized sows artificially inseminated with semen from European PRRSV-infected boars are susceptible to reproductive failure. On Korean swine farms, >90% of sows are bred by artificial insemination (AI). Therefore, the objective of the present study was to determine whether European PRRSV spiked semen can be transmitted via AI to preimmunized sows and induce reproductive failure. The commercial modified live PRRSV vaccine and European strain were used as inoculums in this study. Twenty-one conventional crossbred sows with a second parity were randomly divided into three groups. Group 1 (T01) sows served as negative controls. Group 2 (T02) sows were vaccinated intramuscularly with one 2 ml dose of a commercial modified live PRRSV vaccine. Sows in groups 2 (T02) and 3 (T03) were inseminated with a extended semen spiked with PRRSV. Blood samples and semen from each pig were collected at multiple time points for serologic test, viral isolation, and quantification of PRRSV RNA. *In situ* hybridization was performed for detection of PRRSV RNA from tissues samples of all dead and live-born piglets. Student's t test for paired samples was used to estimate the difference at each time point. The vaccine strain was only detected until -7 dpi in the serum samples from vaccinated sows in group 2. European PRRSV was detected from 7 to 28 dpi in sows in T02 and T03. For the inter-group comparison, the number of genomic copies of the European PRRSV was not significantly different in the serum samples between T02 and T03 sows. European PRRSV was isolated and European PRRSV RNA was detected from several organs in live-born piglets and stillborn fetuses from sows. No North American PRRSV RNA was detected in any tissues of stillborn fetuses and live-born piglets in sows from the 3 groups . The European PRRSV induced transplacental infection of embryos and caused death in sows preimmunized with North American-based modified live vaccine and inseminated with semen spiked with European PRRSV. The present study has demonstrated that vaccinating sows with the North American PRRSV-based modified live vaccine does not prevent reproductive failure after insemination with European PRRSV spiked semen.

Brain Abscess in a White-tailed Deer

Anwar J. Sarah

Animal Disease Research & Diagnostic Laboratory, South Dakota State University, Brookings, SD

Narrative: An adult male white-tailed deer (*Odocoileus virginianus*) was submitted to necropsy of the South Dakota State University - Animal Disease Research and Diagnostic Laboratory with a history of CNS signs ("Disoriented, weak, antlers caught in shrub"). The gross pathology of the deer examined revealed inadequate body fat and a 2.5 cm abscess filled with pus in the center of the left half of the cerebrum. Laboratory results showed that the antigen ELISA for CWD was negative. *Arcanobacterium pyogenes* and *P. multocida* were isolated from the brain, and the result of fecal float showed rare Strongyle-type ova and Strongyloides. No other ancillary tests have been performed on other organs. The histologic examination of the brain collected showed locally extensive necrosis of the nervous tissue containing cellular debris with intralesional bacterial colonization. The necrotic area is surrounded by a wide zone of inflammatory cells - mainly neutrophils and fewer lymphocytes and macrophages. There was diffuse intravascular lymphocytic cuffing. The morphologic diagnosis was encephalitis, locally extensive, necrotizing, subacute, with granulation tissue (capsule) formation and intralesional bacterial colonization. Brain abscess-related mortality is a growing concern of deer managers and biologists across the country. Brain abscesses are caused by a variety of bacteria (primarily *Arcanobacterium pyogenes*) that naturally inhabit the skin of deer, as well as other animals. These bacteria typically enter the brain through lesions and skin abrasions associated with the antler pedicle (where the antler protrudes from the skull) or junctions between cranial bones that are referred to as "sutures". Mortality generally occurs from fall (following velvet shedding) through spring (shortly after antler casting). Thus, the period when bucks are developing antlers or when antlers have hardened is when they are most susceptible to this disease. Certain buck behaviors, such as aggressive sparring and fighting, can cause damage to the antler pedicle and/or other parts of the skull that can predispose them to brain abscesses. The clinical signs include several behavioral characteristics associated with the neurological system, such as "circling" or loss of coordination. Often, deer can become emaciated, which is characterized by excessive weight loss or having a "deteriorated" appearance. Bucks also may have a puss-like substance located around the antler pedicle that leaks through openings in the skull. It is not known whether this disease can be transmitted between deer or other animals through direct contact or other sources. Also, it is important to note that deer with brain abscesses are not recommended for consumption.

Development and Evaluation of a Mobile Diagnostic Computer Application for Veterinary Laboratories

Jacqueline C. Cassady[1], Craig N. Carter[1], Norman Ronald[3], Michelle L. Arnold[1], Cynthia L. Gaskill[1], Jacqueline Smith[1], Wade A. Northington[2]

[1]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY; [2]Breathitt Veterinary Center, Murray State University, Hopkinsville, KY; [3]Medical Knowledge Base Consultant, Bryan, TX

Narrative: Our laboratory was awarded a USDA-NIFA grant to fund the development and trial of a mobile diagnostic laboratory application (Vet Check-Up). The application is designed to provide Kentucky food animal veterinarians with a software tool to help build differential disease lists, select appropriate diagnostic tests, specimen(s) collection information, and output electronic accession forms. The current version associates approximately 40 highly discriminatory disease findings with more than 730 diseases found in bovine, caprine, and ovine species. Diseases are ranked based on the proportional fit of findings to diseases within color coded incidence categories as follows: common (green), intermediate (blue), rare (orange), and foreign animal diseases (red). The Vet Check-Up application enables veterinarians and other users to run practice scenarios and real clinical cases. Use of the "real case" feature allows veterinarians to store baseline electronic medical records for clients by collecting the following information: owner, farm, and animal identification, case descriptions, clinical histories, test(s) requested, specimen type(s) collected, duration of illness, herd size, and herd health details. An electronic accession form is then generated to be submitted with specimen(s) for laboratory testing. Case information can be accessed through both the mobile application and email records of electronic accession forms. The intended use of the mobile application will enhance clinical case and epidemiological data capture and help veterinarians to better utilize the diagnostic laboratory. The application will be available through individual user web-based email account(s) and mobile device platforms, including but not limited to Apple iOS and Android. A field trial of the mobile application is scheduled for late 2012, and will involve at least ten volunteer practicing veterinarians. Volunteers will assess the capabilities of the mobile application on actual farm visits and provide feedback to identify application enhancements. Future versions of the application are planned for other species such as equine and porcine. Ultimately, such an application should integrate directly with Laboratory Information Management Systems (LIMS).

The Prevalence of Potentially Pathogenic Bacteria and Fungi in Bobwhite Quail in the Rolling Plains Ecoregion

Hongwen Su, Shuping Zhang, Jessica McKelvey

Department of Veterinary Pathobiology, Texas A&M University, College Station, TX

Narrative: Bobwhite populations have been declining across their range. In an effort to understand the impact of infectious diseases on population decline, we determined the prevalence rates of potential respiratory and intestinal microbial pathogens in bobwhites in the Rolling Plains Ecoregion. Tracheal tissues, cecal tissues, and cloacal swabs were collected from 52 bobwhites. One set of PCR assays was performed to screen tracheal tissues for *Mycoplasma spp*., *Mycobacterium spp*., and *Ochroconis gallopavum*. A second set of PCR assays was carried out to detect *Salmonella spp*., *Clostridium colinum*, *Clostridium perfringens*, and *Macrorhabdus ornithogaster* in cecal and cloacal samples. Bacterial and fungal cultures were performed on tracheal, cecal, and cloacal swabs. 16s rRNA gene sequencing and rRNA ITS sequencing were used to identify bacteria and fungi, respectively. The prevalence rates of potential respiratory pathogens as determined by culture and PCR were as follows: *Pseudomonas aeruginosa*, 17.3%; *Bordetella avium*, 0.0%; *Ornithobacterium rhinotracheale*, 0.0%; *Pasteurella multocida*, 0.0%; *Mycobacterium spp*., 0.0%; *Mycoplasma spp*., 0.0%; *Aspergillus fumgatus*, 0.0%; and *Ochroconis (Dactylaria) gallopavum*, 0.0%. The prevalence rates of enteric pathogens were *E. coli*, 30.8%; *Clostridium sordellii*, 3.8%; *Salmonella spp*., 0.0%; *Clostridium perfringens*, 0.0%; *Clostridium colinum*, 0.0%; and *Macrorhabdus ornithogaster*, 0.0%. In addition, human commensal organisms were isolated from the cecal and cloacal samples of these bobwhites at the following rates: *Neisseria flavescens*, 7.8%; *Neisseria sicca*, 7.8%; and *Streptococcus mitis*, 17.3%. The results suggest that the bobwhites do not carry bacterial and fungal pathogens traditionally associated with avian respiratory and intestinal illnesses. In contrast, tracheal colonization by *Pseudomonas aeruginosa* and intestinal colonization by *E. coli* and human commensal organisms are prominent which warrants further investigations on the virulence properties and sources of these bacterial isolates.

Bartonella bovis Isolated from a Cow with Endocarditis

Erdal Erol[1], Carney Jackson[1], Ying Bai[2], Stephen Sells[1], Steve Locke[1], Michael Kosoy[2]

[1]Veterinary Diagnostic Laboratory, University of Kentucky, Lexington, KY; [2]Bacterial Diseases Branch Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO

Narrative: A seven year old pregnant Angus cow was found dead in the field. Necropsy was performed at the University of Kentucky Veterinary Diagnostic Laboratory. Microscopically, the aortic valve was expanded by moderate fibrous connective tissue and acidophilic coagulum containing multifocal marked bacteria, mineral, neutrophils, and red blood cells. Numerous tiny colonies were detected on day 7 from the heart of the cow by aerobic culture. The isolate was a gram negative, very small coccobacillus and did not change litmus milk, esculin, phenylalanine, nitrate broth, gelatin, urease, TSI, or SIM. The isolate did not produce acid from glycerol, inulin, lactose, maltose, mannose, raffinose, salicin, sorbitol, sucrose, trehalose, glycogen, ribose, or starch. PCR was performed targeting gltA, tmRNA, fts, ribC, rpoB, and 16S rRNA genes of *Bartonella spp*. and found to be positive for *Bartonella* species. Amplicons were sequenced and gltA, ribC, ssrA and 16S rRNA gene sequences were found to have 100% homology to *Bartonella bovis*, whereas the fts and rpoB sequences showed 99.9% and 99.6% homology to *Bartonella bovis*, respecively. Bacterially-induced valvular endocarditis is often missed or misdiagnosed and often discovered during necropsy or slaughter. *Bartonella spp*. can cause endocarditis in some other animals and humans. Cattle are thought to be the main reservoir for *Bartonella bovis*. This is the first case in the US in which *Bartonella bovis* has been isolated from bovine valvular endocarditis, stressing culture media should be incubated beyond the standard period of time (2 to 3 days) in order to enhance the recovery of *Bartonella* species.

Use of Electronic Nose Technology to Identify Cattle Experimentally Infected with *Mycobacterium bovis* By Detection of Unique Volatile Organic Compounds in Breath: Preliminarily Findings

Christine K. Ellis[3, 1], Randall Stahl[1], Pauline Nol[2], Jack Rhyan[2], Matthew McCollum[2], Kurt VerCauteren[1], Joni Van Sickle[3], Lyndsey Linke[3], Roberta Magnuson[3], M.D. Salman[3]

[1]National Wildlife Research Center, USDA/ APHIS/ WS, Fort Collins, CO; [2]Western Region Wildlife Livestock Disease Investigations Team, USDA/ APHIS/ VS, Fort Collins, CO; [3]Animal Population Health Institute, College of Veterinary Medicine, Colorado State University, Fort Collins, CO

Narrative: Electronic nose technology has historically been utilized for the detection of volatile organic compounds (VOCs) and semi-volatile compounds in air, soil, water, and for quality control in the food, beverage, and cosmetic industries. This technology has also been used to identify unique VOCs produced by microorganisms in culture. Breath analysis has been used in humans to identify bacterial infection, sepsis, and gastrointestinal, neoplastic, respiratory tract, and urinary tract diseases. In animals, VOC analysis has been used to detect sepsis and acute renal failure in rats, and gastrointestinal infection in cats and monkeys. The source of VOCs present in the breath of infected patients is generally unknown, but may include the infectious organism, inflammation, tissue necrosis, or other host responses. The intent of this study was to determine if sorbent-based sample collection and electronic nose technology could be used to differentiate between cattle experimentally infected with *Mycobacterium bovis* and healthy cattle via identification of VOC biomarkers in breath. Results of our study indicate that infected cattle do appear to produce a VOC profile that is different than the VOC profile produced by cattle that are non-infected. This study provides evidence that breath analysis may have value as a diagnostic surveillance tool for detection of *M. bovis* infection in cattle. Further applications of this technology will be discussed including development of applications for long-term remote surveillance of domestic animal herds or wildlife reservoirs of this infection.

Effects of Freeze-thaw Cycle on Urine Values from Bottlenose Dolphins (*Tursiops truncatus*)

Risa Daniels, Cynthia R. Smith, Stephanie Venn-Watson

National Marine Mammal Foundation, San Diego, CA

Narrative: Urinalysis is a valuable tool for assessing renal health of marine mammals. While retrospective and population renal health studies often use frozen urine samples, it has not been determined if the freeze-thaw process alters urine values from bottlenose dolphins. The primary objective of our study was to compare the values of 38 fresh and frozen paired urine samples collected from 20 bottlenose dolphins (*Tursiops truncatus*) at the United States Navy Marine Mammal Program. Paired t tests and X^2 tests were conducted to assess the effects of storage at -80°C and a subsequent thaw on urine specific gravity, pH, creatinine, protein-to-creatinine ratio, quantitative protein, uric acid, uric acid-to-creatinine ratio, and categorical characterizations of color, clarity, glucose, ketones, occult blood, protein levels and crystals. The freeze-thaw cycle decreased urinary pH and increased urinary uric acid (P = 0.04 and 0.02, respectively). There were no other significant changes in urine variable values, including urinary uric acid concentration by grams of creatinine, when comparing fresh and frozen-thawed urine samples. Urinary uric acid concentration by grams of creatinine is the most accurate uric acid measurement when frozen-thawed samples are used. Urinary pH should be measured in fresh samples to avoid falsely decreased pH values.

Dermatitis, Cellulitis and Myositis due to *Serratia marcescens* in a Cat

Jane Kelly[1, 2], Thomas J. Baldwin[1, 2], Aaron Chamberlain[3]

[1]Utah Veterinary Diagnostic Laboratory, Utah State University, Logan, UT; [2]Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT; [3]Lehi Animal Hospital, Lehi, UT

Narrative: *Serratia marcescens* is a Gram-negative, medium-sized, rod-shaped bacterium in the family Enterobacteriaceae that is saprophytic and widely distributed in the environment. It is generally considered an opportunistic pathogen in human beings and animals, particularly affecting immunosuppressed and debilitated patients. This report is of a community-acquired dermatitis, cellulitis, and myositis due to *S. marcescens* in a previously healthy young adult domestic cat. The cat was presented for veterinary care due to lameness and unwillingness to jump. Swelling and abscessation of the right pelvic limb not associated with a foreign body was noted. Three days later an abscess was identified over the right dorsolateral sacrum. The cat was euthanized and ulcerative, suppurative dermatitis and suppurative/abscessing cellulitis and myositis, with intralesional colonies of rod-shaped, Gram-negative bacteria were identified. *Serratia marcescens* was isolated in large numbers on aerobic culture of affected muscle. This case is unique because, to the authors' knowledge, cellulitis and myositis in young healthy cats due to *S. marcescens* infection has not been reported previously.

Report of a New Genotype of *Ehrlichia* species from Cattle and Cervids

Alvin A. Gajadhar[1], Brad Scandrett[1], Vladislav A. Lobanov[1], Batol Al-Adhami[1], Gary A. Wobeser[2], John Campbell[2]

[1]Centre for Food-borne and Animal Parasitology, Canadian Food Inspection Agency, Saskatoon, SK, Canada;
[2]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Narrative: Tick transmitted Ehrlichia and Anaplasma are closely related blood-borne rickettsia. Several species are important pathogens of domestic and wild ruminants, and some infect monogastric hosts, including humans. Although *Anaplasma spp*. are common in livestock in many parts of the world, Ehrlichia infection in cattle occurs only in a few regions, including sub-Saharan Africa and a few Caribbean islands. In a recent investigation of unexpected reactors from a serological survey for bovine anaplasmosis, we detected the first natural ehrlichial infection in cattle that was not due to *Ehrlichia ruminantium*. In a series of two bioassay studies, splenectomised and intact cattle were inoculated with fresh blood collected from naturally or experimentally infected cattle. Serially collected blood samples were analyzed by blood smear examination, PCR, IFA, C-ELISA, and cell culture. Molecular and serological data showed the presence of a novel genotype of rickettsial species that was distinct from *Anaplasma spp*. and *E. ruminantium*. A few of the experimentally infected animals manifested mild clinical disease and histological evidence of mild encephalitis. Bacterial morulae morphologically consistent with Ehrlichia were detected in cultured leukocytes. Phylogenetic analysis of DNA gene sequences showed that the Ehrlichia found in the cattle is a distinct genotype, with *Ehrlichia canis* as the closest clade and *E. ruminantium* as the furthest removed within the genus. The novel Ehrlichia genotype was also detected in blood collected from mule deer in the region where infected cattle were found. Identification was based on DNA sequences from genes of a major surface protein (msp5), ribosomal RNA (16S rRNA) and citrate synthase (gltA). A molecular and serological study of blood collected from the experimentally infected cattle and naturally infected cattle and cervids showed cross reactivity on IFA and a commercial C-ELISA for bovine anaplasmosis. Although the clinical significance for animals infected with the novel Ehrlichia genotype appears to be minimal, the zoonotic potential is unknown, and the implications of the serological cross reactivity are significant for the diagnosis and control of bovine anaplasmosis. Further research is needed to elucidate the biology and transmission of this novel Ehrlichia genotype, and to develop reliable diagnostic tools for rickettsial pathogens.

Understanding the Factors that Influence Low Pathogenic Avian Influenza Virus Infection in Ducks ◊

Justin Brown[1], Monique Franca[2], Taiana Costa[2], Camille Lebarbenchon[1], Deborah Carter[2], Elizabeth W. Howerth[2], David Stallknecht[1]

[1]Population Health, Southeastern Cooperative Wildlife Disease Study, Athens, GA; [2]Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA

Narrative: Certain species of wild ducks are natural reservoirs for low pathogenic avian influenza (LPAI) viruses and an important source of influenza viruses that have transmitted to and produced disease in a variety of avian and mammalian hosts. Characterizing LPAI virus infection in ducks is a crucial step for understanding the risks for LPAI virus spillover into aberrant hosts and guiding efforts to prevent future transmission events or respond more efficiently. Historically, LPAI virus challenge studies in ducks have consisted of stand-alone projects that characterize host susceptibility, pathobiology, patterns of viral shedding, and/or seroconversion. The ability to compare results between studies has historically been limited by variation between experimental designs and a lack of understanding on the influence that host, viral, or other factors can have on LPAI virus infection in ducks. To address this gap, as well as provide a template for future studies, we have conducted a series of related LPAI virus challenge studies in Mallards (*Anas platyrhynchos*) to evaluate the influence of virus subtype, virus host-of-origin, age, route of inoculation, and AI exposure history. Using the data from these studies, we have developed a more defined Mallard LPAI model system that will allow us to effectively evaluate increasingly complex and challenging variables, as well as atypical influenza viral strains (highly pathogenic avian influenza virus). For the former, we are currently examining differences in phenotype between field and laboratory (egg) propagated viruses.

◊ USAHA Paper

A Validated Assay of Carbamate Pesticides in Feeds, Baits, and Tissues using QuEChERS and Gas Chromatography/Mass Spectrometry

Wilson K. Rumbeiha[1], Andreas Lehner[2], Margaret Johnson[2], Asley Couch[2], John Buchweitz[2]

[1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA; [2]Diagnostic Center for Public & Animal Health, Michigan State University, East Lansing, MI

Narrative: Carbamate pesticide poisoning is commonly encountered in veterinary medicine. Causes of poisoning include accidental exposure and malicious intent. Carbamate pesticides are systemic insecticides with broad agricultural applications. Their mode of action is similar to organophosphates in that they inhibit acetylcholinesterase (AchE), an enzyme responsible for breaking down acetylcholine, a neurotransmitter, at cholinergic nerve endings in the central nervous system. Inhibition of AchE allows for the accumulation of acetylcholine, leading to excessive activity of the parasympathetic nervous system. Diagnosis of carbamate poisoning utilizes different extraction procedures. We have developed and validated a qualitative fast extraction QuEChERS method for seven carbamate pesticides commonly encountered in animal intoxications including aldicarb, bendiocarb, carbaryl, carbofuran, methiocarb, methomyl, and propoxur. The method is applicable to baits, feed/food, and rumen contents, stomach contents, and liver samples. Identification and confirmation of the seven carbamate compounds is by gas chromatography/mass spectrometry (GC/MS). The limits of detection for these compounds are 1 ppm for aldicarb, 0.5 ppm for bendiocarb, carbaryl, carbofuran, methiocarb, and propoxur, and 10 ppm for methomyl. Compared to the traditional liquid/liquid extraction and gel permeation chromatography clean-up, the QuEChERS extraction procedure has many advantages, including use of a smaller sample size, enhanced lab safety, greater economy, and shorter turn-around-time. Work is ongoing to extend the QuEChERS approach to quantitation of these compounds in feeds and tissues, matrices of particular relevance to veterinary diagnostic laboratories.

Aquatic Animal Research in a Traditionally Terrestrial Animal Environment: Considerations for Success

Jean Kaptur[2], Richard Clayton[1]

[1]National Veterinary Services Laboratories, USDA-APHIS, Ames, IA; [2]Small Animal Section ARS, USDA-ARS, Ames, IA

Narrative: Many different aquaculture facilities have been built around the world. Construction and management pose many challenges to meet the needs of the investigators as well as the animals that are being studied. The new aquaculture facility built at the United States Department of Agriculture, National Veterinary Services Laboratories (NVSL) faces the same challenges that other research facilities face: how to be successful. These new facilities require many educational interactions to train primary investigators, veterinarians, technicians, and animal care staff. Personnel in these facilities traditionally were trained to work with terrestrial animals, not aquatic species. When transitioning to aquatics, there are many considerations that everyone must pay attention to for success. Challenges from training staff prior to animal arrival, ordering fish, transportation loss, emergency timelines, fomites, and pest control are just a few of the considerations that will be discussed along with how to maintain the excitement levels when the novelty has tapered off. In addition to these challenges, the different aspects of diagnostic procedures used in aquatics can also be a learning process for staff at a new facility. There are few, if any, vendors that sell specific pathogen free fish, unlike ordering terrestrial species for research. Fish purchased from vendors may have preexisting health conditions that need to be considered upon arrival. If a health issue were to arise, diagnosis and treatment is a team effort including the fish biologist, microbiologist, staff veterinarian, and pathologist working in collaboration to determine effective treatment. Though there are similarities between terrestrial and aquatic species, there are many differences that need to be considered to obtain a successful outcome for any project.

Real-time PCR, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and *Mycoplasma meleagridis*

Pablo Lopez, Kristen Roza, Phyllis Tyrrell

IDEXX Laboratories, Inc., Westbrook, ME

Narrative: Pathogenic *Mycoplasma* species are widely prevalent and detrimental to poultry health and production. The pathogenic *Mycoplasma* species for poultry include *M. gallisepticum* (Mg), *M. synoviae* (Ms) and *M. meleagridis* (Mm). Infection with these pathogens can lead to airsaccultis, synovitis, respiratory disease, decreased growth, and decreased egg production. Detection has historically been via hemagglutination inhibition (HI), serum plate agglutination (SPA), and enzyme-linked immunosorbent assay (ELISA), with culture isolation as the gold standard of testing. The use of real-time PCR for the detection of pathogenic Mycoplasmas has emerged as a rapid and highly accurate technique for the recognition or confirmation of common *Mycoplasma* species in a flock. The reagent sets use hybridization probes to allow for the creation of melting curves that provide another level of diagnostic sensitivity and confidence in test results.

Development and Validation of uvrC Gene-based TaqMan Real-time qPCR Assay for Detection of *Mycoplasma bovis*

Hemant Naikare[1], Daniela Resende Bruno[1], Debabrata Mahapatra[1], Alesia Reinisch[1], Russell Raleigh[1], Robert W. Sprowls[1], Tammy Beckham[2]

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

Narrative: Our objective of the present study was to develop and validate a TaqMan real-time qPCR assay for the detection of *Mycoplasma bovis*. Unique primers for the TaqMan assay were designed by targeting the highly conserved house-keeping gene (uvrC), and the probe sequence was derived from a previously published study (Kuo-Hua et al., *JVDI* 2008). The TaqMan assay was developed, optimized, and evaluated by comparing its performance to a conventional PCR assay targeting 16S rRNA (Gonzalez et al., *Vet Microbiol*.1995) and another uvrC based TaqMan assay (Clothier et al., *JVDI* 2010). There was 100% agreement in the outcome of our TaqMan assay and the other two assays. The analytical detection limit of our TaqMan assay using *M. bovis* ATCC 25523 reference isolate was determined to be approximately 10 copies of uvrC gene. This assay was validated using a total of 214 bovine clinical specimens that were submitted to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL). *M. bovis* was detected in 82 of 214 specimens by PCR. Out of these 82 positives, the break-up was as follows: 93.3 % of lung samples were positive (42 of 45), 19.2 % of milk samples were positive (15 out of 78), 28.2% of nasal and ear swabs were positive (22 of 78), 40% of joint fluid samples were positive (2 of 5), and 12.5% of semen specimens were positive (1 of 8). To the best of our knowledge, this is the first report of a PCR confirmed positive detection of *M. bovis* from a semen specimen. The specificity of the assay was assessed with eighteen other bacterial species which could likely be present in infections where *M. bovis* could be found. No cross-reactivity was found with any of these bacterial species. Although culture based detection of *M. bovis* has historically been cited to be the gold standard test in the literature, our findings with a subset of specimens from this study (71 of 214 specimens) indicate the culture test to be less sensitive than our TaqMan qPCR assay for *M. bovis* detection. Overall based on this extensive validation study, we conclude that the uvrC gene serves as a good diagnostic marker for the detection of *M. bovis* from a wider variety of specimen matrices, including semen. Also, we propose the uvrC gene based TaqMan assay to be considered as the 'new' gold standard test for detection of *M. bovis* infections.

Diagnostic Findings from Common Eider (*Somateria mollissima*) Mortality Events in the Northeastern United States Associated with Wellfleet Bay Virus, a Novel Orthomyxovirus ◊

Jennifer Ballard[1], Andrew Allison[1], Justin Brown[1], Samantha Gibbs[2], Chris Dwyer[3], Julie C. Ellis[4], Sarah Courchesne[4], Randall Mickley[5], Kevin Keel[1]

[1]Population Health, Southeastern Cooperative Wildlife Disease Study, Athens, GA; [2]Division of Migratory Birds, United States Fish and Wildlife Service, Arlington, VA; [3]Division of Migratory Birds, United States Fish and Wildlife Service, Hadley, MA; [4]Environmental and Population Health, Tufts Cummings School of Veterinary Medicine, Grafton, MA; [5]Wildlife Services, United States Department of Agriculture - Animal and Plant Health Inspection Service, Sutton, MA

Narrative: Between 1998 and 2011, 11 recognized mortality events occurred in common eiders (*Somateria mollissima*) along the coast of Cape Cod, Massachusetts. The estimated numbers of eiders involved in these outbreaks ranged from 30 to 2800, with total losses exceeding 6,000 birds. Most of the affected eiders were found dead without showing premonitory signs of disease. When sick birds were detected, they displayed nonspecific signs including weakness, lethargy, and ataxia. A multi-institutional disease investigation was initiated and carcasses of dead or moribund common eiders were submitted to the National Wildlife Health Center, the Southeastern Cooperative Wildlife Disease Study (SCWDS), Tufts University, and the University of New Hampshire for postmortem examination. The findings reported herein are from the 24 common eider carcasses received at SCWDS from three of these mortality events occurring between 2009 and 2011. At necropsy, common gross lesions in the birds included emaciation, skeletal muscle congestion, multifocal hepatic necrosis, and splenomegaly. The most common histologic lesions included myositis, multifocal to coalescing hepatic necrosis, splenic necrosis, and renal tubular necrosis and/or hemorrhage. In 2010, a novel orthomyxovirus, tentatively named Wellfleet Bay Virus (WFBV), was isolated from the tissues of four of these birds. Based on initial genetic comparisons of the three polymerase proteins (PB1, PB2, PA), WFBV was demonstrated to be closely related to members of the newly proposed Quarjavirus genus, which includes Quaranfil, Johnston Atoll, and Lake Chad viruses. To date, common eiders are the only species known to be susceptible to WFBV, and more research is currently underway to characterize the genetic composition, pathophysiology, epidemiology, and ecology of this virus, as well as to better understand the long-term implications of WFBV on common eider populations.

◊ USAHA Paper

Novel Solution for Manual and Automated Identification of Pathogen Nucleic Acids in Various Veterinary Sample Types

Lillian Roth[1], Denis Fluegge[1], Sandy Leifholz[1], Melanie Breitbach[1], Kathrin Wolf[1], Dora Quest[2], Holger Engel[1]

[1]Research & Development, QIAGEN GmbH, Hilden, Germany; [2]Marketing, QIAGEN Ltd., Crawley, United Kingdom

Narrative: Veterinary pathogen identification by real-time PCR and RT-PCR is widely replacing classical culture based methods due to higher sensitivity and speed. A key factor for successful amplification and identification of pathogen nucleic acids is an effective and robust sample preparation method providing reliable purification and maximum recovery of pathogen DNA and RNA. The complex and variable matrix characteristics of many animal derived sample materials typically used for veterinary pathogen identification can pose high demands on sample preparation. For most sample types, dedicated solutions for nucleic acid isolation are available. However, sequential analysis of varying sample types using specialized methods costs hands-on time, and handling differences between different protocols increase the error risk. Parallel processing of different samples varying in regard to pathogen type and sample material is a possible way to streamline workflows for veterinary pathogen identification. As a further challenge, the localization of pathogens or workflow demands may require pathogen nucleic acid isolation from animal whole blood samples. The composition of animal blood can cause clogging of silica membranes in current spin column based sample preparation kits. This may result in lower purification efficiency and carry-over of inhibitory substances into the eluate, thereby leading to impaired downstream performance. The QIAamp cador Pathogen Mini Kit has been developed for the processing of a broad range of animal samples including animal whole blood. The method uses proven silica membrane based spin-column technology for nucleic acid isolation and allows for reliable co-purification of RNA and DNA pathogen nucleic acids from a broad range of veterinary sample materials. Using one universal protocol enables parallel processing of various sample types. Furthermore, undiluted animal whole blood can be processed with greatly reduced risk of silica membrane clogging. Viral RNA and DNA and bacterial DNA are efficiently co-extracted from whole blood samples. Efficiency and reliability of pathogen identification can be further increased by automation of sample preparation and PCR set-up, and by use of universal real-time PCR cycling protocols. We demonstrate how the QIAamp cador Pathogen Mini Kit can be applied for parallel processing of various sample types and for reliable processing of animal whole blood samples. We describe an automated workflow for efficient nucleic acid isolation and identification from animal samples.

Performance Evaluation of Biolog GEN III System for Identification of Bacteria of Veterinary Origins

Hui-Min Hsu[1], Stan Stankevitch[1], Ogi Okwumabua[1, 2]

[1]Wisconsin Veterinary Diagnostic Laboratory, Madison, WI; [2]Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI

Narrative: The Biolog GEN III (OmniLog) microbial identification system is a new-generation bacterial identification system used for general identification of bacteria from variety of sources. The system is becoming increasingly used in veterinary diagnostic laboratories, yet there is paucity of information on its performance in accurately identifying organisms of veterinary origins. To this end, we used 89 isolates representing 20 genera and 48 species that were recovered from veterinary clinical samples to determine the system's performance in comparison with the results obtained from 16S rRNA sequence-based identification. Results showed that the use of appropriate protocol is essential for accurate identification using the Biolog system. Sixty-three of the 89 isolates (70.8%) were identified as the same genus and species by both methods. The two methods were in agreement at the genus level for 21 isolates (23.6%) but disagreed at the species level. Three isolates (5.6%) were identified as different genus and species by the two methods. Twenty isolates (22.5%) were unequivocally assigned to genus and species by the Biolog system but 16S rRNA gene sequencing produced questionable results and lacked discriminatory power. We conclude that the success of the Biolog identification system is protocol dependent and that the system has good promise for identification of bacteria of veterinary origins.

Evaluation of the Development of the Bovine Foot in Response to Variation in Management Practices ◊

Julie A. Gard[1], Debra R. Taylor[1], Soren P. Rodning[3], Dewey R. Wilhite[2], Kent Sanders[4], Misty A. Edmondson[1], Thomas Denney[5]

[1]Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL; [2]Anatomy and Physiology, Auburn University College of Veterinary Medicine, Auburn, AL; [3]Animal Science, Auburn University, Auburn, AL; [4]Radiology, University of Utah, Salt Lake City, UT; [5]Engineering, Auburn University, Auburn, AL

Narrative: A total of eight bull calves, four Holstein and four Jersey, were utilized, with random assignment of four in the control group and four in the treated group with equal number of Jerseys and Holsteins in each group. The control group was reared in accordance with standard practices consistent with the dairy industry, in calf hutches on pasture. The treated calves were housed in calf hutches for the first two weeks of life, and then they were allowed free access to a half mile lane where they walked for a total of at least two miles a day on rocky terrain. When all calves reached four months of age, they were humanely slaughtered and legs were collected and evaluated utilizing Computed Topography (CT) scans. The information from the CT scans was evaluated utilizing two software programs: Mimics 14 (Materialise; http://www.materialise.com/micro-CT) and 3-D Studio Max (Discreet; www.discreet.com/3dsmax). A three dimensional analysis of the medial claw, including P2 and P3, and the lateral claw, including P2 and P3, of the right rear foot from each calf was performed. The surface areas of the individual bones were calculated and evaluated for breed and treated verses control comparisons. The surface areas of both medial and lateral of P2 and P3 in the treated group were increased in each calf by an average of 45mm^2 and 81mm^2, and 193mm^2 and 219mm^2, respectively. Additionally, the treated Jersey group had a greater average increase per calf in the surface area of lateral P3 (349mm^2), in comparison to the Jersey control group than the average increase per treated Holstein calf (90mm^2), when compared to the Holstein control group. In summary, this study implicates the environment's role in the development of the boney structures of the bovine foot. However, additional studies with greater numbers of calves managed for a longer time period are necessary to allow for maximum bone remodeling so that the impact of changes in management especially involving rearing practices can more fully be assessed.

◊ USAHA Paper

Isolation of *Actinobaculum*-like Organism from a Goat Abscess

Yan Zhang, Jing Cui, Anne Parkinson, Troy Farrell, Beverly Byrum

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

Narrative: An abscess sample aspirated from a facial abscess of a 5-month-old female Boer goat was submitted to the Animal Disease Diagnostic Laboratory for confirmation of caseous lymphadenitis. After 5 days of incubation, a few colonies (pure growth) of a bacterium were isolated from anaerobic culture. DNA sequencing analysis on the 16S rRNA gene of the isolate indicated the isolate likely to be a new organism that is most closely related to *Actinobaculum schaalii* (94% identical).

Isolation of a *Campylobacter*-like Organism from a Goat Abscess

Yan Zhang, Jing Cui, Anne Parkinson, Troy Farrell, Beverly Byrum

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

Narrative: An abscess sample from a boer goat was submitted to the Ohio ADDL to rule out caseous lymphadenitis. A *Campylobacter*-like organism was isolated from the abscess in pure culture. Phenotypic and phylogenetic studies were performed on the isolate. Comparative 16S rRNA gene sequencing studies demonstrated that the organism represented an unknown subline within the genus *Campylobacter*, most closely related to *Campylobacter mucosalis*, However, the organism differed from *C*. *mucosalis* in morphology, biochemical reaction, and phylogenetic relationship, which indicates that the isolate belonged to a different, unpublished new species.

Idiopathic Generalized Soft Tissue Mineralization in an Appaloosa Filly # * ◊

Stephanie R. Ostrowski[1], Alexandre P. Loretti[1], Snehal N. Tawde[1], James A. Bergum[2]

[1]California Animal Health and Food Safety Laboratory System, University of California, Davis, Davis, CA; [2]Jim Bergum Veterinary Services, Herald, CA

Narrative: A 10-month old Appaloosa filly was submitted for necropsy at the UC Davis School of Veterinary Medicine, CAHFS-Davis veterinary diagnostic laboratory, following treatment for a chronic upper respiratory infection. The animal had a history of inability to stand, subcutaneous edema of the ventral midline and upper hind legs, depression, poor appetite, loss of muscle mass, and fever. At necropsy, there was severe extensive mineralization of the heart affecting the semilunar aortic valves, atrial epicardium, endocardium of the left ventricle and aorta, and lungs. No bone lesions were observed. Microscopically, calcification was observed in the heart, aorta, lung, kidney, stomach, and thyroid gland. Concentrations of Ca and P in the serum were both elevated (Ca = 220ppm, ref. range 100-130ppm; P = 270ppm, ref. range 27-50ppm). Vitamin D concentration in the serum was within normal limits, and slightly above the normal range in the kidney. The parathyroid glands were not examined by histopathology. PTH was not measured in serum. The cause of the severe, generalized soft tissue mineralization could not be determined in this case. Vitamin D toxicosis was considered but could not be confirmed as there was no history of overzealous vitamin D supplementation, access to calcinogenic plants, or hypercalcemic rodenticides. No neoplasia that would cause primary hyperparathyroidism or hypercalcemia of malignancy was found. No granulomatous disease or bone lysis that would cause hypercalcemia was noted. No primary renal disease that would cause renal failure was seen ruling out renal secondary hyperparathyroidism. The possibility of nutritional secondary hyperparathyroidism was not thoroughly investigated as Ca/P analyses were not done on diet and serum of other horses from the farm, and PTH not measured in serum of any of these animals.

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

Avian Encephalomyelitis Virus Outbreak in Pullets from a Colorado Commercial Layer Flock

Chad Frank, Kristy Lynn Pabilonia

Microbiology, Immunology & Pathology, Colorado State University, Fort Collins, CO

Narrative: Avian encephalomyelitis (AE) virus has a high prevalence in poultry flocks worldwide, but clinical disease is usually restricted to 1- to 3-week-old chicks due to a significant age-related resistance to clinical signs after 3 weeks of age. Avian encephalomyelitis is controlled in the US commercial poultry industry through vaccination of breeder flocks, so the incidence of clinical disease is very low, particularly in chickens older than 3 weeks of age. The producer of a large commercial layer flock reported neurologic disease in 9- to 13-week-old pullets characterized by tremors and ataxia. The breeding flock had been vaccinated for avian encephalomyelitis virus. Four pullets were euthanized and submitted to the Colorado State University Veterinary Diagnostic Laboratory. At necropsy, lesions were not observed in the central nervous system. Significant microscopic lesions were predominantly localized to the brain and cervical spinal cord and consisted of disseminated lymphohistiocytic encephalomyelitis. In addition, there was central chromatolysis, perineuronal microglial satellitosis, glial nodules, and rare neuronophagia involving nuclei of the brainstem and degeneration of Purkinje neurons with multifocal gliosis and glial nodules in the molecular layer of the cerebellum. Avian encephalomyelitis was identified in pooled samples of brain by rRT-PCR. Tracheal swabs were negative for avian influenza virus and avian paramyxovirus type 1 (Newcastle disease) by rRT-PCR . The observed microscopic lesions, in addition to the PCR results, confirmed the diagnosis of AE. The affected pullets from this farm were significantly older than expected for outbreaks of AE; therefore, it was crucial to identify and differentiate the microscopic lesions of AE from other avian encephalitides, such as Newcastle disease, avian influenza virus and equine encephalitis viruses. While these other viral infections also result in non-suppurative encephalitis, AE additionally causes the characteristic microgliosis and central chromatolysis in nuclei of the brainstem (nucleus rotundus and ovoidalis).

Botulism Outbreak Associated with Contaminated Soil in Cattle

Jae-Won Byun, Byeongyeal Jung, Hayoung Kim, Albert Byungyun Jeon, Kyunghyun Lee, Ounkyung Moon, Soonseek Yoon, Osoo Lee, Myoungheon Lee, Youchan Bae

Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang, Republic of Korea

Narrative: Botulism is caused by neurotoxins produced by Clostridium botulinum. Type B, C and D neurotoxins out of seven types (A-F) induce infection in cattle. The outbreak in cattle is associated with ingestions of toxins in feed, such as hay and broiler litter. However, the source of outbreak is often unknown because it is difficult to detect the toxin or to isolate the organism from clinical samples. We describe here on botulism in two dairy cattle farms in which the soil was submerged by the flood of an adjacent river. In farm A, ninety one dairy cattle were raised and all died from September to November, 2011. Farm B was a herd with 56 dairy cattle and all cattle also died from October to November. These farms were 5 to 100 meters away from an adjoining river which was severely flooded by heavy rain in July 2011. The affected farms were submerged by the flood. Initially affected cattle in two farms showed flaccid paralysis of hind legs, and then sternal or lateral recumbency, respiratory distress and death. Some cattle showed excessive salivation. Eleven cattle from farm A, and nine cattle from farm B, the feed and water from the farms were submitted for diagnosis. Necropsy, and histopathological, bacteriological, virological and toxicological tests was performed. Necropsy revealed non specific findings, and histopathological finding showed mild to moderate eosinophilic pachy meningitis in spinal cord and other non specific lesions. Botulinum toxin B was identified in serum from farm A, and botulinum toxin B and C was detected in serum from farm B with mouse inoculation test. Additionally botulinum toxin D was identified in soils from the cowshed in farm A and B. No virus was detected in tissues and no organophosphate pesticide was detected in ruminal contents. In this outbreaks, botulinum toxin B, C and D were identified. And this fact is very unusual because only one toxin type was detected in most cases. We assume that these toxins in soil were exposed to cattle because of submersion of the farms. As far as we know, it is very rare that bovine botulism is associated with flood.

Development of a Diagnostic Method to Detect Exposure to the Castor Bean Plant

Christina Wilson[1, 2], Kimberly Meyerholtz[1], Stephen B. Hooser[1, 2]

[1]Indiana Animal Disease Diagnostic Laboratory, Purdue University, W. Lafayette, IN; [2]Department of Comparative Pathobiology, Purdue University, W. Lafayette, IN

Narrative: Ricin, a 64kDa glycoprotein, is the toxin responsible for the inherent toxicity of the castor bean plant (*Ricinus communis*). It is a type II ribosomal-inactivating protein that causes inhibition of protein synthesis, apoptosis, and up-regulation of inflammatory signaling cascades. As a result, tissue damage can occur in several organs systems including the gastrointestinal tract, kidneys, liver, lungs, and spleen. Clinical signs observed in animals exposed to the plant can include vomiting/diarrhea, abdominal pain, hemorrhagic gastroenteritis, hypotension, myocardial necrosis, or death. Currently, diagnosing castor bean plant toxicity is based primarily on a clinical history of exposure, gross observation of the castor bean plant material in the gastrointestinal tract, or in some cases, detection of ricinine in urine or gastric contents. To date, there are no diagnostic methods established to detect ricin or other ricin agglutinin proteins in clinical samples. Exploiting the fact that ricin is a glycoprotein and has a molecular weight > 20 kDa, two fractionation techniques involving size exclusion and lectin affinity selection have been employed to isolate ricin glycoproteins from bovine rumen contents. Materials and Methods: This procedure briefly involves the following: 1) Two grams of plant material or 25 grams total rumen contents containing various percentages of castor bean plant material (0%, 1%, 5%, 10%, 25%, 50%, and 100%) were homogenized with 10 mL phosphate-buffered saline, 2) proteins from the water-soluble portion that were greater than 20kDa were selected using size exclusion fractionation, 3) glycoproteins from this fraction were then isolated using concanavalin A lectin affinity selection, and 4) extracted glycoproteins were then analyzed using a ricin, sandwich ELISA protocol. Results: This method was able to isolate and detect as low as 1% of the castor bean plant material in bovine rumen contents and showed high specificity for detecting ricin glycoproteins from castor bean plants. Conclusion: This dual-fractionation extraction technique provides a means to selectively isolate ricin glycoproteins from rumen contents, which are detectable using a sandwich ELISA. This procedure also simplifies the complexity of proteinaceous clinical samples, rendering the method amenable to future toxicoproteomic studies and mass spectrometric analyses.

Development and Validation of a Novel Real-Time RT-PCR Assay to Distinguish Porcine Reproductive and Respiratory Syndrome (PRRS) Virus Subtypes with High Sensitivity

Rolf Rauh, William M. Nelson, Silvia Pillay

Tetracore, Inc., Rockville, MD

Narrative: Introduction: Due to its potentially high economic impact, Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the most significant diseases in the swine industry. The two PRRS virus genotypes (type 1/European and type 2/North American) display profound variation in their genomic sequence, and recombination between distinct PRRS viruses has been reported, making simultaneous detection and differentiation of each genotype difficult. This difficulty is compounded by the requirement to analyze multiple specimen types including oral fluids, serum, and semen with simple and rapid sample preparation, while maintaining high sensitivity and specificity. Here, the development and validation of an improved PRRS virus real-time RT-PCR detection assay, meeting all of these stringent requirements, is reported. Experimental Design: Tetracore's existing NextGen Real-Time RT-PCR detection assay was modified to include 1) additional multiplexed primers and probes targeting unique regions to differentiate type 1 and type 2 genotypes; 2) newly available reagents to improve speed and inhibition tolerance; 3) and increased sample volume. This novel assay was validated using multiple PRRS type 1 and type 2 viral strains obtained through international collaborations. Samples were extracted in-house using routine extraction methods. The assay was also externally tested by two separate diagnostic laboratories. Results: The newly developed assay was found to have greater sensitivity and specificity, and successfully distinguished both PRRS viral genotypes. Results from two diagnostic laboratories showed the assay also displayed increased sensitivity. Importantly, data from these diagnostic labs further demonstrated successful detection in oral fluid specimens as well as differentiation of type 1 and type 2 genotypes. In serum specimens, the assay detected type 1 PRRS virus (n = 30, comprised of 4 unique subtypes and 10 genetic lineages) with 97% sensitivity, and type 2 PRRS virus (n = 90) with 98.8% sensitivity. In 150 oral fluid specimens, 100% sensitivity was achieved in a sampling taken from a pen of 25 pigs which included 3 pigs vaccinated with PRRS virus. Conclusions: Both in-house and international diagnostic lab testing validates the newly improved PRRS virus real-time RT-PCR detection assay to have high sensitivity and specificity. International lab testing verified the ability of using this improved assay for universal detection of currently circulating PRRS viral strains. Increased cooperation between both public and private diagnostic laboratories and manufacturers of PRRS virus commercial assays is a critical path forward to ensure the PRRS virus does not escape detection.

Pooling of Ear Punch Samples for the Detection of BVDV PI Animals By Real-Time RT-PCR

Quoc Hoang, Darcy Myers, Jeff Zinza, Catherine O'Connell

Life Technologies, Inc., Austin, TX

Narrative: Bovine Viral Diarrhea Virus (BVDV) infection in cattle is a worldwide problem that results in significant economic losses in the dairy and beef industries. Rapid detection of persistently infected (PI) cattle in a herd is essential for BVDV control. Real-time RT-PCR has become a quick way of identifying presumptive PI animals from herds. Pooling individual samples together is desired as a way to reduce the cost of testing entire herds for BVDV PI animals. In a pooling workflow, ear punches from individual animals are collected and re-suspended in PBS. The individual PBS supernatants are then combined into a pool which is then treated as a single sample used for RNA isolation followed by real-time RT-PCR. If the pooled sample tests positive for BVDV, the individual samples are then re-tested to determine which animal or animals caused the positive result. The sensitive nature of real-time RT-PCR allows samples to be pooled without losing the ability to detect a single PI animal from the pool. VetMAX-Gold BVDV Detection Kit was evaluated to determine the kit's performance when pooling up to 24 bovine ear punch samples into a single pool. Ear punches from confirmed PI or TI animals were pooled with 23 negative BVDV ear punches to determine the effect on assay sensitivity and specificity due to pooling. A total of 63 PI pools, 12 TI pools, and 51 negative pools were used for this study. We showed that the sensitivity and specificity was 100% for the detection of a single PI animal in a 24 sample pool while the TI pools were all negative. This indicates that pooling up to 24 samples is a viable method for the screening of PI animals in herds using VetMAX-Gold BVDV Detection Kit.

Examination of Pet Foods for Three Food Safety Pathogens, a Preliminary Study to Prioritize Future Surveillance Testing

Renate Reimschuessel[1], Sarah Yachetti[1], Olgica Ceric[1], Andriy Tkachenko[1], April Hodges[2], Micheal Grabenstein[1], Tara Doran[2], Terri McConnell[2], Kevin Garner[2], Scott Benko[3], Frances Mohnke[3], Karen Pietrzen[3], Sharon Robeson[3], Karen Wilson[3], Ted Gatesy[3], Gregory Danzeisen[4], Deepa Mathur[4], Bryanne Shaw[4], Grisel Rosario[5], Barbara Baker[6], Yong Liu[6], Maria Ishida[7], Amy Bryant[7], Jason Crowe[7], Maya Achen[8]

[1]CVM, FDA, Laurel, MD; [2]CVM, FDA, Rockville, MD; [3]Michigan Department of Agriculture and Rural Development, East Lansing, MI; [4]Laboratory Services Division, Minnesota Department of Agriculture, St. Paul, MN; [5]Food and Drug Protection Division, North Carolina Department of Agriculture and Consumer Services, Raleigh, NC; [6]Washington State Department of Agriculture, Olympia, WA; [7]Bureau of Food Laboratories, Florida Department of Agriculture and Consumer Services, Tallahasse, FL; [8]Ohio Department of Agriculture, Reynoldsburg, OH

Narrative: The Veterinary Laboratory Response Network (Vet-LRN), in collaboration with the Food Emergency Response Network (FERN) and its Microbiology Cooperative Agreement Program (MCAP) Laboratories conducted a study to evaluate the prevalence of selected microbial organisms in various types of pet foods. The goal of this blinded study was to help the Center for Veterinary Medicine (CVM) prioritize potential future pet food testing efforts. The study also increased FERN laboratories' screening capabilities for foodborne pathogens in animal feed matrices as such pathogens can be a significant health risk to not only animals, but also to consumers who come into contact with the food (1-3). Six FDA FERN MCAP laboratories analyzed approximately 1,020 samples over a two year period. Laboratories tested for Salmonella, Listeria, generic E. coli, E. coli O157:H7, shiga-toxin producing strains of E. coli, and aerobic plate counts using FERN methods. During year 1, dry and semi-moist dog and cat food were analyzed. During year 2 we tested we expanded the pet food types to include raw dog and cat food, jerky type treats, and small animal feed such as gerbil chow. Samples were purchased in local stores and on the internet. The year 2 study is currently still in progress but should be concluded by the end of July 2012.

Physical and Histological Characteristics of Chicken Jerky Treats

Renate Reimschuessel[1], Olgica Ceric[1], Andriy Tkachenko[1], Sarah Yachetti[1], April Hodges[2], Lee Anne Palmer[2]

[1]CVM, FDA, Laurel, MD; [2]CVM, FDA, Rockville, MD

Narrative: During 2007, FDA noted a number of consumer complaints involving consumption of chicken jerky treats (CJT). Product testing did not identify a causative agent, thus FDA issued a cautionary warning regarding chicken jerky products to consumers in 2007 and a Preliminary Animal Health Notification in 2008. There were fewer complaints during 2009 and 2010, but increasing numbers in 2011 generated a third FDA warning 11/ 2011. About 54% of the reported cases involve primarily gastrointestinal (GI) signs, sometimes severe with a hemorrhagic gastroenteritis, pancreatitis, and in some cases death. Renal dysfunction or failure accounts for approximately 29% of the cases, with approximately 10% of those reporting Fanconi syndrome. Acquired Fanconi can be the result of toxins affecting renal proximal tubule function. Additionally, some case reports include hepatic signs, elevated liver enzymes, lethargy or neurologic signs, anemia and skin rashes. Despite extensive testing, so far FDA has not identified a specific causative agent related to CJT illnesses. However, during the sample grinding prior to chemical analysis, laboratories reported finding fibrous material in CJT samples. To investigate potential physical irritant properties of various jerky type products, we examined histological morphology and response to soaking in PBS at pH 3, 5, or 7 for various times up to 7 days. Many products remained rubbery and tough even after days in PBS. This tough consistency could explain some of the milder GI signs reported in consumer complaints. Histological appearance varied, some products had striated muscle fibers throughout the section, however others had varying amount of a basophilic amorphous material (BAM) dispersed through the tissue. In general, the BAM was located near the surface, but in some samples it was distributed throughout the section, between muscle bundles. Laboratory-made jerky did not have BAM present in the sections. Further analysis of this unknown material is pending.

Deployment of a Serological Assay Detecting Foot-and-Mouth Disease virus Antibodies to the National Animal Health Laboratory Network

Stacey M. Bucko[1], Patricia Glas[1], Gregory Mayr[1], Moran Karen[1], Fernando Torres-Velez[1], Sarah M. Tomlinson[3], Thomas Struckmeyer[2], Barbara M. Martin[3], Michael T. McIntosh[1]

[1]USDA APHIS VS NVSL Foreign Animal Disease Diagnostic Laboratory, Plum Island Animal Disease Center, Greenport, NY; [2]Prionics AG, Schlieren-Zurich, Switzerland; [3]National Animal Health Laboratory Network, USDA APHIS VS National Veterinary Services Laboratories, Ames, IA

Narrative: Foot-and-Mouth Disease virus (FMDV) is a high consequence pathogen foreign to the US livestock population. In the event of an outbreak, there would be an urgent need for serological surveillance within the National Animal Health Laboratory Network (NAHLN) to help define the boundaries of the outbreak, distinguish infected from vaccinated animals, and reestablish freedom of the disease in the US. The Prionics PrioCHECK® FMDV NS ELISA kit has been in use by the Foreign Animal Disease Diagnostic Laboratory (FADDL) for FMD rule-out diagnostic investigations for several years. Use of the kit was selected based in-part upon a validation study by Brocchi et al. in 2006 that compared the PrioCHECK® kit (formerly known as Ceditest®) to five other FMD serological assays using limited bovine, caprine, porcine, and ovine samples from European, Asian, and North African regions. Additional validation of the PrioCHECK® FMDV NS ELISA kit has been initiated by the NVSL and NAHLN to better predict its suitability for deployment in the NAHLN in an effort to establish diagnostic capacity for post-outbreak FMD serological surveillance if needed and to inform the development of such a post-outbreak surveillance plan. FADDL diagnostic casework and associated studies from 2009 to the present were analyzed for obtaining performance characteristics ahead of an interlaboratory comparison that will be conducted in a subset of NAHLN laboratories. This indicated a 0.84% false positive rate for approximately 2.5 years of diagnostic testing. To ensure safety of imported test kit materials, positive serum controls will be safety treated by irradiation and a collaborative study between FADDL and Prionics will be used to verify the integrity of the positive controls post irradiation. Serological panels suitable for interlaboratory comparisons and proficiency testing were developed using experimentally infected goats, sheep, pigs and cattle along with negative serum from the same species. Sera were tested pre- and post-gamma irradiation treatment to verify performance in the panels. The interlaboratory comparison will be conducted within the NAHLN in summer 2012 in coordination with proficiency testing on the PrioCHECK® FMDV NS ELISA kit. A negative cohort study on the US animal population involving the NAHLN is planned to follow.

Evaluation of an ELISA Assay as an Ancillary Method in a Herd with Natural Tuberculosis Infection

Ignacio Aranaiz[1], C. Eiras[1], M. Luaces[2], J. Fraga[3], M. Rodriguez[3], C. Calvo[1]

[1]Laboratorio de Sanidade e Production Animal de Galicia, Conselleria do Medio Rural e do Mar, Lugo, Spain; [2]Area Veterinaria de Lugo, Servicio Provencial de Ganaderia de Lugo, Lugo, Spain; [3]Area Veterinaria de Villalba, Servicio Provencial de Ganaderia de Lugo, Lugo, Spain

Narrative: Introduction The single intradermal tuberculin (SIT) test and the Interferon-gamma assay (IFN-g) are the tuberculosis diagnostic tools in the tuberculosis eradication program currently used in Spain. These tests based on cell-mediated response can detect animals in the early stages of infection (1), although not all affected animals are detected with these tests. Humoral immune response is found in cattle with natural infection and this response could be detected by the use of an ELISA assay. ELISA testing is not routinely used in bovine tuberculosis control programs mainly due to a reduced sensitivity (2) although it has been suggested that it might be used as a complement to the tuberculin test, especially for the detection of anergic tuberculous cattle (3). The aim of this study is the evaluation of the use of SIT test and the IFN-g assay together with an ELISA assay in order to obtain the most complete detection of tuberculosis infected animals in a herd previously confirmed to have tuberculosis. Material and Methods 314 animals from a tuberculosis infected beef herd from Galicia, in north-west Spain, were tested using the SIT test. The IFN-g detection assay (BovigamTM, Prionics AG, Switzerland) was used in parallel with the SIT test. The 16 positive SIT animals (of which 15 animals were also detected by IFN-g assay) and 17 cattle that were only detected by IFN-g assay were slaughtered and their tissues were collected for *Mycobacterium tuberculosis* complex (MTC) isolation. At the same time, 20 cows older than 10 years were slaughtered too. Tissues from the 53 slaughtered animals were inoculated into the selective media (4). The MTC was recovered and confirmed by real-time PCR (5) in 18 cattle. The plasma collected for the IFN-g assay was also used on the ELISA assay for *M. bovis* antibody (IDEXX Laboratories). Eighteen cattle were detected with the ELISA assay. The agreement and kappa statistic measurements were calculated by comparing the MTC isolation with SIT test, IFN-g and ELISA assays respectively. Results Ten bovine with MTC recovered were SIT test positive. Sixteen were positive in the IFN-g assay, and the ELISA detected 13 of the 18 cattle with MTC recovered (one of them was negative in SIT test and IFN-g assay). One bovine with MTC recovered was negative in the three tests; this animal was slaughtered because it was more than10 years old. The kappa statistic shows a lower agreement for the SIT test (0.39) and the IFN-g assay (0.36) with the MTC isolation than the ELISA assay (0.58). Discussion & Conclusion The results suggest that the use of IFN-g and ELISA assays as ancillary techniques might help to detect a larger number of tuberculosis infected animals in a TB-confirmed herd. These assays complement each other because they may detect animals in different stages of tuberculosis.

Development of an Influenza A Sequencing Workflow on Ion PGM Sequencer for Improved Surveillance

Angela Burrell[1], Craig Cummings[2], Wayne Ge[1], Catherine O'Connell[1]

[1]Life Technologies, Austin, TX; [2]Bioinformatics, Life Technologies, Foster City, CA

Narrative: Influenza A is a negative-sense RNA virus and a major source of economic loss in the animal health field. Complete genome sequencing is crucial for ongoing surveillance of influenza. It provides detailed information about virus origin and evolution, which is particularly important in identifying emerging strains. Sequencing allows for detection of small mutations across the genome as well as monitoring for larger genetic re-assortments. The information can also be used to characterize the virus for vaccine development and to provide information about antiviral resistance. When sequencing viral nucleic acid, high amounts of background host nucleic acids may be co-processed during library preparation, resulting in a sequencing reaction in which a majority of reads are taken up by the host genome. A solution to this problem is to run a pre-amplification RT-PCR on the extracted nucleic acid with primers specifically designed to amplify only the viral nucleic acid prior to library preparation. We have developed the PathAmp FluA Reagents, a set of highly specific, universal primers along with high-fidelity enzymes and buffers for the amplification of all eight Influenza A genomic segments, which range in size from 900 bp to 2.4 kbp. cDNA obtained from the PathAmp workflow is suitable for direct input into the IonXpress Plus library preparation kit. Reagent performance was verified on a diverse panel of 16 influenza A strains from both avian and swine sources. The PathAmp reagent workflow was compared to another published pre-amplification workflow on the same panel of samples. For both workflows, samples were barcoded allowing for all 16 to be run on a single sequencing reaction saving time and money. Greater than 99% coverage of each segment was observed for 95% of the samples tested with the PathAmp reagent workflow compared to just 90% for the published workflow. Mean coverage depth was approximately 700-fold higher for the PathAmp reagents as compared to the published workflow. Host contamination also dropped from 26% down to less than 0.25% when the PathAmp reagents were used. Sensitivity of the PathAmp reagents was tested by serially diluting a swine influenza virus sample in porcine nasal swab and tonsil tissue nucleic acid. Lineage calls for both sample matrices were correct down to 200 viral copies. Greater than 99% of each segment was covered by reads when virus was present at 1000 copies or 200 copies when diluted in tonsil or nasal swab nucleic acid, respectively. In conclusion, the PathAmp FluA Reagents provide a rapid, accurate, and sensitive solution for Influenza A viral sequencing on the Ion PGM Sequencer.

PCR-Based Panel Testing for Simultaneous Detection of Bovine Respiratory Pathogens

Jaeik Han[1, 2], Yongil Cho[1], Vickie Cooper[1], Binu T. Velayudhan[3], Kent Schwartz[1], Karen Harmon[1], Wonil Kim[1], Kyoung-Jin Yoon[1]

[1]Veterinary Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA; [2]Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea; [3]Texas Veterinay Medical Diagnostic Laboratory, Amarillo, TX

Narrative: Bovine respiratory disease complex (BRDC) is one of the major health obstacles in the cattle industry. Multiple infectious agents can cause BRDC and multiple pathogens are often concurrently implicated in the disease complex. Conventional test methods can be laborious, expensive and/or time-consuming. In this study, a panel of 3 multiplex real-time polymerase chain reaction (PCR) assays was developed and evaluated for simultaneous detection of 9 respiratory pathogens: bovine coronavirus (BCoV), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), bovine herpesvirus type 1 (BHV-1), *Mycoplasma bovis*, *Mannheimia haemolytica*, *Histophilus somni*, *Pasteurella multocida*, and *Bibersteinia trehalosi*. The panel consisted of 3 multiplex PCR assays (A, B, and C). PCR A, B and C were designed to target RNA virus only (BCoV, BRSV, BVDV), bacteria only (*M. bovis*, *M. haemolytica*, and *H. somni*), and bacteria/DNA virus (*P. multocida*, *B. trehalosi*, and BHV-1) respectively. Each PCR included an internal control (65bp) containing a non-specific 16S rRNA gene sequence flanked by *M. bovis* uvrC gene sequence. No cross reactivity was observed between each PCR and infectious agents other than the target. The analytic sensitivity of the multiplex PCR panel estimated using serially diluted recombinant vectors (for viral targets) and bacterial suspensions (for bacterial targets) with known copy numbers or colony-forming units per 1ml was: 4.2 for BCoV; 8.9 for BRSV; 8.5 for BVDV; 0.8 for BHV-1; 200 for *M. bovis*; 33 for *M. haemolytica*; 100 for *H. somni*; 200 for *P. multocida*; and 400 for *B. trehalosi*. Testing conducted on 196 lung samples procured from bovine submissions with respiratory disease to Iowa State University VDL or Texas VMDL, showed that the multiplex PCR panel simultaneously detected the target pathogens in less time and at a lower cost than the conventional test methods. The agreement between the panel and conventional test methods ranged from approximately 82% to 100%. Most of the discrepant results were due to samples that tested positive with the panel, but negative when conventional test methods were used, suggesting that the panel has a higher sensitivity than conventional test methods. The newly developed multiplex real-time PCR panel can be a rapid and accurate testing tool for diagnosticians in investigating BRDC cases and could aid bovine practitioners to intervene BRDC in its early stages.

Evaluation of a Panel of Multiplex Real-Time PCR Assays for the Detection of Organisms Associated with Scours in Camelid Feces

Donna M. Mulrooney[1], Wendy Black[1], Ling Jin[2, 1], Kathy O'Reilly[2, 1]

[1]Veterinary Diagnostic Laboratory, Oregon State University, Corvallis, OR; [2]Department of Biomedical Sciences, Oregon State University, Corvallis, OR

Narrative: Cho et al. (2010) reported a panel of multiplex real-time PCR assays for detection of 5 diarrhea agents in calves: Group A rotavirus, coronavirus, Salmonella, *Escherichia coli* K99, and *Cryptosporidium parvum*. Of these, all but *E. coli* K99 have been associated with diarrhea in camelids. Since it is reported that rotavirus and coronavirus isolated from camelids are closely related to bovine strains, we tested the usefulness of the multiplex PCR in the diagnosis of diarrhea in camelids. To date, we have tested 148 archived bovine and camelid fecal samples submitted to the OSU VDL from 2010 to 2012. Samples used in this study were previously examined using standard diagnostic procedures; electron microscopy for rotavirus and coronavirus, culture for Salmonella, culture followed by PCR identification of the K99 adhesion for *E.coli* K99, and acid fast staining for Cryptosporidium. Fecal samples were processed and tested as described by Cho et al. with the following changes. Concentration of fecal material was decreased for camelid samples, primers and probes that had previously been shown to detect camelid coronavirus were substituted for the sequences reported by Cho et al., and cycling parameters were adjusted. K99 primers and probes were excluded when testing camelid samples. In our study, we found the limits of detection to be 10 to 100 cfu/g for *Salmonella spp* and *E. coli* K99 and 1000 to 10,000 oocytes/ g for Cryptosporidium. Of the 128 bovine fecal samples tested, agreement with traditional diagnosis was 96% for rotavirus, 71% for coronavirus, 92% for Salmonella, 99% for *E.coli* K99, and 75% for Cryptosporidium. Of the 20 camelid samples tested, the percent agreement with traditional diagnosis was 60% for coronavirus and 95% for Cryptosporidium. Only one camelid sample was positive for rotavirus and none were positive for Salmonella so no statistical analysis was performed. This study demonstrates that the multiplex PCR assay developed for detection of scours organisms in cattle can be used in the diagnosis of rotavirus, coronavirus and *Cryptosporidium parvum* in camelids; however, in our hands the multiplex assay did not perform as well as what was reported by Cho et al. The lower diagnostic sensitivity of electron microscopy may account for the decreased agreement in the detection of coronavirus, since Cho et al. used an additional PCR assay for comparison rather than EM. We hypothesize that sample age may have played a role in the differences observed in the DNA based PCR assays.

Integumentary Sensory Organs (ISOs) of the *Alligator mississippiensis* and Intradermal Nevus cells (INC). Is There a Histogenetic and Atavistic Correlation Between Them?

Hector A. Rodriguez-Martinez[2], Abelardo A. Rodriguez-Reyes[3], Gizela Maldonado-Hernández[1], Leonora Chavez-Mercado[4], Ofelia Perez-Olvera[2], Alfredo Gómez-Leal[3], Dolores Ríos y Valles-Valles[3]

[1]Pathology, Bronson Animal Disease Diagnostic Laboratory, Kissimmee, FL; [2]Laboratorio de Investigaciones Anatomopatológicas "Roberto Ruiz Obregón". Departamento de Medicina Experimental, Facultad de Medicina, UNAM y Hospital General de Mexico, OD, México, Mexico; [3]Servicio de Patología Oftálmica, Asociación para evitar la ceguera en México, IAP, Hospital Dr. Luis Sanchez Bulnes, México, Mexico; [4]Pathology Department, Facultad de Medicina, UNAM y Hospital General de México, OD., México, Mexico

Narrative: Laidlaw and Murray (1934) thought the Intradermal Nevus Cells (INC) were of ancient origin and were derived from the Integumentary Sensory Organs (ISOs) found in some animals (*Alligator mississippiensis*). They believed the intradermal nevus (IDN) to be an atavistic appearance of the ISOs which could have played a role in the evolution of hair formation. For Smith and Jones (1966) the IDN corresponded to phylogenetic traces of the pigmented sensory organ of amphibians. This organ could have been present in the evolutionary predecessors of humankind during their "amphibian evolution stage"; however it was not until 1875 that Friedrich S. Merkel discovered the ISOs in the skin of frogs. Fifteen cutaneous IDN and ten samples of skin of *Alligator mississippiensis* were examined. Alligator samples were obtained from the ventral region of maxilla. Histochemical stains were prepared that included hematoxylin and eosin, Masson Trichrome, PAS, methenamine silver, Warthin-Starry and Grimelius stains. Immuhohistochemical stains utilized included vimentin and PS100. The ISOs congregated in circumscribed plates composed of multiple layers of fusiform cells which were arranged parallel to the epidermis. The cytoplasm of these cells had a basal lamina and scant melanin pigment, and nuclei were elongated and had a smooth chromatin pattern. Small caliber vessels and amelanotic nerves were localized at the center and edges of the cellular plates. Axons were not seen either between or within the cells. A layer composed of dendritic melanocytes was present under the cellular plate. The epidermis contained enlarged dendritic melanocytes while the keratinocytes were hyperpigmented. The IDN had a predominant organoid morphology composed of three cellular layers (A,B and C). A and B-type cells shared similar cellular features, while C-cells had Schwannoma-like features. In general only A-type cells were found to produce melanin. It has erroneously been considered that the ISOs cells corresponded to melanophores, the same way the fundamental structures were judged to correspond to lamellar mechanoreceptors similar to Pacinian corpuscles. In the authors' opinion, the cells that form the cellular plate do not appear to correspond to melanophores, but rather to sustentacular cells that shed the nerve endings which then congregate to form the pigmented sensory corpuscle. By the means of light microscopy, there did not appear to be an association between the IDN and the ISOs; however a functional correlation between them cannot be ruled out.

Isolation and Characterization of *Streptococcus suis* from Bovine Clinical Cases

Ogi Okwumabua, Hui-Min Hsu

Veterinary Diagnostic Laboratory, University of Wisconsin, Madison, WI

Narrative: *Streptococcus suis* is commonly known as a swine pathogen but in recent years, the organism has increasingly become an important zoonotic agent. Fifteen isolates of unknown Gram-positive, chain-forming, coccus-shaped and α-hemolytic, bacterial organisms recovered from bovine clinical samples were characterized by standard biochemical tests, API 20 Strep, and the OmniLog GEN III system. The three methods identified them as *Streptococcus suis*. Their identity was further confirmed by the polymerase chain reaction (PCR) and full length sequencing and analysis of the 16S rRNA gene. None of the isolates could be assigned to serotypes 1, 2, 7, and 9 and none carried *S. suis* virulence associated genes such as the muramidase released protein (MRP), extracellular protein factor (EPF), suilysin (SLY), and 89K pathogenicity island (PAI). By Pulse Field Gel Electrophoresis (PFGE), the isolates exhibited distinct patterns, indicating a genetic diversity. Dendrogram analysis revealed that PFGE patterns of the isolates clustered into four groups with genetic similarities ranging from 72% to 100%. Antimicrobial susceptibility determination revealed that the S. suis isolates were susceptible to ampicillin, penicillin, and tiamulin. High level of resistance to sulphadimethoxine (100%), chlortetracycline (80%), oxytetracycline (80%), neomycin (73%), tylosin tartrate (47%), tulathromycin (47%), tilmicosin (47%), and clindamycin (47%) were noted. The multiple resistant strains (≥3 antimicrobial agents) were detected in 67% of the isolates. Although the pathogenic potential of the isolates is unknown, we conclude that *S. suis* is an organism to expect in bovine clinical samples and that bovine could be significant reservoir.

Re-evaluation of the IDEXX *Mycobacterium paratuberculosis* Antibody Test to Improve Diagnostic Sensitivity

Hannah Pearse, Christoph Egli

IDEXX Laboratories, Westbrook, ME

Narrative: Johne's disease is a chronic wasting disease in domestic and wild animals worldwide. Data from the Johne's Information Centre estimates that 7.8% of beef herds and 22% of dairy herds in the U.S. are infected with MAP making Johne's disease a significant animal health problem in US herds. Due to the unusually long incubation period of this disease, significant difficulties arise in its control and management. Ultimately the control of Johne's disease requires education, implementation of effective control measures and accurate diagnostic tools to be successful. Lack of sensitivity in existing diagnostic tests is often cited as one of the main reasons for a lack of progress in Johne's disease control to date. The IDEXX *Mycobacterium paratuberculosis* Antibody Test (IDEXX MAP Ab Test, previously the Institut Pourquier Test) is validated for use in both serum and milk samples and is used in control programmes worldwide. This paper describes the evaluation of the test validation data with modified test cut-offs to improve test sensitivity. Data analysis included results for confirmed positive milk samples (n=157), negative milk samples (n=710), positive serum samples (n=360) and negative serum samples (n=862). At the current test cut-off levels, serum samples with an S/P of ≤0.6 are negative, > 0.6 and < 0.7 S/P suspect and ≥0.7 positive. Milk samples with an S/P of ≤0.3 are classified negative, samples > 0.3 and < 0.4 S/P, suspect and samples ≥ 0.4 positive. Test sensitivity for the kit based on these existing cut-off values was 58% for serum and 73% for milk for the populations tested. Test specificity was greater than 99% for both serum and milk samples at the existing cut-off. By changing the test cut-off for serum to ≥0.45 S/P (S/P of ≤0.45 negative, > 0.45 - < 0.55 S/P suspect and ≥0.55 positive) the sensitivity of the ELISA increases by 5% (to 63%). Changing the milk cut-off to ≥0.20 S/P (S/P of ≤0.20 negative, > 0.20 - < 0.30 S/P suspect and ≥0.30 positive) the sensitivity slightly improves to 75%. Specificity remains greater than 99% for both serum and milk samples. By reducing the test cut-offs for the IDEXX MAP Ab Test, sensitivity can be improved offering a better tool for the detection of paratuberculosis in cattle using either serum or milk samples.

IDEXX RealPCR Diagnostic Test System for Livestock Applications

Michael Angelichio[1], Brooke Bivens[2], Andrea Ballagi[1], Peter Welles[1], Valerie Leathers[1], Christian M. Leutenegger[2]

[1]IDEXX Laboratories, Westbrook, ME; [2]IDEXX Reference Laboratories, West Sacramento, CA

Narrative: Real time PCR has created a revolution in diagnostics, with increased speed, sensitivity and specificity over many other offerings. The components of the real time PCR test include the i) mastermix (containing enzyme, buffer, and nucleotides), ii) positive controls, iii) internal positive controls (to ensure proper nucleic acid purification) and iv) detection mix (containing the target-specific primers and probes). IDEXX is developing a real time PCR system, based on hydrolysis probe technology, in which all tests and testing protocols are standardized. For example, a single Internal Positive Control (IPC) will provide a means to ensure proper nucleic acid purification for both RNA and DNA test targets. Additionally, standard cycling conditions for all IDEXX tests allow the user to run both RNA and DNA targets side-by-side. IDEXX RealPCR tests will run in under one hour (instrument dependent). A number of real time PCR tests have been developed on the new platform. Analytical sensitivity analysis for the IDEXX RealPCR *Mycoplasma gallisepticum, M. synoviae*, Bovine Viral Diarrhea Virus (BVDV), Bluetongue Virus (BTV), and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) tests each demonstrate sensitivity of ≤10 copies per reaction. Standardized protocols and components in the IDEXX RealPCR Test System will provide laboratories a more efficient and flexible platform for real time PCR testing.

AAVLD Author Index

Achen, Maya 192
Adaska, John 59, 126
Adkesson, Michael 68
Afonso, Claudio 139
Agasan, Alice 37
Akey, Bruce L. 96
Al-Adhami, Batol 174
Alarcio, Gwendolyne 64, 66
Allen, Andy 94
Allison, Andrew 129, 180
Anderson, Gary A. 50, 52
Anderson, Mark 126
Andrews, Kirk 36
Angelichio, Michael 202
Aranaiz, Ignacio 195
Arnold, Michelle L. 168
Aston, Linda 66
Atashpaz Gargari, Esmaeil 131
Bae, Youchan 188
Bai, Ying 170
Baker, Barbara 192
Balachandran, Aru 60
Balasuriya, Udeni BR 80
Baldwin, Claudia 110
Baldwin, Thomas J. 173
Ballagi, Andrea 48, 49, 50, 202
Ballard, Jennifer 180
Barker, Christopher 107
Barker, Sarah 106
Barr, Bradd 126
Barrette, Roger 79, 150
Baszler, Timothy V. 94, 95
Batonick, Melissa L. 140
Bauermann, Fernando 145
Bazan, Luis R. 131
Beach, Tamara 141
Beckham, Tammy 81, 131, 152, 179
Beckler, Dana 93
Beingesser, Juliann 57
Belanger, Maryse 78
Benko, Scott 192
Bergum, James A. 186
Berman, Lashondra 136
Berninger, Mary Lou 79
Besser, Thomas E. 95
Bivens, Brooke 202
Bjork, Kathe 46, 47
Black, Wendy 198
Blanchard, Patricia C. 59
Bokanyi, Rick 98
Bolin, Carole 29
Borca, Manuel 154
Borst, Luke B. 32
Bounpheng, Mangkey 77
Bounpheng, Mangkey A. 81, 91, 131, 152
Bower, Leslie 119
Bracht, Alexa 150
Bradway, Dan S. 95
Brandt, Eric 98
Breheny, Patrick 80
Breitbach, Melanie 181
Brito, Barbara P. 154
Broes, Andre Ch. 50, 78, 165
Broman, Meaghan 75
Brooks, Andrew 121
Brown, Justin 112, 129, 149, 175, 180
Brown, Stuart 51
Bryant, Amy 192
Bucafusco, Danilo 154
Buchweitz, John 176
Bucko, Stacey M. 194
Buffington, Tina 141
Bull, Leonard 160
Burrell, Angela 134, 196
Burrough, Eric 119
Bursian, Steve J. 62
Butler, Brian 120
Byrne, Barbara 117
Byrum, Beverly 82, 97, 98, 123, 184, 185
Byun, Jae-Won 188
Calcutt, Michael 88
Calvo, C. 195
Camacho, Mark 41
Camenish-Ruby, Tara 60
Campbell, John 174
Carlson, Erin 125
Carman, Suzanne 50
Carrillo, Consuelo 150
Carriquiry, Miguel 159
Carter, Craig N. 51, 90, 168
Carter, Deborah 149, 175
Cassady, Jacqueline C. 168
Casteran, Karissa 81
Cavender, Jacqueline L. 153
Cawley, Doreen 151
Cazarez, Ray 71
Ceric, Olgica 192, 193
Cha, Rami 139
Chae, Chanhee 166
Chamberlain, Aaron 173
Chander, Yogesh 92, 93
Chang, Hsiao Fen Grace 83
Chang, Yung-Fu 96
Chavez-Mercado, Leonora 199
Chigerwe, Munashe 148
Cho, Yongil 197
Christopher-Hennings, Jane 31
Chriswell, Amy 132
Chueh, Ling-Ling 83
Chung, Simon 78
Churgin, Sarah 124
Ciarlet, Max 155
Cigel, Francine 75, 76, 81
Clavijo, Alfonso 77, 81, 91, 111, 131
Clayton, Richard 177
Clemons, Bambi 61
Clothier, Kris 89
Clouse, Timothy L. 46

AAVLD Author Index

Coarsey, Michele 108
Cohen, Noah 51
Collins, James E. 155
Cook, R. Frank 80
Cooper, Jennifer 75, 76
Cooper, Vickie 197
Cornish, Todd 153
Costa, Taiana 175
Couch, Asley 176
Courchesne, Sarah 180
Crews, Amanda 118
Crocker, Dan 122
Cronin, Sheri 71
Crossley, Beate M. 71, 80, 147, 148
Crowe, Jason 192
Cui, Jing 82, 97, 98, 123, 184, 185
Cummings, Craig 196
Daniels, Joshua 123
Daniels, Risa 172
Danzeisen, Gregory 192
Das, Amaresh 81
Davidson, Michelle 44
Davies, Rebecca 113
Davis, Barbara J. 56
Davis, Bill 136
Davis, Margaret 94
DebRoy, Chitrita 87
Deliberto, Thomas J. 131
Deng, Ming Yi 150
Denney, Thomas 183
Di Giacomo, Sebastian 154
Diab, Santiago 125
Donahoe, Judy 90
Donahue, James M. 90
Donnett, Uri 86, 110
Doolittle, Kent 50
Doran, Tara 192
Drolet, Barbara 149
Drumhiller, Kathryn 103
Drury, Jana 106
Dwyer, Chris 180
Edmondson, Misty A. 183
Effinger, Lee J. 105
Egli, Christoph 201
Eiras, C. 195
Ellis, Angela 61
Ellis, Christine K. 171
Ellis, Julie C. 180
Elobeid, Amani 159
Emery, Elizabeth 44
Engel, Holger 181
Engle, Mark 49
Ensley, Steve M. 72
Erol, Erdal 51, 90, 170
Estey, Lisa 109
Evermann, James 94
Fabian, Andrew 79, 150
Fabiosa, Jacinto 159
Fadly, Aly 129
Fales, William H. 88
Farrell, Troy 97, 184, 185
Fenton, Karla A. 62
Filigenzi, Michael 33
Fitzgerald, Scott D. 45, 58
Fluegge, Denis 181
Folley, Mary J. 46
Foster, Celeste B. 147
Fraga, J. 195
Frana, Timothy 39, 119
Franca, Monique 175
Frank, Chad 187
Fratamico, Pina 87
French, Stephanie 58
Fulton, Robert W. 146
Gajadhar, Alvin A. 174
Galeota, Judith 50
Ganjam, Irene 88
Garcia, Jorge 125
Gard, Julie A. 183
Gardner, Dale 72
Gardner, Ian 107
Garner, Kevin 192
Garrison, Elina 61
Gaskill, Cynthia L. 69, 168
Gatesy, Ted 192
Gauger, Phil 132
Ge, Wayne 196
Gebhart, Connie 92, 93
Gehring, Andrew 87
Gerry, Alec 107
Gibbs, Samantha 180
Gidlewski, Tom 60
Girardin, Darryl A. 106
Glas, Patricia 194
Glazier, Mark 136
Gómez-Leal, Alfredo 199
Gonzalez, Wendy 150
Goodell, Christa K. 133, 134, 135
Goodell, Gregory M. 38
Grabenstein, Micheal 192
Gramer, Marie 155
Grau, Frederic R. 79, 152
Gregory, Gene W. 161
Grodi, Heather 45
Guerin, Michele 40
Haldorson, Gary 95
Hall, S. Mark 41
Halvorson, David A. 46, 47
Hammac, Kenitra 95
Hampson, David 42
Han, Jaeik 197
Han, Kiwon 166
Haney, Devon 132
Harding, Cindy 141
Harkin, Kenneth 52
Harmon, Aaron 145
Harmon, Karen 132, 197
Hayes, Dermot 159

AAVLD Author Index

Hayes, Jeffrey R. 123
Hegde, Narasimha 87
Helm, Michelle N. 69
Henney, Pamela J. 80
Herr, Jason 82, 97, 98
Herrick, Adam 86, 110
Higgins, Jamie K. 71
Hill, Ashley 44
Hines, Nichole 138
Hoang, Hai T. 156
Hoang, Quoc 191
Hoar, Bruce R. 147
Hodges, April 192, 193
Holder, Diane J. 81
Hollender, Elena 37
Hooser, Stephen B. 65, 67, 189
House, Jennifer 136
Howerth, Elizabeth W. 149, 175
Hsu, Hui-Min 182, 200
Hull, Noah C. 103
Ilha, Marcia Regina Da Silva 108
Irons, Aaron 132
Ishida, Maria 192
Jackson, Carney 170
Jaggi, Nicole 122
Janke, Bruce 72
Jenkins-Moore, Melinda 131, 141, 150, 151
Jeon, Albert Byungyun 188
Jin, Ling 198
Johnson, Charles D. 131
Johnson, Donna 77, 151
Johnson, Jeremy 49
Johnson, John 50
Johnson, Margaret 68, 70, 176
Johnson, Suzanne 125
Johnson, Teri L. 37
Johnston, Shirley D. 113
Jones, Megan 126
Jung, Byeongyeal 188
Kaneene, John 45
Kantar, Lee 106
Kapczynski, Darrell 139
Kaptur, Jean 177
Karen, Moran 150, 194
Kaushal, Deepak 36
Kedmi, Maor 149
Keel, Kevin 61, 129, 180
Kelleher, Susan 37
Keller, Dominique 124
Kelley, Molly 92
Kelly, Jane 173
Killian, Mary Lea 136, 138, 151
Kim, BongSuk 67
Kim, Hayoung 188
Kim, Wonil 197
Kinde, Hailu 126
Kinyon, Joann M. 119
Kitikoon, Pravina 136
Kittawornrat, Apisit 49, 50, 133
Kiupel, Matti 55, 62
Klement, Eyal 149
Koller, Sheri 48
Komine, Misa 55
Kosoy, Michael 170
Koster, Leo 131, 136, 151
Kreuder, Amanda 86
Kubiski, Steven V. 71
Kuroki, K. 88
Kuzmina, Tetiana 122
Langohr, Ingeborg Maria 55
Lawrence, John 158
Leathers, Valerie 202
Lebarbenchon, Camille 175
Lee, Fu-Chun 83
Lee, Kyunghyun 188
Lee, Myoungheon 188
Lee, Osoo 188
Lee, Pei-Yu 83
Lehmkuhl, Aaron D. 151
Lehner, Andreas 68, 70, 176
Leifholz, Sandy 181
Lenz, Stephen D. 136
Leutenegger, Christian M. 202
Leyva Baca, Ivan 105
Li, Yanqiu 80
Lichtenwalner, Anne 106
Lin, Xue 132
Lindstrom, Steve 136
Linke, Lyndsey 171
Liu, Yong 192
Lizano, Sergio 48, 49, 50, 109
Lloyd, Audra 41
Lobanov, Vladislav A. 174
Locke, Steve 170
Lopez, Pablo 178
Loretti, Alexandre P. 126, 186
Loynachan, Alan 90
Lu, Zhengchun 80
Luaces, M. 195
Luke, Kimberly 36
Lutman, Mark W. 131
Lyman, Doug 116
Lyons, Gene 122
Macias Rioseco, Melissa 57
MacLachlan, James 107
Madson, Darin 119
Maes, Roger K. 62
Magnuson, Roberta 171
Mahapatra, Debabrata 179
Main, Rodger 133, 134, 135
Maldonado-Hernández, Gizela 37, 199
Malladi, Sasidhar 46, 47
Mapes, Samantha 92, 93
Marsh, Bret 136
Marthaler, Douglas 155
Martin, Barbara M. 81, 194
Mathur, Deepa 192
Matthijnssens, Jelle 155

AAVLD Author Index

Maxwell, James 37
Mayo, Christie E. 107
Mayr, Gregory 140, 150, 194
McCollum, Matthew 171
McConnell, Terri 192
McDonough, Patrick L. 96
McDonough, Sean P. 96
McElwain, Terry 94
McGuire, Jessica 112
McIntosh, Michael T. 79, 81, 150, 152, 194
McKelvey, Jessica 169
McKenna, Thomas 81
McKenzie, Karen 37
McKinley, Enid 137
McReynolds, Sara W. 101, 102
Mead, Daniel 149
Meares, Katherine 90
Medhanie, Genet A. 40
Meier, Jennifer 77
Mestek, Anthony 109
Metz, Richard P. 131
Meyerholtz, Kim 67
Meyerholtz, Kimberly 65, 189
Michaud, Shona 109
Michel, Brady 60
Mickley, Randall 180
Miller, Myrna M. 153
Miller, Patti 139
Moeller, Robert 59, 79
Mohamed, Fawzi 79, 150
Mohammed, Hussni O. 104
Mohan, Shipra D. 37
Mohnke, Frances 192
Montgomery, Donald L. 153
Moon, Lori 29
Moon, Ounkyung 188
Morales, Paula 150
Mulhern, Erin 152
Mullens, Bradley 107
Mulrooney, Donna M. 198
Munk, Brandon 61, 112
Mutatu, Washington 70
Myers, Darcy 191
Naikare, Hemant 91, 179
Nara, Peter 110
Nelson, Eric A. 50
Nelson, Jeffrey T. 41
Nelson, William M. 134, 190
Newman, Shelley J. 118
Nezami, Sara 132
Nichols, Tracy 60
Nikooienejad, Amir 131
Nilsson, Stina 40
Nol, Pauline 171
Northington, Wade A. 168
Oberst, Richard D. 105
O'Connell, Catherine 105, 134, 191, 196
Oh, Yeonsu 166
O'hearn, Emily 150
O'Hearn, Emily S. 79
Okwumabua, Ogi 182, 200
Olsen, Chris 49, 133
O'Reilly, Kathy 198
Orlando, Brittany 98
Orloski, Kathleen 41
Ostlund, Eileen 77
Ostrowski, Stephanie R. 71, 186
O'Toole, Donal 103, 153
Overbay, Thomas 133
Pabilonia, Kristy Lynn 187
Palmer, Lee Anne 193
Palmero, Joan 71
Pantin-Jackwood, Mary 139
Panyasing, Yaowalak 133
Pappagianis, Demosthenes 125
Park, Changhoon 166
Parkinson, Anne 97, 98, 123, 184, 185
Patnayak, Devi P. 50, 130
Patterson, Bill 36
Patterson, Jon S. 58
Pearse, Hannah 201
Peddireddi, Lalitha 105
Pedersen, Janice C. 137, 138
Pedersen, Kerri 131
Perez, Andres 154
Perez, Mariano 154
Perez-Olvera, Ofelia 199
Pessavento, Patricia 126
Peters, Rachel 56
Petrosky, Keiko Y. 56
Pietrzen, Karen 192
Pillay, Silvia 190
Pinkerton, Marie E. 124
Plummer, Paul 86
Pogranichniy, Roman 50, 136
Poppenga, Robert H. 64, 66
Powell, Mindy 141
Prickett, John 49
Pronost, Stéphane 80
Puerro, Mario 42
Pugh, Brian 37
Pusterla, Nicola 92, 93
Pyburn, David 150
Quest, Dora 181
Raeber, Alex 42
Raghavan, Ram 52
Rajeev, Sreekumari 108
Raleigh, Russell 179
Ramamoorthy, Sheela 108
Ramsay, Edward C. 118
Rauh, Rolf 134, 190
Raverty, Stephen 122
Redlinger, Michelle 141
Reeves, Aaron 101, 102
Reilly, Christopher M. 71
Reilly, Thomas J. 88
Reimschuessel, Renate 192, 193
Reinisch, Alesia 179

AAVLD Author Index

Resende Bruno, Daniela 179
Rezabek, Grant 146
Rhyan, Jack 171
Rice, Anna 49, 50
Richards, Shawn 136
Ridpath, Julia F. 145, 146
Rinderknecht, Jennifer 81
Ríos y Valles-Valles, Dolores 199
Robbe-Austerman, Suelee 41, 59
Robeson, Sharon 192
Rodning, Soren P. 183
Rodriguez, Luis L. 154
Rodriguez, M. 195
Rodriguez-Martinez, Hector A. 199
Rodriguez-Reyes, Abelardo A. 199
Ronald, Norman 168
Rosario, Grisel 192
Rossow, Kurt D. 155
Roth, Lillian 181
Rowland, Fessica 150
Roza, Kristen 178
Ruder, Mark G. 112, 149
Rumbeiha, Wilson K. 68, 70, 72, 176
Sa E Silva, Mariana 139
Salman, M.D. 171
Samitz, Eileen M. 117
Sanders, Kent 183
Sanderson, Mike W. 101, 102
Sarah, Anwar J. 167
Scandrett, Brad 174
Schaaf, Stephanie L. 104
Schaefer, John J. 104
Scherba, Gail 50
Schlee, Sara 94
Schmitt, Beverly 138
Schneider, Rhiannon 98
Schoenbaum, Mark 41
Schroeder, Megan 77, 152
Schumaker, Brant 103
Schumaker, Brant A. 153
Schwartz, Kent 49, 119, 156, 197
Schwartz, Scott 131
Schwartz, Trevor 49
Sells, Stephen 80, 90, 170
Seo, Hwiwon 166
Shahrokh, Mohammad 131
Shapiro, Jan 121
Shaw, Bryanne 192
Shaw, John 150
Shepherd, Eric 139
Shlosberg, Alan 68
Simunich, Marilyn 105
Slavic, Durda 40
Smith, Cynthia R. 172
Smith, Diane 139
Smith, Jacqueline 51, 168
Smith, Jana 125
Smith, Kathryn 80
Smith, Lori L. 69
Sneed, Loyd 91
Snider, Douglas 72
Sondgeroth, Kerry 94
Song, Yong 42
Songer, J. Glenn 119
Spackman, Erica 137, 139
Spagnoli, Sean 88
Spinato, Maria 121
Spraker, Terry 60, 122
Sprowls, Robert W. 179
Stahl, Randall 171
Stalker, Margaret J. 121
Stallknecht, David 149, 175
Stanek, Danielle 37
Stankevitch, Stan 182
Steinberg, Howard 124
Steinman, Meg 51, 90
Stensland, Wendy 132
Strait, Erin 119, 132
Strasser, Jennifer 136
Struckmeyer, Thomas 194
Stuart, Kirk 68
Su, Bi-Ling 83
Su, Chen 83
Su, Hongwen 169
Sun, Dong 132
Sun, Feng 91
Swayne, David 139
Swenson, Sabrina L. 131, 136, 141, 150
Swinford, Amy K. 111
Szonyi, Barbara 111
Tahara, John 66
Tawde, Snehal N. 71, 186
Taylor, Debra R. 183
Telling, Glenn 60
Teng, Ping-Hua 83
Thomsen, Bruce V. 41, 59
Tift, Mike 122
Timoney, Peter J. 80
Tkachenko, Andriy 192, 193
Tomlinson, Sarah M. 136, 194
Toms, Dawn 141, 150
Toohey-Kurth, Kathy L. 30, 75, 76, 81
Tor, Elizabeth R. 64, 66
Torres-Velez, Fernando 79, 194
Trujillo, Jessie D. 86, 110
Tsai, Yun-Long 83
Tyrrell, Phyllis 178
Uzal, Francisco 57, 125
Uzal, Francisco A. 71
Van der Schraaf, Marian 79
Van Sickle, Joni 171
Vannucci, Fabio 92, 93
Velayudhan, Binu T. 197
Velek, Katherine 109
Venn-Watson, Stephanie 172
Ventura, Angel 150
VerCauteren, Kurt 60, 171
Vincent, Amy 136

AAVLD Author Index

Voges, Hinrich 144
Wade, Susan E. 104
Wang, Chong 49, 50, 133, 134, 135
Wang, Thomas 83
Weaver, Todd 46, 47
Webb, Patrick 159
Weisner, Mary B. 98, 123
Welles, Peter 202
Welsh, Trista 129
Westropp, Jodi L. 117
White, Holly A. 104
Whittington, Lisa 108
Whittney, Daniel 49
Wilberts, Bailey 119
Wilhite, Dewey R. 183
Williams, Neil M. 90
Wilson, Christina 65, 67, 189
Wilson, David J. 38
Wilson, Karen 192
Wise, Annabel G. 62
Witzel, Ini-Isabee 144
Wobeser, Gary A. 174
Wolf, Kathrin 181
Wu, Sih-Ying 83
Yachetti, Sarah 192, 193
Yan, Weiwei 96
Yang, Xiaoping 42
Yant, Paula 71
Yee, JoAnn 36
Yoon, Kyoung-Jin 132, 135, 156, 197
Yoon, Soonseek 188
Zabel, Mark 60
Zhang, Jianqiang 132
Zhang, Michael Z. 131
Zhang, Shuping 169
Zhang, Yan 82, 97, 98, 123, 184, 185
Zhou, Fanghong 135
Zimmerman, Jeff 49, 50, 133, 134, 135
Zinza, Jeff 191
Zurfluh, Angela 42
Zwald, Daniel 42

AAVLD Keyword Index

16S rRNA gene 97, 182, 184, 185
Abortion 88, 89
abortion 72, 90, 91, 125
Actinobacillus 165
Actinobaculum 184
Aflatoxin 67
AJS 167
alpaca 125
Analysis 64
analysis 70
Anaplasma 174
animal 177
Animal agriculture 158, 160
animal health 168
Animal Welfare 161
Antibiotic use 160
Antibody 67
antifungal 117
Antigen Capture ELISA 144
antlerogenesis 61
antleroma 61
aquatic 177
Armadillo 37
atavistic 199
Automation 181
avian 124
Avian Encephalomyelitis 187
Avian Influenza 46, 47, 137, 175
avian influenza 138
Avian Influenza virus 139
Bacteria 89, 169
bacterial indentification 39
Bartonella bovis 170
Beta Toxin type C 57
Biolog GEN III 182
Bluetongue 77, 107
Bobwhite quail 169
bone remodeling 183
Botulinum toxin 188
Botulism 188
botulism 71
Bovine 116
bovine 59, 72, 145
bovine clinical isolates 200
Bovine respiratory disease 197
Bovine tuberculosis 44
bovine tuberculosis 171
bovine viral diarrhea 146
Brachyspira 42, 119
Brachyspira spp. 40
breath analysis 171
Breeder 153
Broiler Breeder 47
Bulk Tank Milk 81
BVD 108, 147
BVDV 30, 109, 144, 191
calf scours 75
Calves 38
Camelid 198
Campylobacter 185
campylobacteriosis 91
cancer metabolism 32
Candida 117
Canine 52, 83, 88
Canine distemper virus 153
Canine Dysautonomia 103
Canine parvovirus 156
Carbamates 176
caseous lymphadenitis 184
Castor bean plant 189
cat173
Cattle 45, 188
cattle 86, 149, 170
cervid 41
chemistry 33
Chicken Jerky Treats 193
Chronic Wasting Disease 60
Classical swine fever 140
Clostridium perfringens 57
coccidioides 125
Co-infections 139
colorectal 120
Common Eider 180
Companion Animals 110
congenital 126
Copper 116
copper deficiency 58
coronavirus 62
Corynebacterium pseudotuberculosis 111
cost analysis 29
coxiella burnetii 94
Culicoides 107
deer 61, 108
dermatitis 123
Detection 67, 190
diagnosis 96
diagnostic 36
Differential panel 76
Disease Investigation 103
Disease Modeling 101, 102
Diseases 122
Dominican Republic 150
Dried blood spots 68
Drugs-of-Abuse 64
Economics 158
Eggs 161
Ehrlichia 174
EHV-1 .....80, 82
Elephant seals 122
eletronic nose technology 171
ELISA 42, 48, 49, 109, 140, 165, 189, 195
emergency exercise 121
emergency management 121
emerging virus 145
endocarditis 170
Enriched Cages 161
enteric disease 75
Enteric viruses 130

AAVLD Keyword Index

Enzootic ataxia 58
Enzyme-linked immunosorbent assay 104
Epidemiology 103, 107
epidemiology 51
Epizootic hemorrhagic disease 77
epizootic hemorrhagic disease virus 149
Equine 69
equine 71
equine herpesvirus 80
esophagus 55
European PRRSV 166
evolution 132
experimental infection 149
experimental reproduction 93
extraction 70
feral swine 131
fetal anomaly 146
fetal infection 146
Florida 37
FMD 194
FMDV 76, 81
Foetus 105
food safety 192
Foot and Mouth Disease 101, 102
Foot-and-mouth disease 154, 194
Friesian horse 55
FTA 147
Fungi 169
gamma interferon 44
gas chromatrography 176
GIS (Geographic Information Systems) 52
gopher tortoise 112
group A rotavirus 155
group B rotavirus 155
group C rotavirus 155
GS Junior 40
Hatching Eggs 47
Hematocrit 68
Hemomycoplasma 86
High Throughput 30
histological grade 56
horse 90
HPLC 66
Human health 160
hydrolysis 66
hypomyelinogenesis 58
Idiopathic 186
IgG 148
immunohistochemistry 56
infectious peritonitis 62
infertility 91
Influenza 133, 134, 135
Influenza A 196
integumentary sensory organs 199
intradermal nevus cells 199
intranasal inoculation 60
Ion PGM Sequencer 196
Iron 68
Johnes 201
lameness 183
Lawsonia intracellularis 92, 93
Leprae 37
Leptospira 96
Leptospirosis 52
leptospirosis 51, 90, 95
Lig protein 96
Listeria monocytogenes 88
liver 69
Livestock 101, 102
Low pathogenic 175
Luminex 31
lung pathology 106
lungworm 106
Lymphoproliferative Disease Virus 129
MALDI 29
Mallards 175
MAP 201
Mass spectrometry 65
mass spectrometry 39
mast cell tumors 56
Maternal Antibodies 144
megaesophagus 55
microarray 87
Milk 38
Mineralization 118, 186
minerals 69
mink 62
mix-antigen 165
mobile application 168
Molecular diagnostics 197
molecular epidemiology 32
molecular typing 92
Mortality 122
multiplex 31
multistate outbreak 98
Mycobacterium smegmatis 123
Mycoplasma 110, 178
Mycoplasma bovis 179
mycotoxins 70
myocarditis 156
myositis 173
Naked mole-rat 118
necropsy laboratory 121
neoplasia 120
new bacterium 185
Newcastle Disease virus 139
next generation 79
Next Generation Sequencing 196
next generation sequencing 131
Nucleic acid extraction 181
Nutrition 118
O serotype 87
Oral Fluid 48
Oral fluid 49, 50
oral fluid 133, 134, 135, 152
Orthomyxovirus 180
Outbreak 159
pandemic influenza 131

AAVLD Keyword Index

panel testing 197
Pasteurization 38
pathogen detection 32
Pathogen identification 181
pathology 120
PCR 78, 83, 110, 134, 179, 198, 202
PCR inhibitor 148
Pelistega europaea 97
pestivirus 140, 145
pet food 192
Pet store 153
picornavirus 151
pigeon fever 111
pine needle 72
POCKIT 83
Pork 159
portosystemic 126
Poultry 137
Production systems 158
proliferative enteropathy 93
PRRS 48, 78
PRRS Virus 190
PRRSV 49, 50
Pyrosequencing 40
pyrosequencing 82
Q-fever 94
qPCR 86
Quality Assurance 113
Quechers 176
rapid antigen detection kit 133
Real Time PCR 178, 202
Real time RT-PCR 81
realtime PCR 82
Real-time PCR 30, 105
real-time PCR 80, 138
Real-Time RT-PCR assay 190
renal health 172
Research 113
research 177
Retrovirus 129
Revenue 159
Ricin 189
Ring test 50
Risk Analysis 46
rota, corona 75
Ruminant 89
Salmonella 98
scoliosis 112
Scours 198
Sequencing 79
Serology 45, 141
serology 36, 41, 194
seroprevalence 94
Serratia 173
shunting 126
SIT 195
SIV 132, 136
skin granuloma 59
Soft Tissue 186
Sow 166
species specificity 92
specimen collection 137
spectrometry 33
storage temperature 172
Streptococcus suis 200
Surveillance 46
surveillance 192
Survey 174
susceptibility 200
swine 151, 152
Swine Dysentery 42
swine dysentery 119
Swine Health 150
Taylorella equigenitalis 97
Teschovirus Encephalomyelitis 150
test comparison 44
Toolkit 113
Toxicity 116
toxicology 33, 124
Toxicosis 64
Toxoplasma gondii 104
toxoplasmosis 104
Tremetone 65
Trichomonas 105
Trypsin 57
Tuberculosis 45, 195
tuberculosis 36, 41, 59
Turkey 129
Turkey enteritis 130
type A 71
typing 156
urinalysis 172
urinary tract 117
uvrC gene 179
Vaccination 154
Vaccine 166
vesicular 76
Vesicular Stomatitis 141
veterinary 31
Veterinary diagnostic software 168
virus 79, 152
virus isolation 135
Vitamin A 66
Wellfleet Bay 180
White snakeroot 65
White-tailed deer 60

American Association of
Veterinary Laboratory Diagnosticians

Exhibit Directory

Greensboro, NC
October 18-24, 2012

Abaxis, Inc

Booth **310**

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

AbD Serotec

Booth **212**

4350 Lassiter at North Hills Ave
Raleigh, NC 27609
www.abdserotec.com
Contact: Melinda Williams
800.265.7376
melinda.williams@abdserotec.com

AbD Serotec is the research antibody division of MorphoSys, one of the world's leading antibody technology companies. AbD Serotec offers over 15,000 antibodies, kits, and accessories. Within our catalog there are more than 2,000 products specific to the needs of Veterinary researchers. Additionally, as an antibody manufacturer, AbD Serotec provides custom monoclonal antibodies as well as large and small scale antibody production and conjugation services.

Advanced Technology Corp. VADDS

Booth **105**

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

It has been an exciting year for Advanced Technology Corp, as VADDS continues to be the #1 comprehensive LIMS system among public veterinary labs in North America and among AAVLD accredited labs.

New in 2012: Release of VADDS Version 9.0 Universa and Open Client / Runs in a web browser

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.

Biochek

Booth **109**

3 Southgate Road, Bldg 1, Unit 2
Scarborough, ME 04074
www.biochek.com
Contact: Tim Goode
207-809-9509
timgoode@biochek.com

BioChek is a worldwide leading company in veterinary diagnostics. Founded in 1996, the company specializes in supplying production animal serology kits for the poultry and swine industry. BioChek's distribution center based in Scarborough, Maine, supplies and supports numerous USDA licensed kits and affiliated equipment. For a full list of diagnostic assays available from BioChek, please contact Tim Goode, VP of Sales and General Manager at 207-809-9509 or go online at www.biochek.com.

Biolog, Inc.

Booth **106**

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.

BIOplastics/CYCLERtest, Inc.

Booth **103**

2933 South Miami Blvd., Suite 121
Durham, NC 27703
Contact: Edward Bogdanski
919.806.8811
edwardb@bpcti.com

BIOplastics/CYCLERtest Inc. is uniquely positioned to assist with two key variables in PCR/qPCR, thermal cyclers and plastic vessels. Our DRIFTCON and MTAS dynamic multichannel temperature validation systems are calibrated to ISO 17025 standards and meet accreditation requirements for thermal cycler temperature validation. The systems measure heat rate, cool rate, overshoots and undershoots, as well as accuracy and uniformity throughout the block, providing easy to use reports. DRIFTCON also recommends where to place your positive and negative controls based on your cycler's unique thermal fingerprint. When joined with our Extreme Uniformity plastic disposables (BIOplastics), you can be assured of the most consistent and reproducible results possible for your assays.

Biovet

Booth **214**

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.

Centaur, Inc.

Booth **305**

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

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

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

Computer Aid, Inc.

Booth **308**

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 3000 associate, $350 million Information Technology (IT) firm based in Pennsylvania. CAI specialized in Application Development & Application Maintenance.

USAHerds and the Michigan Department of Agriculture were proud winners of the 2011 NASCIO Technology Award. Offered by the National Agribusiness Technology Center the AgraGuard software applications assist farmers and food safety regulators in keeping our food supply safe and disease free. CAI is a certified vendor to install, maintain and enhance the AgraGuard suite of applications; USALims / USAHerds / USAPlants / USAFoodSafety. 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.

ECL2

Booth **104**

PO Box 1731
Grapevine, TX 76099
www.ECL2.com
Contact: Keith Ernst
972.983.3956
kernst@ECL2.com

ECL2 is the U.S. preferred provider of the QMS Software, Q-Pulse to the Life Sciences industry. We provide expertise well beyond our competitors by requiring that all of our QMS consultants have been Quality Managers themselves. We provide a complete solution starting from the initial sale, to delivering training and configuration and supporting you in your ongoing needs. Our consultants also provide consulting services to those that require additional guidance in developing their Quality Management Systems.

Regulatory requirements are easily managed with our user friendly application, Q-Pulse. The system comes with all of the modules integrated for a comprehensive solution. The application allows you to stay on top of training requirements, equipment maintenance, provides a document control solution, manages corrective and preventative actions as well as complaints through resolution and trending, audit management and much more. Reporting and workload management are also included.

Please stop by and see us!

GeneReach Biotechnology Corporation

Booth **213**

No 19, Keyuan 2nd 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 **215**

2604 Northridge Parkway
Ames, IA 50010
www.globalvetlink.com
Contact: Kaylen Henry
515.296.0860
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 automatically 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.

Hardy Diagnostics

Booth **309**

1430 West McCoy Lane
Santa Maria, CA 93455
Contact: Christopher A Catani, (ASCP), Rm(ACM)
800.266.2222 ext. 5696
sales@HardyDiagnostics.com

If you are doing any microbiology in your laboratory, please stop by our booth and learn how Hardy Diagnostics can help you with your microbiology supply needs, and pick up a free veterinary microbiology products catalog.

Visit our website www.HardyDiagnostics.com and become a customer today, and experience the Hardy Diagnostics difference. "A Culture of Service"

IDEXX Laboratories

Booths **203-205**

One Idexx Drive
Westbrook, ME 04092
www.idexx.com/production
Contact: Mary Spear
207.556.8059 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 **301-303**

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

Increasing production pressure and growing international trade mean that emerging diseases, new strains and changes in pathogen virulence are major challenges to animal health and production animal businesses. At Life Technologies we are continually developing new and better products so that you can give your customers the information they need to protect animals against specific disease threats and to enhance their businesses. Thanks to this commitment to pushing the boundaries of molecular technology, you have never had access to such fast, precise and reliable animal health diagnostic tools.

To see what's new this year/To learn about the latest developments, and to find out what we are working on next, visit our booth #301-303.

LIMS Pro, Inc.

Booths **201**

Palm Bay, FL
www.LIMSPRO.com
Contact: Anna Ferrari
814.237.3001
anna.ferrari@LIMSPRO.com

LIMS PRO continues to raise the bar with an inovative Ver 11 of our versatile Laboratory Information Management System (LIMS). Experience the power of a multi-tasking, multi-threaded LIMS, the professional looking reports, the power of data mining, the simplicity of conducting ad-hoc queries, the customized worklists and worksheets. You can also associate scanned images like submission forms, photos, voice recordings, even video with your cases and warehouse, track and print photographs along with your reports. All of this plus daily /weekly /monthly /yearly workload reporting, quality assurance reporting, turn-around time metrics, and much more. And let's not forget the customized billing, invoicing, and accounts receivables. We provide you with customized LIMS for your laboratory preconfigured on a server for a set fee. Please stop by our booth for a demo and speak with one of our representatives about how you can take advantage of our all-inclusive one price offering.

National Institute for Animal Agriculture

Booth **207**

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.

Orchard Software

Booth **107**

701 Congressional Blvd #360
Carmel, IN 46032
www.orchardsoft.com
Contact: Kerry Foster, Director of Marketing
800.856.1948
sales@orchardsoft.com

More than 1,000 laboratories have turned to Orchard Software for their laboratory information system. While the majority of our customers are focused on the human patient, independent veterinary laboratories and university veterinary schools also utilize our laboratory information systems.

Featuring a single shared database for clinical laboratory, microbiology, cytology, and anatomic pathology, Orchard's lab system eliminates paper and electronically integrates all departments—no need for separate systems. The shared database provides access to the animal's entire lab history and enables the addition of clinical results to the cytology or pathology report. Image management tools make it easy to link images, annotated diagrams, and scanned documents to case worksheets and incorporate them onto patient reports.

For veterinary laboratories, our software contains features for species, breeds within the species, species/breed-specific reference intervals, and demographic fields for the sex of an animal. Owner information is easily captured and accessed within the system.

Pfizer Animal Health

Booth **312-314**

5 Giralda Farms
Madison, NJ 07960
www.animalhealth.pfizer.com
Contact: Constantina Poga
973.660.5245
constantina.poga@pfizer.com

Synbiotics/Pfizer Animal Health is a leading developer, manufacturer and marketer of immunodiagnostic test platforms for reference laboratories worldwide. With over 25 years of experience, Synbiotics is recognized as a leader in diagnostics with an innovative portfolio of products-of-choice for companion and production animals. Pfizer Animal Health is dedicated to complementing this heritage with our extensive knowledge of the animal health industry and its stakeholders, from veterinarians through to the livestock farmers and pet owners they assist. Together, Synbiotics and Pfizer Animal Health are committed to ensuring the long-term health and sustainability of veterinary medicine by addressing the challenges facing the profession. Our goal is to support veterinarians along the continuum of care for disease management and advance the profession through our research and development and continuing education and training.

Prionics USA, Inc.

Booth **216**

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 the leading provider of bovine TB diagnostic solutions worldwide.

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.

PRI Bio

Booth **307**

700 Industrial Drive
Dupo, IL 62239
www.pri-bio.com
Contact: Jim Laarman
618.286.5000
jlaarman@progressive-recovery.com

PRI Bio is a global leader in Alkaline Hydrolysis Technologies (Tissue Digesters) and Effluent Decontamination Systems servicing Veterinary Research and Diagnostic Labs; Zootonic Research Facilities; Centers for Disease Control; and Pharmaceutical R&D and Manufacturing operations. For more than 29 years, PRI has advanced the science in hazardous liquid and solid waste treatment, specializing in the design and manufacture of sterilization and digester equipment. PRI's thermal products are currently used in24 countries worldwide, with over 2,900 units installed.

PRI Bio's flexible and modular Digester and EDS designs are specified for BSL4 through BSL2 facilities, including BSL3 AG/Enhanced. PRI's equipment can operate in batch or continuous flow modes, vertical or horizontal orientation, thru-the-floor or thru-the-wall configurations, with or without Containment Bioseals, and even with wet or dry discharge options. PRI Bio offers AAVLD facilities the largest array of design and configuration solutions for effluent and tissue treatment.

Explore us at www.pri-bio.com.

QIAGEN, Inc.

Booth **208-210**
19300 Germantown Road
Germantown, MD 20874
www.qiagen.com

QIAGEN is the leading global provider of sample and assay technologies that are used to transform biological materials into valuable molecular information. QIAGEN markets more than 500 products around the world, selling both consumable kits and automation systems to four customer classes: Molecular Diagnostics (human healthcare), Academia (life sciences research), Applied Testing (forensics, veterinary testing and food safety), and Pharma (pharmaceutical and biotechnology companies).

Remel – see Thermo Scientific

SafePath Laboratories, LLC

Booth **209**
5909 Sea Lion Place, Suite D
Carlsbad, CA 92010
www.safepath.com
Contact: Steve Ness
949-436-8854
steve@safepath.com

SafePath Laboratories is a USDA Biologics licensed manufacturing facility that also operates under FDA GMP's. SafePath Laboratories 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, campylobacter antigen and giardia/cryptosporidium direct fluorescent assay.

SAGE

Booth **313**
2455 Teller Road
Thousand Oaks, CA 91320
www.sagepub.com
Contact: Lisa LaMont
805.410.7239
lisa.lamont@sagepub.com

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

Tetracore, Inc.

Booth **111**
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 CSFV, in addition to specific detection reagents for PRRSV, FMDV, *West Nile virus*, ASFV and others. Please visit our booth to see the T-COR 4 – an all-new real-time PCR thermocycler. Featuring four independent sample wells with multiplex capability, it is small, highly portable, completely self contained, and has an 8-hour (rechargeable) battery life. We would also like to announce our brand new qPCR PRRSV reagents, EZ-PRRSV MPX 4.0. A new faster, easier to use assay, with true differentiation between NA and EU PRRSV strains, EZ-PRRSV MPX 4.0 improves on NextGen PRRSV Multiplex's coverage and is available at a lower price.

Thermo Scientific

Booth **211**

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

Thermo Scientific Veterinary Solutions are setting global standards for high quality susceptibility testing products, reagents and automated Johne's Disease detection in veterinary microbiology laboratories, as well as a comprehensive range of media, disks, strips, and diagnostic kits. In fact, Thermo Scientific Sensititre susceptibility testing products are the system of choice for global surveillance programs, including NARMS. Other signature product lines include Thermo Scientific PathoProof Mastitis PCR Assays, the new standard in bovine mastitis testing, and the Thermo Scientific *para*-JEM System for automated Johne's Disease detection.

Trek Diagnostics – see Thermo Scientific

VertiQ Software LLC

Booth **311**

135 E. Main Avenue, Suite 150
Morgan Hill, CA 95037
www.vertiq.com
Contact: Paula Lomanto
Cheryl Rossi
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 **306**

4641 Pullman Albion Road
PO Box 502
Pullman, WA 99163
www.vmrd.com
Contact: Michelle Nay or Ed Felt
800.222.8673
vmrd@vmrd.com

VMRD was founded in 1981 by D. Scott Adams, DVM, PhD, and currently employs approximately 50 researchers, lab technicians and support personnel. From its site in Pullman, 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.

AAVLD/USAHA Upcoming Meetings

2013: October 17-23
San Diego, California

2014: October 16-22
Kansas City, Missouri

Exhibits and Poster Presentations

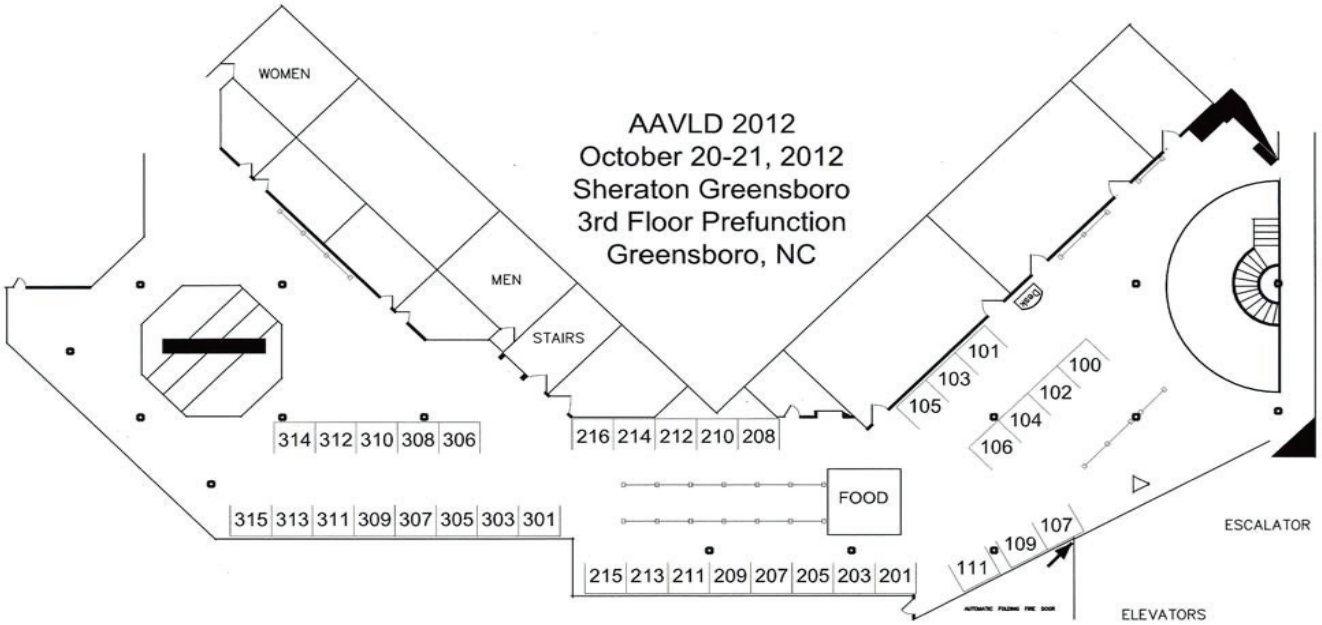


Exhibit Hall Times

Saturday, October 20

9:10 am - 9:40 am
11:30 am - 1:00 pm
3:00 pm - 6:00 pm

Sunday, Saturday 21

9:15 am -10:15 am
11:15 am -2:00 pm

Poster Session

3:00 pm Friday, Oct 19, *through*
2:00 pm Sunday, Oct 21

Saturday, October 20, 2012

3:00-4:00 pm Authors present

Booth #	Company	Booth #	Company
312 / 314	Pfizer Animal Health	315	ISWAVLD 2013 Conference
310	Abaxis, Inc. - Animal Health	313	SAGE
308	Computer Aid, Inc.	311	VertiQ Software, LLC
306	VMRD, Inc.	309	Hardy Diagnostics
216	Prionics	307	PRI Bio
214	Biovet Inc.	305	Centaur, Inc.
212	AbD Serotec	301 / 303	Life Technologies
208 / 210	Qiagen, Inc.	215	GlobalVetLINK
105	Advanced Technology Corp.	213	GeneReach Biotechnology Corp
103	Bioplastics/Cyclertest, Inc	211	Thermo Scientific
104	ECL2/Q-Pulse	209	Safe Path Laboratories, LLC
106	Biolog, Inc.	207	Nat'l Institute for Animal Agriculture
		203 / 205	IDEXX Laboratories
		201	LIMS Pro, Inc.
		111	Tetracore, Inc.
		109	BioChek
		107	Orchard Software Corp.

Sponsor Presentations

Saturday – October 20, 2012

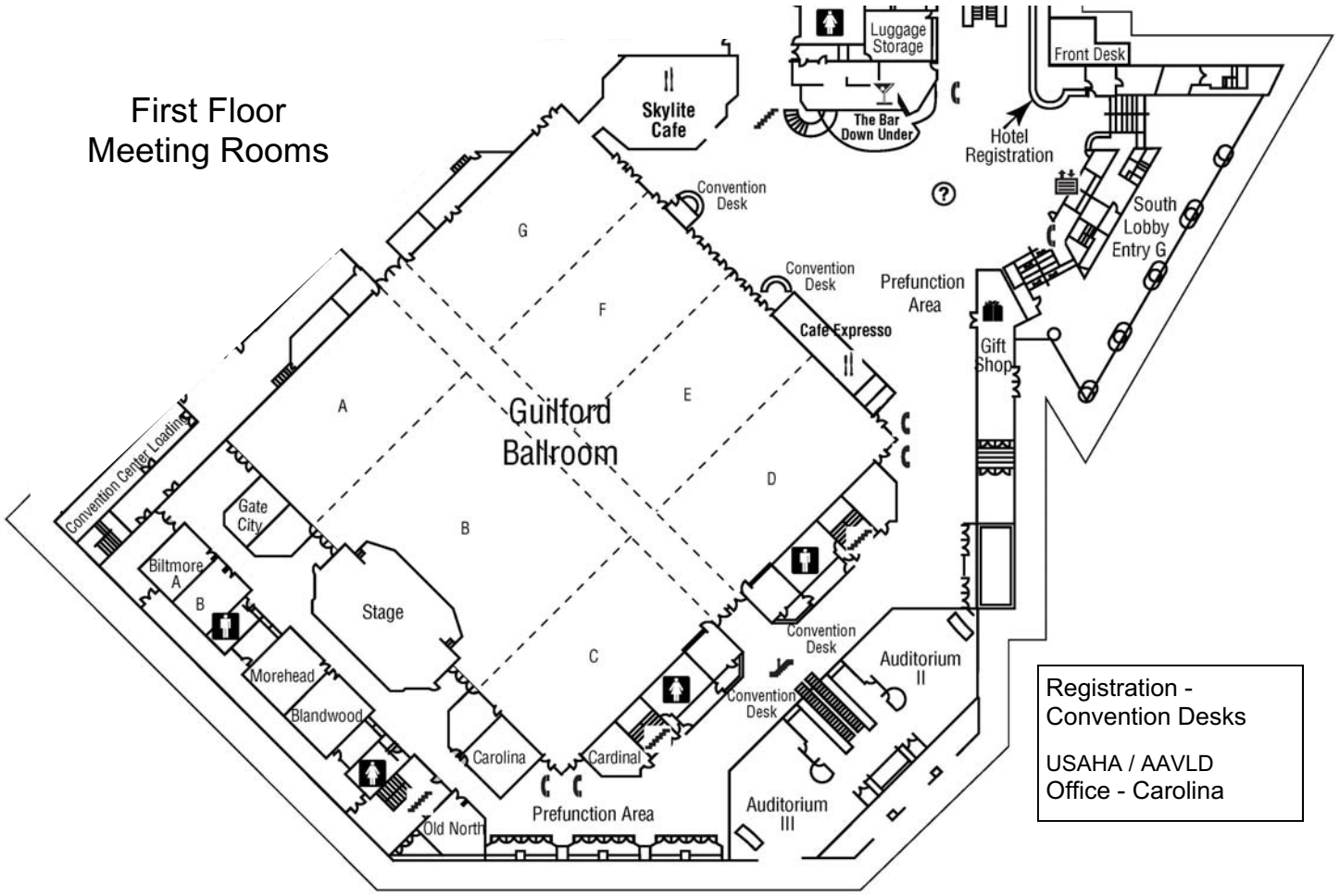
Pfizer Animal Health	6:00-6:30 pm	Guilford D	Rapid On-Farm Detection of Swine Influenza Virus
VMRD, Inc.	6:00-6:30 pm	Guilford E	VMRD Equine Arteritis Virus cELISA: Rapid and Robust Sero-diagnostic Tool for EAV
Prionics	6:30-7:00 pm	Guilford D	PrioCHECK® PregAffirm - A Milk-based Bovine Pregnancy Test
QIAGEN	6:30-7:00 pm	Guilford E	An opportunity to meet and talk with QIAGEN representatives

Upcoming AAVLD/USAHA meetings:

San Diego, CA
October 17-23, 2013

Kansas City, MO
October 16-22, 2014

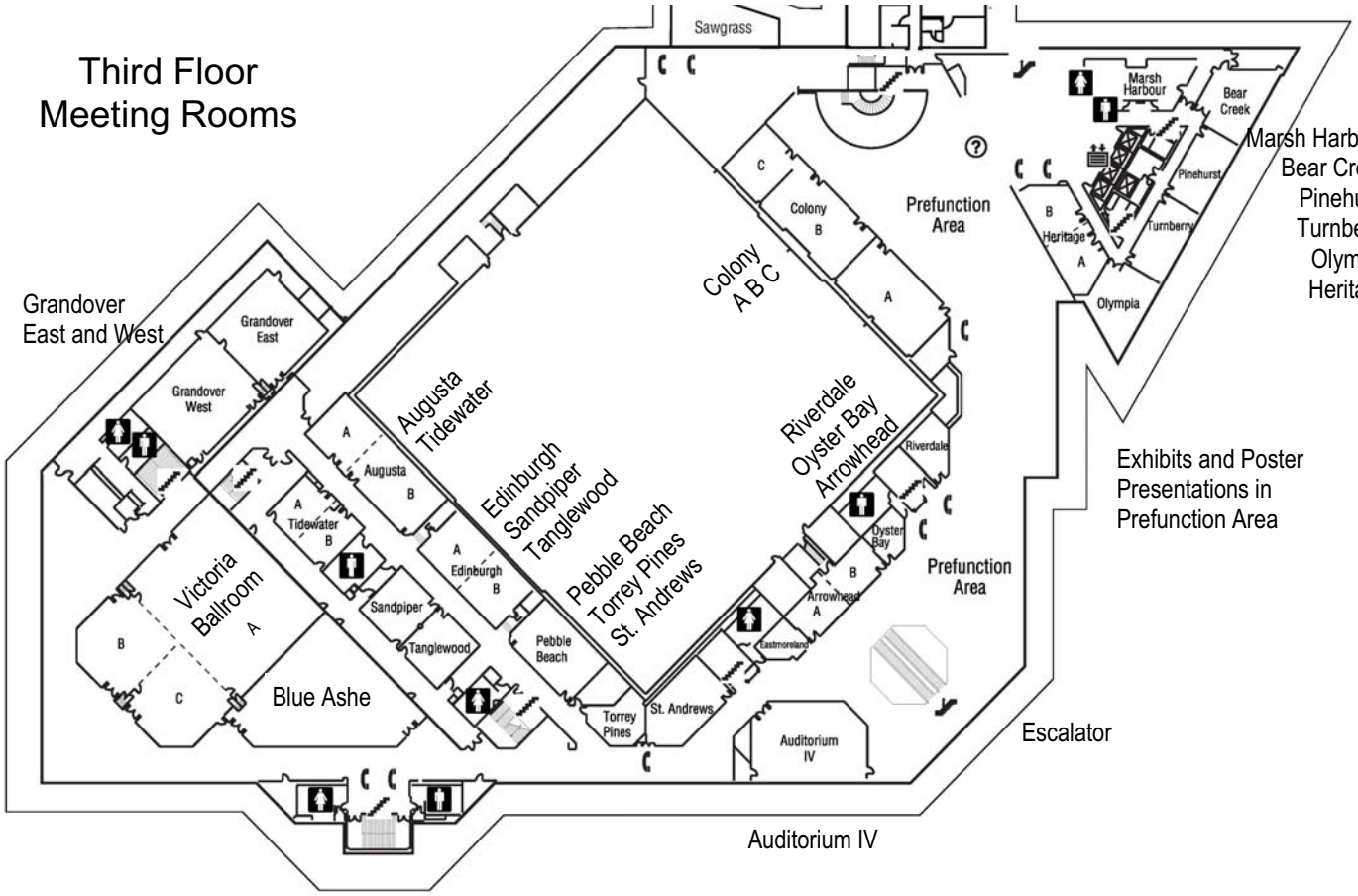
First Floor Meeting Rooms



Registration - Convention Desks

USAHA / AAVLD Office - Carolina

Third Floor Meeting Rooms



Marsh Harbour
Bear Creek
Pinehurst
Turnberry
Olympia
Heritage

Exhibits and Poster Presentations in Prefunction Area

Auditorium IV